97-205-01p
Petition

AgrEvo®

Petition for Determination of Nonregulated Status: Glufosinate Tolerant Canola Transformation Event T45

The undersigned submits this petition under 7 CFR 340.6 to request that the Director, BSS, make a determination that the article should not be regulated under 7 CFR 340.

Submitted by:

Vicki Forster July 18, 1997

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Contains No Confidential Business Information

IMPORTANT!
Before reading this petition document it is recommended that the reviewers/readers first read the entirety of The Companion Summary to this Petition: Glufosinate Tolerant Canola: (N-acetyl-L-phosphinothricin: metabolic product) Canola Lines pHoe 4/Ac. Environmental Safety Assessment Background Volume 1, January 23, 1996, MacDonald, R. Basis for Selectivity. This document was submitted to Agriculture and Agri-Food Canada as the summary document for environmental safety clearance of T45 canola in Canada. The Companion Summary follows the petition beginning on page 37.

SUMMARY

AgrEvo USA Company herewith submits a Petition for Determination of Nonregulated Status to The Animal and Plant Health Inspection Service (APHIS) for Glufosinate Tolerant Canola Transformation Event T45. AgrEvo requests a determination from APHIS that Glufosinate Tolerant Canola Transformation Event T45, and any progeny derived from crosses of event T45 with traditional canola varieties, and any progeny derived from crosses of event T45 with transgenic canola varieties which have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. Event T45 is considered a regulated article because it contains sequences from the plant pests, cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens,* and was transformed using the plant pest *A. tumefaciens.*

Glufosinate-ammonium is in the glutamine synthetase inhibitor class of herbicides. It is a non-systemic, non-selective herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. Glufosinate-ammonium controls weeds through the inhibition of glutamine-synthetase, which leads to the accumulation of phytotoxic levels of ammonia in the plant. Glutamine-synthetase is the only enzyme in plants that can detoxify ammonia released by photorespiration, nitrate reduction, and amino acid degradation.

Transformation event T45 is canola, *Brassica napus*, material containing a stably integrated gene which encodes phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyzes the conversion of L-phosphinothricin (PPT), the active ingredient in glufosinate-ammonium, to an inactive form, thereby conferring tolerance to the herbicide. The *pat* gene in event T45 is a synthetic version of the native gene isolated from *Streptomyces viridochromogenes*, strain Tü 494. The nucleotide sequence has been modified to provide codons preferred by plants without changing the amino acid sequence of the enzyme. The gene was introduced into canola protoplasts using disarmed *A. tumefaciens*. Southern blot and analyses show that event T45 contains a single, stably integrated copy of the *pat* gene. Southern blot analyses also indicate that the incorporation has been limited to DNA sequences contained within the T-DNA borders.

Genetically engineered glufosinate-tolerant canola will provide a new weed management tool to canola growers. Glufosinate-ammonium is currently registered in the United States as a herbicide for both non-crop and crop uses. It is registered as FINALE® for non-crop uses, and it is registered as RELY® for use on trees, nuts and vines, REMOVE™ for seed propagation use, cur-

rently on corn and soybean, and as LIBERTY™ for crop use, currently on corn and soybean. It is biodegradable, has no residual activity, and has very low toxicity for humans and wild fauna. Glufosinate-tolerant canola may positively impact current agronomic practices in canola by, **1)** offering a broad spectrum, post-emergence weed control system; **2)** providing the opportunity to continue to move away from pre-emergent and residually active compounds; **3)** providing a new herbicidal mode of action that allows for improved weed resistance management in canola acreage; **4)** offering the use of an environmentally sound and naturally occurring herbicide; **5)** encouraging herbicide use on an as needed basis; **6)** decreasing cultivation needs; and **7)** allowing the application of less total pounds of active ingredient than used presently in canola.

Transformation Event T45 has been field tested in the United States. In 1996 nineteen (19) field trials were conducted during the growing season in primary canola growing states under USDA permit 96-057-01r. An additional 20 (approximately) trials are being conducted under permit authorizations 97-015-01r and 97-035-05r during the 1997 growing season. Event T45 has also been extensively field tested by AgrEvo Canada, Inc. during 1995-1996 in the primary canola growing regions in Canada. Ninety-five (95) field tests were conducted under Agriculture Canada authorization during 1995. In 1996 an additional 53 field tests were conducted. No authorization was necessary as T45 had environmental clearance in Canada. Transformation event T45 has also been field tested in Chile, Japan, the United Kingdom and Australia.

Data collected from field trials, laboratory analyses, and literature references presented herein demonstrate that glufosinate-tolerant canola event T45:

- exhibits no plant pathogenic properties,
- is no more likely to become a weed than non-modified canola,
- is unlikely to increase the weediness potential of any other cultivated plant or native wild species,
- does not cause damage to processed agricultural commodities,
- is unlikely to harm other organisms that are beneficial to agriculture.

Transformation event T45 has been selected for commercial development. It has been crossed with available traditionally derived canola lines and cultivars. The primary transformation event T45 and its progeny are collectively referred to as glufosinate-tolerant canola T45 in this petition.

AgrEvo USA considers recent actions by the Canadian government relevant to the evaluation of this petition. In 1995 and 1996, the Canadian government cleared seven lines of herbicide-tolerant canola for commercial use. Three glufosinate-tolerant canola lines have received clearance from Agriculture and Agri-Foods Canada (AAFC) and Health Canada: two from AgrEvo Canada; DD95-01 and DD96-11, respectively; and one from, Plant Genetic Systems, male sterile; DD95-04. Three lines of imidazolinone-tolerant canola from Pioneer Hi-Bred (derived from mutation breeding); DD95-03 and one line of glyphosate-tolerant canola from Monsanto; DD95-02 have also been reviewed and cleared. For each of these evaluations, the AAFC considered the following environmental and agricultural issues as per regulatory directive Dir94-08:

- potential of the herbicide tolerant canola to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybird offspring may become more weedy or more invasive,
- potential of the herbicide tolerant canola to become a plant pest,
- potential impact of the herbicide tolerant canola or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

The feeds section of AAFC considered safety for use as animal feed and Health Canada reviewed safety data for human food use. All seven of the herbicide-tolerant canola lines were found to be as safe as their counterparts. The subject of this petition, transformation event T45 was one of the three glufosinate-tolerant canola lines granted clearance by the Canadian agencies.

Consultation with the Food and Drug Administration (FDA) is underway regarding the food and feed safety of event T45. The food and feed safety assessment summary was submitted to the FDA in early June 1997.

A submission to extend the tolerance to canola will be made to the EPA in 1997. LIBERTY™ (glufosinate-ammonium) is already registered with the EPA for use on glufosinate tolerant corn and soybean.

CERTIFICATION

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.

Vicki Forster July 18, 1997

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ACRONYMS AND SCIENTIFIC TERMS

AAFC: Agriculture and Agri--Food Canada

Ac-PPT: acetylated phosphinothricin

CaMV: cauliflower mosaic virus

CODEX: abbreviation for *codex alimentaris* (from the Latin). WHO international code of regulation for residues in food

CO2: carbon dioxide

ELISA: enzyme linked immunosorbant assay

DNA: dioxyribonucleic acid

GRAS: generally regarded as safe

IUPAC: International Union of Pure and Applied Chemistry

NaOH: sodium hydroxide

PAT: phosphinothricin acetyltransferase

***pat*:** phoshinothricin acetyltransferase gene (origin *Streptomyces viridochromogenes*)

PCR: polymerase chain reaction

PPT: phosphinothricin

SDS: sodium dodecylsulfate

T-DNA: transformed DNA

WCC/RRC: Western Canada Canola/Rapeseed Recommending Committee

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I. Overview

IMPORTANT!
Before reading this petition document it is recommended that the reviewers/readers first read the entirety of The Companion Summary to this Petition: Glufosinate Tolerant Canola: (N-acetyl-L-phosphinothricin: metabolic product) Canola Lines pHoe 4/Ac. Environmental Safety Assessment Background Volume 1, January 23, 1996, MacDonald, R. Basis for Selectivity. This document was submitted to Agriculture and Agri-Food Canada as the summary document for environmental safety clearance of T45 canola in Canada. The Companion Summary immediately follows the petition beginning on page 37.

A. Statement of Grounds for Nonregulated Status: Glufosinate Tolerant Canola Transformation Event T45

AgrEvo requests a determination from APHIS that Glufosinate Tolerant Canola Transformation Event T45, and any progeny derived from crosses of event T45 with traditional canola varieties, and any progeny derived from crosses of event T45 with transgenic canola varieties which have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. In this petition, data from extensive field testing in Canada and supplemental testing in the United States, as well as peer reviewed, and published information on other transformation events of glufosinate tolerant canola will be used to support our position that glufosinate tolerant canola is neither a plant pest nor presents a risk to the environment. AgrEvo requests that the USDA take into consideration the Decision Document, DD96-11 (Attachment IV) prepared by Agriculture and Agri-Foods Canada (AAFC) that found the unconfined release of glufosinate tolerant canola, line HCN28 (derived from transformation event T45) to be considered safe. The line has subsequently been given clearance for feed use by AAFC, DD96-11 Supplement (Attachment V), and ruled as safe for food use by Health Canada in a letter of authorization dated February 17, 1997 (Attachment VI).

Transformation event T45 canola has been genetically modified to contain the *pat* gene. This gene confers tolerance to glufosinate-ammonium, the active ingredient in the broad spectrum, non-selective herbicide marketed for selective use on glufosinate tolerant crops as LIBERTY™. Glufosinate tolerant canola, which will be marketed as LIBERTY LINK™ canola, will allow growers to adopt a more sustainable agriculture program by allowing for broad spectrum weed control with one postemergent herbicide treatment during the growing season.

This Petition for Nonregulated Status will demonstrate that glufosinate tolerant T45 canola is neither a plant pest nor presents a risk to the environment because:

- **T45 canola exhibits no plant pathogenic properties,**
- **T45 canola does not demonstrate weediness characteristics,**
- **T45 canola is not harmful to beneficial organisms,**
- **T45 canola may outcross but will not create persistant populations of glufosinate tolerant *Brassica* species hybrids, and**
- **T45 canola will not cause damage to processed agricultural commodities.**

B. Definition of T45 Canola and Field Trial History

In this submission, event T45 is represented by canola line HCN 28. Event T45 was produced through *A. tumefaciens* mediated transformation of variety AC EXCEL with the pHoe4/AC construct. AC EXCEL is a cultivar grown in Western Canada and produced by Agriculture Canada plant breeders in Saskatoon, Saskatchewan. Event T45 was selected on the basis of its tolerance to the herbicide LIBERTY™ (glufosinate-ammonium). HCN28 was derived from T45 by three crosses of the original transformant to the cultivar AC EXCEL. After the last backcross, the segregating material was handled through a breeding procedure called single seed decent for three generations (to the F3 generation). After the F3 generation, breeding proceeded through the pedigree method to result in the final product, HCN28. The original HCN28 plant was selected on the basis of herbicide tolerance, oil content, protein content, erucic acid and glucosinolate levels. HCN28 was advanced on the basis of its agronomic performance.

Other lines derived from transformation event T45 include HCN27 and HCN25. HCN 25 is different from HCN28 in that HCN25 was a selection from the selfed generation of the original transformant (no further crossing) and HCN25 was crossed to AC EXCEL twice followed by another cross to a breeding line called AGO13. HCN27 is very similar to HCN25 differing only in a third cross to AC EXCEL as in the case of HCN28, rather than to AGO13.

The field data presented in this petition are from the 1995, 1996, and beginning 1997 growing seasons. Transformation event T45 has been field tested extensively. During the 1995 and 1996 growing seasons T45 was tested at a combined 150 sites in Western Canada. During the 1996 growing season, T45 was field tested in the USA under permit authorization 96-057-01r at 19 sites, primarily in the northern tier of states which border Canada (see termination report in Attachment II). T45 is also being field tested at approximately 20 sites in the USA during the 1997 growing season under permit authorizations 97-015-01r and 97-035-01r (see 1997 preliminary termination report in Attachment II). In addition, transformation event T45 has been field tested in Chile, Japan, the United Kingdom and Australia. No unexpected results were observed in trials in these countries. In all cases agronomic performance and plant morphology was similar to non-transgenic counterparts.

II. *The Parent Plant: Canola (Brassica napus), and Related Weed Species in the United States*

Canola, *B. napus*, is a recognized generic crop derived from its parent, rapeseed. Canola grown commercially in the United States is comprised of the *napus* species. Canola grown in Canada and other northern climes consists of both the *napus* and *rapa* species. Canola differs from rapeseed grown earlier in this century in that both the erucic fatty acid (22 carbon atom chain) content and the glucosinolate content are much lower compared to that of rapeseed. Glucosinolates are sulfur containing compounds which are found in all cruciferae and give a distinctive flavor and odor found in the related cole vegetables, radishes, mustards, turnips. The lower erucic acid and glucosinolate content give canola nutritional advantages over rapeseed. Canola is a trademark term in Canada that is presently defined as seed, oil and meal from *B. napus* and *B. rapa* plants which contain no more than 2% erucic acid in their seed oil and no more than 30 μmoles of aliphatic glucosinolates in the oil-free, moisture-free meal (Canola Council of Canada, 1990). Commercially harvested *B. napus* canola averages 0.5% erucic acid and 10-15 μmoles of glucosinolates and commercially harvested *B. rapa* canola averages 0.5% erucic acid and 15-20 μmoles of glucosinolate. Canola oil is one of the lowest in saturated fats of all edible oils. It is used in cooking and salad oils, shortening, margarine, coffee creamers and prepared foods. In 1987 the American Heart Foundation named it "Food Product of the Year".

The United States Canola Council reported 333,000 acres planted in the U.S. in 1994, with the two leading states being North Dakota (129,000 acres) and Montana (50,000 acres). It was estimated that the U.S. canola market would be 600,000 acres in 1996, with North Dakota alone growing 400,000 acres. While the majority of canola (spring varieties) grown in the U.S. is in the northern region (bordering to Canada), spring varieties are also grown in the southeast (Alabama, Georgia and North Carolina) during the winter.

According to the USDA, in 1994, ten (10) states grew more than 1% of the total canola (spring variety) grown in the USA for production. These top ten canola growing states were (relative % are given in parentheses following the state):

1. North Dakota (38.7%)
2. Montana (15.1%)
3. Idaho (12.5%)
4. Minnesota (9.3%)
5. Washington (8.6%)
6. Georgia (5.1%)
7. Oregon (3.5%)
8. Alabama (2.2%)
9. Colorado (1.7%)
10. South Dakota (1.6%)

Total: 98.3% of all canola grown in USA for production.

In these states the only weed species related to *B. napus*, which are listed on a state weed list as a noxious, prohibited or secondary weed, are wild mustard (*Sinapis arvensis*, syn. *Brassica kaber*) and wild radish (*Raphanus raphanistrum L.*). Wild mustard is listed as a secondary weed in the state of Minnesota, and wild radish and wild mustard are listed as secondary weeds in Alabama. Hybrids are not formed between *B. napus* and *B. kaber* (Bing, 1991, Olsson, 1960). Field hybrids between *B. napus* and *R. raphanistrum L.* are highly unlikely (Bing, 1991).

A thorough discussion of outcrossing potential of *B. napus* to related species and the consequences thereof can be found in section IV.F.2. on pages 19-31. A comprehensive discussion and review of rapeseed (*B. napus*) taxonomy, biology and weed characteristics can be found in Agriculture and Agri-Food Canada: Regulatory Directive Dir94-09. The Biology of *Brassica napus L.* (Canola/Rapeseed). (Attachment III).

III. Transformation and Molecular Characterization of Transformation Event

A. Transformation System

Protoplasts isolated from the cultivar *B. napus* var. AC EXCEL were transformed using disarmed *A. tumefaciens* carrying the pHoe4/Ac plasmid. A map of the plasmid is given on page 49 of the Companion Summary. These protoplast cultures were then incubated. Following incubation *A.tumefaciens* was washed out of the protoplast cultures and the cultures were reincubated. After several days of the second incubation period, the protoplast cultures were washed with feeder medium. This procedure was followed until microcolonies had formed. At this point protoplast cultures were embedded in agarose. Later, colonies were put into an enrichment medium to selectively enrich for *pat* positive colonies. Surviving colonies were then placed onto regeneration medium. Regenerated shoots were then transferred onto a rooting medium until normal plantlets appeared. Transformed plants were potted in a soilless mix and placed in a growth chamber (Wang, 1996) .

B. Plasmid Used for Transformation and Molecular Characterization of Event T45.

For information about the transformation plasmid, pHoe4/Ac, and its open reading frames and associated regulatory regions, please refer to pages 46-49 of the Companion Summary. The *pat* gene nucleic acid sequence is found on page 47 of the Companion Summary, whereas the PAT amino acid sequence and content are found on pages 46-47 of the Companion Summary. Plasmid pHoe4/Ac has been disarmed. The *pat* gene is regulated by a promoter and terminator from the CaMV *35S* gene. No other DNA from CaMV was inserted. Other molecular evidence support the claims that the: **1)** inserted T-DNA is stable over many generations (Appendix 1, pages 2-8); **2)** only one copy of the T-DNA has been inserted from pHoe4/Ac (Appendix 2, pages 2-9); and **3)** no *Streptomycin/Spectinomycin* antibiotic resistance marker gene or other sequences found outside the T-DNA borders has been inserted (Appendix 3, pages 4-9).

C. Expression of PAT in Seed

Trials were conducted with the *B. napus* nontransgenic variety AC EXCEL and the transgenic line HCN28 at Indian Head, Rosthern, and High Bluff, in Saskatchewan, Canada. Tabular results of locations analyzed are summarized as follows:

Comparison of PAT Levels in *B. napus* Seed

Line	PAT Protein (ug/mg)	Protein/Sample (ug/g)	PAT/Sample (ug/g)	Replications
EXCEL	ND	56 ± 14	ND	13
HCN 28	4.3 ± 1.7	43 ± 12	171 ± 79	11

The PAT content of seed from the glufosinate tolerant canola line HCN28 varied somewhat from location to location. The seed was analyzed for PAT using enzymed linked immunosorbant assay (ELISA) analysis.

IV. Potential for Environmental Impact from Noncontained Use of Event T45

A. Introduction

In this section, we will address the interaction of T45 canola with the environment and show that it is unlikely to have an effect on agriculture or in natural areas which is different from that of growing of nontransgenic canola.

T45 canola has been genetically modified to be tolerant to the non-selective herbicide active ingredient glufosinate-ammonium. This is the only characteristic different between T45 canola and related commercial varieties. With regards to the characteristics of weediness, plant pathogen properties, outcrossing potential, effects on beneficial organisms and nutrition/composition parameters T45 canola is equivalent to other commercial varieties on the market.

For experimental detail, see individual Environmental Impact studies which are referenced in discussions below. No environmental impact concerns have been identified for the U.S. that are different from those addressed in the referenced Canadian documents.

The majority of the data presented in this petition are from Canadian trials. These data are applicable for a petition to the United States Department of Agriculture in support of a Nonregulated Status of Glufosinate-Ammonium Tolerant Canola for the following reasons:

1) The environment (landscape, geography, soil type, climate and weather patterns, plant and weed species, growing season) is essentially the same in Canada where these trials were conducted to the principle canola growing regions of the U.S., including Minnesota, North Dakota, South Dakota, Montana, Idaho, Oregon, Washington and Colorado.

2) For the U.S. regions where spring canola varieties are planted in the Fall (California, Alabama and Georgia) no negative environmental impact can be anticipated as the weed species and

non-target organisms have been addressed in documentation presented here. (See pages 15, non-targets and beneficials, and 19-31, weed species, respectively).

3) Field observations of trials conducted in the U.S., in the major canola growing regions, substantiate data presented in this submission (Attachment II).

B. Current Agricultural Practices and How Glufosinate Ammonium could Alter Them

Canola fits very well into a rotation with other crops such as small grains, corn, alfalfa and potatoes. To avoid potential disease build-up canola should not be planted immediately following a previous canola crop. It is recommended that canola be planted only once in any four year rotation cycle.

Canola is resistant to many of the diseases and insect pests that can affect yield in small grains. Rotating with canola enables growers to break disease and insect cycles that become a problem in many continuous cropping situations. Most diseases are kept under control with proper crop rotation and/or disease resistance being bred into the various canola varieties.
The occurrence of problems due to insect pests has not been a major concern in most regions of the U.S. canola production. Flea Beetle is the most common insect pest that feeds on newly germinated canola causing stunted plants and death of the seedlings. The addition of canola in a cropping rotation will also help eliminate certain weed problems associated with continous cereal grain production. Grass type weeds can be substantially reduced when canola is grown in rotation with cereal grains. Subsequent volunteer canola can also be easily controlled when followed by a cereal grain crop by using broadleaf herbicides (2,4-D, MCPA, and several sulfonylurea type herbicides) normally used in small grains. Producers should avoid close rotations to crops susceptible to sclerotina (sunflowers, dry beans).

In sufficient numbers, weeds can significantly reduce canola yields, quality and ease of harvest. Few herbicides are registered for use in canola in the United States. Treflan is a preemergent herbicide that controls some grass and broadleaf weeds, however, this product has been used so extensively in small grain production some weeds have developed resistance to this herbicide (i.e. foxtail a major weed in northern growing areas). In addition, Treflan does not control wild mustard which is wide spread in cereal growing areas and is one of the weeds that reduces canola quality as it's seeds are similar to canola seed and can not be removed from canola. Two other products registered for use in the United States are Ultima 160 and Assure II, which are herbicides that will control grass weeds in canola. These do not have an effect on broadleaf weeds.

Glufosinate-ammonium will provide control most annual grass and broadleaf weeds in glufosinate-ammonium resistant canola including wild mustard and Treflan resistant foxtail. In addition, glufosinate-ammonium will provide a different herbicide mode of action in the growers crop rotation, which is important in preventing the build up of herbicide resistant weeds. Glufosinate-ammonium is applied like any other postemergent herbicide used in any other crop. Herbicide drift from an application of glufosinate-ammonium will not cause any greater harm than herbicides currently used. Both Ultima 160 and Assure II will severely injure or kill small grains growing

near a sugar beet field if drift occurs. Similarly, a sulfonylurea herbicide application to small grains will kill sugar beets if wind carries the herbicide to a neighboring field of sugar beets.

Canola can be harvested by either direct combining or by first swathing (cutting the standing plants and gathering into rows) and then combining at a later date. The preferred method for any particular grower will depend on prevailing weather conditions and the equipment available for harvesting. After canola has been harvested, the standing stubble would be left to dry before it is worked into the soil with a disc or field cultivator.

From the evidence provided above we conclude that T45 canola will not alter agricultural management practices now carried out in nontransgenic (conventional) canola. Glufosinate tolerant canola could have a positive impact on current agricultural practices since less chemical can be applied to achieve weed control. Potentially one post-emergent application of the broad spectrum LIBERTY™ Herbicide is necessary to achieve weed control in fields containing LIBERTY LINK™ canola. Like its parent, T45 canola should not establish populations outside of agriculturally managed areas.

C. Agronomic Traits and Quality Characteristics

Extensive field evaluations in the United States and in Canada, have shown that glufosinate-tolerant canola is no different from its counterpart (see Companion Summary pages 60-64). Also see Attachment II and Appendix 6 pages 2-3 .

D. Plant Pathogenic Properties

Extensive field evaluations have shown that glufosinate-tolerant canola has no change in its disease and pest susceptibility characteristics (see Companion Summary pages 84-89) and Attachment II.

E. Effects of T45 Canola on beneficial organisms

No negative effects on non-target organisms were observed or were expected since T45 canola expresses a protein which belongs to a family of enzymes which are ubiquitous in nature, and shares no homology with proteins that are known to be toxic (Eckes, 1994). This is substantiated by the following: **1)** no change was observed in honey bee behavior, honey production, hive development and subsequent canola crop, as compared to bees feeding on nontransgenic canola (Appendix 13, pages 1-7); **2)** T45 canola does not exhibit residual effects on rotated crops as evidenced in Canadian residual effects studies (Appendix 12, pages 2-15); and **3)** T45 canola has been determined to be safe for animal feed (AAFC DD96-11, Supplement, Attachment V), and human food (Health Canada letter dated 2/17/97, Attachment VI).

F. Potential to cause damage to processed agricultural commodities

There is a low level of PAT protein in the seed from T45 (Section III. C.). Through processing of seed to provide refined oil for human consumption and seed meal for animal consumption the enzyme is denatured or removed (data not submitted). Should there be any PAT remaining after these treatments the only route of exposure is oral. However, AgrEvo has experimentally confirmed that the PAT enzyme does not have the characteristics of an allergen or toxin. It is acid and heat labile (see page 50 of the Companion Summary) and contains no glycosylation motifs (Eckes, 1994). The protein has no homology to proteins other than *pat* genes from other organisms (Eckes, 1994). The substrate specificity for the PAT enzyme is very strict in that the only substrate is L-PPT. Neither any protein amino acid nor D-PPT is acetylated by PAT (see Companion Summary pages 52-53). Acetyl transferases are abundant and ubiquitous in nature where they share the common function of transferring an acetyl group from acetyl- CoA. Acetyl transferases differ in substrates and the metabolic pathways in which they function (Webb, 1992). Based on: **1)** the substrate specificity of PAT; **2)** the physicochemical properties of PAT; **3)** the rapid degradation of PAT upon ingestion (see Companion Summary page 54); and **4)** the ubiquitous presence of acetyl transferases in nature, no adverse effects are predicted if the PAT enzyme is a minor constituent of human and animal food.

G. Weediness and Gene Transfer Potential

Much of the data referenced under specific points **1.**, **2.** and **3.** below, are discussed on pages 61-81 in the Companion Summary. These data are from Canada and are relevant to the United States because weedy *Brassica* relatives and the potential for outcrossing in Canada are very similar, and in most cases the same as in the USA. (See information given under point **2.** beginning on page 19, and information given on pages 81-86 in the Companion Summary.)

1. Weediness potential

Two (2) sets of extensive data support our conclusion that glufosinate tolerant canola does not present any increased risk of weediness in uncontained release in the canola growing areas of the United States.

These data sets are contained in studies by Crawley and colleagues in the United Kingdom, and studies conducted by MacDonald and colleagues in Canada. Crawley and colleagues have field tested glufosinate tolerant and kanamycin tolerant canola to measure the potential for increased invasiveness of transgenic canola in the United Kingdom (Cherfas, 1991; Crawley, 1992; Crawley et al., 1993). The major conclusions of these studies are: **1)** that transgenic canola is not any more aggressive than the nontransgenic canola; **2)** transgenic rapeseeds do not invade undisturbed habitats; and, **3)** transgenic rapeseeds do not persist in the environment into which they were introduced any more than their parents did. (See Table 1 on page 17 for a summary of these data.

Table 1: Comparison of Weediness Potential of *B. napus* by Crawley and MacDonald

Comparison of the studies completed with glufosinate tolerant canola by Crawley's group in the United Kingdom and MacDonald's group in Canada.

Characteristic	MacDonald	Crawley
1. **Plant material**	glufosinate tolerant AC EXCEL variety canola	glufosinate tolerant Westar variety canola
2. **Types of studies**	seed dormancy invasiveness replacement series residual effects management of volunteers	seed dormancy invasiveness survey of feral populations

From extensive data gathered in western Canada, MacDonald and colleagues have demonstrated that: **1)** glufosinate tolerant canola is noninvasive in environments in western Canada (Appendix 4, pages 4-13; Appendix 5, pages 5-17); **2)** T45 canola is no different than nontransgenic canola in its lack of competitive ability in the presence of common agricultural weeds (Appendix 5, pages 5-17); and **3)** T45 canola is not different from nontransgenic canola in persistence characteristics and agricultural control measures required to manage volunteers (Appendix 11, pages 5-10). These data are all summarized in Table 2 on page 18.

In addition, results of seed studies conducted in Canada (Appendix 7, pages 1-7) and in the UK (Crawley, 1993) have shown no prolonged dormancy characteristics of glufosinate tolerant canola as compared to other *B. napus* canola.

Summarized 1996 U.S. field data provided in Attachment II indicate that growth rate and growth habitat were the same for both transgenic (T45 canola) and nontransgenic canola. After reviewing extensive studies in Canada, the AAFC (Attachment IV) considered the unconfined release into the environment of HCN28 and other *B. napus* lines derived from T45 to be safe.

Table 2: Weediness Potential of T45 Canola vs. that of Nontransgenic Canola

Guide to the data submitted in support of the lack of weediness potential of T45 canola as compared with traditional, nontransgenic canola varieties.

Fitness Character Measured	Performance Compared to Non-transgenic Counterparts	Reference
early germination: – undisturbed seed bed – disturbed seed bed	stand the same or less	Companion Summary, pages 66-69 Appendix 4, pages 4-13
seed production and net replacement value:	less (in one location, no T45 plants survived to maturity)	Companion Summary, pages 66-71 Appendix 4, pages 4-13
competitiveness: – (replacement series) – plant number and biomass	no advantage or inhibition in competitive ability	Appendix 5, pages 5-17
agressivity index:	no difference	Appendix 5, pages 7-11, 17
agricultural characteristics:	maturity later by 4 to 8 days (location dependent) all other traits, no difference	Companion Summary, pages 37-60 Appendix 6, pages 2-3
resistance to disease and insects pests:	not different from counterpart	Companion Summary, pages 84-89 Attachment II Appendix 15, pages 1-8
response to environmental stress:	no difference in response to soil salinity or drought conditions.	Companion Summary, pages 89-90 Appendix 14, pages 1-12
seed dormancy: – germination in volunteer populations – lab study of dormancy	no difference	Companion Summary, pages 76-79 Appendix 7, pages 1-7
residual effects:	no difference	Companion Summary, pages 76-79 Appendix 12, pages 5-18
sensitivity to herbicides volunteer chemical fallow:	no change in susceptibility to herbicides (glyphosate/2,4-D)	Companion Summary, pages 72-74 Appendix 11, pages 5-10

The Agriculture and Agri-Foods Canada (AAFC) review of glufosinate tolerant canola found it to be comparable to its counterpart and safe for unconfined release. A summary of the finding of Decision Document - DD96-11 (Attachment IV) for glufosinate tolerant canola follows:

- it exhibits no altered weed or invasiveness potential compared to currently commercialized canola varieties,
- gene flow to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives,
- it did not display any altered pest potential,
- it will not result in altered impacts on interacting organisms, including humans, compared with currently commercialized counterparts, and
- potential impact on biodiversity is equivalent to that of currently commercialized canola lines.

2. Impact of the Introgression of Transgenes from *B. napus* into Related Species

In 1992 at an international congress on biosafety, the plant breeder who developed canola as a crop for Canada, Dr. Keith Downey, offered the following based on his extensive experience as a plant breeder and wealth of field experimental data: "the natural barriers to gene flow from *B. napus* to the weed species are formidable and would not occur" (Downey, 1992).

While *B. napus* does outcross to some related plants (see Table 3 and the following discussions), it is only in two *Brassica* species, *B. rapa* and *B. juncea*, both crop species, that one may expect to see the new trait introgressed in an agriculturally managed ecosystem, but not in a natural ecosystem.

Below, and on the following pages, AgrEvo has presented information from different sources: **USDA**; **U.S. States**; **literature**; and **AAFC**; which demonstrates that while potential for outcrossing of canola (*B. napus*) to various weedy relatives exists, and thus the possibility for transfer of the glufosinate-tolerant trait, no increased risk of weediness will occur due to the fact that current weed management practices are very effective in controlling glufosinate tolerant *Brassicacea*.

a. USDA

USDA has demonstrated that it regards only two *Brassica* species (*B. rapa* and *B. juncea*) as potential outcrossing concerns with *B.napus*. In its Environmental Assessment of Calgene Inc.'s Lauarate Canola, the USDA concluded that "the potential of a gene movement, at very low level, from *B. napus* to other *Brassica* spp. such as *B. juncea* or *B. rapa*, will be subject to the availability of the target organism and the reduced fertility of the hybrids" (USDA, 1994, pg. 6).

In their May 28-29, 1997, Customer Service Meeting, APHIS presented reference information on examples of commercially important species that can hybridize with wild relatives in the U.S.A., adapted from Snow and Palma, 1997. Snow and Palma identified two wild relatives, in addition to itself, with which *B. napus* (canola) could hybridize. These are *B. campestris* (syn. *B rapa)* and *B. juncea.*

b. U.S. States

As presented in Section II, In the United States, in 1994 , ten (10) states accounted for 98.3% of the total canola-planted acreage. These states and percentages were:

Alabama (2.2%)
Colorado (1.7%)
Georgia (5.1%)
Idaho (12.5%)
Minnesota (9.3%)
Montana (15.1%)
North Dakota (38.7%)
Oregon (3.5%)
South Dakota (1.6%)
Washington (8.6%)

In order to find out which weedy species in each of these ten states could present outcrossing concerns with canola representatives from each of the ten states listed above were contacted. Following conversations with knowledgeable representatives from each of these states, AgrEvo has received the information given below about weeds/plants in each state with which *B. napus* could potentially outcross. Table 3 given on page 21 summarizes weeds/plants that occur in the major (>1%) canola growing states of the U.S. and with which *B. napus* (canola) can outcross, their resulting hybrid fertility characteristics and literature references.

Alabama: On October 17,1996, Dr. Glen Wehtje of Auburn University, Department of Agronomy and Soils, (334) 844-4100, informed AgrEvo that there are only two (2) weeds in Alabama which could interbreed with canola: wild mustard (*B.kaber (DC.) L.*) and wild radish (*Raphanus raphanistrum L.*). Reference Attachment I.

Colorado: On April 22, 1997, Dr. Duane Johnson of Colorado State University, Department of Soil and Crop Sciences, (970) 491-6517, informed AgrEvo that weeds in Colorado which could interbreed with canola are *B. nigra, B. juncea, B. rapa, B. hirta, and B.kaber*. None are exceptionally prevalent in Colorado with the exception of *B. nigra*. Reference Attachment I.

Georgia: On October 17,1996, Mr. Tom Kowalski, Director Entomology and Pesticide Division, Georgia Department of Agriculture, (404) 651-9486, informed AgrEvo that he knows of no weeds growing in Georgia which could outcross with *B. napus*. Reference Attachment I.

Idaho: On April 22,1997, Dr. Rogelio Vega of Division of Plant Industries, Idaho Department of Agriculture, (208) 332-8620, informed AgrEvo that although there are several *Brassica* species produced in Idaho, only wild mustard (*B.kaber (DC.) L.*) is of concern. Also please reference Attachment I which lists weeds considered Noxious in Idaho. No plant/weed with which canola can interbreed is considered noxious in Idaho.
Minnesota: Charles G. Dale, Supervisor of the Seed and Noxious Weed Section of the Minnesota Department of Agriculture, (612) 296-6123, forwarded to AgrEvo the Minnesota Noxious Weeds Bulletin. A copy of which is enclosed in Attachment I. As discussed in the overview, wild mustard (*B. kaber*), is the only species related to *B. napus* which is considered a weed.

Montana: On April 28, 1997, Dr. Barbara Mullen, Weed Specialist, Montana Department of Agriculture, Agricultural Sciences Division, (406)444-2944, faxed a list of the wild *Brassica* species which are recognized as established in Montana and with which *B. napus* can outcross. Dr. Mullen verbally informed AgrEvo that the weed of greatest outcrossing concern is *B. kaber*. Please reference Attachment I for more information.

North Dakota: On May 1, Dr. Bill Barker of the North Dakota State University Agronomy Department, (701) 231-7222, informed AgrEvo that wild Brassica species occurring in North Dakota with which *B. napus* can interbreed are wild mustard (*B. kaber*), wild radish (*raphanus raphanistrum L.*), white mustard (*B. hirta*), Indian mustard (*B. juncea*), wild turnip (*B. campestris*) and black mustard (*B. nigra*). In addition Mr. Cliff Nygard, Burleigh County Weed Officer, North Dakota Department of Agriculture, forwarded the North Dakota Noxious Weed Law and Regulations which lists problematic weeds in North Dakota. There are no weeds on this list which have the potential to interbreed with canola. Reference Attachment I.

Oregon: On April 28, 1997, Dr. Dan Ball, Hermiston Agriculture and Research Extension Center, (541) 278-4186, said that in Oregon the most prevalent weed and, therefore, the greatest concern for outcrossing with *B. napus* is wild mustard, *B. kaber*. Reference Attachment I for an Oregon state weed list.

South Dakota: On May 8, 1997, Dr. Leon Reggie, South Dakota State University Agronomy Extension, (605) 688-4600, informed AgrEvo that the weed/plant species which present the greatest outcrossing concern with *B. napus* is wild mustard (*B. kaber*). See Attachment I.

Washington: On June 6, 1997, Tom Wessells, State Pathologist, Plant Services Division, Washington Department of Agriculture, (509) 786-9275, informed AgrEvo that weedy species occurring naturally in Washington with which *B. napus* could outcross are wild mustard (*B. kaber*), white mustard (*B. hirta*) and *B. rapa*. See Attachment I.

California: On April 28, 1997, Dr. Steve Kafka, (916) 752-8108, told AgrEvo that several wild mustards and radishes occur in California (see Table 3 for listing).

Although California grows <1% of the total canola acreage for production in the United States (336 acres in 1994), California does grow other *Brassica* species, such as *B. olrecea* in agriculturally managed areas for crop production, and does grow canola for seed production. Therefore

experts in California were consulted regarding the possible impact of *B. napus* to outcross with relatives in California.

Also included in Attachment I are distribution maps of the occurrence of *Brassicacea* across the Great Plains of the United States taken from, The Great Plains Flora Association , 1997. Atlas of the Flora of the Great Plains. Iowa State University Press. Ames, IA, 600 pp.

Current Weed Management practices in these states as they relate to the control of weedy *Brassicacea* are treatment with the chemical families of phenoxys (2,4-D, dicamba), glyphosate, bromoxinil and sulfonylureas (chlorsulfuron, metasulfuron). The sulfonylureas are especially effective against *Brassicacea*, very low doses result in complete weed control.

Table 3: Outcrossing Potential of *B. napus* with Related Species in the United States

Summary of interspecific crossing results under field conditions between various *Brassicae* member species and ***B. napus*** **(pollen donor).**

Pollen Recipient	Occurs in Agriculturally unmanaged areas	State (>1% of U.S. canola production)[1]	Field Hybrids Produced?	Fertility of Hybrids	Reference
B. napus	Yes	CA	Yes	normal	Bing et al., 1991
B. rapa	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.7-1.3%) Yes (56-93%)	< 10% viable 21-86% pollen viable	Bing et al., 1991 Jorgensen & Anderson, 1994
B. juncea	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.1-0.3%)	< 10% pollen viable	Bing et al., 1991 Calgene, 1994
B. nigra (black mustard)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (extremely low numbers);	male sterile	Bing et al., 1991 Calgene, 1994 Brown et al., 1994
B. oleracea[2] (cabbage family)	No	CA	No	n/a	Calgene, 1994 Kerlan et al., 1992 Downey, 1992
B. carinata[1]	No		No	n/a	Calgene, 1994
B. elongata	Yes	NV	No	n/a	Calgene, 1994
B. tournefortii	Yes	CA	No	n/a	Calgene, 1994
B. adpressa, syn. Herschfeldia incana (hoary mustard)	Yes	CA, NV, OR	Yes (extremely low numbers)	mostly sterile	Lefol et al., 1991 Eber et al., 1994
Raphanus raphanistrum (wild radish)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.2%) Yes (but only under sp. circumstances)	very low (0.16 seeds/ plant) very low (4-14%)	Baranger, et al., 1995 Eber et. al., 1994
Sinapis arvensis syn. *B. kaber* (wild mustard)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	No	n/a	Lefol et al., 1994 Lefol et al, 1996 Bing et al., 1991 Bing et al., 1995
Sinapsis alba syn. *B. hirta*	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	No	n/a	Calgene, 1994 Warwick, 1993
Diplotaxis muralis	Yes	CA, OR, SD	No	n/a	Ringdahl, 1987 Calgene, 1994

n/a = not assessed

[1] Warwick, 1993.

[2] In North America, does not naturally occur in the wild and is not taken to seed.

Table 4 below gives data obtained by Kerlan and colleagues in 1992 using embryo rescue technique to attempt to fertilize related species using *B. napus* as the male parent. It is important to note that these are laboratory data. When Kerlan et al. went to the field they could not produce seed with *B. napus* as the pollen donor for any of these species. They did report fertilization of male sterile *B. napus* by pollen from *R. raphanistrum* and *H. incana* under field conditions. The male sterile *B. napus* is a special "Ogura" type with cytoplasm derived from *R. raphanistrum*.

Table 4: Crosses of *B.napus* (Pollen Donor) via Ovary Culture and Embryo Rescue

Species	number of ovaries in culture	number of plantlets obtained	plants produced per fertilized ovary
B. oleracea var. acephala	445	3	0.002
B. oleracea var. capitata	585	1	0.006
H. incana (hoary mustard)	1117	15	0.013
B. nigra	916	0	0
S. arvensis (wild mustard)	732	0	0
R. raphanistrum (wild radish)	583	9	0.015

c. Literature

Below are synopses from literature regarding the potential for outcrossing to and gene introgression, and their subsequent consequences, into the species listed in Table 3.

Brassica napus

MacDonald, R., 1996. Glufosinate Tolerant Canola: (N-acetyl-L-phosphinothricin: metabolic product) Canola Lines pHoe 4/Ac. Environmental Safety Assessment Background Volume 1, Basis for Selectivity.

Self-pollination characteristics of T45 canola (B. napus) were no different than self-pollination of nontransgenic canola varieties. Findings of low outcrossing (0.6% beyond 4 m) were observed under field conditions (see Companion Summary page 80 and Appendix 8, pages 2-9).

Brassica rapa syn. *Brassica campestris*

Jørgensen, Rikke and Bente Andersen, 1994. Spontaneous Hybridization between Oilseed Rape (*Brassica napus*) and Weedy *B. campestris* (*Brassicaceae*): A risk of growing genetically modified oilseed rape, Am. J. 81, 1620-1624.

Research completed in Denmark has shown that under field conditions, where *B. rapa* has long been cultivated, that it has become a persistent weed because proper weed management practices have not been followed. *Brassica rapa* is not grown commercially in the U. S. due to lower yields and its tendency to cultivate weed banks due to a prolonged seed dormancy. AgrEvo have no plans to introduce a transgenic *B. rapa* hybrids into the U.S. for commercial canola production due to the associated commercial disadvantages in comparison with *B. napus*. In *B. napus* production, the introgression of herbicide tolerant genes does occur where the two species are in close proximity and flowering periods overlap. This is not a surprising result, since these two species have been shown to outcross and produce hybrids of <10% fertility. (Bing et al., 1991).

In data presented in the Companion Summary, pages 81-82 and in Appendix 9, pages 2-5 (note: *B. rapa* is referred to as "tame mustard".), no outcrossing was observed between *B. napus* (T45 canola) and *B. rapa*.

Indian/brown mustard *(Brassica juncea)*

Calgene, Inc., 1994, Petition for Determination of Nonregulated Status for Laurate Canola (*Brassica napus*).

"*B. napus* is capable of acting as the pollen donor in crosses with *B. juncea*, cultivated as Indian or brown mustard although fertility of the hybrids is less than 10% (Bing, 1991; Dhillon et al., 1985; Heyn, 1977; Roy, 1980). Under field conditions in western Canada with *B. napus* and *B. juncea* interplanted, an average of 4 hybrid seed per plant (4.7% of seeds tested) were produced on the maternal *B. juncea* plants. Many of these F1 plants were completely infertile and produced no seed, 50% produced only 5 seed, 10% produced up to 25 seed and the remainder produced intermediate amounts of seed (6 to 15 seed per plant) under open pollinating conditions in a greenhouse (Bing, 1991). Using herbicide tolerant *B. napus* as the pollen parent, 0.3% and 0.1% of seed were hybrid in two years of field trials. Fertility of the hybrids was very low, but actual values were not given (Bing, 1991). The distribution of naturalized *B. juncea* is sparse (although widespread) throughout temperate North America." (Calgene, 1994)

No published reprints of natural field hybrids being formed were found.

black mustard (*Brassica nigra*)

Calgene, Inc., 1994, Petition for Determination of Nonregulated Status for Laurate Canola (*Brassica napus*).

"Crosses (of *B. napus*) with *B. nigra* under field conditions produced either no hybrids (Baranger, et al., 1992) or were produced in very low numbers and were male sterile (Bing, 1991)." (Calgene, 1994).

Brown, A.P. Brown, J. Thill, D. C., Brammer, T. A., Nair, H.S., 1995, Gene Transfer between Canola (*Brassica napus* and *Brassica campestris*) and related weed species. Proceedings GCIRC 9th International Rapeseed Congress, Cambridge, 4, 1040-1043.

Brown et al. (1995) attempted crosses in the greenhouse to wild mustard (*Sinapis arvensis*, syn. *B. kaber*) and black mustard (*B. nigra*) pollinating immature buds with pollen from glufosinate tolerant canola. No fertile hybrids were made, however the authors proposed bridge crosses across the *Brassica* genomes as a potential means to introgress the glufosinate tolerant gene into related species. The work published by Bing, Doweny and Rakow (1991) and Bing (1995) showed that such introgression did not occur under field conditions in Western Canada.

wild radish (*R. raphanistrum*)

Baranger A., Chevre A.M., Eber F.; Renard M., 1995, Effect of Oilseed Rape Genotype on the Sponaneous Hybridization Rate with a Weedy Species- An Assessment of Transgene Dispersal. Theoretical and Applied Genetics, V91, N6-7:956-963.

Westar T5 from Plant Genetics Systems was crossed into 5 male sterile lines, all with the Ogura cytoplasm (derived from *Raphanus raphanistrum*). The resulting hybrid seed gave rise to male sterile plants, as Westar does not carry the restorer gene for fertility. The canola plants were interplanted with wild radish (*R. raphanistrum*) and seed was set by pollen from the wild radish and a canola field some distance away. The resulting seed was in two sizes, large seed from the rapeseed pollinations and small seed from wild radish pollinations. The small seed were triploid and produced mostly sterile plants (86 to 96% of the plants). Under normal conditions, male sterile plants would be planted with male fertile plants in the adjacent row. Thus, rapeseed pollen would be much more abundant and the likelihood of pollination by wild radish would be extremely remote.

Therefore, based on the observations of Baranger et al., 1995, it can be concluded that the likelihood of introgression of the transgene into populations of wild radish is extremely low because:

1) Crosses are only possible in the field under special circumstances; when pollen from the wild radish can successfully pollinate a male sterile canola using the Ogura cytoplasm (derived from wild radish). Hybrid seed production fields are planted with a large supply of pollinator plants and care is taken to isolate a seed production field from contaminating weeds,
2) The fertility of the resulting triploid plants is reduced,

3) The resulting triploid plants must survive in the field in subsequent generations, and backcross into the existing populations of wild radish. The triploid chromosome structure will make such backcrossing difficult, and
4) The only selective advantage would be resistance to the herbicide.

wild mustard (*Synapis arvensis L.*, syn. *Brassica kaber*)

Lefol E.; Danilou V.; Darmency H., 1996, Predicting Hybridization between Transgenic Oilseed Rape and Wild Mustard. Field Crops Research, V45, N1-3:153-161.

Quote from abstract: "No hybrid was found among 2.9 million seeds produced by wild mustard grown in a garden in the presence of a herbicide-resistant transgenic cultivar." The herbicide resistant rapeseed was glufosinate tolerant, supplied by Plant Genetics Systems. Wild mustard is (*Sinapis arvensis* syn *B. kaber*)

Bing, D.J.; Downey, K.; and Rakow, G.F.W., 1995, An Evaluation of the Potential of Intergeneric Gene Transfer between *Brassica napus* and *Sinapis arvensis*. Plant Breeding, V115:481-484.

To summarize this article: the likelihood of introgression of the transgene into populations of wild mustard is nil because, crosses between canola and wild mustard do not occur under field conditions.

Brown, A.P. Brown, J. Thill, D. C., Brammer, T. A., Nair, H.S., 1995, Gene Transfer between Canola (*Brassica napus* and *Brassica campestris*) and related weed species. Proceedings GCIRC 9th International Rapeseed Congress, Cambridge,4, 1040-1043.

Brown et al. (1995) attempted crosses to wild mustard (*Sinapis arvensis*, syn. *B. kaber*) and black mustard (*B. nigra*) pollinating immature buds with pollen from glufosinate tolerant canola in the greenhouse. No fertile hybrids were made, however the authors proposed bridge crosses across the *Brassica* genomes as a potential means to introgress the glufosinate tolerant gene into related species. The work published by Bing, Doweny and Rakow (1991) and Bing (1995) showed that such introgression did not occur under field conditions in Western Canada.

cabbage family (*Brassica olracea*)

Kerlan, M.C., Chevre, A.M., Eber, F., Baranger, A. and Renard,M. 1992. Risk assessment of outcrossing of transgenic rapeseed to related species: I. Interspecific hybrid production under optimal conditions with emphasis on pollination and fertilization. Euphytica 62: 145-153.

Downey, R.K., Biosafety of Transgenic Oilseed Brassica Species, 1992, Proceedings of 2nd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Goslar, Germany.

B. oleracea was not identified by Dr. Keith Downey as a potential recipient of *B. napus* pollen under field conditions (Downey, 1992). Neither did the USDA recognize *B. oleracea* as a potential recipient of *B. napus* pollen under field conditions (USDA, 1994, pg. 6).

Several biological facts prevent such gene flow and potential for environmental consequence:

1) hybrids may be formed only under laboratory conditions (manual pollinations and embryo rescue) between *B. napus* and *B. oleracea* , (Kerlan et al., 1992)
2) crosses between *B. napus* and *B. oleracea* are especially difficult when *B. napus* is the pollen parent (Kerlan et. al. 1992 reported 0.002-0.0067 plants produced per fertilized ovary using hand pollination and embryo rescue techniques, (see Table 4 on page 24), and
3) there is little opportunity for field crossing since *B. oleracea* is not naturalized in North America and geographic isolation is used for the production of seed (Kerlan et al., 1992).

***B. carinata, B. elongata*, wild turnip (*B. tournefortii*), white mustard (*Synapis alba*)**

Calgene, Inc., 1994, Petition for Determination of Nonregulated Status for Laurate Canola (*Brassica napus*).

Warwick, S.I., 1993. Guide to the Wild Germplasm of *Brassica* and Allied Crops, part IV. Agriculture Canada Research Branch Technical Bulletin, 17E, 19.

"Crosses between *B. napus* and *B. carinata* would be possible in the field (although very unlikely due to incompatibility, Fernandez-Serrano et al., 1991; Kerlan et al., 1992; Downey et al., 1980) except that neither species occur in the wild (are naturalized) in the U.S. Standard isolation practices prevent hybrid production. There is no significant production of *B. carinata* anywhere in the U.S. The vegetable *Brassicas* (e.g. broccoli) are not taken to seed intentionally, except in geographically isolated seed production areas." (Calgene, 1994)

B. elongata is not cultivated in the U.S. nor do naturalized forms occur. (Calgene, 1994; Warwick, 1993).

B. tournefortii is not cultivated in the U.S. (Calgene, 1994). No crosses between *B. napus* and *B. tournefortii* have been documented in literature (Calgene, 1994; Warwick, 1993).

No field hybridization between *B. napus* and *Synapis alba* (*B. hirta*) has been documented (Warwick, 1993). Manual hybridization was attempted with no success (Calgene, 1994).

wild radish and hoary mustard

Lefol, E. Dantelou, V., Darmarcy, H., Boucher, F., Maillet, J and M. Renard, 1995, Gene Dispersal from Transgenic Crops. I. Growth of Interspecific Hybrids between Oilseed Rape and the Wild Hoary Mustard. Journal of Applied Ecology. V32: 803-808.

Research in France has shown that field hybrids can be made under special circumstances between male sterile *B. napus* and hoary mustard (*Herschfeldia incana* syn. *B. adpressa*) a weed of Mediterranean regions. Hoary mustard is found as an occasional weed in North America in roadside and waste areas of California, Oregon and Nevada. It is not likely to be in the proximity of commercial canola production. (Warwick, 1993).

Eber F.; Tanguy X.; Chevre A.M.; Baranger A.; 1994, Spontaneous Hybridization between a male Sterile Oilseed Rape and two Weeds. Theoretical and Applied Genetics, V88 N3-4:362-368.

Eber et al., 1994, used the two weeds hoary mustard (*Herschfeldia incana* syn. *B. adpressa*) and wild radish (*Raphanus raphanistrum*). The male sterile rapeseed was the Ogura cytoplasm (derived from *Raphanus raphanistrum*).

To quote from the discussion section of this paper (p. 367):
" The R1 interspecific hybrids produced were vigorous and well adapted to natural conditions, but some difficulties arose for the BC1 seed production, particularly with the diploid species as the recurrent parent. It seems that it is difficult to return to the diploid level, which is in agreement with the results of Bing et al. (1991). Even if that difficulty could be overcome, gene introgression will depend on chromosome rearrangement in the 2x genome."

"We have demonstrated that interspecific crosses can occur using male-sterile rapeseed. However, we may expect that the pollen competition due to the co-cultiviation of a male-fertile rapeseed variety will result in rare pollinations involving wild species, except where the female parent flowers earlier than the male parent."

The likelihood of introgression of the transgene into populations of hoary mustard is nil in the USA because:

1) Hoary mustard does not grow in the same location as canola which is grown for production in the United States. Hoary mustard (*H. incana*, syn. *B. adrepressa*) grows in ditches and roadside areas of California, Nevada and Oregon. It does not occur in the canola producing areas of these states (Warwick, 1993),
2) In the possible cases of hybrid seed production in the Imperial Valley of California where hoary mustard may be present, the opportunity for hybridization is extremely small due to the management practices of seed production, such as isolation distances of several meters (AgrEvo internal communication), and
3) Introgression of the transgene into the hoary mustard population is not likely due to chromosome incompatibilities. (Eber et al., 1994).

Diplotaxis muralis

Calgene, Inc., 1994, Petition for Determination of Nonregulated Status for Laurate Canola (*Brassica napus*).

"Crosses of *B. napus* with *Diplotaxis muralis* have only been reported from laboratory studies (Ringdahl et al., 1987; Salisbury, 1988). Field crosses with *D. muralis* are extremely unlikely since it is not a common agricultural weed (based on a description of distribution in Rollins, 1980; also, the species is not listed in the Weed Control Manual, 1992). Further, *D. muralis* is highly self-compatible and most fertilization is complete before emasculation (Ringdahl et al., 1987), which is normally done 24-48 hours before the flower would open." (Calgene, 1994).

d. AAFC

After reviewing the data submitted by AgrEvo Canada, Inc. as well as reviewing literature references submitted in support of HCN28 being recommended for Environmental Clearance, AAFC concluded that gene flow from HCN28 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives (Attachment III, pg 8). *Brassica napus* plants are known to outcross with other plants of the same species. Studies show that introgression of the herbicide tolerant gene is most likely to occur with *B. rapa*, the other major canola species and occasional weed of cultivated land especially in the eastern provinces of Canada. If glufosinate-ammonium tolerant individuals rose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate-ammonium. This would only occur in managed ecosystems where glufosinate-ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate-ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate-ammonium tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would easily be controlled using mechanical and available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate-ammonium as a tool to control these species. This, however, can be minimized by the use of sound crop management practices (Attachment IV, pg. 4). A discussion of the potential impact of introgression is provided on pages 80-84.

Summary of Canola (*B.napus*) Outcrossing Potential and Consequences Thereof

The potential of T45 canola to outcross to related plant/weed species, and produce hybrid species which potentially could express the trait of herbicide tolerance to glufosinate-ammonium can be summarized as follows: As described above, it has been referenced in literature and acknowledged by USDA, that the only plant/weed species with which there is potential for T45 canola to outcross and produce fertile hybrids are *B. rapa* and *B. juncea.* Should fertile hybrids of *B.rapa* and/or *B. juncea* be produced with the capability of backcrossing with their respective natualized parent, and which could potentially express tolerance to glufosinate-ammonium as a result of outcrossing between T45 canola and naturalized *B.rapa* and/or *B. juncea,* no increased risk of weediness of either of these naturalized species will occur because current weed management practices now in place to control weedy *Brassica* species would effectively control glufosinate tolerant *Brassica* species! These practices include: treatment with the chemical families of phenoxys (2,4-D, dicamba), glyphosate, bromoxinil and sulfonylureas (chlorsulfuron, metasulfuron). The sulfonylureas are especially effective against *Brassicacea*, very low doses result in complete weed control.

3. Gene Transfer to Organisms with which *B. napus* cannot Interbreed

Movement of transgenes from genetically engineered plants to microorganisms has been suggested as a risk if such plants are released into the environment. As initially stated in the USDA's Interpretive Ruling on Calgene, Inc. Petition for Determination for Nonregulated Status of FLAVR SAVR™ Tomato (USDA, 1992), and subsequently repeated in other USDA Determination documents, "There is no published evidence for the existence of any mechanism, other than sexual crossing" by which genes can be transferred from a plant to other organisms. As summarized in these Determination documents, evidence suggests that, based on limited DNA homologies, transfer from plants to microorganisms may have occurred in evolutionary time over many millennia. Even if such transfer were to take place, transfer of the *pat* gene to a microbe would not pose a plant pest risk. Genes encoding both PAT enzymes and acetyl transferases are found in microbes in nature. Indeed as described earlier in this document , the synthetic *pat* gene present in T45 canola is derived from a pat gene isolated from a naturally occurring soil microbe.

V. Statement of Grounds Unfavorable

No unfavorable information and data has been demonstrated for glufosinate-tolerant canola transformation event T45.

VI. Literature Cited

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VII. Attachments and Appendices

Attachment I.

Documentation from states growing >1% of Canola for Production.

Attachment II.

1996 USA Field Termination Report; 1997 USA Preliminary Field Termination Report

Attachment III.

Agriculture and Agri-Food Canada: Agriculture and Agri-Food Canada: Regulatory Directive Dir 94-09. The Biology of *Brassica napus L.* (Canola/Rapeseed).

Attachment IV.

Agriculture and Agri-Food Canada: Decision Document DD 96-11. Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate-Ammonium Tolerant Canola Line HCN 28, May 6, 1996.

Attachment V.

Agriculture and Agri-Food Canada: Decision Document DD 96-11, Supplement. Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate-Ammonium Tolerant Canola Line HCN 28, May 6, 1996.

Attachment VI.

Health Canada: Health Protection Branch: authorization letter stating, "no objection" to the sale of refined canola oil from canola lines derived from the transformation event T45 as human food in Canada, February 17, 1997.

Appendices 1-13.

AgrEvo Internal Reports referenced in this document.

1. Bennett, C.F., Southern Analysis of Two Lines of Transformation Event to Determine T-DNA Stability. AgrEvo Report Reference AC195-10.

2. Bennett, C.F., Southern Analysis of Two Lines of Transformation Event T45 to Determine T-DNA Copy Number. AgrEvo Report Reference AC195-22.

3. Bennett, C.F., Southern Analysis of Two Lines of Transformation Event T45 for the Presence of the Streptomycin/Spectinomycin Antibiotic Resistance Marker. AgrEvo Report Reference AC195-12.

4. Belyk, M. and MacDonald, R., Invasive Potential of Transgenic *B. napus* (pHoe4) Under Disturbed and Undisturbed Field Conditions. AgrEvo Report Reference C195-16.

5. Belyk, M. and MacDonald, R., Invasive Potential of Transgenic pHOE4 *B. napus* (HCN28) Under Agronomic Conditions. AgrEvo Report Reference AC195-18.

6. Belyk, M., Agronomic Characteristics (mean and coefficient of variance) of *Brassica napus* varieties grown at two Saskatchewan Locations in 1995. AgrEvo Report Reference AC196-09.

7. Drexler, D.M., Comparison of Standard Canola to Glufosinate Tolerant Canola Survival Adaptations - Seed Dormancy. AgrEvo Report Reference HC193-21.

8. MacDonald, R.L., Assessment of Outcross Frequency of Glufosinate Tolerant *Brassica napus* cv. HCN-92. AgrEvo Report Reference HC193-01.

9. MacDonald, R.L., Estimation of the Influence of Distance on the Outcrossing Frequency of Glufosinate Tolerant Canola with Tame Mustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions. AgrEvo Report Reference HC193-03.

10. MacDonald, R.L., Assessment of Outcrossing of Glufosinate Tolerant Canola to Related Species. AgrEvo Report Reference HC193-18.

11. Belyk, M. and MacDonald, R., Assessment of Volunteer Glufosinate Tolerant Canola Under Chemical Fallow Conditions. AgrEvo Report Reference HC193-04.

12. Belyk, M. and MacDonald, R., Residual Effects of Glufosinate Tolerant Canola on the Growth and Productivity of Grain, Forage and Pulse Crops. AgrEvo Report Reference HC193-02.

13. Belyk, M. and MacDonald, R., Behaviour of Honey Bees Foraging on Transgenic Canola (*Brassica napus L.*). AgrEvo Report Reference HC193-14.

14. Redmann, R.E., Qi, M.Q., and Belyk, M., The Response of Transgenic Canola (*Brassica napus L.* cv. HCN92) to Soil Salinity under Growth Chamber Conditions. AgrEvo Report Reference HC193-15.

15. Belyk, M. and AG-QUEST Inc., Susceptibility Glufosinate-Tolerant Canola to Flea Beetles). AgrEvo Report Reference HC193-12.

Companion Summary for T45 Canola:

Petition for Determination of Nonregulated Status

AgrEvo

Glufosinate Tolerant Canola

Environmental Safety Assessment Background

Prepared by:

AgrEvo Canada Inc.
295 Henderson Drive
Regina, Saskatchewan
Canada S4N 6C2

Date:

January 18, 1996

Report Number

Canadian reference: ACI96-03

International reference: A55196

Preface

This document summarizes the environmental safety data for the second series of glufosinate tolerant canola lines developed by AgrEvo Canada Inc. These new lines represent advances in technology over presently registered glufosinate tolerant canola lines as the vector which is used contains only the gene required for glufosinate tolerance. The purpose of this document is to provide the reader with a thorough overview of the data generated to substantiate the safety of these new recombinant lines. Specific data for the event T45 and subsequent breeding lines derived by transformation of the cultivar AC Excel with the vector pHoe4/Ac is presented. The full reports of the pHoe4/Ac data are included in the appendix of this document. In addition, data is also presented from studies with glufosinate tolerant lines originating from the transformant event Topas 19/2 which has previously been reviewed by AAFC. Trial results from Topas 19/2 support the safe use of the T45 derived transformant lines in that the pat gene is controlled by the same regulatory sequences in both constructs and the same transformation techniques are utilized.

Table of Contents

Introduction

Background on Canola

Canola, is a distinct *Brassica* cultivar that produces oil with nutritionally superior quality than rapeseed. *Brassica napus* together with closely related oilseed species (*B. rapa, B. juncea)* provide approximately 15% of the world's edible vegetable oil. In the USA, canola oils make up approximately 4% of the edible oil market (Downey, 1992).

The host plant *Brassica napus* L. var. *oleifera* of the tribe Brassicaceae and family Cruciferae is commonly known as rapeseed or colza. However, the transformed plant belongs to a distinct class of rapeseed called canola that produces seed with nutritionally superior qualities. In 1994, over 14 million acres of canola were sown in western Canada, rivalling wheat in terms of crop value. Approximately 55% of this acreage was sown with *B. napus* canola varieties, the remaining area being sown to canola varieties of the *B. rapa* species. Seed from both species is harvested and marketed as a single commodity.

Traditionally, Canada has utilised about half its annual production domestically. The remainder is exported as whole seed, primarily to Japan, with smaller quantities of oil and meal exported to the US. The major use is as a salad and cooking oil. However, it is also used in the manufacture of margarines and shortenings.

The small (1000 seeds = 4 g) black seed, containing 40 to 45% oil, is produced by the plant within slender pods that are borne on long racemes. Modern prepress-solvent extraction facilities remove the oil which is then degummed, deodorised and refined before commercial use. The meal remaining after oil extraction contains 36-38% nutritionally well-balanced protein and is marketed as a high quality animal feed.

Canola Quality

Erucic Acid

When rapeseed oil began to enter European and Canadian diets in the 1940's and early 1950's, its nutritional properties were investigated by Health and Welfare Canada and other nutritionists since it was a new oil in the diet and its fatty acid composition differed markedly from other commonly consumed edible oils. Further nutritional investigations in Canada and Europe, using a wide array of young laboratory animals, were reported at the 1970 International Rapeseed Congress and confirmed the assessment that high erucic acid oils were nutritionally undesirable (Sauer and Kramer, 1983).

Alerted by early nutritional studies, Canadian plant breeders were able to genetically manipulate the *B. napus* plant using conventional procedures so as to block the biosynthetic pathway for erucic acid synthesis in the developing embryo. This was achieved by preventing the formation of eicosenoic and erucic acids from their precursor oleic acid. Canada completed its conversion to varieties that produce this oil in 1974. *B. napus* cultivars must be less than 2% erucic acid to be registered for use in Canada as an edible oil source. Commercially harvested seed in Canada, averages 0.5% erucic acid (DeClercq *et al.*, 1993). Low erucic oil (<2% erucic), also known as canola oil, was granted "GRAS" status in the US in 1986. Canola oil, received the 1987 Food Product of the Year from the American Heart Foundation of New York, and the 1989 Product Acceptance Award from the American College of Nutrition. Glufosinate tolerant canola meets or exceeds the standards established for canola oil.

Glucosinolates

All species of the Cruciferae contain sulphur compounds known as glucosinolates. These compounds, of which about 90 are known, give the distinctive flavour and odour to the related cole vegetables, mustards, radishes, turnips, etc., and are reported to be dietary protectants against colon cancer (Zhang *et al.*, 1992).

Plants of this family concentrate these compounds in their seed and *B. napus* rapeseed plants once contained about 140 to 150 µmoles of glucosinolates per gram of oil- and moisture-free meal. Such high levels limited the nutritional value as well as the palatability of rapeseed meal as an animal feed for non-ruminant animals and poultry (Bell, 1984). When the cells of the seed are broken and moisture is present, the seed-borne enzyme myrosinase hydrolyses the glucosinolates to release sulphur, glucose isothiocyanates, oxazolidinethiones and if the pH is low, nitriles (Bell, 1993). The nitriles are toxic compounds while the isothiocyanates and oxazolidinethiones are active goitrogens that interfere with iodine uptake by the thyroid gland in non-ruminant animals. To overcome this constraint to meal usage, Canadian plant breeders using conventional plant breeding methods, were able to genetically block the biosynthetic pathway between the amino acid precursor, methionine, and the aliphatic glucosinolates. Present Canadian cultivars of *B. napus* contain some 10 to 15 µmoles of aliphatic glucosinolates plus approximately 10 µmoles of indolglucosinolates. Canola is a trademark term that is presently defined as seed, oil and meal from *B. napus* and *B. rapa* plants that contain no more than 2% erucic acid in their seed oil and no more than 30 µmoles of aliphatic glucosinolates in the oil-free, moisture-free meal (Canola Council of Canada, 1990).

In the processing of canola, seed is dry heated to deactivate the myrosinase enzyme and the small amount of the glucosinolates that remain. Myrosinase and glucosinolates are removed from the oil during processing because they can inactivate the catalysts used during the hydrogenation of margarine and shortening

Many nutritionists consider canola oil to have an almost ideal fatty acid composition for human consumption. Canola oil has the lowest level of saturated fatty acids of any edible oil (6%), an adequate content of the polyunsaturated fatty acid linoleic (20%), plus a small amount of the essential w-3 fatty acid linolenic (9%), with nearly all the remainder being the nutritionally neutral oleic acid. A new canola variety cannot be registered for use in Canada unless it meets canola standards for erucic acid and glucosinolate content. Evaluation is conducted through a public CO-OP field evaluation program over three years at multiple locations in the growing region.

Canola Production

Growers across Canada are adopting sustainable agronomic practices to minimize soil erosion and conserve moisture. Practices like minimum tillage, direct seeding, trash management and chemical fallow are all part of a sustainable management system.

Weed competition is one of the most limiting factors in canola production. Canola is not a strong competitor if weeds emerge before the crop. In Western Canada, the estimated loss from potential canola production due to weeds range from 10 to 13 %. Weeds that are closely related to canola are particularly difficult to control in canola because no effective herbicide is available.

Currently, grassy weed control products are applied on virtually 90% of the Canadian canola crop. The majority of these products are applied pre-emergent and require soil incorporation. The grower must either till the field excessively or burn the stubble to eliminate the trash, or accept poor incorporation and subsequently poor weed control. Since weed control in canola is essential, attempts to control weeds may take precedence over soil and moisture conservation practices.

Recent advances in tissue culture and transformation technologies using *Brassica napus* have allowed plant breeders to introduce novel traits to cultivars. These advances have allowed for the development of a *B. napus* lines such as Innovator that are tolerant to the non-selective herbicide glufosinate ammonium. When applied, the tolerant plant rapidly converts glufosinate ammonium to a non-toxic metabolite. Consequently glufosinate can provide broad spectrum weed control with a single postemergent application.

The availability of canola varieties tolerant to glufosinate ammonium will allow growers to continue their progressive move towards sustainable agriculture. An additional benefit of this production system would be an overall reduction in the total dosage of herbicide products currently applied for broad spectrum weed control. Applications need only be made when necessary, allowing farmers the option to wait and see what weed

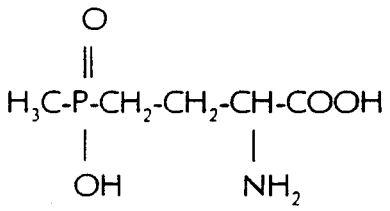
populations arise. In addition, glufosinate is not registered for postemergent weed control in any other field crops thus the grower will now have a new tool available to manage weed problems.

As well, glufosinate ammonium represents a unique mode of action. This provides growers with a new rotational option for weed control in canola reducing the occurrence of herbicide resistant weed populations.

Glufosinate ammonium

Glufosinate ammonium (L - phosphinothricin), the active ingredient in the herbicides Liberty™, Harvest® and Ignite®, is a broad spectrum, non-selective herbicide (Figure 1). The molecule is a synthetic version of a naturally occurring compound and has favorable environmental, health and safety characteristics. Glufosinate an analogue of L-glutamic acid, is a potent inhibitor of glutamine synthetase. Until recently, glufosinate ammonium could not be applied to emerged crops without causing serious injury. Recent advances in biotechnology have allowed AgrEvo Canada Inc. to insert a gene into canola, which acetylates glufosinate ammonium, rendering it inactive. This advance has made it possible for growers to apply glufosinate ammonium, post emergent for effective broad spectrum weed control. Glufosinate tolerant canola will allow growers to adopt more sustainable production practices by allowing for broad spectrum weed control with a single postemergent herbicide.

Figure 1 Glufosinate (PPT)



Chemical name: DL-homoalanin-4-yl(methyl)-phosphinic acid (IUPAC)
Empirical formula: $C_5H_{12}NO_4P$
Molar mass: 181.15

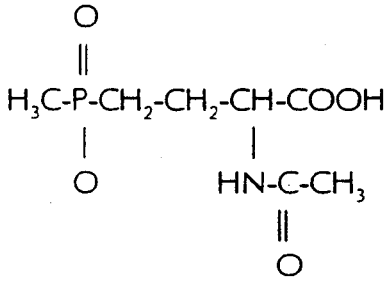
Glufosinate tolerant canola

Basis for selectivity

Phosphinothricin acetyltransferase (PAT) is a highly specific enzyme which catalyses the acetylation of glufosinate (phosphinothricin) while not affecting its analogue, L-glutamic acid. Phosphinothricin (PPT) (Figure 1) is the active form of the herbicides Harvest®, Ignite® and Liberty™. The *pat* gene encodes the enzyme phosphinothricin acetyltransferase (PAT) (Wohlleben et al, 1988).
The acetylation of the PPT molecule renders the compound herbicidally inactive (Figure 2). This effectively blocks the action of the herbicide as the transformed plants contain the PAT enzyme in sufficient quantities to tolerate the herbicidal action of glufosinate ammonium.

Transformed canola lines containing the PAT gene are tolerant to doses as high as 5 times the maximum use rate of 750 g ai/ha. Therefore postemergent application of the herbicide will provide broad spectrum weed control with little or no injury to the crop.

Figure 2 N-acetyl-glufosinate (N-acetyl-PPT)



Chemical name: L-2-acetamido-4-methylphosphinato-butyrate (IUPAC)
Empirical formula: $C_7H_{13}NO_5P$
Molar mass: 268.2 g/mol

Transformation System

Construct: pHoe4/Ac
Transformant T45

Agrobacterium tumefasciens

The glufosinate tolerant transformant T45 was produced by *Agrobacterium tumefaciens* mediated transformation of a protoplast culture of a canola cultivar A.C. Excel using the construct pHoe4/Ac. A synthetic version of the *pat* gene was introduced into the disarmed vector using standard cloning techniques was utilised in the transformation process. Further crosses of the transformant line followed by pedigree selection, has resulted in the development of a series of glufosinate-tolerant lines which include HCN27 and HCN28.

The introduced gene has been sequenced and its function is well characterized. The amino acid sequence and composition of the PAT protein is provided in Figure 3 and 4. Southern analysis of DNA digested with a battery of restriction enzymes has indicated that a single copy of the T-DNA have been stabily incorporated at a single locus in the *Brassica* genome (ACI95-22). Segregation data confirms the stable integration of the T-DNA in the Brassica genome (data not presented). The plasmid map illustrates the organization of the construct (Figure 5).

Figure 3

I. FORMATTED SEQUENCE

p1 : n>u 1>~~~~~~ patpep (183 aa)~~~~~~>u 183>C

1 11
met ser pro glu arg arg pro val glu ile arg pro ala thr ala
21
ala asp met ala ala val cys asp ile val asn his tyr ile glu
31 41
thr ser thr val asn phe arg thr glu pro gln thr pro gln glu
51
trp ile asp asp leu glu arg leu gln asp arg tyr pro trp leu
61 71
val ala glu val glu gly val val ala gly ile ala tyr ala gly
81
pro trp lys ala arg asn ala tyr asp trp thr val glu ser thr
91 101
vai tyr val ser his arg his gln arg leu gly leu gly ser thr
111
leu tyr thr his leu leu lys ser met glu ala gln gly phe lys
121 131
ser val val ala val ile gly leu pro asn asp pro ser val arg
141
leu his glu ala leu gly tyr thr ala arg gly thr leu arg ala
151 161
ala gly tyr lys his gly gly trp his asp val gly phe trp gln
171
arg asp phe glu leu pro ala pro pro arg pro val arg pro val
181
thr gln ile

Figure 4

COMPOSITION OF SEQUENCE

p1 : n>u 1>~~~~~~ patpep (183 aa)~~~~~~>u 183>C

Amino Acid	Occurrences	% of Total	Amino Acid	Occurrences	% of Total
ala	18	9.84	asx	0	0.00
cys	1	0.55	asp	9	4.92
glu	12	6.56	phe	4	2.19
gly	13	7.10	his	7	3.83
ile	7	3.83	lys	4	2.19
leu	13	7.10	met	3	1.64
asn	4	2.19	pro	14	7.65
gln	7	3.83	arg	15	8.20
ser	8	4.37	thr	12	6.56
val	18	9.84	trp	6	3.28
tyr	8	4.37	glx	0	0.00
Acidic	21	11.48	Basic	26	14.21
Polar	53	28.96	Nonpolar	83	45.36

Calculated Molecular weight = 20621.13

Figure 5 **Plasmid Map of pHoe4/AC**

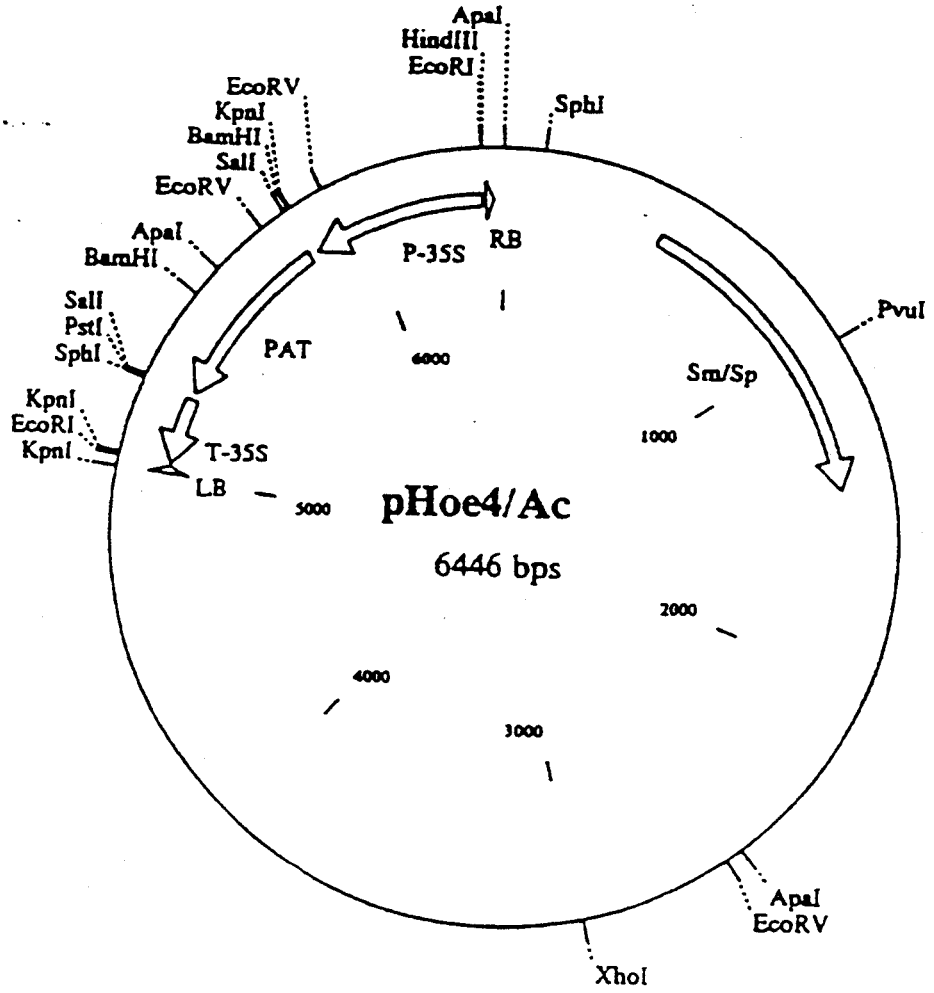


Table 1

Identity and function of DNA elements

Line Description

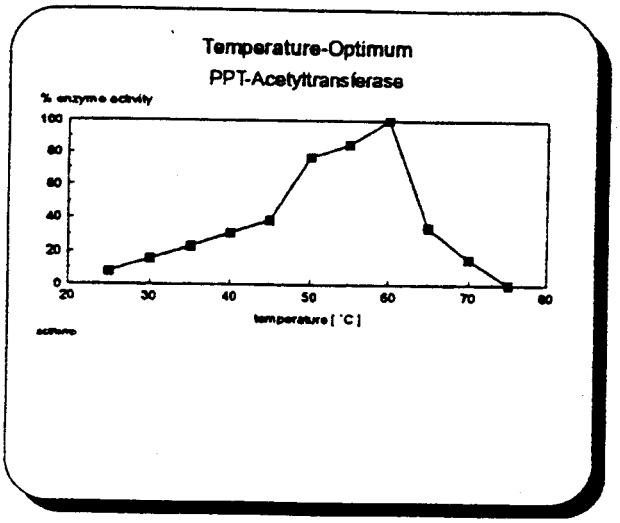
Cultivar Identification:	
Species name:	*Brassica napus* L.
Crop:	Canola
Transformation Method:	*B.napus* obtained through disarmed *Agrobacterium tumefaciens* mediated transformation
Vector :	pHOE4/Ac
Trait 1:	tolerance to glufosinate ammonium
Gene 1:	phosphinothricin acetyltransferase (*pat*) gene
Donor 1:	*Streptomyces viridochromogenes*
Promoter 1/Donor:	35S gene promoter /Cauliflower Mosaic Virus (CaMV)

Terminator 1/Donor:35S gene terminator /Cauliflower Mosaic Virus (CaMV)

Characterization of PAT Enzyme

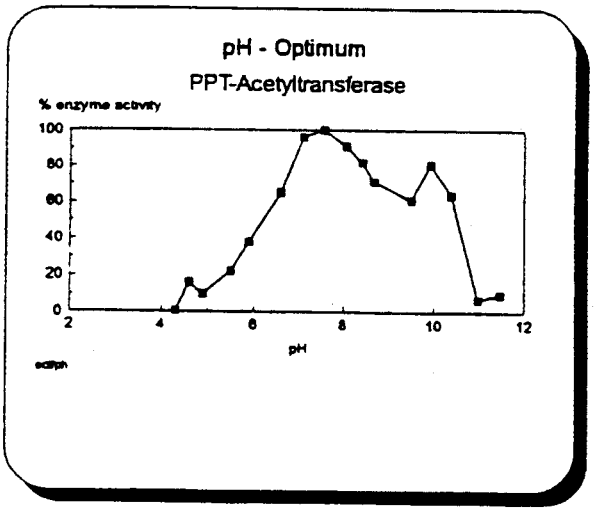
The PAT enzyme has been well characterized with regard to kinetics and substrate specificity. There is no evidence that PAT is toxic, and it has no sequence homology with known toxins. The enzymatic behaviour of the PAT protein was examined in a series of controlled laboratory studies. The influence of temperature and pH on enzymatic activity was determined.

Figure 6 Temperature Optimum Of The Phosphinothricin Acetyltransferase



PAT enzyme activity is very low at temperatures below 20°C, increases with temperature and reaches its maximum at 60°C. At temperatures above 75°C the protein is enzymatically inactive

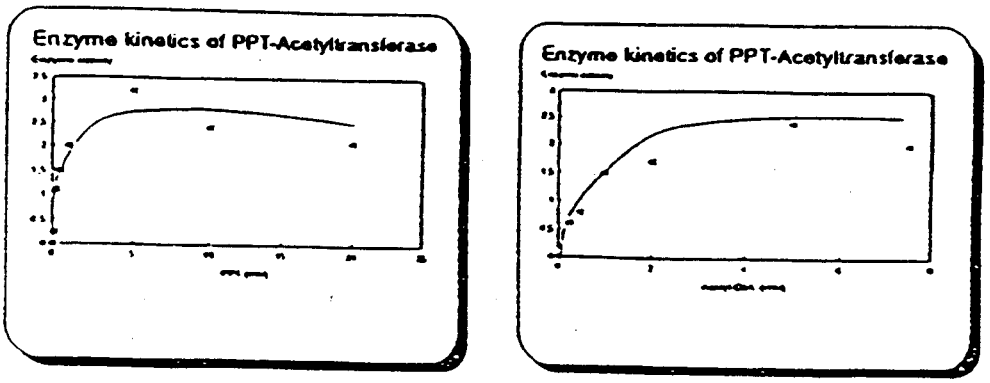
Figure 7 pH Optimum Of The Phosphinothricin Acetyltransferase



Phosphinothricin acetyltransferase has a broad optimum of activity in the pH range from 7 to 10. At pH values less than 4, the enzyme is inactive.

Enzyme kinetics

Figure 8 Phosphinothricin Acetyltransferase Activity.



PAT activity follows a simple Michaelis-Menton kinetic when both substrate concentrations (PPT and Acetyl-CoA) are varied (Figure 9). The K_M - values were determined by double reciprocal transformations of the data:

$K_{M(Acetyl\text{-}CoA)}$ = 0.6 mM and $K_{M(L\text{-}PPT)}$ = 0.3 mM.

Substrate Specificity

Studies on the specificity of PAT have clearly demonstrated that the enzyme is highly specific for the substrate L-PPT. The protein amino acid and D-PPT were not acetylated by the PAT enzyme.

^{14}C-L-PPT (0.035 mM) was incubated with Acetyl-CoA and PAT at an amount sufficient to give approximately 50% conversion of PPT to Acetyl-PPT (Ac-PPT). PPT and Ac-PPT were separated by thin layer chromatography (TLC). The influence of 20 mM amino acid on this acetyltransferase reaction was tested: (Figure 10).

Figure 9 Influence Of An Excess Of Unlabelled Amino Acids Ala, Arg, Asn, Asp, Cys, Cystin, Glu, Gln, Gly, His, OH-Pro, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val On The Conversion Of ^{14}C-L-PPT To ^{14}C-Acetyl PPT By Phosphinothricin Acetylase. (The Sample At The Extreme Right Is The Control)

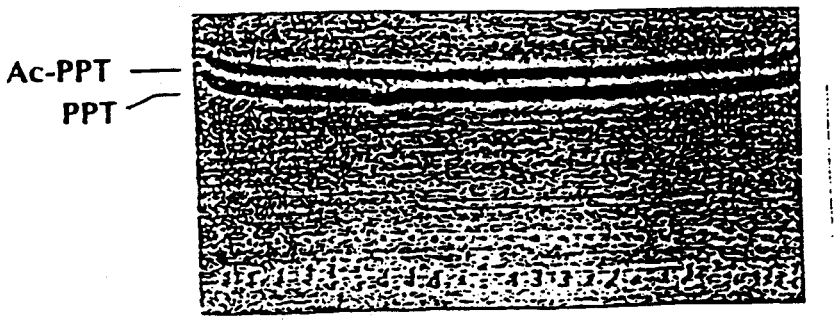
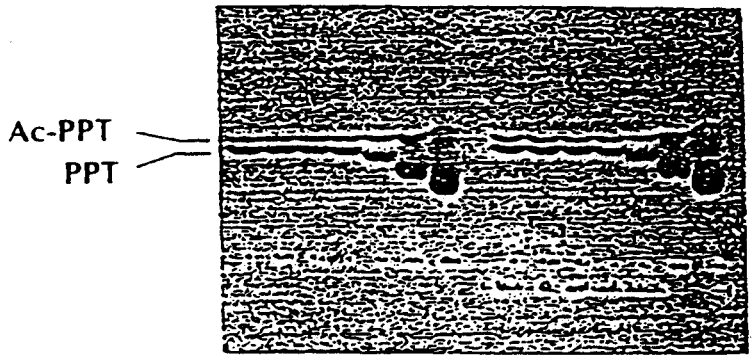


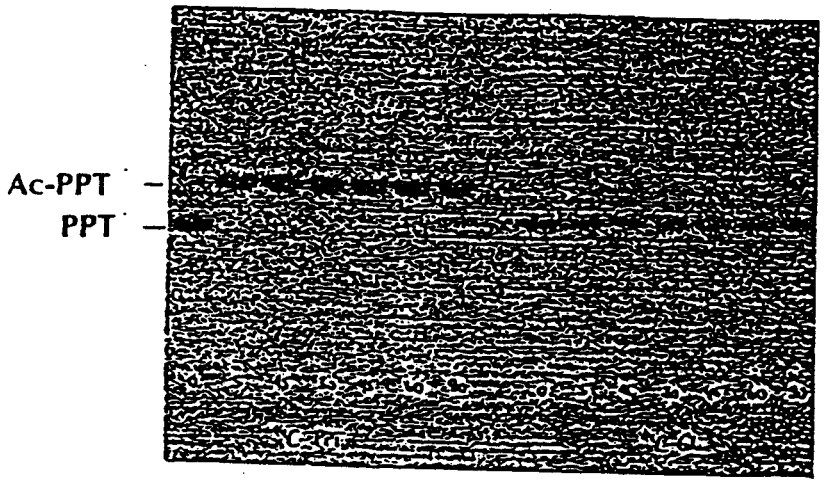
Figure 9 indicates that none of the 22 tested amino acids were acetylated by PAT. Thompson et al. (EMBO J.;1987;6 (9) pp. 2519 - 2523) reported that PAT has a low affinity toward L-glutamate. This was further investigated by studying the effect of very high concentrations of glutamate on the acetylation of ^{14}C-PPT by the acetyltransferase. ^{14}C-PPT was supplied at 0.3 mM, while the concentrations of glutamate ranged from 0 to 166 mM. A control set of samples was co-chromatographed. Figure 10 shows that even a high excess of glutamate is not able to block the PPT-acetyltransferase reaction.

Figure 10 Influence of Glutamate On The Acetylation Of ^{14}C-PPT By Phosphinothricin Acetyltransferase. Glutamate Concentrations 0, 0.0033, 0.033, 0.33, 3.3, 33 And 166 mM are shown From Left To Right. A Control Set Of Samples is presented (Right Panel).



To directly compare the enzyme's affinity towards L-PPT and L-glutamate, ^{14}C-PPT and L-[1-^{14}C]-glutamate were used in equimolar concentrations sufficient to convert all the L-PPT to N-Ac-PPT within 0.1 minutes. Figure 11 illustrates that ^{14}C-PPT was completely transformed to N-Ac-PPT. The enzyme was not able to acetylate glutamate even when incubated for 90 minutes demonstrating the high specificity of the enzyme.

Figure 11 Transformation Of ^{14}C-PPT And ^{14}C-Glutamate By PAT Incubated With Equimolar Concentrations Of Phosphinothricin And Glutamate For 0 To 90 Min.

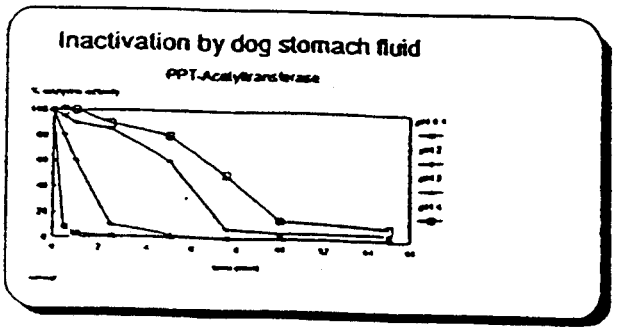


A further experiment measured the enzyme's affinity towards other amino acids with the aid of ^{14}C-Acetyl-CoA (during the acetyltransferase reaction the labelled acetyl group of ^{14}C-Acetyl-CoA is transferred to the α-amino group of the acceptor amino acid). Unlabelled α-amino acids were incubated with ^{14}C-Acetyl-CoA in the presence of PAT. After the incubations the samples were separated by TLC. The autoradiography revealed that ^{14}C-Acetyl-CoA was relatively unstable and several degradation products were seen. Only in the presence of PPT (second sample from right end) was the enzyme able to form a radioactively labelled product (Ac-PPT) Figure 11. The radioactive products formed in the samples containing cystein or cystin were also formed in the absence of PAT and are probably products of chemical reactions of the thioether-S- of Acetyl-CoA with the -SH or -S-S- groups of cystein and cystin. The results demonstrate that the PAT enzyme has a very low affinity for both related compounds and amino acids.

Inactivation of the phosphinothricin acetyltransferase by stomach fluid

To test the stability of the PAT enzyme in gastric juice, the enzyme was incubated at 37°C in stomach fluid from beagle dogs for up to 15 minutes. Samples were taken at different time intervals and the reactions were halted by diluting in a buffer of pH 8.0. Enzyme activity of the sample was measured immediately. The PAT was completely inactivated by the stomach fluid (original pH = 1.1) (Figure 12), within 1 minute. When the pH of the stomach fluid was adjusted to higher pH values the enzyme was inactivated more slowly (10 minutes for inactivation at pH = 4) indicating a rapid degradation in a gastric environment even under buffered conditions.

Figure 12 Inactivation Of Phosphinothricin Acetyltransferase By Gastric Juice Of Beagle Dogs. The pH Of The Gastric Juice (pH 1.1) Was Varied By The Addition Of NaOH.



In summary, the phosphinothricin acetyltransferase protein is inactivated by high temperatures or extremes in pH. The enzyme displays kinetics typical of those found in the plant kingdom. This highly specific enzyme catalyses the acetylation of PPT while not affecting L-glutamic acid or other amino acids. In addition the enzyme is rapidly inactivated by gastric juices. Collectively these results indicate that the enzymatic properties of the PAT protein do not raise any safety concerns

Analysis of Transformants

Southern Analysis

T45 and two daughter lines HCN27 and HCN28 genomic DNA was characterized in order to determine the orientation of the integrated T-DNA and the number of insertion events. The characterization was done by using Southern blot analysis, digested with restriction enzymes. The 550 bp *PAT* gene was used as a probe.

The original transformant line T45 and two daughter lines HCN27 and 28 were assayed to determine stability of the inserted DNA. The inserted T-DNA has remained stable over several generations as demonstrated by a single 1.3 kb band in all transformants on the gel (ACI95-10). Restriction analysis has also determined that a single copy of the T-DNA has been incorporated into the *Brassica* genome (ACI95-22).

Southern analysis for the presence of the antibiotic resistance marker located outside the T-DNA of the vector pHoe4/Ac indicated that the marker has not been transfered to the *Brassica* genome (ACI95-12).

Mendelian Inheritance

Both the nature and the stability of transformed line T45 were assessed by observing the expression of the *pat* gene over multiple generations. A single dominant characteristic such as glufosinate tolerance will segregate according to a defined pattern as described by Mendel. Segregation analysis of both self and backcrosses was used to assess the stability of transformed materials.

The segregation analysis of T45 derived breeding lines clearly shows that the segregation of the inserted construct behaves according to the Mendelian single gene model:

1) No segregation was observed in the breeder seed generation which indicated that the plant was homozygous for the *pat* insert.
2) A 3:1 segregation in the F_2 indicated a single, dominant gene.

The segregation data obtained from the transformant line T45 and its descendants, clearly indicates that the T-DNA insertion locus is stably inherited.

Expression levels

The expression of the *pat* in the transformant canola line HCN92 (Topas 19/2) were determined in the seed and leaf tissue by an enzyme linked immunosorbant assay (ELISA) Both

the transformants T45 and Topas 19/2 have the *pat* gene controlled by the same regulatory sequences taken from cauliflower mosaic virus and it is expected that they will exhibit similar expression levels.

PAT Protien

PAT Enzyme Assay

The PAT assay allows us to determine the presence and activity of the enzyme phosphinothricin acetyltransferase in variety of matrices. By using a radiolabelled ^{14}C L-phosphinothricin substrate, the acetylated PPT complex can be visualized and semi-quantitated by thin layer chromatography.

The assay was used to determine the amount of PAT enzymatic activity found in various matrices within the transformant line HCN92 as compared to its nontransformed parent, Excel®.

The presence of PAT enzymatic activity was detected in roots, leaves, buds and seeds of glufosinate-tolerant canola. Activity in these tissues was determined to be equivalent of 200 - 1000 ng PAT protein/mg total plant protein by comparison to a series of standards spiked with purified PAT protein. Activity was generally greatest in tissue colected from the buds and leaves of glufosinate tolerant plants. Activity levels in these tisuues corresponded to 1000 ng/mg total protien. PAT activity was not detected in protein extracts from the pollen of glufosinate-tolerant line HCN92.

The presence of PAT activity was not detected in unprocessed honey collected from a hive which had foraged in the glufosinate-tolerant canola line. The limit of detection of the assay was established at 10 ng PAT protein/mg plant protein.

The activity levels of phosphinothricin acetyltransferase in the various tissues were comparable between the original transformant 19/2 and the daughter HCN92.

PAT enzymatic activity is expressed constitutively throughout most of the tissues in the transgenic plants 19/2 and HCN92 (Chua, et al., 1990). The levels of enzyme expression were very similar in both 19/2 and HCN92, indicating that the gene is stably integrated into the plant genome.

As mentioned above, the pat gene in the transformant T45 is controlled by the same regulatory sequnces which are presnt in the the transformant Topas 19/2, therefore we expect that the expression of the protien between transformant lines of the same species would be similar.

ELISA
Canola meal

Enzyme linked immunosorbent assay (ELISA) analysis of the meal indicates the presence of denatured PAT protein in the meal at 2-5 PPM. This represents approximately 0.005% of the protein present in the meal.

Seed from HCN92 and a commercial cultivar (Legend) were processed in a simulated commercial crush at Texas A&M University under GLP conditions. Resulting samples were assayed for the presence and activity of the PAT protein. Processed fractions analysed included untoasted canola meal, toasted canola meal, crude oil, refined oil, refined bleached oil, and refined bleached deodorized oil. The PAT immunoassay was validated at a level limit of quantitation of 250 ng/g sample (1 ng/ml).

Levels of PAT protein detected by ELISA were highest in the untoasted canola meal of HCN92. The PAT immunoassay indicated levels as high as 38 µg/g of PAT protein in untoasted canola meal. The presence of the PAT protein was expected in the meal due to the use of a constitutive promoter (CaMV 35S Promoter) which drives PAT expression throughout the plant, but only minimally in pollen. The untoasted canola meal shows detectable enzymatic activity. After the meal is toasted, all activity is destroyed indicating that the enzyme is denatured during the first stages of processing.

A background signal equivalent to 0.093 µg/g was detected in the untoasted meal of the negative canola (Legend). Detection of the PAT protein was not expected in the control material. This trace amount can be attributed to non-specific binding resulting in higher background levels than established using the phosphate buffered saline control. Therefore, the trace amounts detected are not indicative of the presence of PAT in the Legend material. The levels were below the validated limit of quantitation of 250 ng/g of sample.

Levels of PAT protein detected in toasted canola meal were much lower than those in untoasted meal. According to ELISA studies, the PAT protein level in toasted meal ranged from approximately 2 - 5 µg/g of canola meal. The level of PAT found in the toasted meal was approximately one tenth that of the untoasted meal. This is likely due to the destruction of the enzyme epitope during the meal toasting process where temperatures in excess of 90• C are encountered. A study of the enzyme activity of PAT in these processed fractions revealed no activity.

Canola Oil:

Crude Oil:
PAT protein was not detected in crude oil from the nontransgenic Legend control. Low levels of PAT were detected in HCN92 crude oil samples. Amounts detected ranged from 0.296 to 0.460 ng/g, well below the validated limit of quantitation of 250 ng/g. The protein was not

expected to be found in the oil phase due to the polar characteristics of the enzyme. These findings most probably reflect the presence of particulate remnants of the meal in the crude oil samples. The Bradford protein assay (Bradford, 1976) did not detect any protein in any of the crude oil samples. No PAT enzyme activity was detected in the crude oil from HCN92 seed.

Refined Oil:
The PAT protein was not detected by ELISA in any of the refined oils of Legend or HCN92. There was not any detectable PAT activity associated with any of the refined oils. Refined canola oil generally contains no protein of any kind. Consequently we would not expect any PAT protien to be present in the oil of the transformant line T45 or any of its descendants.

Pleitropic Effects

A gene exhibiting pleiotropic effects is one which influences the expression of a number of different characteristics in a plant. Genes can have pleiotropic effects by acting as modifiers or suppressors of other genes. Pleiotropy generally results in positive correlations between traits. If the insertion of the pat gene resulted in pleiotropic effects, they would become apparent when evaluating agronomic traits during the course of cultivar development. To evaluate new transformants for the presence of pleitropic effects the following traits are examined:

a. Plant Height (centimetres) - at crop maturity
b. Yield - adjusted dry weight - grams per plot
c. Maturity - Days from time of planting to 50% pod turn
d. Quality characteristics of the seed
    Percent oil - % oil in seed (dry basis) via N.M.R.
    Protein - % protein in oil-free meal (dry basis)
    Fatty acid composition - erucic acid as percent of total
    Glucosinolates - umoles/g (oil and moisture free basis)

After several years of testing, the glufosinate tolerant canola varieties typically fall well withing the range of variability seen in the *Brassica napus* germplasm pool for all of the above listed traits. By examining the lines in terms of agronomic and quality traits, there were no positive correlations between the presence of the gene and the level of expression of the traits examined.

Brassica napus Agronomic Characteristics - 1995

Trait	Innovator	HCN28	Cyclone	Excel
Cotyledon width (mm)	9.20	9.26	10.33	10.66
Days to 50% Flowering	51.50	57.25	52.50	52.75
Days to Finish Flowering	71.75	78.50	72.50	73.00
Days to Maturity	92.50	100.75	97.50	97.25
Plant Height (cm)	102.50	119.00	112.00	115.00
Lodging Score (0-5)*	0.00	1.00	0.50	0.25
Thousand Kernel Seed Weight (g)	2.80	2.79	3.14	2.83
Yield (g/m2)	21.41	26.75	28.31	23.11
Leaf Width (cm)	9.05	12.70	12.10	11.45
Leaf Length (cm)	19.40	27.60	27.15	24.70
Pedicel Length (cm)	2.20	2.05	2.10	1.80
Silique Length (cm)	4.50	6.45	6.10	6.15
Beak Length (mm)	12.00	15.00	12.00	13.50
Pod Width (mm)	5.00	4.50	5.00	5.00
Protein Content (%)	47.55	47.35	48.75	48.75
Oil Content (%)	47.65	46.75	45.25	47.10

* 0 = no lodging, 5 = flat

In terms of the quality parameters, no identifiable trends were observed for the level of protein, oil, erucic acid or glucosinolates. For example, in the case of oil, protein erucic acid and glucosinolate content,the HCN28 line is essentially equivalent to the commercial varieties evaluated in the study. Subtle differences between cultivars for these parameters are not the result of plietropic effect but rather a result of the efforts of the selection of lines by the breeder against antinutritional factors.

Fatty Acid Composition

Fatty acid analysis was conducted on raw, harvested seed collected from 1994 and 1995 (ACI96-02). An example of the fatty acid profiles for each variety is presented in Table 3. The data represent the mean value of 2 replicates. With the exception of a single result (the linoleic acid content of Excel at Outlook in 1995, ACI96-02), the fatty acid profiles of all varieties at all sites were within CODEX standards. In all instances erucic acid (C22:1) was below the established 2% limit.

Therefore, with respect to fatty acid content, HCN28 glufosinate resistant canola is substantially equivalent to the commercially available glufosinate resistant variety Innovator and to commercially available non-transgenic canola varieties.

Glucosinolate Composition

Glucosinolate analysis was conducted on raw, harvested seed collected in 1994 and 1995 (ACI96-02). An example of the results for the glucosinolate content for each variety is presented by site in Table 4. All canola varieties were below the required concentration of 30 mmoles/g, on an oil and moisture free basis. With respect to glucosinolate content, HCN28 glufosinate resistant canola is substantially equivalent to Innovator glufosinate resistant canola and to non-transgenic commercially available varieties.

Quality analysis of commercial canola varieties, including the glufosinate resistant variety Innovator, and the glufosinate resistant variety HCN28 were conducted on raw seed collected from several locations across Western Canada in 1994 and 1995. Overall, HCN28 was substantially equivalent to the commercial varieties.

Quality analysis of HCN28 glufosinate resistant canola seed confirmed that the levels of erucic acid and total glucosinolate compounds were below the mandatory concentrations for commercial canola varieties.

Proxmate Analysis

Seed moisture, oil, protein, ash, crude fibre, phytosterol contents and gross energy levels were not substantially different among the transgenic canola lines (HCN28 and Innovator) and the commercial varieties evaluated at the three field locations (Table 2) (ACI95-27). Only the protein contents of Cyclone and Legend grown at High Bluff and the ash content of Legend grown at Rosthern were significantly greater ($p<0.05$) than either HCN28 (pHoe4/Ac) and Innovator (HCN92).

Based on the presented data there are no substantial difference in the moisture, oil, protein, crude fibre, ash, phytosterol and gross energy among HCN28 (pHoe4), Innovator (HCN92) and the non-transgenic canola varieties Excel, Legend and Cyclone.

Table 2.

Location	Variety	Moisture g/100g	Oil g/100g	Protein g/100g	Ash g/100g	Crude Fibre g/100g	Phytosterol mg/100g	Energy KJ[1]/100g seed
High Bluff,MB	HCN28	5.7 a*	35.1 a	24.8 a	4.7 a	6.9 a	NA	2233 a
	Innovator	5.6 a	37.4 a	25.0 a	4.5 a	6.6 a	NA	2288 a
	Excel	6.1 a	36.4 a	25.8 ab	4.7 a	6.8 a	NA	2255 a
	Cyclone	6.2 a	32.7 a	26.7 b	4.8 a	6.9 a	NA	2176 a
	Legend	5.6 a	35.9 a	26.9 b	4.6 a	6.5 a	NA	2254 a
Rosthern, SK	HCN28	4.6 a	39.7 a	21.7 a	3.4 a	5.8 a	NA	2370 a
	Innovator	4.6 a	40.2 a	22.1 a	3.3 a	5.9 a	NA	2383 a
	Excel	4.6 a	40.9 a	22.4 a	3.3 a	5.5 a	NA	2396 a
	Cyclone	4.8 a	41.0 a	22.7 a	3.4 a	6.4 a	NA	2395 a
	Legend	4.9 a	39.8 a	23.1 a	3.5 b	5.8 a	NA	2383 a
Indian Head, SK	HCN28	4.4 a	40.4 a	22.8 a	4.2 a	6.0 a	62.6 a	2374 a
	Innovator	4.1 a	40.3 a	23.0 a	3.9 a	5.8 a	66.0 a	2382 a
	Excel	4.2 a	41.0 a	22.5 a	3.9 a	6.7 a	72.7 a	2396 a
	Legend	4.2 a	38.6 a	22.9 a	4.0 a	6.1 a	63.2 a	2345 a

[1] KJ indicates kilojoules; NA indicates not analyzed;
* By location, mean values with the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range comparison.

and would enhance weediness. Plant emergence in the spring of 1993 was not significantly different among HCN92 and commercial canola varieties which were cultivated and harvested in 1992 (HCI93-21). This suggests that there is no appreciable dormancy difference between the volunteer HCN92 and commercial canola varieties. The pat gene is controlled by the same regulatory sequences in both Topas19/2 and T45.

The primary means of canola seed dispersal is by wind and to a lesser extent by water. The distance of dispersal by wind is dependent upon the size of the seed. Results obtained indicate that HCN27 and HCN28 would not be dispersed by wind to a greater or lesser extent than related commercial canola varieties as Mean 1000 seed weight for HCN27 and HCN28 was not significantly different than Excel and Cyclone. Furthermore no phenotypic modification to the seed has occured which would facilitate dispersal.

Invasive Potential of Transgenic *B. napus* (HCN28) Under Disturbed and Undisturbed Field Conditions

One of the identified environmental risks associated with transgenic crops is that the crop itself will become a weed (Rissler and Mellon, 1993). A weed is broadly defined as an unwanted plant which is objectionable or interferes with the activities or welfare of humans. While no plant can be said to be a weed, some characteristics are often associated with weediness. Some plants may possess those phenotypic characteristics which enable them to quickly adapt to a different or new habitat. This may result in a competitive advantage over desirable plants.

A net replacement (invasiveness) potential compares the ecological performance of a population of plants to produce viable, fertile off-spring. Depending on the habitat, the net replacement of a particular phenotype can either increase or decrease over time.

A net replacement rate was calculated for each canola variety grown on disturbed and undisturbed soil at Indian Head and Rosthern. The calculated rates were based on the following equation (Rissler and Mellon, 1993):

Net Replacement Rate = number of seeds collected ÷ number of seeds sown

A summary of mean heights, counts, seed yield and net replacement are presented in Tables 5 and 6.

Table 5. Mean plant counts, seed number and net replacement of various canola varieties grown on disturbed and undisturbed soil, Indian Head, SK, 1995.

Location	Seed bed[5]	Variety	Early Counts	Seed No.	Net Replace
Indian Head,SK	U	LEGEND	34.3 ab	22036 b	77.1 b
Indian Head,SK	U	HCN28 (pHoe4)	27.5 a	5281 a	16.9 a
Indian Head,SK	U	CYCLONE	26.8 a	2875 a	10.9 a
Indian Head,SK	U	INNOVATOR	67.8 bc	1699 a	5.8 a
Indian Head,SK	U	EXCEL	81.8 c	49856 c	164.5 c
Indian Head,SK	D	LEGEND	70.0 bc	39257 bc	137.4 bc
Indian Head,SK	D	HCN28 (pHoe4)	25.5 a	3063 a	9.8 a
Indian Head,SK	D	CYCLONE	93.5 c	30737 b	116.8 b
Indian Head,SK	D	INNOVATOR	78.5 c	28316 b	96.3 b
Indian Head,SK	D	EXCEL	104.8 c	13841 ab	45.7 ab

mean values followed by the same letter are not significantly different at a 5% level (Duncan's Multiple Range Test).

[5] U indicates undisturbed; D indicates disturbed plots.

Table 6. Mean plant counts, seed number and net replacement of various canola varieties grown on disturbed and undisturbed soil, Rosthern, SK, 1995.

Location	Seed bed[5]	Variety	Early Counts	Late Counts	Seed No.	Net Replace
Rosthern,SK	U	LEGEND	26.3 ab	1.3 a	300 a	1.1 a
Rosthern,SK	U	HCN28 (pHoe4)	17.5 ab	0 a	0 a	0 a
Rosthern,SK	U	CYCLONE	36.8 b	10.3 a	1119 a	4.3 a
Rosthern,SK	U	INNOVATOR	20.8 ab	2.0 a	1051 a	3.6 a
Rosthern,SK	U	EXCEL	34.8 ab	7.0 a	674 a	2.2 a
Rosthern,SK	D	LEGEND	30.0 ab	30.0 b	1400 a	4.9 a
Rosthern,SK	D	HCN28 (pHoe4)	8.3 a	0 a	0 a	0 a
Rosthern,SK	D	CYCLONE	65.0 c	61.0 c	572 a	2.2 a
Rosthern,SK	D	HCN92	36.3 b	7.3 a	853 a	2.9 a
Rosthern,SK	D	EXCEL	37.3 bc	3.5 a	1743 a	5.8 a

mean values followed by the same letter are not significantly different at a 5% level (Duncan's Multiple Range Test).

[5] U indicates undisturbed; D indicates disturbed plots.

HCN28 (pHoe4/Ac) had the lowest net replacement values (0-16.9) among all canola varieties tested at Indian Head and Rosthern, Saskatchewan. Typically, commercially grown canola will yield a 300 fold increase in seed.

Due to strong weed competition at Rosthern, both the transgenic canola plants and their non-transformed counterparts could not establish adequately to yield seed. The low net replacement values for all canola varieties grown at both Indian Head and Rosthern indicates a very poor invasiveness potential. Generally, canola is not identified as being invasive in natural habitats.

Regardless of variety, only a small fraction of the ~300 canola seeds (1 g) spread over both disturbed and undisturbed plots matured to produce seed. The early plant counts of the transgenic and non-transgenic canola varieties for both undisturbed and disturbed habitats was 8-35% and 4-21% at the Indian Head and Rosthern locations, respectively.

At Rosthern, all canola varieties competed poorly for nutrients, water and sunlight against grassy and broadleaf weeds commonly found in the area.

Overall, the net replacement value calculated for HCN28 was lowest compared with the values calculated for the commercial varieties INNOVATOR, CYCLONE, EXCEL and LEGEND when grown on disturbed soil at Indian Head. There was no substantial difference in the net replacement among all canola varieties when seeded onto disturbed and undisturbed seedbeds at Rosthern.

The ecological performance of the glufosinate tolerant canola variety HCN28 (pHoe4/Ac) was not affected by the insertion of the phosphinothricin acetyl transferase (PAT) gene. Based on these findings, there was no evidence that HCN28 was more invasive or persistent in disturbed or undisturbed habitats compared with commercial canola varieties over one growing season. The net replacement potential of HCN28 was lowest among the canola varieties tested at Indian Head. There were no substantially differences among all canola varieties tested at Rosthern.

Invasive Potential of Transgenic *B. napus* (HCN28) Under Agronomic Conditions

The replacement series design has been used widely to study interactions between two species of plants. The design maintains a constant total plant density while varying the relative proportions of the two species. Some researchers have criticized the series design as it does not address the contributions made by intra and interspecific competition. However, the present study design is ideal because only one species is evaluated in the series and all comparisons are between different cultivars. The presence of the herbicide resistance gene in the transgenic cultivar HCN28 serves as a useful marker for distinguishing plants from one another in the field study. Without the presence of the resistance gene such a study of intraspecific competition would be impossible under field conditions.

The results of the replacement series can be used to define and contrast any differences in the competitiveness and aggressivity of the transgenic canola cultivar HCN28 and standard commercially available cultivars. Relative biomass yield and aggressivity indices were determined by the following formula:

Relative Yield	$r_a = x_{ab}/x_{aa}$ $r_b = x_{ba}/x_{bb}$
Relative Yield Total	$RYT = r_a + r_b$
Aggressivity	$A = (r_a - r_b)/RYT$

where r_a and r_b are relative yields of cultivar a and b, respectively; x_{ab} is the yield of cultivar a grown in the mixture with cultivar b; x_{aa} and x_{bb} are the yields of the cultivars a and b grown in monoculture. Plots were seeded in warm soil and plants established rapidly at all locations. The distribution of transgenic and nontransgenic plant densities in each of the mixtures is presented in Figure 13. Plots were seeded at a target rate of 100 seeds m^{-2}; the sum of seeding densities indicated a population of 80 to 90 % of target seeding rate.

The glufosinate tolerant transgenic canola line HCN28 and three commercial canola cultivars (Excel, Legend and Cyclone) were investigated in a replacement series experiment under field conditions at three locations in western Canada. Above-ground biomass, collected just prior to bolting, was used to evaluate the competitive ability and aggressivity of HCN28 with its non-transgenic counterparts. Results from this study demonstrated that the presence of the gene coding for phosphinothricin acetyl-transferase (PAT) does not enhance or inhibit the competitive ability of canola under agronomic conditions. Calculated aggressivity values indicated HCN28 was not significantly different when seeded with the commercial canola varieties. Mean aggressivity values calculated across all planting densities for all three locations were 0.12, 0.06 and 0.04 for Excel, Cyclone and Legend, respectively. Therefore, it

is not anticipated that the glufosinate tolerant canola line HCN28 has an increased invasive potential over commercial varieties, even in fields which were not treated with Liberty™ (glufosinate ammonium).

Environmental Interaction of HCN92 (Innovator)

The following data has been summarized form a series of trials which examined the environmental interaction of glufosinate tolerant canola. All trial were conducted with the transgenic line HCN92. The data is provided to support the safe use of another glufosinate tolerant transfrormant line T45. The rational for providing this data is that both the introduced gene and its associated reguilatory sequences are identical between the two transformed material. Furthermore the objective of the trials was to provide an understanding of the interaction of the herbicide tolerance trait and the environment which it has been introduced, therefore, althought the submitted data summary pertians to a diiferent event /construct than the T45 lines it is clearly relevant to its environmental safety assement.

Primary and Secondary Seed Dormancy Characteristics of Glufosinate Tolerant and Susceptible Canola Cultivars

Canola pods that shatter at harvest can result in volunteer canola weeds in subsequent years if secondary dormancy is expressed in them. Canola as a volunteer species has been frequently associated with reduced-tillage systems (Derksen et al., 1993), indicating that the shattered canola seeds may persist in the soil seed bank.

The impact of the *pat* gene on seed dormany has been previously been examined using the pOCA/Ac derived transfromant line HCN92. The objective of the study was to compare, under controlled conditions, the primary and secondary dormancy characteristics of selected glufosinate-susceptible canola cultivars and a glufosinate-tolerant variety, HCN-92.

A series of detailed growth chamber and laboratory studies were conduct to evaluate the impact of the pat gene on the seed dormancy characterisitics of transformed canola lines. Seeds of both transgenic and non-transformed varieties tested germinated completely after 35 days of incubation at 20 °C. Less than 4 % dormancy was observed in all cultivars after 35 days. No treatment effects were significant 14 days after incubation. In contrast, at day 4, the effects of variety and germination temperature, and their interactions were highly significant at $P < 0.001$.

Seed germination of all cultivars was reduced when incubated at 10/5°C as compared to 20°C. In the first experimental run, the germination of cultivars was reduced significantly by reduced storage temperatures. In the subsequent run there was no significant effect ($p < 0.05$) of storage temperatures on germination. The HCN(+) seeds incubated at 10°C and stored at room temperature had significantly lower germination at days 4 through 14 as compared to HCN(-). However, no differences were detected at day 35.

The germination response of HCN92 under all regimes evaluated was never significantly higher or lower than all other cultivars evaluated. Therefore, it can be concluded from the trial data that the dormancy characteristics under these conditions are substantially equivalent to the non-transgenic cultivars.

The seed dormancy characteristics of this glufosinate-resistant variety are similar to those glufosinate-susceptible commercial canola cultivars used in this study. Some 2-4 % of HCN-92 seeds possess primary dormancy and cold, wet seed burial at 10/5 °C for 7 days does not induce secondary dormancy in these seeds. Therefore, the transgenic line HCN92 does not exhibit any unique dormancy characteristics which might result in an increase in the potential weediness of the species.

The transformant T45 carries the same herbicide tolerance gene and associated regulatory sequences as its predecessor HCN92. The impact of the introduction of the gene in the new transformant will be equivalent to the former. Therefore, the transformant T45 and its descendants including HCN27 and HCN28 would not possess any unique dormancy characterisitics.

Assessment of Volunteer Glufosinate-Tolerant Canola Under Chemical Fallow Conditions

Concern has been raised as to the potential risk for a glufosinate-tolerant crop becoming a weedy pest. It has previously been demonstrated that the introduction of the pat gene into the glufosinate tolerant line HCN92 has not influenced the susceptibility of the canola to other commercial herbicides. We would expect that the response of the T45 derived transformant lines would be comparable to that of the Topas 19/2 derived lines response to herbicides. The behaviour of glufosinate tolerant canola HCN92 was contrasted to commercial non-transgenic varieties (Table 7).

Table 7. Mean plant counts (#/m²) prior to and following a 1993 application of glufosinate-ammonium and glyphosate/2,4-D Amine for Standard Canola, Treated and Untreated Transgenic Canola.

Treatment (1993)	Standard Canola		Transgenic Canola (untreated)		Transgenic Canola (Treated)	
Pre-Spray	212		235		257	
Post-Glufosinate	19*	(91%)	157	(33%)	161	(36%)
Post-Glyphosate/2,4-D	0.7	(0.3%)	0.2	(0.1)	0.1	(0.03%)

*Indicates significant difference where $p < 0.05$

Plant counts collected prior to the 1993 application of glufosinate ammonium were not significantly different among the transgenic and non-transgenic plots and ranged between 212- 257 per m². This indicates that volunteer plant populations were equivalent when transgenic and non-transgenic plots were compared.

Non-transgenic canola plants were very susceptible to glufosinate ammonium and were controlled (90%) in the study area after the 1993 application of glufosinate ammonium. Mean plant counts collected 15 DAT were 157, 161 and 19 per m² for untreated HCN92, treated HCN92 and standard canola plots, respectively . As a result, volunteer transgenic and non-transgenic plants were associated with their respective plots.

Tolerance to glufosinate ammonium was expressed in a high number of plants that emerged in the following year. Visual observations indicated that these volunteer, transgenic canola plants were healthy and vigorously growing. However, not all canola plants in transgenic plots were tolerant to glufosinate ammonium. Plant counts in the transgenic canola plots were reduced by approximately 33% compared with the pre-spray counts.

The treatment of glyphosate/2,4-D amine provided excellent (99-100%) weed control. Plant counts collected 10 days after the chemical fallow treatment were below 1 per m² for all three treatments. This indicates that glufosinate-tolerant canola plants were controlled by the traditional chemical fallow treatment. The survival of less than 1% of the population could likely be attributed to a lack of spray coverage on the target or on the emergence of the plants after the herbicide application as their is no residual activity.

Tolerance to the herbicide glufosinate ammonium can be transferred and expressed in volunteer, transgenic-canola plants that emerge in the following growing season. An

application of glyphosate and 2,4-D amine, at recommended rates as a chemical fallow application, provided excellent control of these volunteer canola plants. This study confirms that glufosinate-tolerant canola plants are susceptible to other herbicidal active ingredients with different modes of action. Therefore, glufosinate-tolerant canola plants do not exhibit characteristics which would lead them to become a weedy pest under chemical fallow.

NON-TARGET PLANTS AND ORGANISMS

The PAT gene was modelled from soil bacterium, therefore the gene product can be considered as naturally occurring. No adverse effects to date have been associated with these naturally occurring enzymes.

Equivalence of the gene product produced in the canola plants with the gene product produced by bacteria has been demonstrated in an SDS gel where the bacterial and plant expressed gene products migrate with the same apparent molecular weight (ACI94-16).

The apparent similarity in molecular weights would suggest that no post transcriptional events have occurred, such as glycosylation. Glycosylation of either gene product is unlikely as both proteins lack potential glycosylation sites. Furthermore both proteins are present in the cytosol and are unlikely to be transported to the endoplasmic reticulum where glycosylation would take place.

Proteins as a class are rarely toxic (Pariza, 1989). All plants and animals contain enzymes, with no adverse effects, that are similar in action to the phosphorylating enzyme NPTII. Similarly the PAT protein is an acetyltransferase and is also ubiquitous in nature. The specificity of this enzyme for the substrate phosphinothricin has been demonstrated to be very high (AGR94-10).

The PAT protein does not possess any properties which distinguishs itself from other enzymes. Consequently it does not poses any toxicological risk to nontarget organisms which might interact with transformed canola plants in the environment.

Furthermore, ingestion of PAT protein by nontarget organism poses no safety concern. PAT is rapidly inactivated in both stomach and intestinal fluids by a combination of enzymatic degradation as well as pH mediated proteolysis. It is improbable that the enzyme could survive ingestion. Although unlikely, if the enzyme were to survive ingestion, it is unlikely that any adverse effect would result. As mentioned above, enzymes of similar action are ubiquitous in plants and have not been associated with adverse effects in animals (ACI94-15).

DNA is rapidly destroyed at gastric pH's, therefore it is unlikely that sufficient intact DNA would be available to allow for horizontal transfer of the gene and the subsequent expansion of antibiotic resistant pathogens. In the case of ruminants, DNA is also rapidly degraded in the rumen to its nucleotides and nucleosides. (McAllan, 1973)

The FDA agreed with the conclusion of Calgene in its petition for the FLAVR SAVR tomato that the potential of horizontal transfer of DNA to gut microorganisms or gut epithelial cells is not of significant concern.

The product of the introduced gene in the glufosinate resistant canola lines do not have characteristics which would result in adverse effects on nontarget organisms. Since 1993, the transgenic canola line HCN92 has been evaluated in over 20 field trials conducted across Canada. Careful monitoring of trial areas during and post harvest have shown no evidence of adverse effects towards nontarget organisms including deer, rodents, birds, bees and other insects that frequent canola fields.

Behaviour of Honey Bees Foraging on Transgenic Canola (*Brassica napus*)

The recent development of transgenic canola has raised a number of questions as to the impact of the introduction of this plant on other organisms. Canola flowers produce an abundant supply of nectar for insect pollinators. Cross pollination of flowers often occurs when bees are searching for nectar. Because introduction of a novel gene may elicit a biochemical change in plants, it is important to evaluate the impact of the transgenic on the crop on the behaviour of honey bees. The introduction of the pat gene has previously been investigated using the glufosinate tolerant lie HCN92 over a period of several years. The results of these studies are summarized below. This data is provided as evidence of the safe use of this gene in an agronomic environment.

This study represents a second field experiment. The results obtained from this year's study will be compared with those from the previous year. The 1993 results, contained in an earlier report HCI93-14, indicated that trangenic canola had no effect on honey production or bee behaviour. The objective of the study is to evaluate the impact of transgenic canola on the behaviour of honey bees (*Apis mellifera*) under field conditions.

After transporting the hives to Indian Head, the first inspection took place when fresh honey supers were introduced to the colony when canola flowering commenced. Inspection of brood chamber revealed the queen actively laying with both eggs and larvae present in abundance with minimal honey stores in the brood chambers. The bees were flying on the day of inspection and were observed to be actively foraging in the transgenic crop.

Colony was again inspected approximately two weeks after the commencement of canola flowering. The lower honey super was approximately 75% filled, while the upper super was 25% filled. Cells were partially filled with a very light honey characteristic of canola. Many of the frames were fully capped. Frames towards the centre of supers were fully capped with less capping evident on the outer frames. Bee population in the honey supers as well as larvae in the brood chamber had increased markedly from the commencement of canola flowering. The presence of drones near the hive entrance was noted. The queen was observed to be actively laying and both eggs and developing brood were observed.

The honey supers from each site were removed after the canola flowering had terminated. The lower super was completely filled and the upper super was approximately 80% capped. Honey production was equal between hives. Approximately 60 kg of honey was extracted from each hive. The extracted honey was light in colour coloured, characteristic of canola, and highly viscous. Fresh supers were introduced to both colonies.

A fall inspection of the hives was conducted on September 22. The top three honey super of both hives at each location were 100% filled with a very light coloured honey. All frames were fully capped. Bee population in both hives were above average. Honey storage was excellent for both hives with approximately 90 kg per hive. This honey was not extracted and left for the bees as an overwinter food supply. Brood and pollen supplies were normal for both hives. There was very little difference in honey production and worker bee population. No behaviour differences were observed between the two hives.

Bee behaviour of the colony located near the transgenic canola field was no different from the colony located near the non-transgenic canola field. Results from this study indicate that honey bees will actively forage on glufosinate resistant canola and produce a light coloured honey. Hive development was observed to be normal during and subsequent to flowering of both transgenic and non-transgenic canola crops. Prior to overwintering, the health of both hives was rated as above average condition.

Residual Effects of Glufosinate-Tolerant Canola on the Growth and Productivity of Grain, Forage and Pulse Crops

The application of this new technology has raised questions regarding the impact of environmental releases of transgenic plants. Residual effects of transgenic plants on soil productivity can be addressed by comparing a number of typical rotational crops (ie. grains, forage and pulses) at locations where transgenic and non-transgenic plants were grown.
A

The objective of this study was to determine the residual effects of glufosinate-tolerant canola (HCN-92) on typical rotational crops grown in the following year. Plant performance was evaluated in the plots which previously grew transgenic canola and non-transgenic canola varieties. Crop vigour, growth and yield were used as indicators of the productivity of the soils.

Residual effects of glufosinate-tolerant canola (HCN92) on soil productivity was assessed by comparing the performance of a number of typical rotational crops (ie. grains, forage and pulse) at locations where transgenic and non-transgenic plants were previously grown (HCI93-02). Crop vigour, growth and yield were used as indicators of the productivity of the soils (Table 8).

The effect of transgenic canola residue on agronomic performance was examined for wheat, barley, lentils, peas, flax and alfalfa. In 1993, plant counts, mid-season biomass and yield were measured at Edgeley and Rosthern, Saskatchewan and Rosebank, Manitoba. Although some differences were statistically significant on an individual location basis, these effects were not consistent across the three study locations. Performance differences in Manitoba were attributed to the cool, wet growing season; in Saskatchewan, excessive weed pressure at early stages of development, particularly in the less competitive crops (ie. flax), reduced productivity. Therefore, results from these trials indicate no residual effect of transgenic canola on rotational crops grown in the following year (Table 21).

Table 8 Table of Means

Crop	Edgely		Rosthern		Rosebank	
Wheat	S	T	S	T	S	T
Plant count	71.15	72.05	97.13	100.44	141.75	140.75
Dry weight	149.50	161.06	177.63	205.19	242.38	235.88
Yield	313.93	319.19	407.95	376.51	388.86	400.61
Barley	S	T	S	T	S	T
Plant count	70.89	71.43	83.62	84.44	77.19	75.00
Dry weight	153.88	169.12	172.75	183.00	207.31	213.61
Yield	362.27	323.00	466.11	415.74	315.29	314.29
Flax	S	T	S	T	S	T
Plant count	73.06	74.37	20.61	21.95	155.44	155.78
Dry weight	*91.88	*106.88	*80.13	*86.60	161.74	159.19
Yield	50.69	56.73	-	-	263.44	280.00
Lentils	S	T	S	T	S	T
Plant count	39.00	36.75	20.75	12.50	79.50	76.25
Dry weight	107.88	112.43	97.75	100.75	196.45	195.78
Yield	276.80	301.98	-	-	29.88	35.08
Alfalfa	S	T	S	T	S	T
Plant count	107.50	129.13	-	-	-	-
Dry weight	91.88	99.81	-	-	-	-
Yield	254.65	265.86	-	-	-	-
Peas	S	T	S	T	S	T
Plant count	-	-	*11.25	*6.33	13.75	13.38
Dry weight	-	-	101.88	104.31	205.78	216.64
Yield	-	-	-	-	131.08	122.69

* signifies a significant difference at 5% confidence level between the standard and transgenic canola
S = Standard Canola Variety ; T = Transgenic (HCN92) Canola

Results obtained from the trials conducted in 1994 confirm those obtained in 1993 (Belyk and MacDonald, 1994). Each rotational crop was examined individually at both locations in 1994. Statistical analysis of the data indicated that there were no significant differences in plant counts, dry matter weights or grain yield for flax, wheat, barley, lentils, peas and alfalfa when grown on either transgenic or non-transgenic crop residue. Poor emergence of the lentil plots at Rosthern was the result of a substantial rainfall immediately following seeding which

This apparent lack of residual effects is supported by the work of Stotsky (1989) in which soils were amended with high concentrations of transgenic *E. coli* expressing various antibiotic resistance markers. No impact on gross metabolic activity, CO_2 evolution, nitrogen transformation and soil enzyme activity was detected as a result of the addition of the antibiotic resistant plasmid. This indicated that there was no negative impact on soil micro-organisms.

Gene Outcrossing

Brassica napus canola is a self compatible species which exhibits a variable level of outcrossing depending on environmental conditions. Field outcrossing studies with glufosinate tolerant canola (HCN92) demonstrated outcrossing with nearby (< 8m) *B. napus* plants; the transfer of tolerance to glufosinate was observed in as much as 25% of the progeny (HCI93-01, HCI93-03). No outcrosses between glufosinate tolerant HCN92 and *Sinapsis arvensis, Brassica campestris* or *Brassica nigra* was observed in either greenhouse or field trials (HCI93-03, HCI93-18).

The major vector for pollen transfer of *B. napus* in Western Canada is the honey bee. In a field study monitoring honey bee behaviour, bees were observed to actively forage on HCN92 canola (HCI93-14). Colony development was observed to be normal during and subsequent to canola flowering.

Overall, the reproductive characteristics of the glufosinate-tolerant canola were determined to be equivalent to the non-transformed counterpart Excel.

Impact of the Introgression of Transgenes from *B. napus* (HCN92) into Related Species

One of the principal prominent issues regarding the use of genetically engineered crops is the likelihood and possible consequences of the transgenes being introgressed by cross pollination into wild populations of plants. The concern is that some genes could confer some adaptive advantage if they were introduced into wild populations.

The wild relatives of *B. napus* are numerous and widely distributed, but botanical barriers do exist to prevent most interspecific crossing (Bing, 1991). However, *B. napus* is relatively easy to cross with *B. rapa* (ACI94-08) and spontaneous crosses have been observed in the field when the two species were grown adjacent (Downey, 1980).

Therefore, it is necessary to assess the potential impact of hybridization between the glufosinate tolerant canola line HCN92 and related species. With the exception of tolerance to the herbicide glufosinate ammonium, introgression of the PAT gene would confer no selective advantage to hybrid progeny. Since the herbicide glufosinate ammonium is unlikely to be used on natural plant populations the selection pressure would be limited to the agricultural environment where it is used (Dale, 1992).

Selection pressure is magnified when a herbicide is used exclusively and continuously. A current recommendation to reduce the occurrence of black leg and white mold diseases is to exclude canola in a four year rotation by growing nonsusceptible species such as cereals. Glufosinate ammonium can not be used in crops which have not been engineered for tolerance. Consequently, the selective pressure of the herbicide on a hybrid populations would be very limited. In addition, glufosinate resistant canola progeny can be managed easily by mechanical and chemical means (HCI93-04, HCI93-05).

The hybrid population is unlikely to proliferate in the absence of selection pressure caused by the herbicide. Many *B. napus* hybrids have reduced fertility when compared to the wild type. Hybrid crosses may lose weedy characteristics such as a reduction in seed dormancy as observed in hybrids of *B. napus* x *B. adpressa* (Lefol et al, 1991).

It has been speculated that the introduction of transgenes might change the normal physiology either as a consequence of the transgene or an insertional effect. Physiological changes might result in aggressive "weedy" characteristics in the phenotype. To address this issue, the interaction of T45 derived *B. napus* line HCN28 with its growing environment was examined intensively. Field studies demonstrated quantitatively that HCN92 canola is equivalent with common commercial canola varieties in terms of invasiveness and aggressiveness (data

presented above). Based on our findings, and on the work of Crawley et al. (1993), the ecological performance of *B. napus* was not affected by the presence of the PAT gene.

The recent interest to monitor gene flow in the environment has increased dramatically with the development of a number of plants with novel traits. Consequently, it has been necessary to evaluate novel traits on a case by case basis to establish their impact on the fitness of plants. However, the selection for novel traits in plants is not new. Plant breeders have been developing techniques and selecting plants with novel traits for generations. Resistance to symmetric-triazine herbicides was introduced into canola approximately 15 years ago using classical plant breeding techniques. To date, there is no evidence to report that this novel trait has resulted in any adverse effects on related plants species.

With respect to genetically modified HCN27 and HCN28 canola, all experimental results indicate that the introgression of the PAT genes into related species would have no impact on their ecological performance.

Spontaneous, Interspecific Crosses Between *B. napus* and Related Species Literature Review

Under field conditions, the transfer of pollen among related *Brassicaceae* member plants can occur either by beneficial insects, physical contact, or by wind. However, successful interspecific crosses have proven difficult even under ideal growing conditions. Many important environmental and physiological factors must first be met before a viable hybrid develops between *B. napus* and wild relatives. As well, once these hybrid plants reach maturity, they must be fertile or sufficiently fertile to maintain themselves by self-pollination, and/or able to backcross to their weedy parent. A hybrid that lacks fertility or can not reproduce will soon become extinct.

With respect to glufosinate resistant canola (*B. napus*), there are only two species (*B. rapa* and *B. juncea*) that are likely to produce fertile hybrids after receiving pollen from genetically modified *B. napus* (Bing, 1991). However, it is possible that volunteer *B. rapa* and *B. juncea* to come in close physical proximity to receive pollen from cultivated *B. napus*. Natural field hybrids between *B. napus* and *B. rapa* are often identified in western Canada (Downey, 1992). Hybrids between *B. napus* and *B. rapa* or *B. juncea* have been generated in field experiments although fertility of the hybrids is <10% (Bing, 1991).

Hybridization with several *Brassicaceae* member species has been investigated under field conditions. Hybrids between *B. napus* and *B. adpressa, B. nigra, Sinapis arvensis,* or *Raphanus raphanistrum* were either not produced or sterile (Bing, 1991; Kerlan et al., 1992; HCI93-03; ACI94-08). An extremely low number of hybrids between *B. adpressa* and *B. napus* occurred,

however, under very atypical field circumstances (Lefol et al. 1991). However, these hybrids were found to be sterile and would neither selfcross or backcross. Hybrids produced with maternal *Raphanus raphanistrum* (ACI94-08), *Sinapis arvensis* (HCI93-03; Bing, 1991) and *B. nigra* (HCI93-03) were not observed in the field.

Field crosses with *Diplotaxis muralis* are extremely unlikely since it is not commonly found in agricultural fields. Spontaneous outcrosses with *D. muralis* are very unlikely since it is highly self-compatible and most fertilization is complete before the flower opens (Ringdahl et al., 1987). Spontaneous crosses between *B. napus* and either *B. carinata* or *B. oleracea* are highly unlikely in the field since the latter species do not naturally occur in the wild and commercially they are not taken to seed (Calgene, 1994).

In conclusion, Table 10 summarizes the interspecific crosses between *B. napus* with various *Brassicaceae* member species. Overall, the possibility of *B. adpressa, B. nigra, S. arvensis, R. raphanistrum* or *D. muralis* to hybridize with *B. napus* is extremely low. Hybrids may be formed with commercial *B. napus, B. rapa* and *B. juncea.*

Table 10 Summary of interspecific crossing results under field conditions between various *Brassicaceae* member species and *B. napus* (pollen donor).

Pollen Recipient	Field Hybrids Produced?	Fertility of Hybrids	Reference
B. napus	Yes	normal	Bing, 1991
B. rapa	Yes (0.7-1.3%) Yes (3.3%)	< 10% viable	Bing, 1991; ACI94-08, 1994
B. juncea	Yes (0.1-0.3%)	< 10% pollen viable	Bing, 1991
B. nigra	Yes (extremely low numbers); No	male sterile; n/a	Bing, 1991; HCI93-03, 1993
B. oleracea†	No	n/a	Calgene, 1994
B. carinata††	No	n/a	Calgene, 1994
B. adpressa	Yes (extremely low numbers)	mostly sterile	Lefol et al., 1991
Raphanus raphanistrum	No	n/a	ACI94-08, 1994
Sinapis arvensis	No	n/a	Lefol et al., 1994; HCI93-03, 1993
Erucastrum gallicum	No	n/a	ACI94-08, 1994
Diplotaxis muralis	No	n/a	ACI94-08, 1994

† *B. oleracea* does not naturally occur in the wild and is not taken to seed in Canada.

†† *B. carinata* does not occur in the wild and is not commercially grown in North America.

Response of Glufosinate Tolerant Canola to Pests and Diseases

Flea Beetles

Flea beetles are pests to canola, mustard, flixweed and other cruciferous weeds (Thomson and Hughes, 1986). The most serious damage is caused by over-wintering adults which feed on the cotyledons and first true leaves. The "shot-holes" are an early sign of damage. Seedlings

that are severely damaged may die, while less serious damage can result in yield loss. Once a plant gets beyond the seedling stage, serious damage does not usually occur because the plant material has increased many-fold and the adult flea beetle population has often begun to decline. Flea beetles have one generation per year in Western Canada. The overwintered beetles mate and lay their eggs during May and June, and the adult population die off by the end of June.

Numerous flea beetles in a field can act as a biological control agent for cruciferous species. Therefore, any tolerance to flea beetle damage can enhance the invasiveness of an adaptive weed. Below, the susceptibility of a glufosinate tolerant canola (HCN92) to flea beetle damage is compared with commercial varieties of canola.

For all canola varieties (Excel, Legend, Cyclone and HCN92), induced flea-beetles caused greater plant damage compared with the natural occurring flea-beetle population. At the cotyledon to 1 leaf stage (June 6), an injury rating representing up to 75% and 25% damage were observed for caged and natural flea-beetles, respectively (Table 11). It was assumed that greater injury occurred with caged flee-beetles as a result of the limited number of plants available to feed upon. Flea-beetle damage decreased rapidly as the canola plants grew. By June 18, very little damage (0.5 rating) was observed by the 3-4 leaf stage (Table 11).

HCN92 exhibited similar characteristics to the other commercial varieties with respect to emergence, plant counts, plant vigor, number of days to first and last flower, maturity and grain yield. There was no significant difference in emergence, plant counts, plant vigor and yield for all canola varieties tested (Table 12). There was a significant difference in the number of days to first flowering (3 day difference), last flowering (2 day difference), duration of flowering (1 day difference) and maturity (17 day difference) between all varieties. HCN92 was observed to be intermediate among the lines evaluated in terms of the number of days to first flower and last flower. While HCN92 displayed the highest percentage of seed turn at maturity. These differences are attributable to the lineage of HCN92 which have been selected for using traditional plant breeding methodologies.

Table 11. Summary of the Flea Beetle impact on Transgenic and Conventional Canola.

Variety	Caged Beetle Damage[a] 06-06-93	Natural Beetle Damage 06-06-93	Caged Beetle Damage 06-18-93	Natural Beetle Damage 06-18-93	Emerg-ence Percent 06-11-93	Plant Counts per m2 06-28-93
Excel	2.9 a[b]	1.0 a	1.7 a	1.0 a	53 ab	117 a
Cyclone	2.8 a	1.0 a	1.5 a	1.0 a	65 a	119 a
Legend	3.1 a	1.0 a	1.6 a	1.0 a	50 b	96 a
HCN92	3.2 a	1.0 a	2.0 a	1.0 a	55 ab	148 a
LSD (.05)	1	0	0.4	0	14	52

[a]Damage rating ranges from 0 (no damage) to 4 (severe damage).

[b]Treatment means followed by the same letter are not significantly different ($p < .05$).

Table 12. Summary of the Flea Beetle impact on Transgenic and Conventional Canola.

Variety	Visual Vigor 0-10 06-22-93 30 DAE	# days to first flower	# days to last flower	Duration of flowering, days	Maturity Seedturn Percent 09-14-93 Maturity	Grain Yield kg/ha 09-17-93 Maturity
Excel	7.3 ab[a]	55 a	85 a	31 ab	49 b	1911.6 a
Cyclone	8.3 a	54 b	83 c	31 b	41 c	2363.4 a
Legend	7.0 b	52 c	83 c	32 a	50 b	1986.6 a
HCN92	7.3 ab	53 b	84 b	32 a	58 a	2078.8 a
LSD (.05)	1.1	1	1	1	7	423.4

[a]Treatment means followed by the same letter are not significantly different ($p<.05$).

There was no difference between the glufosinate tolerant canola variety and the standard commercial canola varieties Legend, Excel, and Cyclone in their susceptibility and reaction to flea beetle feeding. Therefore introduction of the glufosinate tolerance trait has not effected the susceptibility of the canola lines to flea beetles.

Leptosphaeria maculans

Blackleg or stem canker is caused by the organism *Leptosphaeria maculans* and results in yield losses of canola of 13-50 percent on a world wide basis (Saharan, 1993) In Western Canada, the problem of blackleg disease has been alleviated to some extent through the use of longer crop rotations, however, the development of blackleg tolerant lines remains a major breeding objective. Testing for blackleg tolerance can be performed on greenhouse material at the seedling or more mature stages or under field conditions[1]. Concerns may exist that a new variety with limited blackleg resistance might serve as a host for the distribution of disease, if it became volunteer. Much emphasis is placed by the WCC/RRC (Western Canada Canola/Rapeseed Recommending Committee) on levels of resistance in new cultivars being equal or greater than existing cultivars.

Results from the WCC/RRC trials to date have indicated that the T45 derived transformant lines HCN27 and HCN28 have comparable resistance to blackleg to the commercial standards used as check in the evaluation program. Both HCN27 and HCN28 possess adequate blackleg resistance and would not pose a threat to agriculture if it was a volunteer weed, as it will not serve as a source of inoculum. The level of resistance to this disease is superior to older varieties and similar to newer varieties being developed.

Sclerotinia sclerotiorum

Sclerotinia sclerotiorium is a fungus that occurs throughout most of agricultural areas of Western Canada. *S. sclerotiorium* affects a variety of important oilseed and pulse crops such as canola, sunflower, field pea, alfalfa, and lentils. Sclerotia (resting bodies) of *S. sclerotio*rum in the upper layers of the soil (top 3-5 cm) germinate under cool, moist conditions to produce apothecia (fruiting bodies). The disease varies in intensity from year to year and does not occur uniformly throughout a growing area. *Sclerotinia* infections can lead to pre-mature ripening, reductions in seed quality and yield.

The earliest symptom is a soft, watery rot on the stem. When a stem is completely girdled by a lesion the plant wilts and dies. Plants infected at early flowering produce little or no seed.

Those infected at late flowering or podding will set seed and may suffer little yield loss. However, shattering of plants which have ripened pre-maturely due to infection can result in yield loss.

Tolerance to *Sclerotinia* would be an asset to a crop variety but might enhance hardiness as a volunteer crop. The impact of the glufosinate tolerance gene on the susceptibility of the canola cultivar to *Sclerotinina* was evaluated using the line HCN92. The results of this stud are summarized below and are provided to demonstrate that the impact of this trait of susceptibility to this disease for all glufosinate tolerant cultivars.

The objective of this study was to compare the susceptibility to *Sclerotinia* infection on glufosinate tolerant canola with commercial varieties of canola.

Throughout the monitoring period, disease infection was not significantly different for Cyclone, Excel, Sprayed (glufosinate ammonium @ 750 g/ha) and Unsprayed HCN92 canola varieties. Disease infection symptoms during early flower consisted of leaf lesions only on 6-12 % of the plants assessed for each variety (Table 13.). During mid and late flowering, disease infection was slight to moderate and detected on 30-40% of the plants assessed for each variety (Table 13). Symptoms at this time consisted of leaf and stem lesions. Even under a disease stress condition, all varieties displayed good to excellent vigor.

At maturity, Cyclone was observed to have the lowest disease severity rating (2.0), while sprayed, unsprayed HCN-92 and Excel did not differ significantly ($P < 0.05$) from each other having disease rating of 3.1, 2.9 and 2.9, respectively. Grain yield was not significantly different among all varieties; all varieties produced a mean grain yield greater than 2500 kg/ha (Table 13).

Susceptibility of sprayed and unsprayed HCN92 to *Sclerotinia* was not significantly different compared to Cyclone and Excel. Disease measurements and plant vigor for early, mid and late flowering stages indicated no substantial differences among the transgenic and non-transgenic varieties. At maturity, grain yield was not significantly different among all varieties.

Table 13. Sclerotinia Susceptibility Evaluation Trial

Ag-Quest, Inc.
Minto, Manitoba

Character rated		Grain	Disease	Disease	Disease	Disease	Emergence	Plant
Rating data type		Yield	Infect'n	Infect'n	Severity	Severity	Plot	Count
Rating unit		Kg/ha	Percent	Percent	0-5	0-5	Percent	Per m2
Rating date		09-17-93	07-20-93	07-31-93	09-17-93	09-17-93	06-07-93	06-11-93
Trt-Eval Interval			GS. 4.1	GS. 4.2	GS.4.3	Maturity	GS. 1-2.1	14 DAE
PRM Data Type								
Trt #	Treatment Name							
1	HCN92	2627.1a	10.0a	30.0a	35.8a	3.1a	65.0a	123a
2	Cyclone	2631.5a	9.2a	33.3a	36.7a	2.0b	40.0b	112a
3	Excel	2525.3a	11.7a	35.0a	40.0a	2.9a	40.0b	103a
4	HCN92 Sprayed	2552.1a	5.8a	34.2a	35.0a	2.9a	50.0b	131a
LDS (.05)		378.5	5.6	6.9	5.7	0.7	11.6	40
Standard Dev.		236.615	3.51285	4.30516	3.54916	.442923	7.26483	24.8031
CV		9.16	38.37	13.00	9.62	16.29	14.90	21.22
Block F		3.805	2.541	11.797	1.095	4.752	2.684	0.141
Block Prob(F)		0.0518	0.1218	0.0018	0.4003	0.0298	0.1097	0.9329
Treatment F		0.204	1.979	1.038	1.527	4.965	10.579	0.971
Treatment Prob(F)		0.8908	0.1877	0.4216	0.2733	0.0266	0.0026	0.4483

Means followed by same letter do not significantly differ (P=.05, Duncan's MRT

The Response of Glufosinate Tolerant Canola to Environmental Stress

Soil Salinity

Some environmental factors that can adversely affect plant growth have been investigated to ensure that herbicide tolerant plants will not show unintended genetic changes in hardiness that could lead to altered weediness when compared to their unmodified counterparts. The object of this study was to compare a glufosinate ammonium tolerant canola variety *(Brassica*

napus L. cv. HCN92) to a commercial standard canola variety *(Brassica napus* L. cv. Legend) when grown in soils with a wide range of salinities.

HCN92 and Legend canola are more salt tolerant at seedling emergence than at later stages of development. Moderate and severe salinity levels did not reduce total seedling emergence, but significantly reduced plant growth parameters. HCN92 had higher shoot weight, greater plant height, and larger number of leaves than Legend, but in general the two varieties responded similarly to soil salinity stress. We conclude that vegetative growth of the genetically transformed variety, HCN92 is substantially equivalent to that of variety Legend under salt stress conditions. Therefore, the herbicide tolerance trait has not influenced the salt tolerance of the transgenic cultivar.

Moisture Stress

Below, the influence of moisture stress on an glufosinate tolerant canola variety HCN92 is compared to five other commercial canola cultivars (Westar, Legend, Delta, Crusher and Excel).

Several canola growth parameters, agronomic characteristics as well as canola yield were evaluated to evaluate the response of canola cultivars to moisture stress. Moisture stress had a significant effect ($p < 0.05$) on canola vigour, lodging and yield. There was also a significant difference amongst the cultivars evaluated (main effect of cultivar). However, no significant interaction occurred between the effects of moisture regime and cultivars were found. Therefore only main effects upon canola growth and yield were summarized.

No lodging was observed during bloom. Lodging occurred at the onset of crop maturity. No diseases were present on site at sufficient pressures to result in crop injury or permit rating. The trial was harvested on October 11, 1993

The effect of the two moisture regimes upon canola growth and yield were summarized. Overall, irrigation was beneficial to canola growth. Irrigation significantly increased seedling vigour later in the season and increased canola yield by 15%. Irrigation, however, did increase crop lodging. Crop lodging was probably enhanced by the increased seed burden in the higher yielding irrigated plots.

The cultivar differences for the growth parameters analyzed. The cultivars significantly differed in their bloom and maturity dates, the amount of lodging expressed (stem strength) and their seed yield at the 5 % level of the test.

Lodging resistance was greatest in the cultivars Crusher, Delta and HCN-92, only fair in Excel and poorest in the cultivars Westar and Legend.

The best canola yield was produced by Delta followed by Excel, HCN92, Crusher, Westar and Legend with harvested yields of 232, 215, 213, 211, 187 and 172 g/m^2, respectively.

Irrigation was beneficial to all canola cultivars increasing yield on average by 15%. All cultivars responded equally to the two moisture regimes as no significant interactions were present. Irrigation, however, did tend to increase the incidence of canola plant lodging.

The glufosinate tolerant variety HCN92 is a relatively early cultivar with maturity (110 days) between the cultivars Westar and Legend. The lodging resistance of HCN92 is good. HCN92 yields favourably compared to the other six cultivars. HCN92 ranked third in yield after Delta and Excel but the differences in yield amongst these cultivars were not significant at the 5% level of the test. Canola seed yield of HCN92 was slightly but not significantly better than Crusher and Westar and significantly better than Legend.

The glufosinate tolerant canola variety HCN92 is not better adapted to drought stress than the other widely grown canola cultivars tested as its response to drought was not significantly different to that of the other cultivars tested.

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Attachment I:

Documentation from States growing >1% of Canola for Production

October 17, 1996

To: Vickie Forster

Hello,

I check with my colleagues, and we are of a consensus that only two weeds in Alabama have any potential to inter breed with genetically- engineered rape or canola. These are 1) wild mustard [Brassica kaber (DC.) L.] and wild radish [Raphanus raphanistrum L.].

Sincerely

Glenn

Glenn Wehtje
University of Auburn
Auburn University

Colorado State University

MEMORANDUM

Department of Soil and Crop Sciences
Fort Collins, Colorado 80523-1170
(970) 491-6517
FAX: (970) 491-0564

to: Vicki Forster
from: Duane Johnson
subject: Colorado mustards
date: April 22, 1997

We have identified the following growing in Colorado. None are exceptionally prevalent with the exception of Brassica nigra which is in several ecosystems.

Brassica nigra (Eastern Colorado 4,500-8,000 ft elev.)
Brassica juncea (Northcentral Colorado 4,500-7,500 ft elev)
Brassica rapa (Brassica campestris) (Central Colorado 7,000 to 8,000 ft elev)
Sinapis alba (Brassica hirta) (Northern Colorado 3,800 to 7,500 ft elev)
Sinapis arvensis (Brassica kaber) (Northern and Western Colorado 4,500 to 8,500 ft elev)

Colorado canola production would be limited primarily to Northeastern Colorado, South Central and Southeastern Colorado and Southwestern Colorado. *The primary types of interest to Colorado growers will be high oleic types.* Winter canolas will predominate production in but the South central region. Currently spring canola production in the South Central region accounts for essentially 100% of the Colorado crop.

Tel: 970-491-6438

1995 SWSS REPORT: MOST COMMON, MOST TROUBLESOME WEEDS IN GEORGIA

SM Brown, GE MacDonald, JM Moore, TR Murphy

	COTTON	PEANUTS	SOYBEANS	TOBACCO
	Most Common	**Most Common**	**Most Common**	**Most Common**
1.	Texas panicum	Florida beggarweed	sicklepod	yellow nutsedge
2.	sicklepod	Texas panicum	pigweed spp.	sicklepod
3.	cocklebur	sicklepod	crabgrass	cocklebur
4.	nutsedge spp.	cocklebur	morningglory spp.	Florida beggarweed
5.	pigweed spp.	nutsedge spp.	Texas panicum	morningglory spp.
6.	morningglory spp.	morningglory spp.	cocklebur	bristly starbur
7.	Florida beggarweed	bristly starbur	yellow nutsedge	sandbur spp.
8.	bristly starbur	crabgrass	Florida beggarweed	burgherkin
9.	crabgrass	pigweed spp.	Florida pusley	Texas panicum
10.	coffee senna	prickly sida	common ragweed	crabgrass
	Most Troublesome	**Most Troublesome**	**Most Troublesome**	**Most Troublesome**
1.	nutsedge spp.	Florida beggarweed	sicklepod	yellow nutsedge
2.	sicklepod	nutsedge spp.	morningglory spp.	sicklepod
3.	coffee senna	bristly starbur	pigweed spp.	cocklebur
4.	Texas panicum	sicklepod	coffee senna	Florida beggarweed
5.	pigweed spp.	morningglory spp.	cocklebur	morningglory spp.
6.	cocklebur	burgherkin	johnsongrass	bristly starbur
7.	morningglory spp.	tropic croton	crabgrass	sandbur
8.	wild poinsettia	prickly sida	Texas panicum	annual sedge spp.
9.	bristly starbur	wild poinsettia	Florida beggarweed	Texas panicum
10.	bermudagrass	Florida pusley	yellow nutsedge	crabgrass

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To Brenda Fink	From Sm Brown
Co.	Co. UGA
Dept.	Phone #
Fax #	Fax # 912 386-7308

IDAPA 02
TITLE 06
Chapter 22

IDAHO DEPARTMENT OF AGRICULTURE
NOXIOUS WEEDS RULES

000. -- 099. (RESERVED).

100. NOXIOUS WEEDS. (7-1-93)

01. Designation of Noxious Weeds. The following weeds are hereby officially designated and published as noxious: (7-1-93)

a. Buffalo bur (Solanum rostratum). (7-1-93)

b. Canada thistle (Cirsium arvense) (L.) Scop. (7-1-93)

c. Common crupina (Crupina vulgaris) (Cass.). (7-1-93)

d. Dalmatian toad flax (Linaria dalmatica) (L.) Mill. (7-1-93)

e. Diffuse knapweed (Centaurea diffusa) Lam. (7-1-93)

f. Dyers woad (Isatis tinctoria) L. (7-1-93)

g. Henbane (Hyoscyamus niger) L. (7-1-93)

h. Johnsongrass (Sorghum halepense). (7-1-93)

i. Jointed goatgrass (Aegilops cylindrica). (7-1-93)

j. Leafy spurge (Euphorbia esula) L. (7-1-93)

k. Loosestrife (Lythrum salicaria) L. (7-1-93)

l. Matgrass (Nardus stricta). (7-1-93)

m. Meadow knapweed (Centaurea pratensis). (7-1-93)

n. Milium (Milium vernale). (7-1-93)

o. Orange Hawkweed (Hieracium aurantiacum). (7-1-93)

p. Musk or nodding thistle (Carduus nutans) L. (7-1-93)

q. Perennial pepperweed (Lepidium latifolium) L. (7-1-93)

r. Perennial sowthistle (Sonchus arvensis) L. (7-1-93)

s. Poison hemlock (Conium maculatum). (7-1-93)

t. Puncture vine (Tribulus terrestris) L. (7-1-93)

u. Rush skeleton weed (Chondrilla juncea) L. (7-1-93)

v. Russian knapweed (Centaurea repens) L. (7-1-93)

w. Scotch broom (Cytisus scoparius). (7-1-93)

x. Scotch thistle (Onopordum acanthium) L. (7-1-93)

y. Silver-leaf nightshade (Solanum elaeagnifolium) Cav. (7-1-93)

z. Skeletonleaf bursage (Franseria discolor) Nutt. (7-1-93)

aa. Spotted knapweed (Centaurea maculosa) Lam. (7-1-93)

bb. Syrian bean caper (Zygophyllum fabago) L.. (7-1-93)

cc. Tansy ragwort (Senecio jacobaea). (7-1-93)

dd. Toothed spurge (Euphorbia dentata). (7-1-93)

ee. White-top (Cardaria draba) (L.) Desv. (7-1-93)

ff. Yellow hawkweed (Hieracium pratense). (7-1-93)

gg. Yellow star thistle (Centaurea solstitialis) L. (7-1-93)

hh. Yellow toad flax (Linaria vulgaris) Hill. (7-1-93)

02. Designation of Articles Capable of Disseminating Noxious Weeds. The following articles are designated by the Director as capable of disseminating noxious weeds: (7-1-93)

a. Construction equipment, road building and maintenance equipment, and farm machinery. (7-1-93)

b. Trucks and motorized vehicles. (7-1-93)

c. Grain or seed. (7-1-93)

d. Hay, straw or other material of similar nature. (7-1-93)

e. Nursery stock. (7-1-93)

f. Feed, seed and seed screenings. (7-1-93)

g. Fence posts, fencing or railroad ties. (7-1-93)

h. Sod. (7-1-93)

i. Manure, fertilizers or material of similar nature. (7-1-93)

j. Soil, sand or gravel. (7-1-93)

101 -- 199. (RESERVED).

200. TREATMENT OF ARTICLES. (7-1-93)

01. Duty. It shall be the duty of every person, before removing any article from any place that is infested with noxious weeds or before moving the article onto any public roadway, to enclose, clean, or treat the article in a manner that will prevent the spread of noxious weeds. (7-1-93)

02. Treatment. No article containing noxious weed propagules shall be sold or furnished to any person within this state, until it has been treated in a manner sufficient to eliminate all noxious weed propagating capability except when sold or furnished to a person for the purpose of destroying the viability of the noxious weed propagules. (7-1-93)

201. -- 299. (RESERVED).

300. SPECIAL MANAGEMENT ZONES. Special management zone designation shall define the geographical location of the zone, identify noxious weeds which will receive modified control, and delineate the modified control. (7-1-93)

301. -- 999. (RESERVED).

"MINNESOTA NOXIOUS WEED RULES"

1505.0730 NOXIOUS WEEDS. The following plants are deemed by the Commissioner of Agriculture to be injurious to public health, public roads, crops, livestock, and other property as noxious weeds:

COMMON NAME	BOTANICAL NAME
Field bindweed	Convolvulus arvensis
Hemp	Cannabis sativa
Loosestrife, purple	Lythrum salicaria, virgatum, or any combination
Poison ivy	Rhus radicans
Spurge, leafy	Euphorbia esula
Sowthistle, perennial	Sonchus arvensis
Thistle, bull	Cirsium vulgare
Thistle, Canada	Cirsium arvense
Thistle, musk	Carduus nutans
Thistle, plumeless	Carduus acanthoides

1505.0740 SECONDARY WEEDS. A weed or weeds may be selected from the following list to be placed on a county noxious weed list by following the procedure outlined in (3).

COMMON NAME	BOTANICAL NAME
Alyssum, hoary	Berteroa incana
Artichoke, Jerusalem	Helianthus tuberosus
Buckwheat, wild	Polygonum convolvulus
Buffalobur	Solanum rostratum
Burdock	Arctium minus
Buttercup, tall	Ranunculus acris
Bracken	Pteridium aquilinum
Carrot, wild	Daucus carota
Catchfly, nightflowering	Silene noctiflora
Cockle, white	Lychnis alba
Cocklebur, common	Xanthium pensylvanicum
Daisy, oxeye	Chrysanthemum leucanthemum
Dock, curly	Rumex crispus
Flixweed	Descurainia sophia
Foxtail, giant	Setaria faberii
Gumweed	Grindelia squarrosa
Hawksbeard, narrowleaf	Crepis tectorum
Hawksbeard, smooth	Crepis capillaris
Hawkweed, orange	Hieracium aurantiacum
Jimsonweed	Datura stramonium
Knapweed, Russian	Centaurea repens
Knapweed, spotted	Centaurea maculosa
Kochia	Kochia scoparia
Lambsquarters, common	Chenopodium album
Mallow, venice	Hibiscus trionum
Marshelder	Iva xanthifolia
Milkweed, common	Asclepias syriaca
Muhly, wirestem	Muhlenbergia frondosa
Mustard, wild	Brassica kaber

COMMON NAME	BOTANICAL NAME
Nightshade, black	Solanum nigrum
Nutsedge, yellow (nutgrass)	Cyperus esculentus
Oat, wild	Avena fatua
Panicum, fall	Panicum dichotomiflorum
Panicum, wild proso millet	Panicum miliaceum
Pigweed, redroot	Amaranthus retroflexus
Pigweed, prostrate	Amaranthus blitoides
Quackgrass	Agropyron repens
Radish, wild	Raphanus raphanistrum
Ragweed, common	Ambrosia artemisiifolia
Ragweed, giant	Ambrosia trifida
Sandbur, field	Cenchrus pauciflorus
Smartweed, Pennsylvania	Polygonum pensylvanicum
Smartweed, (ladysthumb)	Polygonum persicaria
Sorghum-almum	Sorghum almum
Sunflower, common (except cultivars)	Helianthus annuus
Tansy	Tanacetum vulgare
Thistle, Russian	Salsola kali
Velvetleaf	Abutilon theophrasti
Yellow rocket	Barbarea vulgaris
Woolly cupgrass	Eriochloa villosa
Wormwood, absinth	Artemisia absinthium

1505.0750 The Minnesota Commissioner of Agriculture may without further hearing, take a weed or weeds from the secondary list (2) above and add it to the noxious weed list (1) on a county basis if:

(a) A majority of the Township Boards and City Mayors in a county petition the Commissioner of Agriculture, on forms provided by the department, to add a weed or weeds to the primary noxious list on the grounds that the weed or weeds is injurious to public health, public roads, crops, livestock or other property;

(b) The petition is approved by that county's Board of County Commissioners; and,

(c) The Commissioner of Agriculture deems the weed or weeds to be injurious to public health, public roads, crops, livestock, or other property.

1505.0760 Qualification Guidelines. As of March 26, 1971, the following qualifications have been established to serve as guidelines for County Commissioners to consider for applicants for the position of County Agricultural Inspector:

(a) Must be physically able to perform the duties connected with the position. May be asked to have a physical examination at county expense.

(b) Must submit legible required reports pertaining to the position.

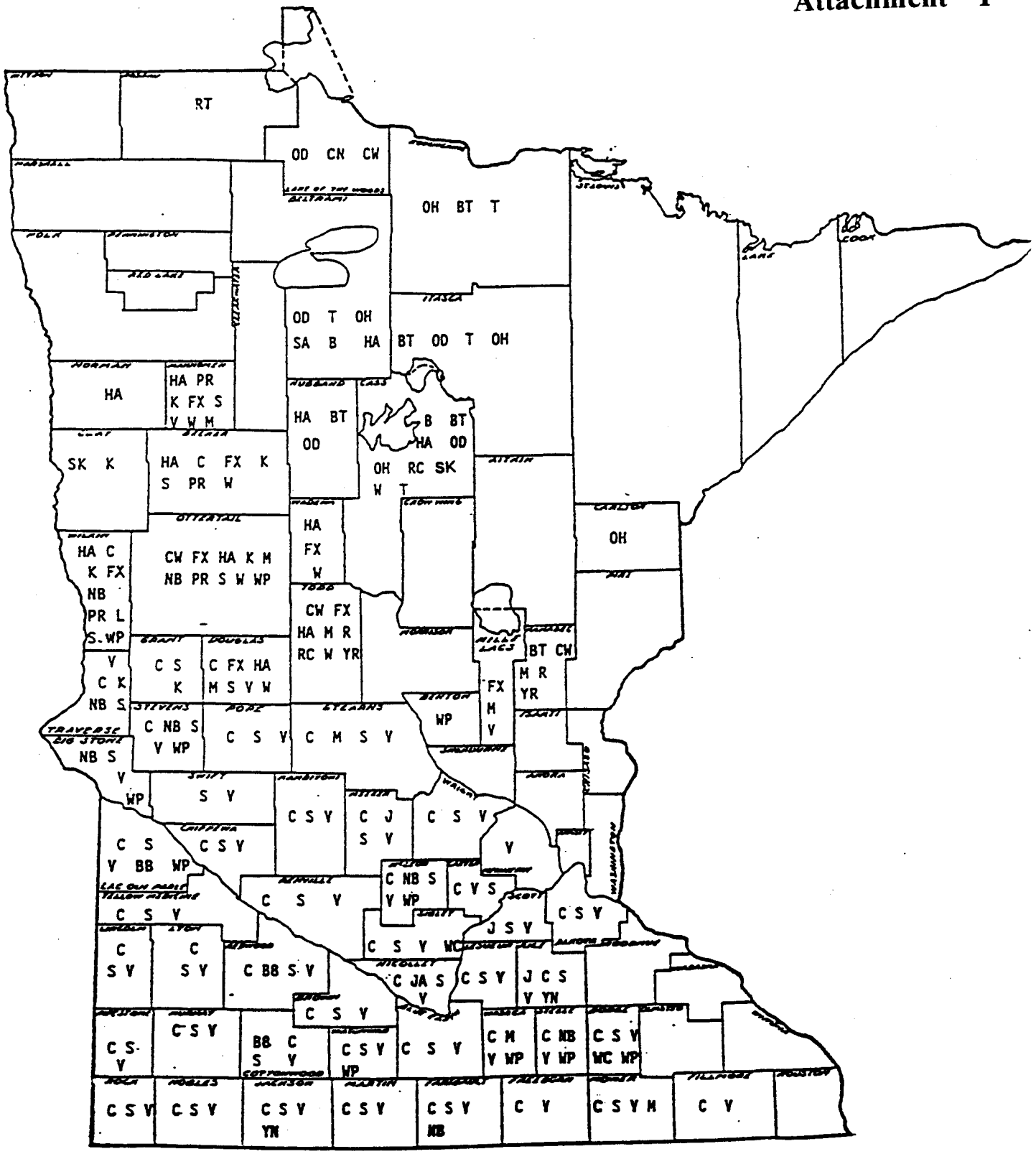
(c) Must have a valid drivers license and a car at his disposal or be able to obtain one.

(d) Must devote necessary time to the position as determined by the Minnesota Department of Agriculture.

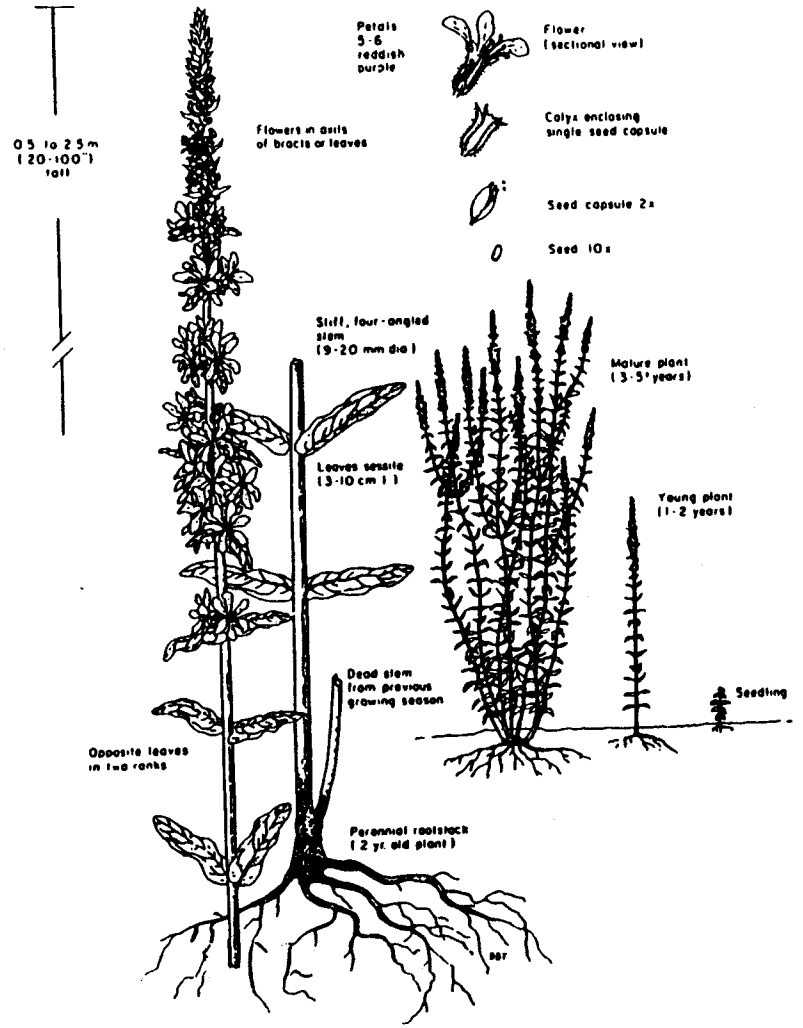
(e) Must not engage in activities which may be construed by the Minnesota Department of Agriculture as being a conflict of interest with the duties of the position.

SECONDARY NOXIOUS WEEDS BY COUNTY PETITION - JANUARY 1994

Attachment I

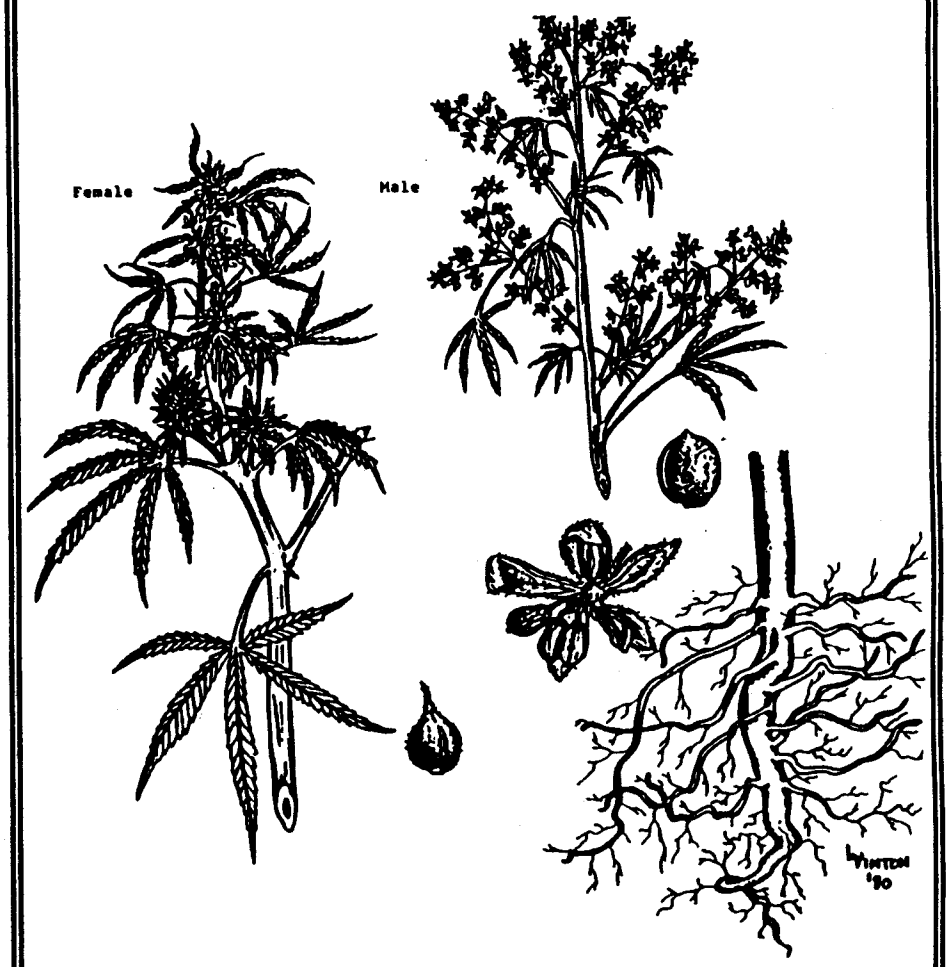


B	BURDOCK	K	KOCHIA	RC	RAGWEED, COMMON
BB	BUFFALOBUR	L	LAMBSQUARTERS	RT	RUSSIAN THISTLE
BT	BUTTERCUP, TALL	M	MUSTARD, WILD	S	SUNFLOWER, WILD
BW	BUCKWHEAT, WILD	MW	MILKWEED	SA	SANDBUR
C	COCKLEBUR	NB	NIGHTSHADE, BLACK	SK	SPOTTED KNAPWEED
CN	CATCHFLY, NIGHTFLOWERING	O	OAT, WILD	T	TANSEY
CW	COCKLE, WHITE	OD	OXEYE DAISY	V	VELVETLEAF
FX	FOXTAIL, GIANT	OH	ORANGE HAWKWEED	W	WORMWOOD
HA	HOARY ALYSSUM	PP	PIGWEED, PROSTRATE	WC	WOOLEY CUPGRASS
J	JIMSONWEED	PR	PIGWEED, REDROOT	WP	WILD PROSO MILLET
JA	JERUSALEM ARTICHOKE	Q	QUACKGRASS	YN	YELLOW NUTSEDGE
		R	RADISH, WILD	YR	YELLOW ROCKET



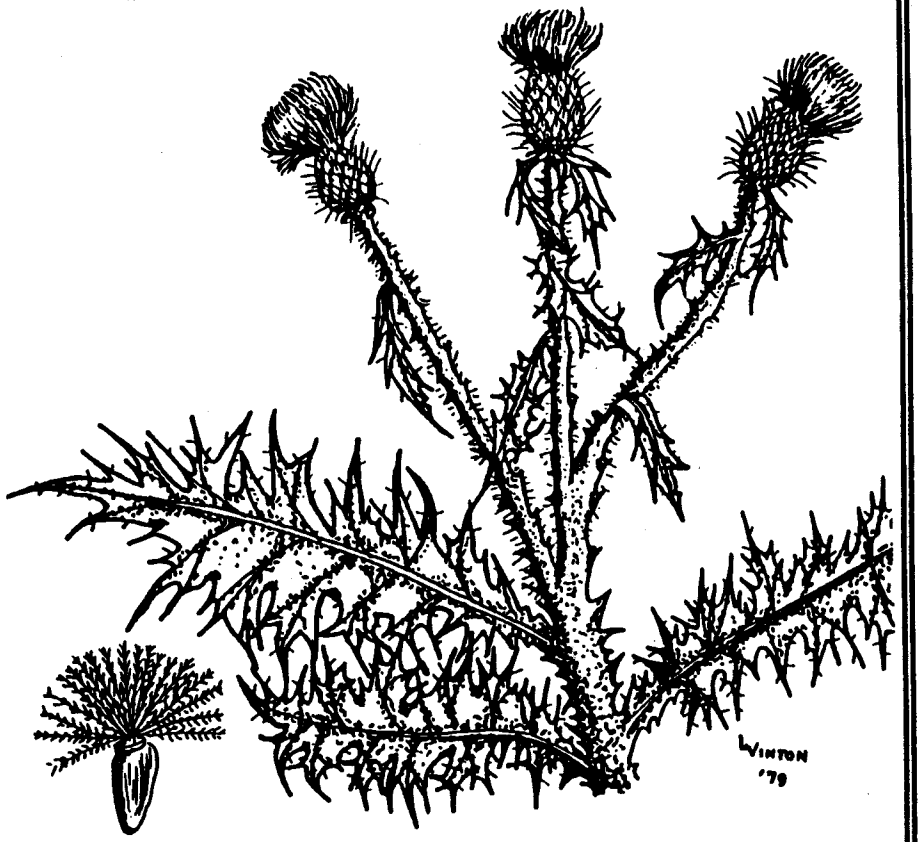
PURPLE LOOSESTRIFE
(Lythum salicaria or virgatum)

Purple loosestrife is an aquatic perennial that reproduces by seed, underground roots, and can spread by sprouting from pieces broken off the plant. It grows in wet soils in wet meadows, cattail marshes, streams, riverbanks, lake shores and ditches. The stems of a mature plant, which can reach 7 feet, are somewhat square, covered with fine hairs, and branched. The leaves are opposite or whorled, linear shaped, and have smooth edges, hairy, and attached directly to the stem. Flowers are in dense terminal spikes at the top of the plant, and are purple-magenta in color with 5 to 7 petals. The seeds are flat, light tan to golden brown and are produced in seed pods. Each plant can produce 120,000 to 2.7 million seeds in a single growing season.



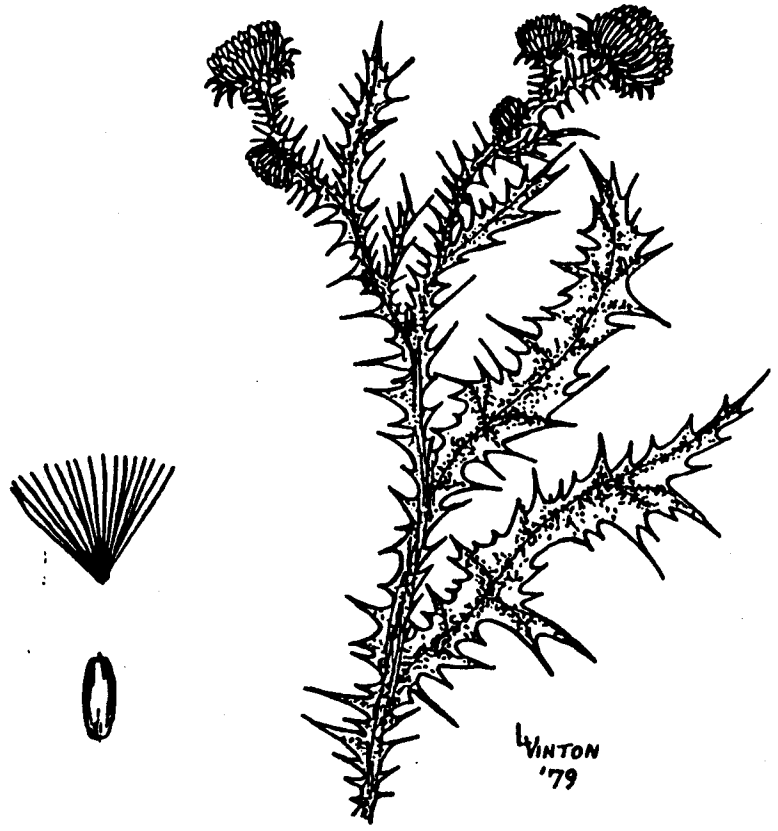
HEMP
(Cannabis sativa)

Hemp is an introduced annual, reproducing by seed. It is found in waste places, pastures, barnyards, drainage ditches, roadsides and occasionally cropland. The seeds are oval, mottled brown, about 1/8 inch long. Hemp grows from 3 to 14 feet high with rough hairy stems. The leaves are palmately compound with 5 to 9 hairy leaflets that have notched or uniformly jagged edges. Plants are usually bushy unless crowded. Hairs on the upper part of the plant exude a characteristic odor. The flowers consist of two kinds--male and female flowers on separate plants. Neither flower has any petals. Both flowers are borne from the axils of the upper leaves. The male plants turn yellow and die after shedding pollen while female plants remain green until frost.



BULL THISTLE
(Cirsium vulgare)

Bull thistle is an introduced biennial, reproducing by seed. It is found in pastures, meadows, waste places, fence rows and roadsides. Bull thistle grows 3 to 6 feet high. The first year it produces a large tap root and a rosette of flat leaves. The second year it produces an upright stalk, blooms, produces seed and then dies. The stems are stout and spiny, with the margins extending down stems. The leaves are spiny, alternate, 3 to 6 inches long, dark green on the upper surface and pale green on the lower surface, and have irregular, spiny margins. The flowers are a deep purple or rose color and measure about 1-1/2 to 3 inches across. The buds or bolls also are covered with a number of stiff, sharp yellow spines. The seeds are straw-colored, striped lengthwise with brown, and tipped with a parachute of soft branched bristles.



PLUMELESS THISTLE
(Carduus acanthoides)

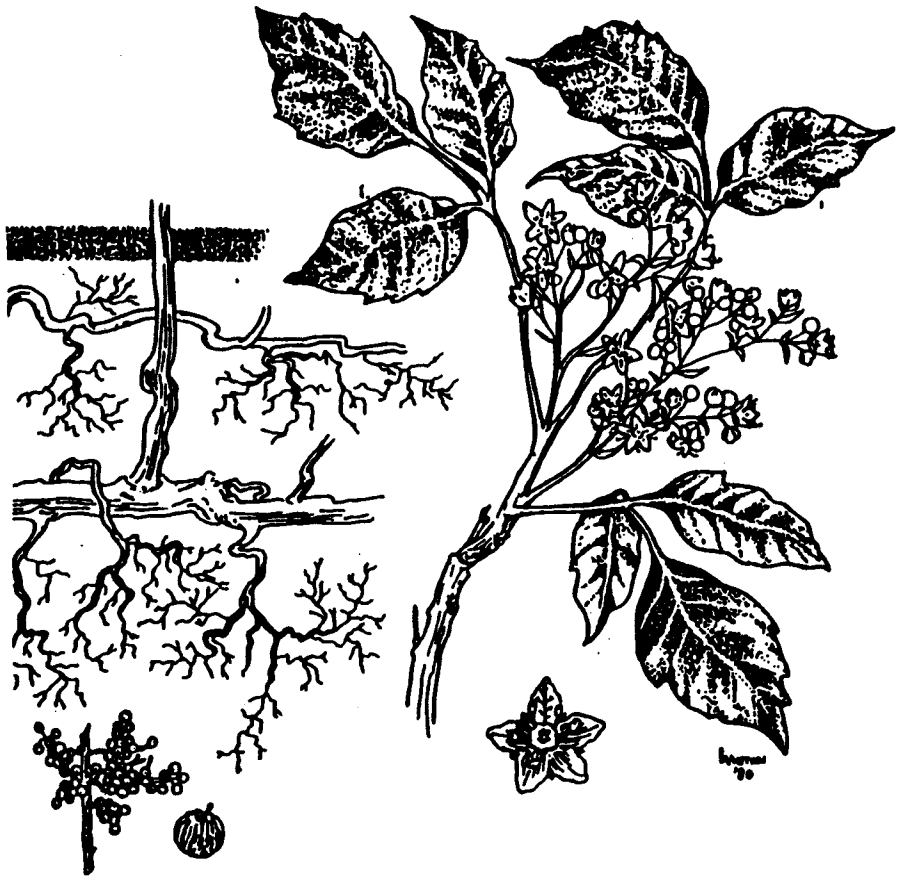
Plumeless thistle is an introduced biennial, reproducing by seed. It is found in pastures, waste places, feed lots, along roadsides and fence rows and has been spreading rapidly in recent years. A heavy taproot and rosette of leaves are produced in the first year. The second year a rough, hairy, flowering stalk 2 to 4 feet high is produced. The leaves are very spiny, deeply cut and extend down the stems. The heads are spiny, 1 to 2 inches across and are pink to purple. The plumes attached to the seed are not branched--hence the name plumeless. This plumeless feature distinguishes this thistle from the other thistles--Canada, bull, Flodman, etc.

MUSK THISTLE
(Carduus nutans)

Musk thistle is an introduced biennial reproducing only by seed. It is found in pastures, meadows, along roadsides and in woodlots. It produces only a rosette of leaves the first year and a flowering stalk the second year. It grows 2 to 4 feet high and has large spiny dark green leaves that have the leaf margins extending down the stems. The heads are solitary on a stem or branch. The large 2 to 3 inch diameter heads are nodding on a rather slender stalks. This thistle, as well as the plumeless thistle, is distinguished by the unbranched plumes attached to the flowers and seeds. The flower has an unpleasant odor from which it gets its name.

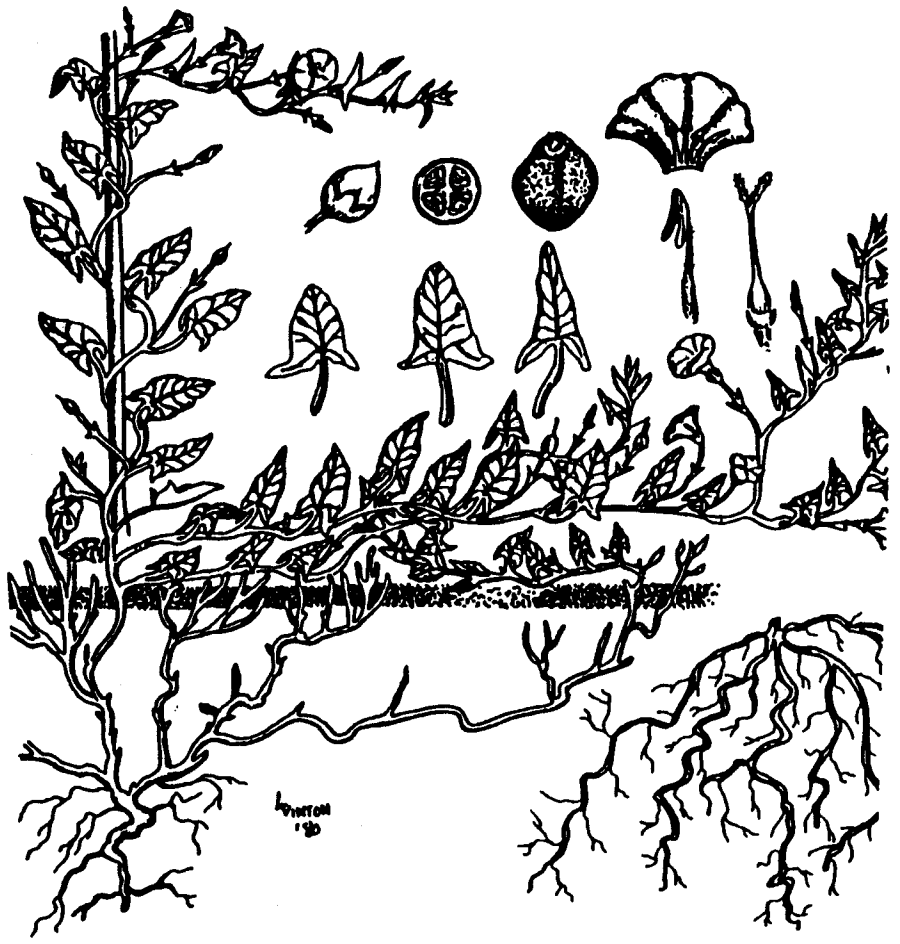
PERENNIAL SOW THISTLE
(Sonchus arvensis)

Perennial sow thistle is an introduced perennial, reproducing by airborne seeds and creeping rootstocks. Roots may penetrate soil to a depth of several feet and spread horizontally, producing shoots from buds on the roots. The stems are smooth, 2 to 5 feet tall, erect and contain a milky sap. The leaves are 4 to 8 inches long, alternate, irregularly toothed, lobed and have spiny edges. The flower heads are about 1-1/2 inches across with a deep yellow ray type of flower. (The annual sow thistle's flower is about 3/4 inch across and light yellow in color.) The seed is dark reddish-brown, about 1/8 inch long, slightly flattened, 5 to 7-ribbed with smaller cross wrinkles. It is found in cultivated fields, pastures, wastelands and roadsides. It is a persistent weed that can spread rapidly.



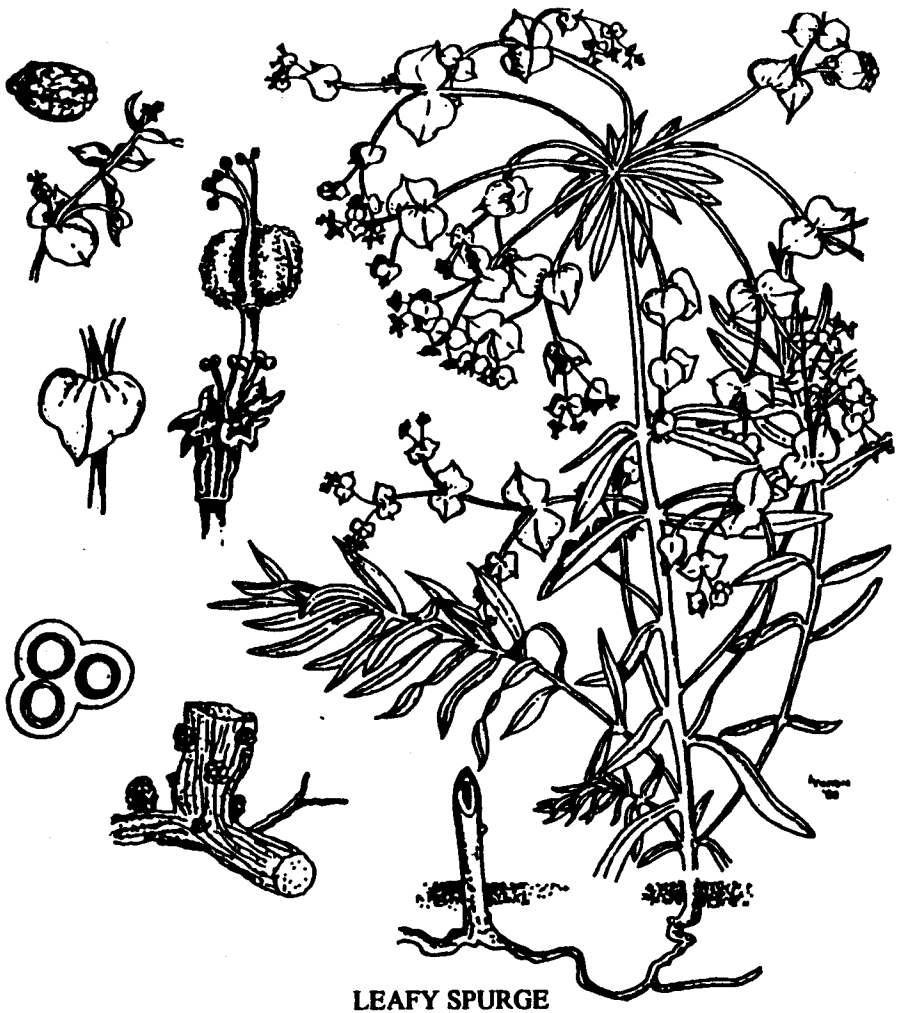
POISON IVY
(Rhus radicans)

Poison ivy is a native woody perennial, reproducing by seed and underground rootstocks. It is found growing in woodlands and meadows and along streams or low places, fence rows, roadsides and wastelands. All parts of this plant contain a poisonous material. The stems are from 8 to 18 inches high. The leaves are smooth, shiny or waxlike in appearance and divided into 3 parts (leaflets). Each leaflet is from 1 to 4 inches long. The flowers are small, green 5-petaled and arranged in a clustered head about 1 to 3 inches long. The berries are small, white, hard, round and arranged in clusters. The plant changes from a bright green in summertime to a reddish-yellow in late summer or fall.



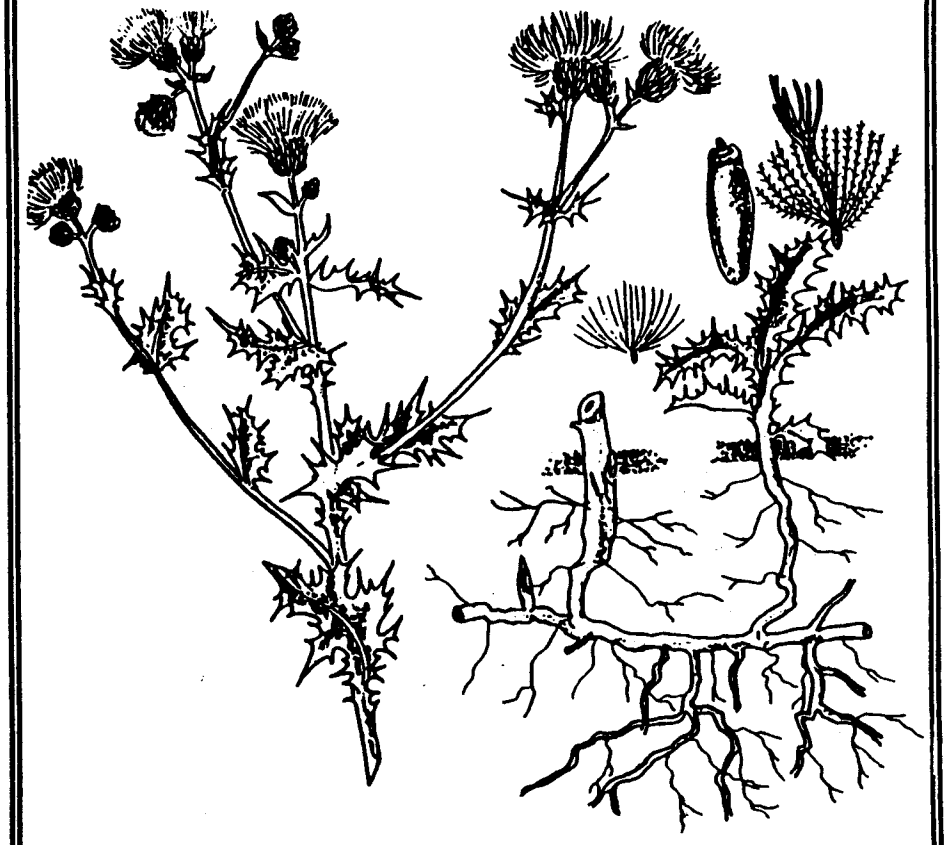
FIELD BINDWEED
(Convolvulus arvensis)

Field bindweed is an introduced perennial, reproducing by seeds and rootstocks. The root system can be quite extensive with roots going down 20 to 30 feet. A two to three year food reserve can be stored in the root system. Seeds are dark brownish-gray, roughened, about 1/8 inch long with one rounded and two flattened sides. The stems are smooth, slender, 2 to 7 feet long, and twining or spreading over the surface of the ground. The leaves are ovate with spreading basal lobes. The flowers are white or pink, trumpet shaped, 1 inch across and usually borne singly in the leave axils. The flower stalk has two bracts about 1/2 to 2 inches below the flower. Seeds are borne in an egg-shaped pod containing four seeds. It is found in and able to persist and spread in all non cultivated areas and under most cropping systems



LEAFY SPURGE
(Euphorbia esula)

Leafy spurge is an introduced perennial, reproducing by rootstocks and seeds. The root system may penetrate into the soil to a depth of 15 feet or more. The reddish-brown roots have numerous buds which can produce a new plant when tops are destroyed. The root system can store 2 to 3 years supply of food to carry the plant through adverse conditions. The stems are erect, smooth, branched toward the top, 1 to 2 feet tall and contain a milky juice. The leaves are arranged alternately on stems and are strap-shaped, 1/4 inch wide and usually drooping. The flowers are small, pale greenish-yellow and have 2 heart-shaped floral bracts. The seeds are smooth, light gray, and resemble a small beetle, and are contained in a 3-lobed pod. It is found in pastures, waste areas, roadsides and in cultivated fields. It is a very persistent weed.



CANADA THISTLE
(Cirsium arvense)

Canada thistle is an introduced perennial, reproducing by airborne seeds and creeping roots which can extend several feet deep and for some distance horizontally. The seeds are easily scattered by wind and are capable of long dormancy periods. Each piece or segment of the root system is capable of giving rise to a new plant. The flower heads are numerous, compact, about 3/4 inch or less in diameter and lavender or pink in color. The flower is surrounded by bracts with spiny tips. Male and female flowers are usually on separate heads and borne on different plants. The leaves usually have crinkled edges, spiny margins, and are somewhat lobed and smooth. While this description fits most Canada thistle, there are a number of varieties differing slightly in appearance and relative maturity. Canada thistle can be found in cropland, roadsides, fence rows, pastures, woodlands and waste areas. It is a troublesome and persistent weed--making it often very difficult to control.

MARC RACICOT
GOVERNOR

MONTANA DEPARTMENT OF AGRICULTURE

AGRICULTURAL SCIENCES DIVISION
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GARY GINGERY
ADMINISTRATOR
(406) 444-2944

2 pages

April 28, 1997

FAX TO: Vickie Forester

FROM: Barbra Mullin, Weed Specialist

RE: Mustards in Montana Canola Fields

Five wild Brassica species are recognized and established in Montana. They are:

1. Brassica hirta white mustard
2. Brassica juncea India mustard
3. Brassica kaber charlock
4. Brassica niger black mustard
5. Brassica rapa common mustard

Mustards found associated with canola in Montana in a 1991 survey, with their relative frequency, are listed below.

Table 1. A Summary of Plants Encountered During a Survey of 13 Canola Fields, 1991.

		Number of Fields (out of 13) with the following Abundance Rating			
		1	2	3	4
GRASSES					
Agropyron repens*	quackgrass	–	4	3	2
Avena fatua	wild oat	3	1	4	–
Bromus inermis*	smooth brome	–	–	–	2
Bromus tectorum	cheatgrass	–	2	2	2
Hordeum/Triticum	volunteer grain	2	1	4	1
Lolium temulentum	Persian darnel	–	–	1	–
Phleum pratense*	timothy	–	–	–	1
Setaria viridis	green foxtail	2	1	2	2
FORBS					
Amaranthus album	white pigweed	–	1	3	–
A. graecizans	prostrate pigweed	–	–	1	3
A. retroflexus	redroot pigweed	1	1	2	4
Asclepias syriaca*	common milkweed	–	–	2	–
Brassica hirta	white mustard	–	–	–	1
Brassica kaber	wild mustard	1	1	–	–
Brassica rapus	field mustard	–	2	–	–
Capsella bursa-pastoris	shepard's purse	–	1	2	1
Carduus nutans	musk thistle	–	–	1	–

Serving Montana

Chenopodium album	lamb's quarter	1	1	5	2
Chenopodium glaucum	oakleaf goosefoot	–	–	–	1
Cirsium arvense*	Canada thistle	2	1	2	4
Convolvulus arvensis*	field bindweed	–	–	–	1
Cruciferae sp.	mustard	–	–	1	1
Descurainia sp.	flixweed/tansy	–	2	7	1
Erodium cicutarium	alfilaria	–	–	1	–
Euphorbia esula*	leafy spurge	–	–	1	–
E. glyptosperma	ridgeseed spurge	–	–	–	2
Helianthus annuus	annual sunflower	–	–	1	2
Kochia scoparium	kochia	–	1	7	2
Lactuca serriola	prickly lettuce	–	–	–	5
Lappula redowskii	western sticktight	–	–	2	
Malva parviflora	smallflower malva	–	–	1	1
Medicago lupulina	black medic	–	–	2	1
Medicago sativa*	alfalfa	3	–	1	1
Melilotus officinalis	yellow sweetclover	–	1	2	
Monolepis nuttalliana	povertyweed	–	1	1	2
Polygonum aviculare	prostrate knotweed	–	–	2	–
Polygonum convolvulus	wild buckwheat	–	1	6	2
Potentilla sp.*	cinquefoil	–	–	–	1
Rosa acicularis*	wild rose	–	–	1	–
Rumex crispus	curly dock	–	–	–	1
Salsola kali	Russian thistle	–	2	3	2
Sisymbrium sp.	tumble mustard	–	–	1	3
S. altissimum	tumblemustard	1	–	–	–
S. loeselii	small tumblemustard	–	–	3	–
Sonchus sp.	sowthistle	–	–	–	2
Solanum nigrum	black nightshade	–	–	1	1
Solanum sarrachoides	hairy nightshade	–	–	1	–
Solanum triflorum	cutleaf nightshade	–	–	1	4
Taraxacum officinale*	common dandelion	–	–	2	3
Thlaspi arvense	field pennycress	2	3	–	1
Tragopogon dubius	salsify	–	–	–	3
Vaccaria segetalis	cow cockle	–	1	1	–
Vicia sp.*	vetch	–	–	1	1

* - Denotes perennial species

[1] Abundance Ratings:

1 = abundant 3 = incidental

2 = common 4 = rare

COUNTY NOXIOUS WEED CONTROL ACT

Title 7, Chapter 22

Sections

7-22-2101 through 7-22-2153

M C A

Amended 1991

AND RULES

Rules 4.5.201 through 4.5.203

State of Montana
Department of Agriculture
Agricultural and Biological Sciences Division
Capitol Station
Helena, MT 59620-0205
(406) 444-2944

TABLE OF CONTENTS

COUNTY NOXIOUS WEED CONTROL ACT

RULES
COUNTY NOXIOUS WEED LIST

COUNTY NOXIOUS WEED CONTROL ACT

7-22-2101. Definitions. As used in this part, unless the context indicates otherwise, the following definitions apply:

(1) "Board" means a district weed board created under 7-22-2103.

(2) "Commissioners" means the board of county commissioners.

(3) "Department" means the department of agriculture provided for in 2-15-3001.

(4) "District" means a weed management district organized under 7-22-2102.

(5) "Native plant" means a plant endemic to the state of Montana.

(6) "Native plant community" means an assemblage of native plants occurring in a natural habitat.

(7) (a) "Noxious weeds" or "weeds" means any exotic plant species established or that may be introduced in the state which may render land unfit for agriculture, forestry, livestock, wildlife, or other beneficial uses or that may harm native plant communities and that is designated:

(i) as a statewide noxious weed by rule of the department; or

(ii) as a district noxious weed by a board, following public notice of intent and a public hearing.

(b) A weed designated by rule of the department as a statewide noxious weed must be considered noxious in every district of the state.

(8) "Person" means an individual, partnership, corporation, association, or state or local government agency or subdivision owning, occupying, or controlling any land, easement, or right-of-way, including any county, state, or federally owned and controlled highway, drainage or irrigation ditch, spoil bank, borrow pit, or right-of-way for a canal or lateral.

(9) "Supervisor" means the person employed by the board to conduct the district noxious weed management program and supervise other district employees.

(10) "Weed management" or "control" means the planning and implementation of a coordinated program for the containment, suppression, and, where possible, eradication of noxious weeds.

7-22-2102. Weed Management Districts Established. A weed management district shall be formed in every county of this state and shall include all the land within the boundaries of the

county, except that a weed management district may include more than one county through agreement of the commissioners of the affected counties.

7-22-2103. District Weed Board -- Appointment and Term.

(1) The commissioners shall appoint a district weed board.

(2) The commissioners shall, at a public meeting, pass a resolution establishing the number of members of the district weed board and the terms of the appointments. The board must consist of at least three members and no more than nine members, and the members of the board must be residents of the district. A majority of the board members must be rural agricultural land owners.

(3) The county extension agent in each county and other interested individuals may be appointed to serve as nonvoting members of that district's weed board.

(4) The board members are public officers.

(5) The board may call upon the county attorney for legal advice and services as it may require.

7-22-2104. Term of Office.

(1) Except as provided in subsection (2), a member of a district weed board serves a term of 3 years and until the qualification of his successor. The term of office begins January 1.

(2) When a three-member weed board is established, the initial board members serve terms of 1, 2, and 3 years, respectively, as designated by the commissioners. When a five-member weed board is established, two of the initial members serve terms of 1 year, two serve terms of 2 years, and one serves a term of 3 years. After expiration of an initial term of office, the successor serves a 3-year term as provided in subsection (1).

7-22-2105. Organization of District Weed Board and Compensation.

(1)T The board shall organize by choosing a chairman and a secretary. The secretary may or may not be a member of the board.

(2) Salary, per diem, and mileage of such board members shall be set by resolution of the commissioners.

(3) A majority of the board constitutes a quorum for the conduct of business.

7-22-2106. Renumbered 7-22-2115 by Code Commissioner, 1985.

7-22-2107. Renumbered 7-22-2116 by Code Commissioner, 1985.

7-22-2108. Renumbered 7-22-2117 by Code Commissioner, 1985.

7-22-2109. Powers and Duties of Board.

(1) The board may:

(a) employ a supervisor and other employees as necessary and provide for their compensation;

(b) purchase such chemicals, materials, and equipment and pay other operational costs as it determines necessary for implementing an effective weed management program. Such costs must be paid from the noxious weed fund.

(c) determine what chemicals, materials, or equipment may be made available to persons controlling weeds on their own land. The cost for such chemicals, materials, or equipment must be paid by such person and collected as provided in this part.

(d) enter into agreements with the department for the control and eradication of any new exotic plant species not previously established in the state which may render land unfit for agriculture, forestry, livestock, wildlife, or other beneficial use if such plant species spreads or threatens to spread into the state; and

(e) perform other activities relating to weed management.

(2) The board shall:

(a) administer the district's noxious weed program;

(b) establish management criteria for noxious weeds on all land within the district;

(c) make all reasonable efforts to develop and implement a noxious weed program covering all land within the district owned or administered by a federal agency.

7-22-2110. Administrative Hearing – Appeals.

(1) A person adversely affected by any notice, action, or order of the board may request an administrative hearing before the board. The board shall hold a hearing within 30 days of the request. Participants may be represented by legal counsel. The board shall make a record of the proceeding and enter its order and findings within 7 days after the hearing.

(2) An order of the board may be appealed to the commissioners within 30 days from the time the order is entered. The commissioners shall hear such appeal within 30 days after the notice of appeal and shall render their order and findings within 7 days after such hearing. Participants may be represented by legal counsel.

(3) Within 30 days after the commissioners render their order and findings, the person adversely affected may file a petition in district court requesting that the order and findings of the commissioners be set aside or modified. The court may affirm, modify, or set aside the order complained of, in whole or in part.

7-22-2111. (Temporary) Liability Restrictions. A district, as defined in 7-22-2101, is liable for damages caused by its use of herbicides only for an act or omission that constitutes gross negligence. The provisions of 2-9-305 apply to board members, supervisors, and employees of a district. (Terminates July 1, 1995 -- sec. 7, Ch. 530, L. 1991.)

7-22-2112. (Temporary) Information on Herbicide Use. The district must provide information on protective clothing, health hazards, and proper application techniques to mixers, loaders, and applicators of herbicides and make information available for review by the public at the district office. (Terminates July 1, 1995 -- sec. 7, Ch. 530, L. 1991.)

7-22-2113 and 7-22-2114 reserved.

7-22-2115. Noxious Weeds and Seeds Declared Nuisance. Noxious weeds and the seed of any noxious weed are hereby declared a common nuisance.

7-22-2116. Unlawful to Permit Noxious Weeds to Propagate. It is unlawful for any person to permit any noxious weed to propagate or go to seed on his land, except that any person who adheres to the noxious weed management program of his district or who has entered into and is in compliance with a noxious weed management agreement is considered to be in compliance with this section.

7-22-2117. Violations.

(1) Any person who in any manner interferes with the board or its authorized agent in carrying out the provisions of this part or who refuses to obey an order or notice of the board is guilty of a misdemeanor, and upon conviction thereof, he shall be fined not to exceed $100 for the first offense and not less than $100 or more than $200 for each subsequent offense.

(2) All fines, bonds, and penalties collected under the provisions of this part, except those collected by a justice's court, shall be paid to the county treasurer of each county and placed by him to the credit of a fund to be known as the noxious weed fund.

7-22-2118 through 7-22-2120 reserved.

7-22-2121. Weed Management Program.

(1) The noxious weed management program must be based on a plan approved by the board.

(2) The noxious weed management plan must:

(a) specify the goals and priorities of the program;

(b) review the distribution and abundance of each noxious weed species known to occur within the district and specify the locations of new infestations and areas particularly susceptible to new infestations;

(c) specify pesticide management goals and procedures, including but not limited to water quality protection, public and worker safety, equipment selection and maintenance, and pesticide selection, application, mixing, loading, storage, and disposal; and

(d) estimate the personnel, operations, and equipment costs of the proposed program.

(3) The board shall provide for the management of noxious weeds on all land or rights-of-way owned or controlled by a county or municipality within the confines of the district. It shall take particular precautions while managing the noxious weeds to preserve beneficial vegetation and wildlife habitat. Where at all possible, methods for such control shall include cultural, chemical, and biological methods.

(4) The board may establish special management zones within the district. The management criteria in such zones may be more or less stringent than the general management criteria for the district.

7-22-2122. Repealed. Sec. 32, Ch. 607, L. 1985.

7-22-2123. Procedure in a Case of Noncompliance.

(1) Where complaint has been made or the board has reason to believe that noxious weeds described in this part are present upon a person's land within the district in violation of the law, that person must be notified by mail or telephone of the complaint and the board may request inspection of such land. The board or its authorized agent and the landowner or his representative shall inspect the land at an agreeable time, within 10 days of notification of the landowner. If after reasonable effort the board is unable to gain cooperation of the person, the board or its authorized agent may enter and inspect the land to determine if the complaint is valid.

(2) If noxious weeds are found, the board or supervisor shall notify the person or his representative and seek voluntary compliance with the district weed control program. If voluntary compliance is not possible, notice of noncompliance must be sent to the person by certified mail.

(3) The notice must specify:

(a) the basis for the determination of noncompliance;

(b) the geographic location of the area of noncompliance, by legal description or other reasonably identifiable description;

(c) measures to be undertaken in order to comply with the district's management criteria;

(d) a reasonable period of time, not less than 10 days, in which compliance measures must be initiated; and

(e) the right of the person to request, within the time specified in subsection (3) (d), an administrative hearing as provided by 7-22-2110.

(4) A person is considered in compliance if he submits and the board accepts a proposal to undertake specified control measures and is in compliance for so long as he performs according to the terms of the proposal. If the measures proposed to be taken extend beyond the current growing season, the proposal and acceptance must be in writing.

(5) In accepting or rejecting a proposal, the board shall consider the economic impact on the person and his neighbors, practical biological and environmental limitations, and alternative control methods to be used.

7-22-2124. Destruction of Weeds by Board.

(1) If corrective action is not taken and no proposal is made and accepted or no request for an administrative hearing is made within the time specified in the notice, the board may forthwith enter upon the person's land and institute appropriate control measures.

In such case the board shall submit a bill to the person, itemizing man-hours of labor, material, and equipment time, together with a penalty not exceeding 10% of the total cost incurred. Labor and equipment must be valued at the current rate paid for commercial management operations in the district. The bill must specify and order a payment due date of 30 days from the date the bill is sent.

(2) A copy of the bill must also be submitted by the board to the county clerk and recorder.

(3) If a person receiving an order to take corrective action requests an administrative hearing, the board may not institute control measures until the matter is finally resolved, except in case of an emergency. In such a case, the person is liable for costs as provided in subsection (1) only to the extent determined appropriate by the board, commissioners, or court that finally resolves the matter.

7-22-2125. Repealed. Sec. 32, Ch. 607, L. 1985.

7-22-2126. Embargo. The board may establish voluntary embargo programs to reduce the spread of noxious weeds within the district or the introduction of noxious weeds into the district.

7-22-2127. Repealed. Sec. 32, Ch. 607, L. 1985.

7-22-2128 and 7-22-2129 reserved.

7-22-2130. Weed District Supervisor Training. Within the limitations of available funds, the board shall ensure that the weed district supervisor obtains training to properly implement the noxious weed management program described in 7-22-2121. The department shall specify through rulemaking the level and type of training necessary to fulfill this requirement.

7-22-2131 through 7-22-2140 reserved.

7-22-2141. Noxious Weed Fund Authorized.

(1) The commissioners of each county in this state shall create a noxious weed management fund, to be designated the "noxious weed fund".

(2) This fund shall be kept separate and distinct by the county treasurer.

7-22-2142. Sources of Money For Noxious Weed Fund.

(1) The commissioners may create the noxious weed fund and provide sufficient money in the fund for the board to fulfill its duties, as specified in 7-22-2109, by:

(a) appropriating money from the general fund of the county;

(b) at any time fixed by law for levy and assessment of taxes, levying a tax not exceeding 2 mills on the dollar of total taxable valuation in the county. The tax levied under this subsection must be identified on the assessment as the tax that will be used for noxious weed control; and

(c) levying a tax in excess of 2 mills if authorized by a majority of the qualified electors voting in an election held for this purpose pursuant to 7-6-2531 through 7-6-2536.

(2) The proceeds of the noxious weed control tax must be used solely for the purpose of managing noxious weeds in the county and must be designated to the noxious weed fund.

(3) Any proceeds from work or chemical sales must revert to the noxious weed fund and must be available for reuse within that fiscal year or any subsequent year.

(4) The commissioners may accept any private, state, or federal gifts, grants, contracts, or other funds to aid in the management of noxious weeds within the district. These funds must be placed in the noxious weed fund.

7-22-2143. Determination of Cost of Weed Control Program. Based on the board's recommendations, the commissioners shall determine and fix the cost of the control of noxious

weeds in the district, whether the same be performed by the individual landowners or by the board.

7-22-2144. Payment of Cost of Weed Control Program. The total cost of such control shall be paid from the noxious weed fund. The cost of controlling such weeds growing along the right-of-way of a state or federal highway shall, upon the presentation by the board of a verified account of the expenses incurred, be paid from the state highway fund in compliance with 7-14-2132 and any agreement between the board and the department of highways. Costs attributed to other lands within the district shall be assessed to and collected from the responsible person as set forth in 7-22-2116.

7-22-2145. Expenditures From Noxious Weed Fund.

(1) The noxious weed fund must be expended by the commissioners at the time and in the manner as is recommended by the board to secure the control of noxious weeds.

(2) Warrants upon the fund must be drawn by the board. Warrants may not be drawn except upon claims duly itemized by the claimant, except payroll claims that must be itemized and certified by the board, and each claim must be presented to the commissioners for approval before the warrant is countersigned by the commissioners.

7-22-2146. Financial Assistance to Persons Responsible For Weed Control.

(1) The commissioners, upon recommendation of the board, may establish cost-share programs with any person, specifying costs that may be paid from the noxious weed fund and costs that must be paid by the person. Cost-share programs may be established for special projects and for established management zones.

(2) (a) When under the terms of any voluntary agreement, whether entered into pursuant to 7-22-2123 or otherwise, or under any cost-share program entered pursuant to this section a person incurs any obligation for materials or services provided by the board, the board shall submit a bill to the person, itemizing man-hours of labor, material, and equipment time. The bill must specify and order a payment due date not less than 30 days from the date the bill is sent.

(b) A copy of the bill must be submitted by the board to the county clerk and recorder. If the sum to be repaid by the person billed is not repaid on or before the date due, the county clerk and recorder shall certify the amount thereof, with the description of the land to be charged, and shall enter the sum on the assessment list as a special tax on the land, to be collected in the manner provided in 7-22-2148.

7-22-2147. Repealed. Sec. 32, Ch. 607, L. 1985.

7-22-2148. Tax Liability For Payment of Weed Control Expenses.

(1) The expenses referred to in 7-22-2124 shall be paid by the county out of the noxious weed fund, and unless the sum to be repaid by the person billed under 7-22-2124 is repaid on or before the date due, the county clerk shall certify the amount thereof, with the description of the land to be charged, and shall enter the same on the assessment list of the county as a special tax on the land. If the land for any reason is exempt from general taxation, the amount of such charge may be recovered by direct claim against the lessee and collected in the same manner as personal taxes. When such charges are collected, they shall be credited to the noxious weed fund.

(2) In determining what lands are included as land covered by the special tax and are described in the certificate of the county clerk, it is presumed that all work done upon any of the land of any one landowner is for the benefit of all of the land within the district belonging to the owner, together with the parcel upon which the work was done, and the amount certified becomes a tax upon the whole thereof.

7-22-2149. Responsibility For Assessments And Taxes For Weed Control Levied on Leased State Lands. The lessee of agricultural state land is responsible for assessments and taxes levied by the board of county commissioners for the district as provided in 77-6-114.

7-22-2150. Cooperation With State And Federal-Aid Programs. The board is empowered to cooperate with any state or federal-aid program that becomes available. Under such a plan of cooperation, the direction of the program shall be under the direct supervision of the board of the district in which the program operates.

7-22-2151. Cooperative Agreements.

(1) Any state agency controlling land within a district, including the department of highways; the department of state lands; the department of fish, wildlife, and parks; the department of institutions; the department of natural resources and conservation; and the university system, shall enter into a written agreement with the board. The agreement must specify mutual responsibilities for noxious weed management on state-owned or state-controlled land within the district.

(2) The board and the governing body of each incorporated municipality within the district shall enter into a written agreement and shall cooperatively plan for the management of noxious weeds within the boundaries of the municipality. The board may implement management procedures described in the plan within the boundaries of the municipality for noxious weeds only. Control of nuisance weeds within the municipality remains the responsibility of the governing body of the municipality, as specified in 7-22-4101.

(3) A board may develop and carry out its noxious weed management program in cooperation with boards of other districts, with state and federal governments and their agencies, or with any person within the district. The board may enter into cooperative agreements with any of these parties.

7-22-2152. Revegetation of Rights-Of-Way And Disturbed Areas.

(1) Any state agency or local government unit approving a mine, major facility, transmission line, solid waste facility, highway, subdivision, or any other development resulting in significant disturbance of land within a district shall notify the board.

(2) Whenever any person or agency disturbs vegetation on an easement or right-of-way within a district by construction of a road, irrigation or drainage ditch, pipeline, transmission line, or other development, the board shall require that the disturbed areas be seeded, planted, or otherwise managed to reestablish a cover of beneficial plants.

(3) (a) The person or agency disturbing the land shall submit to the board a written plan specifying the methods to be used to accomplish revegetation. The plan must describe the time and method of seeding, fertilization practices, recommended plant species, use of weed-free seed, and the weed management procedures to be used.

(b) The plan is subject to approval by the board, which may require revisions to bring the revegetation plan into compliance with the district weed management plan. Upon approval by the board, the revegetation plan must be signed by the chairman of the board and the person or agency responsible for the disturbance and constitutes a binding agreement between the board and such person or agency.

7-22-2153. Voluntary Agreements For Control of Noxious Weeds Along Roads.

(1) Any person may voluntarily seek to enter into an agreement for the management of noxious weeds along a state or county highway or road bordering or running through his land. The supervisor may draft such an agreement upon the request of and in cooperation with the person; however, the agreement must, in the board's judgment, provide for effective weed management. The weed management agreement must be signed by the person and, upon approval of the board, by the chairman. An agreement involving a state highway right-of-way must also be signed by a representative of the department of highways.

(2) The agreement must contain a statement disclaiming any liability of the board and, if applicable, the department of highways for any injuries or losses suffered by the person in managing noxious weeds on the state or county highway right-of-way. The signed agreement transfers responsibility for managing noxious weeds on the specified section of right-of-way from the board to the person signing the agreement. If the board later finds that the person has failed to adhere to the agreement, the board shall issue an order informing the person that the agreement will be void and that responsibility for the management of noxious weeds on the right-of-way will revert to the board unless the person complies with the provisions of the agreement within a specified time period.

RULES
COUNTY NOXIOUS WEED LIST
Sub-Chapter 2
Designation of Noxious Weeds

4.5.201. Designation of Noxious Weeds. The department designates certain exotic plants listed in these rules as statewide noxious weeds under the County Weed Control Act 7-22-2101 (5), MCA. All counties must implement management standards for these noxious weeds consistent with weed management criteria developed under 7-22-2109 (2)(b) of the Act. The department established three categories of the noxious weeds. (History: Sec. 7-22-2101 MCA; IMP, Sec. 7-22-2101 MCA; NEW 1986, p. 337, Eff. 3/14/86; AMD, 1991 MAR p. 511, Eff. 4/26/91.)

4.5.202. Category 1.

(1) Category 1 noxious weeds are weeds that are currently established and generally widespread in many counties of the state. Management criteria includes awareness and education, containment and suppression of existing infestations and prevention of new infestations. These weeds are capable of rapid spread and render land unfit or greatly limit beneficial uses.

(2) The following are designated as category 1 noxious weeds:

(a) Canada Thistle (Cirsium arvense)

(b) Field Bindweed (Convolvulus arvensis)

(c) Whitetop or Hoary Cress (Cardaria draba)

(d) Leafy Spurge (Euphorbia esula)

(e) Russian Knapweed (Centaurea repens)

(f) Spotted Knapweed (Centaurea maculosa)

(g) Diffuse Knapweed (Centaurea diffusa)

(h) Dalmatian Toadflax (Linaria dalmatica)

(i) St. Johnswort (Hypericum perforatum)

(j) Sulfur (erect) cinquefoil (Potentilla recta)

(History: Sec. 7-22-2101 MCA; IMP, Sec. 7-22-2101 MCA; NEW 1986 MAR p. 337, Eff. 3/14/86; AMD, 1991 MAR p. 511, Eff. 4/26/91; AMD, 1994 MAR p. 93, Eff. 3/18/94.))

Part Cross References:

Weed Control - Department of Agriculture, Title 80, Chapter 7, Part 7.
Municipal Weed Control, 7-22-4101.
Noxious Weed Management Funding, Title 80, Chapter 7, Part 8.
Embargo against introduction of noxious weed seed from other state, 80-7-701.
General authority of county commissioners, 7-5-2101.
County officers - term of office, 7-4-2205.
Nuisance, Title 27, Chapter 30.
Classification of offenses, 45-1-201.
Department of State Lands, general powers and duties, Title 77, Chapter 1, Part 3.
Mining on State Lands, Title 77, Chapter 3.
Department of Fish, Wildlife and Parks, general powers and duties, Title 87, Chapter 1, Part 2.
Department of Highways, general powers and duties, Title 60, Chapter 2, Part 7.
Highways, acquisition and disposition of property, Title 60, Chapter 4.
Highway maintenance agreements with local government, 60-2-204.
Montana Environmental Protection Act, Title 75, Chapter 1.
Montana Solid Waste Management Act, Title 75, Chapter 10, Part 2.
County Taxation, Title 7, Chapter 6, Part 25.
Department of Institutions, general powers and duties, Title 53, Chapter 1, Part 2.
University system, Title 20, Chapter 5.
Department of Natural Resources and Conservation established 2-15-3301.
Major Facility Siting Act, Title 75, Chapter 20.
Subdivisions, Title 76, Chapter 2 and 3.
Coal mining, Title 82, Chapter 3.
Oil and gas conservation, Title 82, Chapter 11.
Hard rock mining impact, Title 90, Chapter 6, Part 3.
Role and duties of county clerk, 7-4-2611.
Employment of personnel by county commissioners. 7-5-2107.

North Dakota's Noxious Weeds

ABSINTH WORMWOOD: (*Artemisia absinthium*) is a perennial reproducing by seeds and branching rootstocks. It grows 2 to 4 feet high with many branched stems. The plant is covered with fine hairs giving it a grayish appearance like sagebrush. The yellow flowers are numerous, drooped and short-stalked. Seeds are about 1/16 of an inch long, flattened, narrow at the base, rounded at the tip and light gray-brown.

CANADA THISTLE (*Cirsium arvense*) is a deep-rooted perennial reproducing by seeds and horizontal roots. It grows 1 to 5 feet high with slightly hairy stems branching near the top. The leaves are dark green, spiny and very crinkly. The numerous flowerheads and are about 3/4 of an inch or less in diameter. The seeds are about 1/8 of an inch long, smooth, oblong, ringed and pointed on top and dull brown.

FIELD BINDWEED (*Convolvulus arvensis*) is a perennial that spreads by horizontal root branches and seeds. Its vines grow from 2 to 7 feet long, spreading over the surface of the ground. The numerous leaves may vary in shape and size; but are usually arrowhead-shaped. The flowers are trumpet-shaped, white to pinkish and about 1 inch in diameter. The seeds are about 1/8 of an inch with one rounded and two flattened sides and are dark brownish-gray.

HEMP (*Cannabis sativa*) is an annual reproducing by seed. It grows from 3 to 10 feet high. The leaves are divided into five to seven leaflets with notched edges. The seeds are about 1/8 of an inch long, slightly oblong and are yellow to olive brown. Hemp, commonly known as marijuana, is listed as a noxious weed because it is an illegal drug. Treatment is most effective in early spring when plants are 6 to 8 inches tall, but before seed set occurs.

HOARY CRESS (*Cardaria draba*) is a perennial that reproduces by deep roots and seeds. It grows up to 20 inches high. The leaves are oval or oblong with toothed or almost smooth edges. The numerous white flowers are in flat-topped clusters. The seeds are about 1/16 of an inch long, oval to somewhat pointed in shape and are reddish-brown to brown.

LEAFY SPURGE (*Euphorbia esula*) is a perennial reproducing by seeds and an extensive root system. It grows from 1 to 3 feet tall, and contains a milky juice called latex. The long, narrow leaves are 1 to 3 inches long. The yellowish-green flowers cluster at the top of the plant. Seeds are about 1/16 of an inch long and are light to dark grayish-brown. **NOTE:** The showy, yellow bracts appear in late May to early June; the true flowers do not emerge until mid-June.

MUSK THISTLE (*Carduus nutans*) is a biennial reproducing by seeds. It grows from 2 to 6 feet tall. The leaves are deep lobed, very prickly and 3 to 6 inches long. The rose-purple flowers appear almost naked at the ends of the long stems and are about 1.5 to 2.5 inches in diameter. Seeds are about 3/16 of an inch long and are yellowish brown.

PERENNIAL SOWTHISTLE (*Sonchus arvensis*) is a perennial reproducing by creeping roots and seeds. It grows from 3 to 7 feet tall. The leaves have a spiny middle with prickly edges and are 4 to 8 inches long. The deep yellow flowers are 1.5 inches in diameter and appear in July. The dark reddish-brown seeds are about 1/8 of inch long, ribbed and cross wrinkled. Apply chemicals when the plant is actively growing but before flowering. **NOTE:** Annual Sowthistle and Prickly Lettuce are similar, but are annuals without creeping roots.

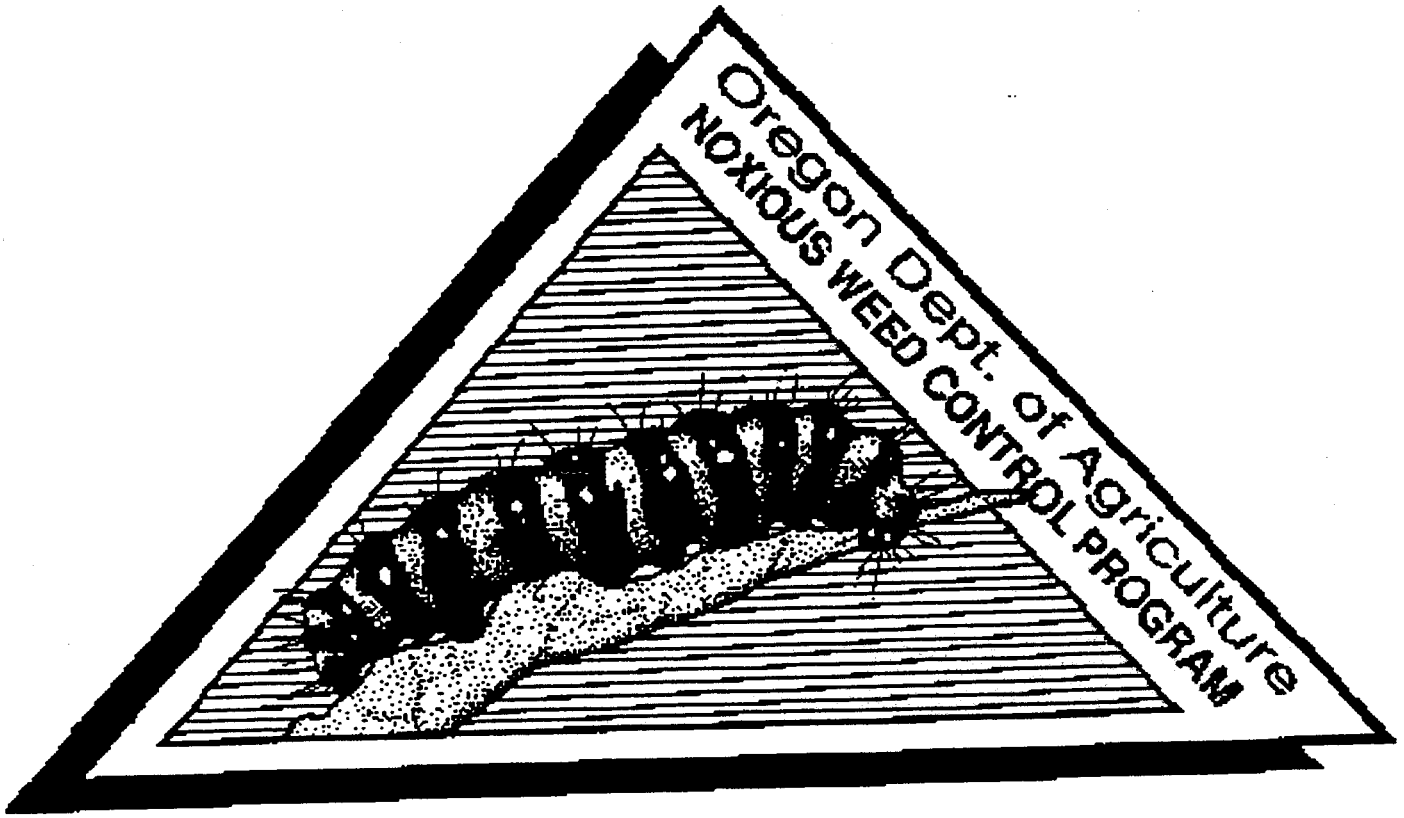
RUSSIAN KNAPWEED (*Centaurea repens)* is a perennial reproducing by creeping roots and seeds. It grows from 2 to 3 feet tall. The grayish green leaves are larger at the base of the plant, smaller and narrower towards the top of the plant and are 1 to 3 inches long. The thistle-like flowers vary in color from white to light rose to light blue and are about an inch in diameter. The seeds are about 1/8 of an inch long and grayish or yellowish.

SPOTTED KNAPWEED (*Centaurea maculosa*) is a biennial or short-lived perennial reproducing by seeds. It grows from 1 to 3 feet tall. The leaves are alternate, spotted, pale green in color and are 1 to 3 inches long. The flowers are white to light-purple and are produced on the end of the branches. The brownish seeds are about 1/8 of an inch long and oblong with a hook at the bottom. Twenty-one counties reported a statewide total of more than 81 infestations in 1995. Two of those infestions exceeded 100 acres in size. **NOTE:** Spotted Knapweed flowerheads have bracts with hair-like bristles, while Russian Knapweed has smooth bracts.

Not much of a problem

Attachment 1

Noxious Weed Policy and Classification System



Oregon Department of Agriculture
Noxious Weed Control Program

1995

OREGON DEPARTMENT OF AGRICULTURE
NOXIOUS WEED CONTROL POLICY
AND
CLASSIFICATION SYSTEM

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OREGON DEPARTMENT OF AGRICULTURE
NOXIOUS WEED CONTROL POLICY AND CLASSIFICATION SYSTEM

The Weed Policy and Classification System is an advisory document of the Oregon Department of Agriculture. This document is a guideline for prioritization and implementation of integrated weed control measures.

The Weed Policy and Classification System has been reviewed and approved by the Oregon Department of Agriculture and the Oregon State Weed Board.

Lona Young (for)
Director, Oregon Department of Agriculture — 2/16/95 Date

Administrator, Commodity Inspection Division — 2/16/95 Date

Supervisor, Noxious Weed Control Program — 2/16/95 Date

William S. Harrell
Chairman, Oregon State Weed Board — 2/14/95 Date

NOXIOUS WEED CONTROL POLICY AND CLASSIFICATION SYSTEM

"Noxious Weed" means any weed designated by the Oregon State Weed Board that is injurious to public health, agriculture, recreation, wildlife, or any public or private property.

Noxious weeds have become so thoroughly established and are spreading so rapidly on state, county, and federally-owned lands, as well as private land, that they have been declared by ORS 570.505 to be a menace to public welfare. Steps leading to eradication, where possible, are necessary. It is further recognized that the responsibility for such eradication and/or intensive control rests not only on the private landowner and operator, but also on the county, state, and federal government.

WEED CONTROL POLICY

THEREFORE, IT SHALL BE THE POLICY OF THE OREGON DEPARTMENT OF AGRICULTURE (ODA) TO:

1. Rate and classify weeds at the state level.
2. Prevent the establishement and spread of noxious weeds.
3. Encourage and implement the control or containment of infestations of designated weed species and, when possible, eradicate them.
4. Develop and manage a program of biological weed control.
5. Increase awareness of potential economic losses and other undesirable effects of existing and new invading noxious weeds, and to act as a resource center for the dissemination of information.
6. Encourage and assist in the organization and operation of noxious weed control programs of other government units.
7. Cooperate with county weed control officers, Oregon State University, and others in developing weed control methods.
8. Conduct statewide noxious weed surveys and weed control efficacy studies.

WEED CLASSIFICATION SYSTEM

THE PURPOSE OF THE CLASSIFICATION SYSTEM IS TO:

1. Act as the Department of Agriculture's official guideline for implementing and prioritizing noxious weed control programs.
2. Assist the Department of Agriculture in the distribution of available funds for county requests.
3. Serve as a model for the private and public sectors in developing noxious weed classification systems.

THE CRITERIA FOR DETERMINING THE ECONOMIC SIGNIFICANCE OF A NOXIOUS WEED ARE BASED UPON:

1. Detrimental Effects

   a. A plant species that is causing or has the potential of causing severe production losses or increased control costs to the agricultural and/or horticultural industries of this state.
   b. A plant species that is or has the potential of endangering native flora and fauna by its encroachment in forest and conservation areas.
   c. A plant species that is or has the potential of hampering the full utilization and enjoyment of recreational areas.
   d. A plant species that is poisonous, injurious, or otherwise harmful to humans and animals.

2. Plant Reproduction

   a. A plant species that reproduces by seeds capable of being dispersed over wide areas.
   b. A plant species that reproduces by tubers, creeping roots, stolons, rhizomes or other natural vegetative means.

3. Difficulty of Control

   a. A plant species that is not easily controlled with accepted management practices such as chemical, cultural, biological and physical methods.

4. Distribution

   a. A weed of known economic importance which occurs in the state in small enough infestations to make eradication/containment possible; or not known to occur, but its presence in neighboring states makes future occurrence seem imminent.
   b. A weed of economic importance and of limited distribution in the state.
   c. A weed which has not infested the full extent of its potential habitat in the state.

NOXIOUS WEED CONTROL RATING SYSTEM

Noxious weeds, for the purpose of this system, shall be designated "A", "B", and/or "T", according to the ODA Noxious Weed Rating System.

1. "A" designated weed—a weed of known economic importance which occurs in the state in small enough infestations to make eradication/containment possible; or is not known to occur, but its presence in neighboring states make future occurrence in Oregon seem imminent (Table 1).

   RECOMMENDED ACTION: Infestations are subject to intensive control when and where found.

2. "B" designated weed—a weed of economic importance which is regionally abundant, but which may have limited distribution in some counties (Table 2). Where implementation of a fully-integrated statewide management plan is infeasible, biological control shall be the main control approach ("B" weeds for which biological control agents are available are identified with an asterisk).

   RECOMMENDED ACTION: Limited to intensive control at the state or county level as determined on a case-by-case basis.

3. "T" designated weed—a priority noxious weed designated by the State Weed Board as a target weed species on which the Department will implement a statewide management plan (Table 3).

Table 1. "A" designated weeds as determined by the Oregon Department of agriculture.

Common Name	Scientific Name
African rue	*Peganum harmala*
Barbed goatgrass	*Aegilops triuncialis*
Bearded creeper (Common Crupina)	*Crupina vulgaris*
Big-headed knapweed	*Centaurea macrocephala*
Bulbed goatgrass	*Aegilops ventricosa*
Camelthorn	*Alhagi pseudalhagi*
Coltsfoot	*Tussilago farfara*
Feather-headed knapweed	*Centaurea trichocephala*
Giant Hogweed	*Heracium mantegazzianum*
Hydrilla	*Hydrilla verticillata*
Iberian starthistle	*Centaurea iberica*
Kudzu	*Pueraria lobata*
Lepyrodiclis	*Lepyrodiclis holosteoides*
Matgrass	*Nardus stricta*
Ovate goatgrass	*Aegilops ovata*
Purple nutsedge	*Cyperus rotundus*
Purple starthistle	*Centaurea calcitrapa*
Short-fringed knapweed	*Centaurea nigrescens*
Silverleaf nightshade	*Solanum elaegnifolium*
Skeletonleaf bursage	*Ambrosia tomentosa*
Smooth cordgrass	*Spartina alterniflora*
Smooth distaff thistle	*Carthamus baeticus*
Spartina	*Spartina densiflora*
Spartina	*Spartina anglica*
Squarrose Knapweed	*Centaurea virgata*
Syrian bean-caper	*Zygophyllum fabago*
Tausch's goatgrass	*Aegilops tauschii*
Texas Blueweed	*Helianthus ciliaris*
Whitestem distaff thistle	*Carthamus leucocaulos*
Wild safflower	*Carthamus oxycantha*
Woolly distaff thistle	*Carthamus lanatus*

Table 2. "B" designated weeds as determined by the Oregon Department of Agriculture.

Common Name	Scientific Name
Austrian peaweed (Swainsonpea)	*Sphaerophysa salsula*
Buffaloburr	*Solanum rostratum*
*Bull thistle	*Cirsium vulgare*
*Canada Thistle	*Cirsium arvense*
Creeping yellow cress	*Rorippa sylvestris*
*Dalmation Toadflax	*Linaria dalmatica*
*Diffuse knapweed	*Centaurea diffusa*
Dodder	*Cuscuta spp.*
Dyers woad	*Isatis tinctoria*
Eurasian watermilfoil	*Myriophyllum spicatum*
Field bindweed	*Convolvulus arvensis*
French broom	*Cytisus monspessulanas*
Giant horsetail	*Equisetum telmateia*
Giant knotweed	*Polygonum sachalinense*
*Gorse	*Ulex europaeus*
Halogeton	*Halogeton glomeratus*
Himalayan knotweed	*Polygonum polystachyum*
Houndstongue	*Cynoglossum officinale*
*Italian thistle	*Carduus pycnocephalus*
Japanese knotweed (Fleece flower)	*Polygonum cuspidatum*
Johnsongrass	*Sorghum halepense*
Jointed goatgrass	*Aegilops cylindrica*
Kochia	*Kochia scoparia*
*Leafy spurge	*Euphorbia esula*
*Meadow knapweed	*Centaurea pratensis*
*Mediterranean sage	*Salvia aethiopis*
Medusahead rye	*Taeniatherum caput-medusae*
*Milk thistle	*Silybum marianum*
*Musk thistle	*Carduus nutans*
Perennial pepperweed	*Lepidium latifolium*
*Poison hemlock	*Conium maculatum*
Portugese broom	*Cytisus*
*Puncturevine	*Tribulus terrestris*
*Purple loosestrife	*Lythrum salicaria*
Quackgrass	*Agropyron repens*
Ragweed	*Ambrosia artemisiifolia*
*Rush skeletonweed	*Chondrilla juncea*
*Russian knapweed	*Centaurea repens*
*Scotch broom	*Cytisus scoparius*
Scotch thistle	*Onopordum acanthium*
*Slender-flowered thistle	*Carduus tenuiflorus*

South American waterweed (Elodea)	*Elodea densa*
Spartina	*Spartina patens*
Spanish broom	*Spartium junceum*
Spikeweed	*Hemizonia pungens*
Spiny cocklebur	*Xanthium spinosum*
*Spotted knapweed	*Centaurea maculosa*
*St. Johnswort (Klamath weed)	*Hypericum perforatum*
Sulfur cinquefoil	*Potentilla recta*
*Tansy ragwort	*Senecio jacobaea*
Velvetleaf	*Abutilon theophrasti*
Western horsetail	*Equisetum arvense*
White top (Hoary cress)	*Cardaria spp.*
Wild proso millet	*Panicum miliaceum*
Yellow nutsedge	*Cyperus esculentus*
*Yellow starthistle	*Centaurea solstitialis*
*Yellow toadflax	*Linaria vulgaris*

Table 3. The Oregon Department of Agriculture "T" or target list.

The Oregon Department of Agriculture annually develops a target list of weed species that will be the focus of control by the Weed Control Program, sanctioned by the Oregon State Weed Board. Because of the economic threat to the state of Oregon, action against these weeds will receive priority.

Common Name	**Scientific Name**
Bearded creeper (Common Crupina)	*Crupina vulgaris*
Gorse	*Ulex europaeus*
Leafy spurge	*Euphorbia esula*
Rush skeletonweed	*Chondrilla juncea*
Squarrose knapweed	*Centaurea virgata*
Tansy ragwort	*Senecio jacobaea*
Woolly distaff thistle	*Carthamus lanatus*
Yellow starthistle	*Centaurea solstitialis*

COUNTY GUIDELINES FOR COST ASSISTANCE

I. It is recommended that counties rate noxious weeds and designate them as "A" or "B" according to the guidelines below. Designation of noxious weeds may vary from county to county and geographical areas, particularly east and west of the Cascade Mountains. The Oregon State Weed Board shall assist ODA in the distribution of funds to qualified county programs.

County "A" designated weed: A weed that causes economic loss and is not known to occur in the county, or occurs in small numbers, or is restricted in distribution, making eradication/containment possible.

Recommended Action: Infestations should be subject to intensive control by the county with state assistance as funds are available.

County "B" designated weed: A more common noxious weed that causes substantial economic loss on which control measures are directed at protecting crops and resources.

Recommended Action: Infestations are subject to moderate control at the county level with state assistance as funds are available.

II. Counties may apply to participate in distribution of cost share/assistance funds as approved by ODA. In order to receive cost share/assistance funding, a county shall:

A. Annually update county noxious weed list.
B. Designate noxious weeds as "A" and "B".
C. Maintain a weed advisory board.
D. Provide matching funds.

III. Counties may also apply for special grants from the State Weed Board to work on a specific noxious weed project. Applications will be reviewed and considered on an individual basis and funded as resources are available.

South Dakota State University
SDSU

COOPERATIVE EXTENSION SERVICE

College of Agriculture and Biological Sciences

Department of Plant Science

Box 2207A, Agricultural Hall 219
SDSU
Brookings, SD 57007
Phone 605-688-4600
FAX 605-688-4602

May 22, 1997

Vickie Forster
AgrEvo
FAX: (302) 892-3099

Dear Vickie:

The report regarding *Brassica spp.* in South Dakota is attached. *B. kaber* is the only species commonly found. Others noted were collected several years ago; there are no recent records based on herbarium collections.

Sincerely,

Leon J. Wrage

Leon J. Wrage
Extension Agronomist - Weeds

LJW:pe
Attachment

Creating Opportunities for a Lifetime

Post-it™ brand fax transmittal memo 7671 | # of pages ▸ 1

To Dr. Leon Reger	From Vichie Foster
Co. SDSU	Co.
Dept. Agronomy Extension	Phone # 302-892-3034
Fax # 605-688-4602	Fax # 302-892-3099

Pollen Recipient	Occurs in Agriculturally unmanaged areas	State (>1% of U.S. canola production)	Field Hybrids Produced?	Fertility of Hybrids	Reference	
B. napus	Yes	CA	Yes	normal	Bing, 1991	
B. rapa	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.7-1.3%) Yes (56-93%)	< 10% viable 21-86% pollen viable	Bing, 1991 Jorgensen & Anderson, 1994	
B. juncea	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.1-0.3%)	< 10% pollen viable	Bing, 1991	Uncommon in SD
B. nigra (black mustard)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (extremely low numbers);	male sterile	Bing, 1991	Uncommon
B. oleracea (cabbage family)	No	CA	No	n/a	Calgene, 1994	
B. carinata	No		No	n/a	Calgene, 1994	
B. elongata	Yes	NV	No	n/a	Calgene, 1994	
B. tournefortii	Yes	CA	No	n/a	Calgene, 1994	
B. adpressa, syn. *Herschfeldia incana* (hoary mustard)	Yes	CA, NV, OR	Yes (extremely low numbers)	mostly sterile	Lefol et al., 1991	
Raphanus raphanistrum (wild radish)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	Yes (0.2%) Yes (but only under sp. circumstances)	very low (0.16 seeds/ plant) very low (4-14%)	Darmency et al, 1995 Baranger, et al., 1995 Eber et. al.,	
Sinapis arvensis syn. *B. kaber* (wild mustard)	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	No	n/a	Lefol et al., 1994 Lefol et al, 1996	Frequent in ea + ne SD
Sinapis alba syn. *B. hirta*	Yes	AL, CO, GA, ID, MN, MN, ND, OR, SD, WA	No	n/a	Calgene, 1994 Warwick, 1993	Uncommon
Diplotaxis muralis	Yes	CA, OR, SD	No	n/a	Ringdahl, 1987	

n/a = not assessed

Washington State Department of Agriculture
24106 N Bunn Road
Prosser, WA 99350

Fax Cover Sheet

DATE:	June 6, 1997	**TIME:**	2:58 PM
TO:	Vicki Foster		
FROM:	Tom Wessels WSDA-Lab Services	**PHONE:** **FAX:**	509/786-9275 509/786-9370
RE:	Brassica spp.		

Number of pages including cover sheet: 2

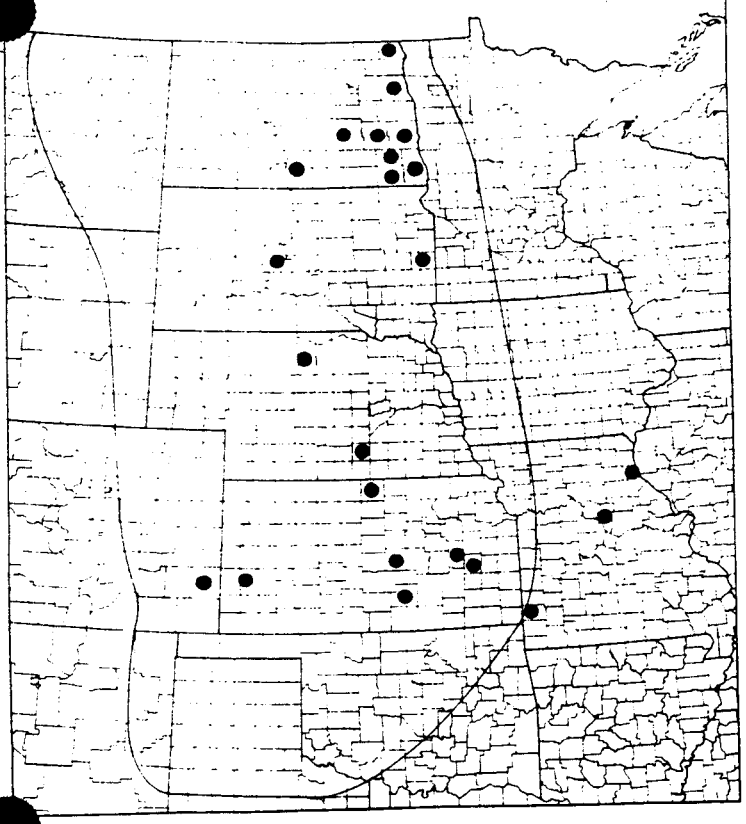
From Weeds Of The West

Brassicaceae (Mustard Family)

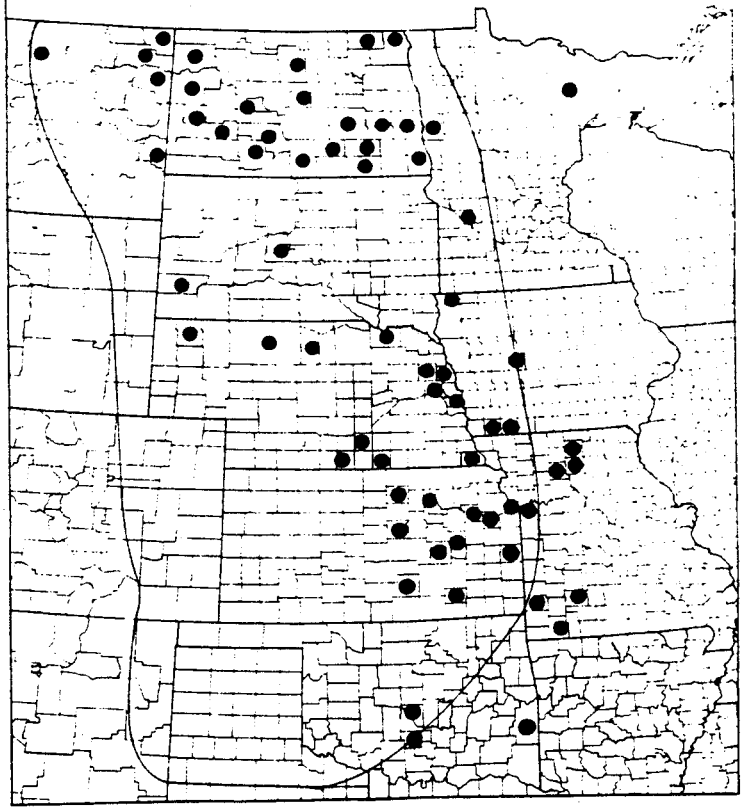
Alyssum alyssoides	Yellow alyssum
A. desertorum	Dwarf alyssum
A. minus	Field alyssum
Brassica kaber	Wild mustard
B. hirta	White mustard
B. arvensis	Charlock mustard, Kaber mustard
B. rapa	Birdsrape mustard, Birds rape, etc.
Camelina microcarpa	Smallseed falseflax
Cardaria spp.	Hoary cress, Whitetop
chalepensis	
draba	
pubescens	
repens	
Chorispora tenella	Blue mustard
Descurainia sophia	Flixweed
D. pinnata	Pinnate tansymustard
Isatis tinctoria	Dyers woad
Lepidium latifolium	Perennial pepperweed
L. perfoliatum	Clasping pepperweed
Raphanus sativus	Wild radish
Sisymbrium altissimum	Tumble mustard
S. irio	London rocket
Thlaspi arvense	Field pennycress

(Brassicaceae)

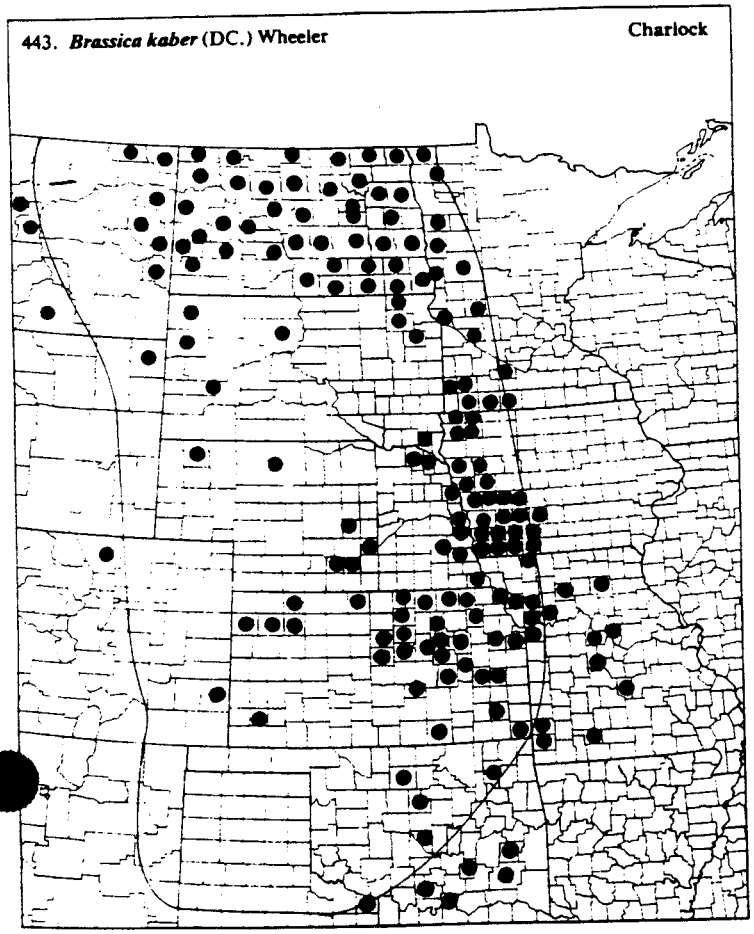
441. ***Brassica hirta*** Moench — White Mustard



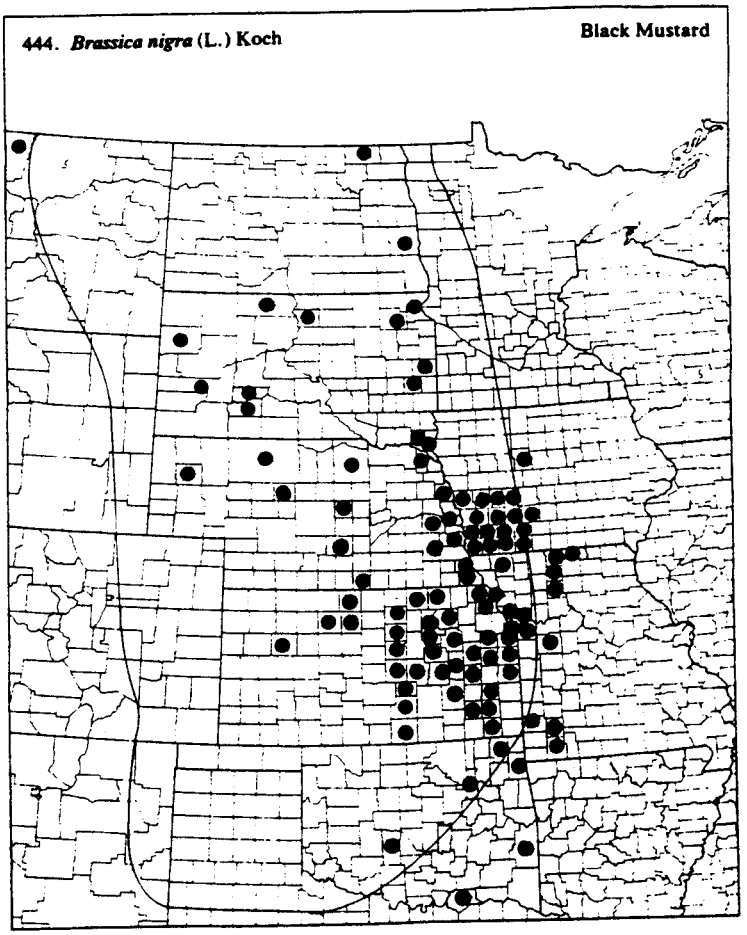
442. ***Brassica juncea*** (L.) Coss. — Indian Mustard

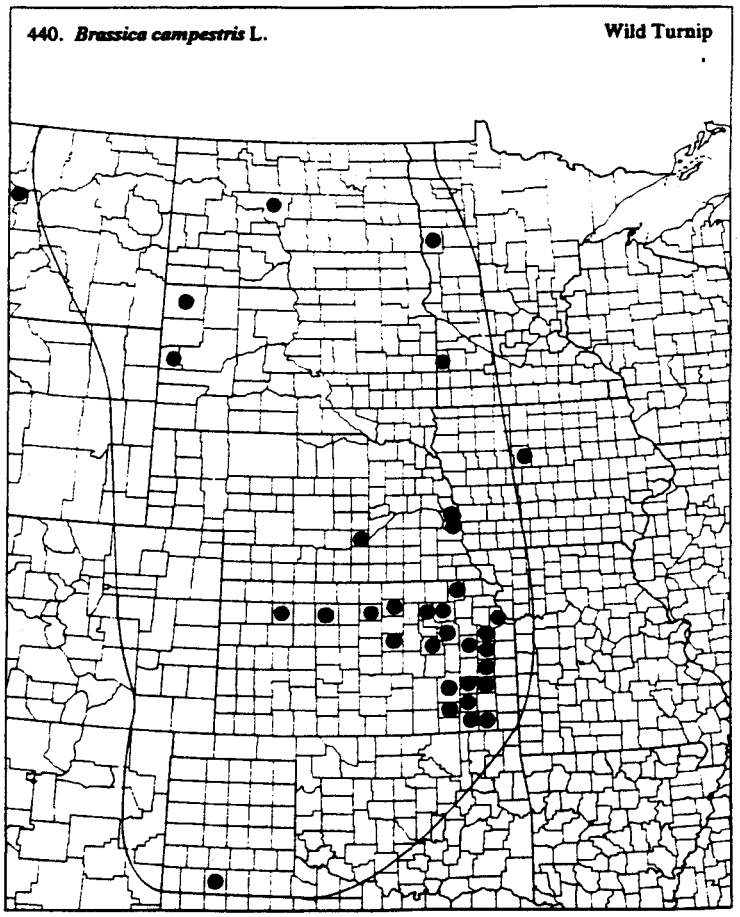


443. ***Brassica kaber*** (DC.) Wheeler — Charlock

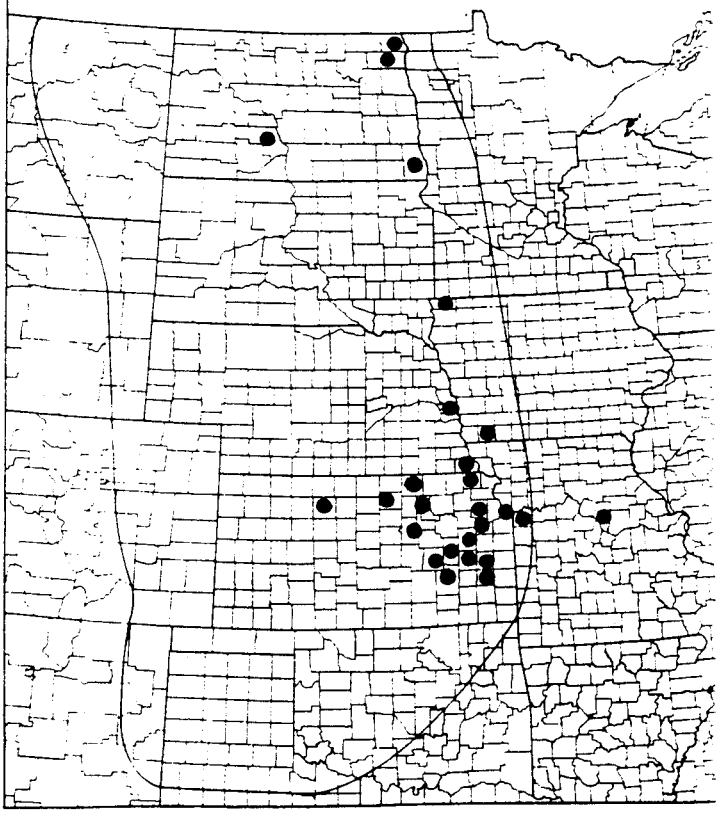


444. ***Brassica nigra*** (L.) Koch — Black Mustard




440. *Brassica campestris* L.
Wild Turnip

488. ***Raphanus sativus*** L. Wild Radish



Attachment II:

1996 U.S.A. Field Termination Report
1997 U.S.A. Preliminary Field Termination Report

Summary Report to the Field Release of Transgenic Canola Expressing Resistance to the Herbicide Glufosinate-Ammonium

Date of Report: October 28, 1996

Permit Number: 96-057-04r

Applicant: Vickie Forster, Registration Specialist
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dates of Release: May-June and October-November, 1996

Dates of Termination: July-September, 1996 and February-March 1997

Sites of Release (States/Number per State): Georgia/2, Iowa/1, Idaho/3, Minnesota/5, North Dakota/7, Washington/2, Wisconsin/1

Purpose of Release
To evaluate weed control and crop tolerance with glufosinate-ammonium herbicide applied to canola (Brassica napus) containing the *pat* gene which confers resistance to the herbicide glufosinate-ammonium. Two varieties of transformed canola were evaluated over a wide range of environmental conditions in several states.

Results
Glufosinate-ammonium herbicide applied at use rates of 400 g/ha or greater were required to provided good control of all weeds germinated at the time of the herbicide application. Crop tolerance was good.

Observations
The frequency of observations differed with each location. Each location was visited 3 or more times during the duration of the release. The area planted to transgenic canola was less than 0.25 acres per site. The transgenic canola plant population ranged from 7 to 17 plants per square foot.

Herbicide Tolerance: Crop tolerance was adequate up to the maximum use rates (1000 g/ha) used in these experiments. The canola variety AAFC 44 showed better tolerance than canola variety HCN 27 to applications of glufosinate-ammonium at the maximum use rate applied. Transgenic canola plants were tolerant to other herbicides currently registered in canola. Transgenic canola plants were killed by an application of 2,4-D herbicide. 2,4-D is a common herbicide used to control volunteer canola in the crops grown the year following canola.

Insect Susceptibility: Bertha armyworm and flea beetles were observed in the trial areas but were not at levels higher than commercial fields in the same general area. Beneficial insects (ladybugs) were noted in some trials.

Disease Susceptibility: Disease resistance in transformed canola is not different from its non-transformed counterpart. Observations in the transgenic canola trials indicate disease tolerance was similar to surrounding commercial fields.

Weather Related Conditions: Most trial locations experienced near normal growing conditions through out the growing season. One location was partially flooded after germination resulting in some stand loss. The remain plants did compensate and produce a good seed yield. Non-transformed canola is similar in its compensatory ability.

Physical Characteristics: Transgenic canola plants were observed from emergence through maturity. No differences were observed from typical commercial canola grown in the general area in plant emergence, seedling vigor, and stand establishment.

Weediness Characteristics: Growth rate and growth habit were identical in both transgenic and non-transgenic plants.

Means of Plant Destruction

The destruction of plants at each site was carried out by cultivation or a combination of mowing and cultivation. Cultivation consisted of either disking or rototilling.

Time and Methods of Monitoring for Volunteers

Sites will be visited one or more times in the spring of 1997 when soil temperatures reach a level at which canola emergence would be expected. If any volunteer canola plants are observed, the numbers and action taken will be reported to APHIS at that time.

Number of Volunteers Observed and Action Taken

The number volunteer canola plants will be observed and recorded in 1997. All volunteer canola plants will be destroyed by mechanical means, removed by hand or destroyed with herbicides other than glufosinate-ammonium.

1997 Interim Field Report for Glufosinate Ammonium Resistant Canola

Planting of the seed was at a normal timing and good stands were established at most locations. In locations with dry conditions this spring, good stands were not established until rain was received. At these dry locations, the canola plants germinated at two different times resulting in two different growth stages of the canola. At all the locations, the canola growth rate is normal and similar to other canola grown in the area.

Weed populations have been normal in the canola growing areas. Weed control with glufosinate ammonia (GA) has been good to excellent. The GA resistant canola has shown no injury from applications of GA, however, approximately 2-5% of the canola plants have been killed by the applications of GA due to the occurrence of non-transgenic types in the seed source.

The trial locations from last year continue to be monitored and any volunteer plants are controlled with tillage or herbicide applications.

Attachment III:

Agriculture and AgriFood Canada: Regulatory Directive Dir 94-09. The Biology of *Brassica napus L.* (Canola/Rapeseed).

Regulatory Directive **Dir94-09**

The Biology of *Brassica napus L.* (Canola/Rapeseed)

A companion document to the Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits

This document replaces Regulatory Proposal 94-02

(publié aussi en français) **December 16, 1994**

This bulletin is published by the Information Division of the Plant Industry Directorate. For further information, please contact a Plant Biotechnology Officer at the following address:

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Government of Canada Gouvernement du Canada

Canada

Table of Contents

Part A - General Information

A1.0 Background

Since 1988, Agriculture and Agri-Food Canada (AAFC) has been regulating the field testing in Canada of agricultural and horticultural crop plants with novel traits (PNT's). "Plants with novel traits" are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. "Familiarity" is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. "Substantial equivalence" is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

The PNT's can either be derived from recombinant DNA technologies or from traditional plant breeding. Regulated field testing is necessary when the PNT's have traits of concern, i.e., the traits themselves, their presence in a particular plant species or their use are: (1) considered unfamiliar when compared with products already in the market; (2) not considered substantially equivalent to similar, familiar plant types already in use, and regarded as safe.

Before PNT's may be authorized for unconfined release, they must be assessed for environmental safety. Regulatory guidelines entitled: *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* have been developed to define criteria and information requirements that must be considered in the environmental assessment of PNT's to ensure environmental safety, in the absence of confinement conditions.

A2.0 Scope

The present document represents a companion document to the regulatory guidelines *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*. It is intended to provide background information on the biology of *Brassica napus* (L.), its centres of origin, its related species and the potential for gene introgression from *Brassica napus* into relatives, and details of the life forms with which it interacts.

Such species-specific information will serve as a guide for addressing some information requirements of Part D of the regulatory guidelines. Specifically, it will be used to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of *B. napus* only relate to plants of this species with no novel traits. Novel traits of concern might confer new characteristics to the plant, that could impact on the environment pursuant to their unconfined release.

Part B - The Biology of *Brassica napus*

B1.0 General Description, Use as a Crop Plant and Origin of Species

Brassica napus L., an ancient crop plant, belongs to the Cruciferae (Brassicaceae) family, also known as the mustard family. The name crucifer comes from the shape of flowers, with four diagonally opposite petals in the form of a cross. *Brassica napus* has dark bluish green foliage, glaucous, smooth, or with a few scattered hairs near the margins, and partially clasping. The stems are well branched, although the degree of branching depends on variety and environmental conditions; branches originate in the axils of the highest leaves on the stem, and each terminates in an inflorescence. The inflorescence is an elongated raceme, the flowers are yellow, clustered at the top but not higher than the terminal buds, and open upwards from the base of the raceme (Musil, 1950).

There are two types, the oil-yielding oleiferous rape, often referred to in Canada as Argentine Rape, of which canola is a type having specific quality characteristics, and the tuber-bearing swede or rutabaga. The oleiferous type can also be subdivided into spring and winter forms. Indian Sanskrit writings of 2000 to 1500 BC directly refer to oilseed rape and mustard, as do Greek, Roman and Chinese writings of 500 to 200 BC (Downey and Röbbelen, 1989). In Europe, domestication is believed to have occurred in the early middle ages and commercial plantings of rapeseed were recorded in the Low Countries as early as the 16th century. At that time rapeseed oil was used primarily as an oil for lamps. Later it became used as a lubricant for steam engines. Although used widely as an edible oil in Asia, only through breeding for improved oil quality, and through the development of improved processing techniques, has rapeseed oil become important in western nations. Since the Second World War, as a result of improved oil and meal quality, rapeseed production in Europe and Canada has increased dramatically. China, India, Europe and Canada are now the top producers, although there is potential for the crop to be successfully grown in Australia, the United States and South America.

Within Canada, the primary production areas are the prairie provinces of Manitoba, Saskatchewan, Alberta and the Peace River area of both Alberta and British Columbia, although there is also some production in Ontario and Québec. Today, two species of *Brassica* have varieties of canola quality: *B. napus*, the species considered in these guidelines, and *B. rapa*. The former species requires more frost-free days than the latter to mature. Whereas *B. napus* varieties may require on average 105 days from seeding to harvest, *B. rapa* varieties require on average only 88 days. Consequently, *B. napus* varieties tend to be grown south of the areas in which *B. rapa* is grown: the central parts of Alberta and Saskatchewan, and the southern part of Manitoba.

B2.0 Brief Outlook of Agronomic Practices for the Oleiferous *B. napus* (based on the Canola Growers Manual of the Canola Council of Canada, 1994 edition)

The oleiferous *B. napus*, a cool-season crop, is not as drought-tolerant as the cereals. It is widely adapted, and performs well in a range of soil conditions, providing that moisture and fertility levels are adequate. Air and soil temperatures influence canola plant growth and productivity. The optimum temperature for maximal growth and development is just over 20°C, and it is best grown between 12°C and 30°C. After emergence, seedlings prefer relatively cool temperatures up to flowering; high temperatures at flowering will hasten the plant's development, reducing the time from flowering to maturity.

Due to an increased awareness of soil conservation issues, minimal or no till canola production is advised, where most of the crop residue and stubble are left on the soil surface to trap snow, reduce snow melt run-off, stop erosion and increase soil water storage. Reduced tilllage techniques, however, are only effective when they are combined with a good systematic weed control program.

Weeds can be one of the most limiting parameters in rapeseed production. The closely related cruciferous weeds (wild mustard, stinkweed, shepherd's purse, ball mustard, flixweed, wormseed mustard, hare's-ear mustard and common peppergrass) are often problematic. Oilseed rape does not compete with weeds in the early growth stages, because it is slow growing and slow to cover the ground. Weeds must be controlled early to avoid yield loss due to competition. Although rapeseed crops can be attacked by a number of insect pests, insect control must be carefully designed to reduce unnecessary and costly pesticide applications, chances of resistance buildup in insects, and damage to honeybees and native pollinating insects. Flea beetles are the most important pests of oilseed rape. Diseases can be severe in large production areas, and are greatly influenced by cultivation practices and environmental factors, so that disease management programs are advisable.

When the first pods begin to shatter, *B. napus* is usually cut just below the level of seed pods and swathed. The use of dessicants allows a reduction of shattering, thus allowing direct combining.

Oilseed rape should not be grown on the same field more often than once every four years, to prevent the buildup of diseases, insects, and weeds. Volunteer growth from previous crops (buckwheat for example), and chemical residues from herbicides, are also important factors to consider when selecting sites.

B3.0 The Reproductive Biology of *B. napus*

Most *B. napus* cultivars grown in Canada are of the annual type, the species showing poor survival at temperatures lower than -6°C, although there is some production of fall-sown winter hardy types in the warmest part of southern Ontario. Fertilization of ovules usually result from self pollination, although outcrossing rates of 20 - 30%

have been reported (Rakow and Woods, 1987). The pollen, which is heavy and sticky, is moved from plant to plant primarily through insect transmission. Bees are the primary pollen vector, because the pollen is heavy and sticky and is not carried great distances by wind. Cross pollination of neighbours can also result from physical contact of the flowering racemes. Successive generations of *B. napus* arise from seed from previous generations. There are no reports of vegetative reproduction under field conditions in Canada.

B4.0 The Centres of Origin of the Species[1]

The origins of *B. napus* (an amphidiploid with chromosome n=19) are obscure but were initially proposed to involve natural interspecific hybridization between the two diploid species *B. oleracea* (n = 9) and *B. rapa* (syn. *campestris*)[2] (n = 10), (U 1935). Recent evidence (Song and Osborn, 1992) through analyses of chloroplast and mitochondrial DNA suggests that *B. montana* (n = 9) might be closely related to the prototype that gave rise to both cytoplasms of *B. rapa* and *B. oleracea*. It also suggests that *B. napus* has multiple origins, and that most cultivated forms of *B. napus* were derived from a cross in which a closely related ancestral species of *B. rapa* and *B. oleracea* was the maternal donor.

B4.1 Geographic Origin of *B. oleracea*

First collected as a food in neolithic times (Prakash and Hinata, 1980), it is believed that all cultivated forms of the cabbage group originated from the wild species through mutation, human selection and adaptation. Although the origin of the various cultivar types is not fully understood, the conclusion that could be arrived at is that wild kale was the ancestral progenitor. Chromosome structural changes do not seem to have played an important part in the development of the many different cultivar types because they are similar in genetic architecture to the wild type (Harberd, 1972).

The wild forms of *B. oleracea*, a suffrutescent (low, shrubby plant with woody lower parts of stems and herbaceous upper parts) perennial, grow along the coast of the Mediterranean from Greece through to the Atlantic coasts of Spain and France, around the coast of England and to a limited extent in Helgoland (Snogerup et al., 1990). Typically, the wild type is found on limestone and chalk cliffs in situations protected from grazing. Individuals are often found below cliffs in scree where they grow among other shrubs, and some populations are found on steep grassy slopes. In Helgoland, populations are found on open rocky ground.

---

[1] This section draws heavily on discussions with, and a review paper prepared by, Dr. S. I. Warwick and A. Francis of Centre for Land and Biological Resources Research, Agriculture and Agri-Food Canada.

[2] Toxeopus et al. (1984) recommended the name *Brassica rapa* rather than *B. campestris* because it was used first to describe the species by Metzger in 1833.

In Europe and North America, domesticated types have been reported as escapes, but do not form self sustaining populations outside of cultivation. *B. oleracea* is a recent introduction into North America.

B4.2 Geographic Origin of *B. rapa*

Wild *B. rapa* (subspecies *sylvestris* L.) is regarded as the species from which the subspecies *rapa* (cultivated turnip) and *oleifera* (turnip-rape) originated. It is native throughout Europe, Russia, Central Asia and the Near East (Prakash and Hinata, 1980), with Europe proposed as one centre of origin. There is some debate as to whether the Asian and Near Eastern type arose from an independent centre of origin in Afghanistan which then moved eastward as it became domesticated. Prakash and Hinata (1980) suggest that oleiferous *B. rapa* subspecies developed in two places giving rise to two different races, one European and the other Asian.

Typically, *B. rapa* is found in coastal lowlands, high montane (the slopes of high valleys of mountain ranges) and in alpine and high sierras. In Canada, where it is a recent introduction, it is found in disturbed land, typically in crops, fields, gardens, roadsides and waste places (Warwick and Francis, 1994).

B4.3 Geographic Origin of *B. montana*

Brassica montana, possibly a progenitor species of *B. napus*. (see above), also a suffrutescent perennial, originates from the Mediterranean coastal area between Spain and Northern Italy (Snogerup et al., 1990).

It is found typically in or below limestone cliffs and rocks, walls, etc., often in disturbed ground. It is usually found in coastal areas and on rocky islets, but has been recorded at 1000m somewhat inland of the coast.

B4.4 Geographic Origin of *B. napus*

Brassica napus is thought to have multiple origins resulting from independent natural hybridization events between *B. oleracea* x *B. rapa*. In Europe, predominantly the winter form has become a common yellow crucifer of roadsides, waste and cultivated ground, docks, cities and towns, tips, arable fields and riverbanks. In the British Isles, for instance, it has been naturalized wherever oil-seed rape is grown. It is a relatively recent introduction into Canada and the United States, and is described as an occasional weed, escape or volunteer in cultivated fields (Munz, 1968; Muenscher 1980). It is found typically in crops, fields, gardens, roadsides and waste places.

B5.0 Cultivated *B. napus* as a Volunteer Weed

As with all crops cultivated and harvested at the field scale, some seed may escape harvest and remain in the soil until the following season when it germinates either

before or following seeding of the succeeding crop. In some instances the volunteers may give considerable competition to the seeded crop and warrant chemical and/or mechanical control.

The problem of volunteer plants in succeeding crops is common to most field crop species. Much depends on the management practices used in the production of the crop, e.g., whether the plants have disbursed seed at the time of harvest, the setting of the harvesting equipment, and speed of the harvesting operation which will determine whether more or less seed is lost by the harvester. With crops of the *Brassica* family, because of the small seed size and large number of seeds produced by the crop, poor management practices can result in severe volunteer problems in succeeding crops. Similar problems may be encountered with cultivated *B. juncea* and *B. rapa* varieties.

B6.0 Summary of Ecology of *B. napus* and its Progenitors

Brassica napus and its progenitors are plants of "disturbed land" habitats. In un-managed ecosystems these species may be considered "primary colonizers," i.e., plant species that are the first to take advantage of disturbed land where they would compete against plants of similar types for space. Unless the habitats are disturbed on a regular basis, such as on cliff edges, river edges and the edges of pathways made by animals, populations of these types of plants will become displaced by intermediaries and finally by plants that will form climax ecologies such as perennial grasses on prairies and tree species and perennial shrubs in forests.

In managed ecosystems, including roadsides, industrial sites and waste places, as well as crop lands, there is potential, because of their "primary colonizing" nature, for these species to maintain ever present populations, and it is in these habitat types that these species are recorded in the various flora of Canada and North America. Their success will be dependent on their ability to compete for space with other primary colonizers, in particular with successful weedy types. This, in turn, will depend on how well suited they are to the particular climate, soil conditions, etc. of individual sites.

In crop production systems, poor management practices may result in large numbers of seed of *B. napus* not being harvested, that may cause volunteer "weed" problems in succeeding crops, especially at high density.

Brassica napus is not listed as a noxious weed in the Weed Seed Order (1986). It is not reported as a pest or weed in managed ecosystems in Canada, nor is it recorded as being invasive of natural ecosystems. In summary, there is no evidence that in Canada *B. napus* has weed or pest characteristics.

Part C - The Close Relatives of *B. napus*

C1.0 Inter-species/genus Hybridization

Important in considering the potential environmental impact following the unconfined release of genetically modified *B. napus* is an understanding of the possible development of hybrids through interspecific and intergeneric crosses with the crop and related species. The development of hybrids could result in the introgression of the novel traits into these related species and resulting in:

- the related species becoming more weedy
- the introduction of a novel trait with potential for ecosystem disruption into the related species.

This section will be subject to updating, as more data become available. Based on background information provided in the present document, applicants will need to consider the environmental impacts of potential gene flow.

While many interspecific and intergeneric crosses have been made between *B. napus* and its relatives (Warwick and Black, 1993), many have necessitated intervention in the forms of ovary culture, ovule culture, embryo rescue and protoplast fusion. Reported here from the extensive review by Warwick and Black (1993) are *B. napus* and related species interspecific and intergeneric identified hybrids obtained sexually.

(Note: apart from the *B. juncea* x *B. napus*, *B. napus* x *B. rapa* and *B. napus* x *B. juncea* hybridizations from field outcrossing studies reported by Bing et al. (1991), resulting hybrids were achieved through hand pollination (usually through emasculation of the female plant followed by transfer of pollen from the male plant using a paint brush).

B. napus x *B. carinata*	Alam et al. 1992
B. napus x *B. juncea*	Alam et al. 1992, Bing et al. 1991
B. juncea x *B. napus*	Alam et al. 1992, Bing et al. 1991
B. napus x *B. nigra*	Bing et al. 1991
B. nigra x *B. napus*	Bing et al. 1991
B. napus x *B. rapa*	Bing et al. 1991
B. rapa x *B. napus*	Bing et al. 1991
Diplotaxis erucoides x *B. napus*	Ringdahl et al. 1987
D. muralis x *B. napus*	Ringdahl et al. 1987
B. napus x *Hirschfeldia incana* (*Brassica adpressa*)	Lefol et al. 1991
H. incana x *B. napus*	Lefol et al. 1991
B. napus x *Raphanus raphanistrum*	Lefol, E., R. K. Downey and G. Séguin-Swartz 1993 personal communication
B. napus x *Erucastrum gallicum*	Lefol, E., R. K. Downey and G. Séguin-Swartz 1993 personal communication

Sexual hybrids derived through crosses between the various relatives of *B. napus* listed above, are as follows:

B. carinata x *B. juncea*	Alam et al. 1992
B. juncea x *B. carinata*	Alam et al. 1992
B. carinata x *Sinapis arvensis*	Bing et al. 1991
B. juncea x *B. nigra*	Bing et al. 1991
B. nigra x *B. juncea*	Bing et al. 1991
B. juncea x *Sinapis arvensis*	Bing et al. 1991
B. juncea x *S. arvensis*	Bing et al. 1991
B. oleracea x *B. rapa*	Wojciechowski 1985
B. rapa x *B. oleracea*	Wojciechowski 1985
B. rapa x *B. nigra*	Bing et al. 1991
D. muralis x *B. rapa*	Salisbury 1989
B. rapa x *Raphanus sativus*	Ellerström 1978
R. sativus x *B. rapa*	Ellerström 1978
H. incana x *B. nigra*	Mattson 1988
B. nigra x *H. incana*	Mattson 1988
R. sativus x *B. oleracea*	Harberd and McArthur 1980

For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediaries, and survival and fertility of the resulting offspring, is necessary.

C2.0 Potential for Introgression of Genetic Information from *B. napus* into Relatives.

Sinapis arvensis is perhaps the worst of the weedy Brassica relatives, especially in the major canola growing areas of Manitoba, Saskatchewan and Alberta. A plant reported from the cross between *B. juncea* x *S. arvensis* was backcrossed into *B. juncea*, and into *S. arvensis* (Bing et al. 1991). The resulting plants were weak or sterile and produced no seed on open pollination suggesting that this cross would not result in the natural transfer of traits from either species being stably inserted into the other species.

Two other weedy species, *Raphanus raphanistrum* (wild radish) recorded to be more abundant in eastern Canada than in the prairie region, and *Erucastrum gallicum* (dog mustard) which may be locally quite abundant in croplands in the prairie provinces, formed hybrids with *B. napus* as the female parent. Work is ongoing in Saskatoon (G. Séguin-Swartz, 1993, personal communication) to determine whether F_1 hybrids are viable, produce fertile pollen which may be backcrossed onto either parent and whether stable populations result. Studies are also planned to determine if natural outcrossing under field conditions occurs.

Hybrids resulting from the *D. muralis* x *B. napus* and *D. erucoides* x *B. napus* crosses were male sterile (Ringdahl et al. 1987).

The same outcome was reported for backcrosses resulting from the hybrids produced from the *B. nigra* x *B. napus* cross.

Bing et al. (1991) suggested that of the crosses they attempted, there was potential for hybrids between *B. napus, B. juncea* and *B. rapa* to produce viable seed that could survive to the next generations. These three species are widely grown as crops for the production of both canola and mustard.

Hybrid combinations that are successfully created using *B. napus* as a female parent might still be relevant to gene flow considerations, because they can potentially act as genetic bridges.

C3.0 Occurrence of *B. napus* and Related Species in Canada

Of the above listed crosses, *B. carinata* and *Hirschfeldia incana* are not reported as present in Canada (Warwick, 1993), and *Diplotaxis erucoides* is reported as being rare in the Gaspé peninsula of Québec. *Brassica oleracea,* apart from the wild types in their original habitats in Europe, is rarely found outside of cultivation. Of the other species:

- *B. napus* is recorded in the Northwest Territories, District of Mackenzie (NT-M), Labrador (LB), Newfoundland (NF), Prince Edward Island (PE), Nova Scotia (NS), New Brunswick (NB), Québec (PQ), Ontario, (ON), Manitoba (MB), Saskatchewan (SK) Alberta (AB) and British Columbia (BC). *B. napus* is not listed in Weeds of Canada nor in Weeds of Ontario.

- *B. juncea* is recorded in NT-M, NF, NS, PE, NB, PQ, ON, MB, SK, AB and BC. Weeds of Canada reports that it occurs in every province and reaches its greatest abundance in the western provinces. Weeds of Ontario indicates its distribution is similar to that of *S. arvensis* although it is generally less common;

- *B. nigra* is recorded in NF, NS, PE, NB, PQ, ON, SK, AB and BC. Weeds of Canada suggests that it is not very common in western Canada. In Weeds of Ontario it is listed as occurring in a few localities in the south of the province especially in fields and waste areas bordering river valleys, and along railways;

- *B. rapa* is recorded in NT-M, YT (Yukon Territory), LB, NF, NS, PE, NB, PQ, ON, MB, SK, AB and BC. Weeds of Canada suggests it is sometimes abundant and that in some parts of the East, bird rape, the wild form, supplants *S. arvensis* over large areas. Weeds of Ontario indicates it occurs in a few grainfields and waste areas in southern Ontario;

- *Diplotaxis muralis* is recorded in NS, PE, NB, PQ, ON, MB, SK, AB, and BC. Weeds of Canada does not list this species. Weeds of Ontario indicates it usually occurs in coarse soils along roads, railways, beaches and around buildings and waste places in southern Ontario;

- *Erucastrum gallicum* is recorded in NF, NS, PE, NB, PQ, ON, MB, SK, AB and BC. Weeds of Canada states that it reaches its greatest abundance in Manitoba and Saskatchewan where it inhabits fields, waste places, along railways, gardens, and orchards. It is very common on roadsides, and is an abundant field weed in many localities in Western Canada. In Ontario, it occurs throughout the province but is more common in southern Ontario where it is frequently found around railway yards, waste places, orchards, gardens, roadsides and occasionally in grainfields;

- *Raphanus raphanistrum* is recorded in LB, NF, NS, PE, NB, PQ, ON, MB, SK, AB and BC. Weeds of Canada states that this species is very abundant in all provinces on the Atlantic seaboard. In Québec and Ontario, it is of less importance and is reported to occur in the moister parts of Manitoba and Saskatchewan. Weeds of Ontario indicates that it is present in only a few scattered localities in Ontario where it infests cultivated fields and waste places;

- *Raphanus sativus* is recorded in NF, NS, PE, NB, PQ, ON, MB, and BC. Weeds of Canada indicates that this species is occasionally persistent in gardens (as a result of cultivation);

- *Sinapis arvensis* is recorded in NT-M, YT, LB, NF, NS, PE, NB, PQ, ON, MB, SK, AB and BC. Weeds of Canada lists it as one of the commonest annual weeds. It occurs in all provinces where the most serious infestations are probably in the rich river valleys of the West. Its habitats include grainfields, cultivated fields, waste places, fence rows and roadsides. Weeds of Ontario indicates that it occurs throughout Ontario being most frequent in cultivated fields and gardens but occasionally appearing in fence lines, along roadsides and in waste areas.

C4.0 The Agro-ecology of Weedy Relatives of *B. napus*

Of the relatives discussed, *S. arvensis*, *R. raphanistrum* are listed as primary noxious weeds in the *Weed Seeds Order*, 1986 and *E. gallicum* is listed as a secondary noxious weed. These three species are potentially the weediest in agricultural crop lands. All are relatively easily controlled in crops of species other than brassica by the use of selective herbicides.

The abundance of these three species in agricultural croplands is partly determined by the cropping practices. Weed species prominence can be dramatically affected by cropping systems and cultivation practices. The recent adoption of minimum and no till crop production systems, and the abandonment of cultivated summerfallow practices as a means of soil conservation, have caused a shift in the prominence of different weed species.

The above listed species are all plants of "disturbed land" habitats. Their success will be dependent on their ability to compete for space with other primary colonizers, in particular with other successful weedy plant types. This in turn will depend on how well suited they are to the particular climate, soil conditions, etc. of individual sites.

Part D - Potential Interactions of *B. napus* with Other Life Forms

Table 1 is intended to be used to guide applicants in their considerations of potential impacts of the release of the PNT on non-target organisms.

The intention is not to require comparison data between the PNT and its *B. napus* counterpart(s) for all interactions. Depending on the novel traits, applicants might decide to submit data for only some of the interactions. Sound scientific rationale will be required to justify the decision that data would be useless or irrelevant for the remaining interactions. For example, the applicant might chose not to provide data on the potential for gene transfer from the PNT to related species if it can be clearly shown that the novel trait will not affect reproductive characteristics of *B. napus*, either directly or indirectly.

Some of the life forms are listed as categories (i.e., pollinators, mychorrhizal fungi, animal browsers, birds, soil microbes, and soil insects). When, because of the novel traits, a concern is perceived for these specific categories, applicants will be required to provide detailed information on interactions with indicator species in each category.

Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may need to be considered.

TABLE 1. Potential interactions of *B. napus* with other life forms during its life cycle.
"X" indicates the type of interaction between the listed organisms and *B. napus* (information requirements may be waived if valid scientific rationale is provided).

	Interaction with *B. napus*			
Other life forms	**Pathogen**	**Symbiont or Beneficial Organism**	**Consumer**	**Gene transfer**
Albugo candida	X			
Alternaria spp.	X			
Botrytis cinerea	X			
Erysiphe spp.	X			
Leptosphaeria maculans	X			
Peronospora parasitica	X			
Plasmodiophora brassicae	X			
Pythium debaryanum	X			
Rhizoctonia solani	X			
Sclerotinia sclerotiorum	X			
Xanthomonas spp.	X			
Turnip mosaic virus	X			
Aster yellows mycoplasma	X			
Flea beetle			X	
Pollinators		X	X	
Mychorrhizal fungi		X		
Birds			X	
Animal browsers			X	
Soil microbes		X		
Earthworms		X		
Soil insects			X	
other *Brassica napus*				X
Brassica rapa				X
Brassica juncea				X
Brassica nigra				X
Raphanus raphanistrum				X
Erucastrum gallicum				X
Others				

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Attachment IV:

Agriculture and AgriFood Canada: Decision Document DD 96-11.

Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate-Ammonium Tolerant Line HCN 28, May 6, 1996.

Agriculture and Agri-Food Canada
Food Production and Inspection Branch
Plant Products Division

Agriculture et Agroalimentaire Canada
Direction générale, Production et inspection des aliments
Division des produits végétaux

Decision Document DD96-11

Determination of Environmental Safety of Agrevo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola Line HCN28

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document Dir94-09 *The Biology of* Brassica napus *L. (Canola/Rapeseed).*

The Plant Biotechnology Office of the Plant Products Division has evaluated information submitted by AgrEvo Canada Inc. regarding a glufosinate ammonium-tolerant canola line. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties.

Unconfined release into the environment of HCN28 and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

Please note that, while determining the environmental safety of plants with novel traits is a critical step in the commercialization of these plant types, other requirements still need to be addressed, such as for Variety Registration (AAFC) and for the evaluation of feed (AAFC) and food safety (Health Canada).

(publié aussi en français)

May 6, 1996

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Table of Contents

I. Brief Identification of the Plant With Novel Traits (PNT)

Designation of the PNT:	HCN28
Applicant:	AgrEvo Canada Inc.
Plant Species:	Canola (*Brassica napus* L.)
Novel Traits:	Glufosinate ammonium (herbicide) tolerance
Trait Introduction Method:	*Agrobacterium tumefaciens*-mediated transformation
Proposed Use of PNT:	Production of *B. napus* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo has developed a *Brassica napus* canola line tolerant to glufosinate ammonium, a broad spectrum non-residual herbicide. This *B. napus* line, referred to as HCN28 in the present document, will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides.

The development of HCN28 was based on recombinant DNA technology, by the introduction of a bacterial gene into a line of *B. napus*. This gene codes for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation, thus conferring tolerance to glufosinate ammonium. It is the same as the gene inserted in HCN92, a glufosinate ammonium tolerant canola line that was authorized for unconfined release and feed use on March 10, 1995 (see DD95-01).

HCN28 has been field tested in Canada under confined conditions in Saskatchewan (1993, 95), Alberta (1994, 95), Manitoba (1995) and Ontario (1995).

AgrEvo has provided data on the identity of HCN28, a detailed description of the modification method, data and information on the gene insertion, the role of the inserted gene and of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel protein was identified and characterized, including its levels of expression in seed, potential toxicity to non-target organisms and allergenicity.

Agronomic characteristics such as cotyledon width, pod and leaf length, flowering period, time to maturity, plant height, lodging score, seed yield and thousand seed weight, and resistance to white rust and blackleg, were compared to those of unmodified *B. napus* counterparts.

Agriculture and Agri-Food Canada (AAFC) has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of the PNT to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

III. Description of the Novel Trait

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (L-PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonia in susceptible plants within hours of application.

- The phosphinothricin tolerance gene engineered into HCN28 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- The gene is linked to the same constitutive promoter as for line HCN92. Expression levels were quantified in seeds and ranged from 95 to 246 µg/mg of seed tissue; seed expression levels from HCN92 ranged from 150 to 223 µg/mg of seed tissue.

- Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions.

- The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology the toxins or allergens entered in to GENEBANK DNA database.

2. Development Method:

- *Brassica napus* cultivar AC Excel was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and replaced with the gene coding for glufosinate ammonium tolerance. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was shown to be the case in HCN28.

- The original transformant was backcrossed twice with *B. napus* line AC Excel; HCN28 was derived from single seed descent.

3. Stable Integration into the Plant's Genome:

- The provided data showed that there was no incorporation of any coding region from outside the T-DNA borders and that gene integration occurred at only one insertion site.

- HCN28 is at least four generations removed from the original transformant.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to Become a Weed of Agriculture or to Be Invasive of Natural Habitats

AAFC evaluated data submitted by AgrEvo on the reproductive and survival biology of HCN28, and determined that vegetative vigor, flowering period, time to maturity, seed production, and overwintering were within the normal range of expression of characteristics in unmodified *B. napus* counterparts. HCN28 has no specific added genes for cold tolerance or winter hibernation. Based on the molecular characterization of the plants and their agronomic performance, AAFC concurs with AgrEvo that there is no reason to believe that line HCN28 would behave differently than HCN92 in its interactions with the environment.

The biology of *B. napus*, described in Dir94-09, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by AgrEvo, HCN28 was determined not to be different from its counterpart in this respect. No competitive advantage was conferred to glufosinate ammonium-tolerant plants, other than tolerance to glufosinate ammonium.

Glufosinate ammonium is not used in normal crop rotation cycles, and resistance is therefore not an issue of concern in weed management control. Glufosinate-resistant *B. napus* volunteer plants can easily be managed by mechanical means and other available chemicals used to control *B. napus*.

The above considerations, together with the fact that the novel trait has no intended effect on weediness or invasiveness, led AAFC to conclude that HCN28 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

NOTE: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

Brassica napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa* (oilseed rape, Polish canola, turnip, rutabaga), *B. juncea* (brown mustard, Indian mustard), *B. carinata* (Ethiopian mustard), *B. nigra* (black mustard), *Diplotaxis muralis* (sand rocket, stinking wall rocket), *Raphanus raphanistrum* (wild radish), and *Erucastrum gallicum* (dog mustard) (Dir 94-09). Studies show that introgression of the herbicide tolerance gene is most likely to occur with *B. rapa*, the other major canola species and an occasional weed of cultivated land especially in the eastern provinces of Canada.

If glufosinate ammonium-tolerant individuals arose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate ammonium. This would only occur in managed ecosystems where glufosinate ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate ammonium-tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would be easily controlled using mechanical and other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This, however, can be minimized by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from HCN28 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effect of the novel trait is unrelated to plant pest potential, and *Brassica napus* is not a plant pest in Canada (Dir94-09). In addition, agronomic characteristics of HCN28, including *Albugo candida* (white rust) and *Leptosphaeria maculens* (blackleg) resistance, were shown to be within the range of values displayed by currently commercialized *B. napus* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that HCN28 did not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

The PAT enzyme is rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and pH-mediated proteolysis. It does not contain potential glycosylation sites nor does it possess proteolytic or heat stability, indicating that it is not a likely allergen. A search of the GENEBANK DNA sequence database revealed no significant homology with the toxins or allergens entered in that database.

Based on the above, and on the agronomic properties of HCN28, AAFC has determined that the unconfined release of this line will not result in altered impacts on interacting organisms, including humans, compared with currently commercialized counterparts.

5. Potential Impact on Biodiversity

HCN28 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Since outcross species are only found in disturbed habitats, transfer of novel traits would not impact unmanaged environments.

AAFC has therefore concluded that the potential impact on biodiversity of HCN28 is equivalent to that of currently commercialized canola lines.

V. Regulatory Decision

Based on the review of data and information submitted by AgrEvo Canada Inc., AAFC has concluded that neither the novel gene, nor its resulting gene product and associated novel trait, confer any intended or unintended ecological advantage to HCN28. Should these traits be transferred through outcrossing to related plants, these would not result in any ecological advantage.

Unconfined release into the environment of HCN28 and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

Please note that, while determining the environmental safety of plants with novel traits is a critical step in the commercialization of these plant types, other requirements still need to be addressed, such as for Variety Registration (AAFC) and for the evaluation of feed (AAFC) and food safety (Health Canada).

Attachment V.

Agriculture and AgriFood Canada: Decision Document DD 96-11, Supplement.

Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate-Ammonium Tolerant Line HCN 28, May 6, 1996.

Attachment V

Agriculture and Agri-Food Canada
Food Production and Inspection Branch
Plant Products Division

Agriculture et Agroalimentaire Canada
Direction générale, Production et inspection des aliments
Division des produits végétaux

Supplement to the Decision Document:

DD96-11 Suppl.

Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola Line HCN28

Feed Assessment:

This supplement to Decision Document DD96-11 has been prepared to explain the regulatory decision reached under the guidelines Dir95-03 *Guidelines for the Assessment of Livestock Feed From Plants with Novel Traits*.

The Plant Biotechnology Office of the Plant Products Division has evaluated information submitted by Agrevo Canada Inc. regarding the glufosinate ammonium-tolerant canola line HCN28. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties as explained in decision document DD96-11.

The Feed Section of the Plant Products Division, AAFC, has evaluated information submitted by AgrEvo Canada Inc., regarding the glufosinate ammonium-tolerant canola line HCN28 and has determined that it is substantially equivalent to canola currently approved for use as livestock feed.

Feed use of HCN28 and its byproducts, its descendants and any derived sister lines, but without the introduction of any other novel trait, is therefore authorized.

(publié aussi en français)

July 2, 1996

This bulletin is published by the Plant Products Division, Agriculture and Agri-Food Canada. For further information, please contact the Feed Section at:

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Food Production and Inspection Branch
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Table of Contents

I. Brief Identification of the Plant With Novel Traits (PNT)

Designation of the PNT: HCN28

Applicant: AgrEvo Canada Inc.

Plant Species: Canola (*Brassica napus* L.)

Novel Traits: Glufosinate ammonium (herbicide) tolerance

Trait Introduction Method: *Agrobacterium tumefaciens*-mediated transformation

Proposed Use of PNT: Production of *B. napus* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo has developed a *Brassica napus* canola line tolerant to glufosinate ammonium, a broad spectrum non-residual herbicide. This *B. napus* line, referred to as HCN28 in the present document, will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides.

The development of HCN28 was based on recombinant DNA technology, by the introduction of a bacterial gene into a line of *B. napus*. This gene codes for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation, thus conferring tolerance to glufosinate ammonium. It is the same as the gene inserted in HCN92, a glufosinate ammonium tolerant canola line that was authorized for unconfined release and feed use on March 10, 1995 (see DD95-01).

AgrEvo has provided data on the identity of HCN28, a detailed description of the modification method, data and information on the gene insertion, the role of the inserted gene and of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel protein was identified and characterized, including the levels of expression in seed, its potential toxicity and allergenicity.

Data to support the suitability of line HCN28 as livestock feed was provided. Results from proximate analyses, including crude protein, crude fat, the fatty acid profile, crude fibre, and ash were supplied.

For further information and a more detailed description of the novel trait, please refer to decision document DD96-11.

AAFC has reviewed the information submitted by the company in light of the assesment criteria for determining the safety and efficacy of livestock feed as described in Dir95-03 *Guidelines for the Assesment of Livestock Feed Derived From Plants with Novel Traits*. We have considered :

- potential impact on livestock and
- potential impact on livestock nutrition

III. Nutritional Composition

Analyses of the nutritional composition including protein, fat, fatty acid profile, fibre and ash were conducted on samples of line HCN28, line HCN92 (Innovator) and three non-transformed commercial canola varieties (Excel, Cyclone, Legend). Overall, nutritional composition of HCN28 was shown to be substantially equivalent to non-transformed canola varieties. There were no differences among lines in crude fat, fatty acid profile, crude fibre or ash content. At one location, protein content was significantly lower in line HCN28 than two of the non-transformed controls while at the other two locations, there were no significant protein content differences among lines. In all locations, protein content was within the normal range for canola.

IV. Anti-Nutritional Factors

The phytosterol, erucic acid and glucosinolate content of line HCN28 was substantially equivalent to the levels determined for the non-transformed controls. All values were below the prescribed maximum levels for these anti-nutritional factors in canola as set out in the Feeds Regulations

V. Regulatory Decision

Based on the review of submitted data and information, the Feed Section of the Plant Products Division has concluded that the novel trait does not in itself raise any concerns regarding the safety or nutritional composition of line HCN28. Canola oil, seed and meal are currently listed in Schedule IV of the Feeds Regulations and are, therefore, approved for use in livestock feeds in Canada. As line HCN28 has been assessed and found to be substantially equivalent to traditional canola varieties, HCN28 and its byproducts are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

Feed use of the HCN28 line and its byproducts, its descendants and any derived sister lines, but without the introduction of any other novel trait, is therefore considered safe.

Attachment VI.

Health Canada: Health Protection Branch:

authorization letter stating, "no objection" to the sale of refined canola oil from canola lines derived from the transformation event T45 as human food in Canada, February 17, 1997.

Health Canada | Santé Canada
Health Protection Branch | Direction générale de la protection de la santé

Tunney's Pasture
Ottawa, Ontario
K1A 0L2

February 17, 1997

Mr. Conor Dobson
Manager, Government Affairs
AgrEvo Canada Inc.
#213 - 1600 James Naismith Drive
Gloucester, Ontario
K1B 5N4

Dear Mr. Dobson:

This will refer to the Novel Food Submission concerning transgenic canola (*Brassica napus* L.) lines derived from a transformation event designated T45 which is tolerant to glufosinate ammonium herbicides. Officers of the Health Protection Branch have reviewed the information that AgrEvo Canada Inc. provided for assessment of the acceptability of oil from this canola for sale as human food in Canada.

According to the submitted information, the procedure used in developing the subject canola transformation event T45 involved the introduction of a *pat* gene derived from *Streptomyces viridochromogenes*. As a result of this genetic modification, the canola lines derived from transformation event T45 contain the following novel constituents:

(1) the *pat* gene; and,

(2) the enzyme phosphinothricin acetyl transferase which is encoded by the *pat* gene.

The result of this genetic modification is the expression of the phosphinothricin acetyl transferase which confers tolerance to glufosinate ammonium herbicides.

.../2

Canada

Based on our evaluation of the submitted data, we have no objection to the sale of refined canola oil from canola lines derived from the transformation event T45 as human food in Canada.

It should be noted that this opinion is solely with respect to the suitability for sale as human food of refined canola oil from canola lines derived from the transformation event T45. It is the continuing responsibility of AgrEvo Canada Inc. to ensure that its products are in compliance with all applicable statutory and regulatory requirements.

Please note that we are providing our colleagues in Agriculture and Agri-Food Canada (AAFC) with a copy of this letter in regard to that Department's responsibility respecting variety registration, animal feeds, environmental release and labelling issues. We are also providing our colleagues in the Pest Management Regulatory Agency (PMRA) with a copy of this letter for their information.

Yours truly,

George M. Paterson

George M. Paterson, Ph.D.
Director General
Food Directorate

c.c. Dr. A. MacKenzie, AAFC
Ms. C. Franklin, PMRA

AgrEvo

Southern Analysis of Two Lines of Transformation Event to Determine T-DNA Stability

AgrEvo Canada Inc.
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Regina, Saskatchewan
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Tel: (306) 721-4500

ACI95-10
September 25, 1995

Title

Southern Analysis of Two Lines of Transformation Event to Determine T-DNA Stability

Author

C.F. Bennett

Report No.

Canadian Reference ACI95-10

International Reference A56367

Date

September 25, 1995

Study Submitted By

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AgrEvo

Southern Analysis of Two Lines of Transformation Event to Determine T-DNA Stability

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ACI95-10
September 25, 1995

Abstract

Brassica napus c.v. A.C. Excel protoplasts were used via *Agrobacterium tumafaciens* mediated transformation to produce the herbicide tolerant canola transformation event T45. The T45 transformant contains the construct pHoe4/Ac. The T-DNA from this vector contains a 1.3 Kb cassette consisting of a 35S promoter, the herbicide tolerance *pat* gene, and a 35S terminator.

This study demonstrates that the T-DNA, containing the *pat* cassette, from pHoe4/Ac has been stabily introduced into the transformation event T45 and has maintained integrity over generations as observed in the breeding lines HCN27 and HCN28.

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ACI95-10
September 25, 1995

Introduction

Agrobacterium tumafaciens was used to transform *Brassica napus* protoplasts isolated from the cultivar A.C. Excel along with the vector pHoe4/Ac were utilized for the transformation process.

In order to show that the introduced T-DNA has remained stable over several generations, the genomic DNA was restricted with Eco RI and Southern analysis performed, using the *pat* gene (figure 2) as a probe on the original transformant T45 and on two subsequent breeding lines HCN27 and HCN28.

Materials and Methods

Plant genomic DNA was isolated[2] from leaf tissues of the original transformant T45 and the latest generation of the transgenic lines HCN27 and HCN28. The canola cultivars Innovator and Excel were used as positive and negative controls, respectively.

The DNA was digested with restriction enzyme Eco RI, then separated by gel electrophoresis. The restricted DNA was then transferred onto Gene Screen Plus[3] following the capillary transfer method described by Sambrook[4].

Southern hybridizations[5] were performed using the 550 bp *pat* fragment as a probe[6].

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Results

Figure 3 shows the Southern analysis of the earliest (R_1) transformant compared to the latest breeding line (F_4) of HCN28, along with positive and negative controls.

The expected 1.3 Kb fragment appears in all of the transgenic plant samples. No band was found in the lane containing the negative control (non-transformed) plant material.

Discussion/Conclusion

As the results in Figure 3 show, the T-DNA from the breeding line HCN28 has been maintained as an intact unit over a total of five generations.

References:

Ditta, G., Stanfield, S., Corbin, D., Helinski, D. 1980. Broad Host Range DNA cloning system for gram negative bacteria: construction of a gene bank of *Rhizobium meliloti.* Proc. Natl. Acad. Sci. 77:7347[1]

1

1. Ditta, G., Stanfield, S., Corbin, D., Helinski, D. 1980. Broad Host Range DNA cloning system for gram negative bacteria: construction of a gene bank of *Rhizobium meliloti.* Proc. Natl. Acad. Sci. 77:7347
2. Dellaporta, S., Wood, J., Hicks, J., 1983 Molecular Biology Report, Volume I, p.19
3. NEN Research Products.
4. Molecular Cloning; Sambrook, Fritsch, Maniatus, p. 9.3-4
5. Method and reagents by Quikhyb™, Stratagene.
6. Random Primers Kit, BRL

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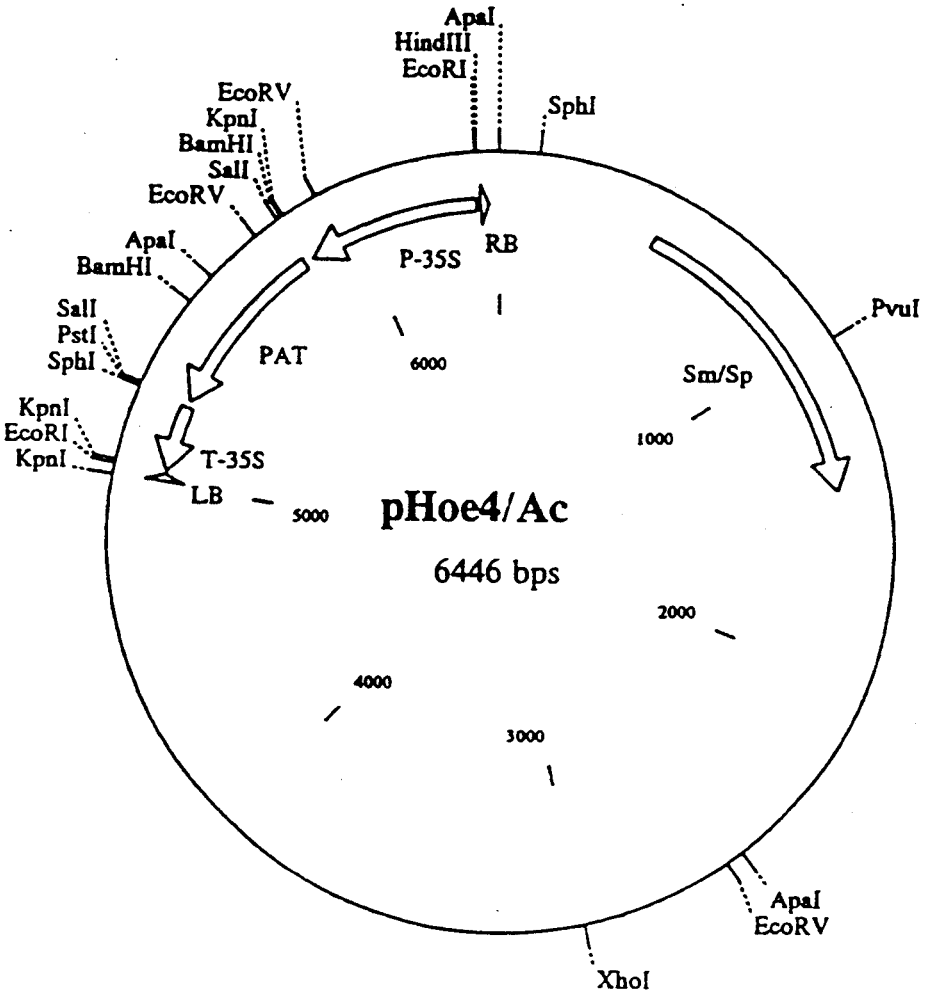
Figure 1. Plasmid Map of pHoe4/Ac



AgrEvo

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Figure 2. Sequence of the *pat* gene

```
   1  ATGTCTCCGG AGAGGAGACC AGTTGAGATT AGGCCAGCTA CAGCAGCTGA TATGGCCGCG
      TACAGAGGCC TCTCCTCTGG TCAACTCTAA TCCGGTCGAT GTCGTCGACT ATACCGGCGC

  61  GTTTGTGATA TCGTTAACCA TTACATTGAG ACGTCTACAG TGAACTTTAG GACAGAGCCA
      CAAACACTAT AGCAATTGGT AATGTAACTC TGCAGATGTC ACTTGAAATC CTGTCTCGGT

 121  CAAACACCAC AAGAGTGGAT TGATGATCTA GAGAGGTTGC AAGATAGATA CCCTTGGTTG
      GTTTGTGGTG TTCTCACCTA ACTACTAGAT CTCTCCAACG TTCTATCTAT GGGAACCAAC

 181  GTTGCTGAGG TTGAGGGTGT TGTGGCTGGT ATTGCTTACG CTGGGCCCTG GAAGGCTAGG
      CAACGACTCC AACTCCCACA ACACCGACCA TAACGAATGC GACCCGGGAC CTTCCGATCC

 241  AACGCTTACG ATTGGACAGT TGAGAGTACT GTTTACGTGT CACATAGGCA TCAAAGGTTG
      TTGCGAATGC TAACCTGTCA ACTCTCATGA CAAATGCACA GTGTATCCGT AGTTTCCAAC

 301  GGCCTAGGAT CCACATTGTA CACACATTTG CTTAAGTCTA TGGAGGCGCA AGGTTTTAAG
      CCGGATCCTA GGTGTAACAT GTGTGTAAAC GAATTCAGAT ACCTCCGCGT TCCAAAATTC

 361  TCTGTGGTTG CTGTTATAGG CCTTCCAAAC GATCCATCTG TTAGGTTGCA TGAGGCTTTG
      AGACACCAAC GACAATATCC GGAAGGTTTG CTAGGTAGAC AATCCAACGT ACTCCGAAAC

 421  GGATACACAG CCCGGGGTAC ATTGCGCGCA GCTGGATACA AGCATGGTGG ATGGCATGAT
      CCTATGTGTC GGGCCCCATG TAACGCGCGT CGACCTATGT TCGTACCACC TACCGTACTA

 481  GTTGGTTTTT GGCAAAGGGA TTTTGAGTTG CCAGCTCCTC CAAGGCCAGT TAGGCCAGTT
      CAACCAAAAA CCGTTTCCCT AAAACTCAAC GGTCGAGGAG GTTCCGGTCA ATCCGGTCAA

 541  ACCCAGATCT GA
      TGGGTCTAGA CT
```

AgrEvo
Southern Analysis of Two Lines of Transformation Event to Determine T-DNA Stability

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ACI95-10
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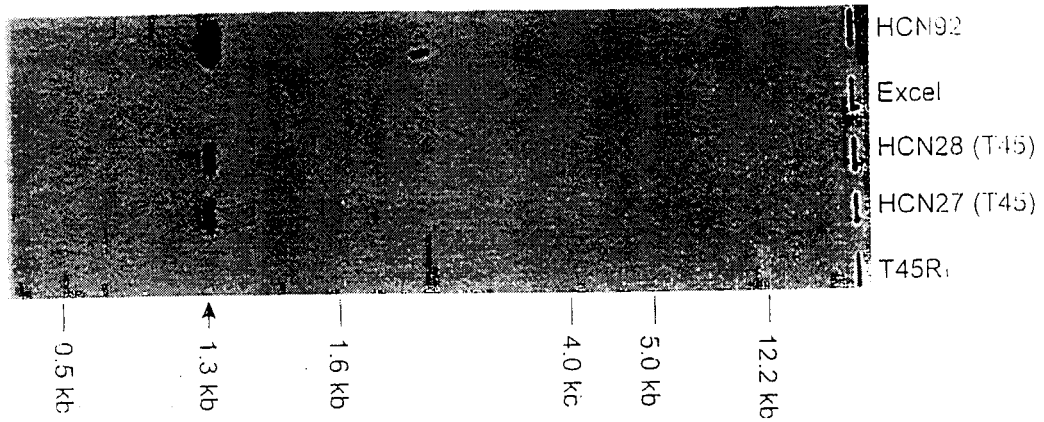
Figure 3: Southern Analysis of DNA digested with restriction enzyme EcoRI as stated in text. The blot was hybridized with the *pat* coding region (see text).



T45 Genomic DNA
pat probe

Figure Legend:
HCN92: Positive Control DNA, HCN92 (INNOVATOR) is derived from transformation event Topas 19/2
(A.C.) EXCEL: Negative Control DNA, nontransgenic canola
HCN28 and HCN27: DNA from lines derived from transformation event T45.
T45R1: DNA from transformation event T45

See Page 8a for Southern Analysis of Positive and Negative Controls as compared to the transforming plasmid vector pHoe4Ac.

HCN92 was used as a positive control in southern blots due in part to historical reasons. HCN92 was the first line developed for commercialization by AgrEvo with the *pat* gene in it. The use of HCN92, which contains the plasmid pOCA/AC, as a positive control demonstrates that even if a different plasmid is used the same insert (same molecular weight) shows up on the southern blot as for the pHoe4/AC plasmid.

See Page 9 for a plasmid map of pOCA/AC, the vector in line HCN92; Page 10 for a description of line HCN92, derived from transformation event Topas 19/2; and, Pages 11-16 for AAFC Decision Document DD95-01 regarding line HCN92.

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Figure 4: Southern Analysis of DNA digested with one of the restriction enzymes EcoRI, Hind III, Nco I, Bam HI or EcoRV, respectively, prior to electrophoresis. The blot was hybridized with the *pat* coding region (see text).

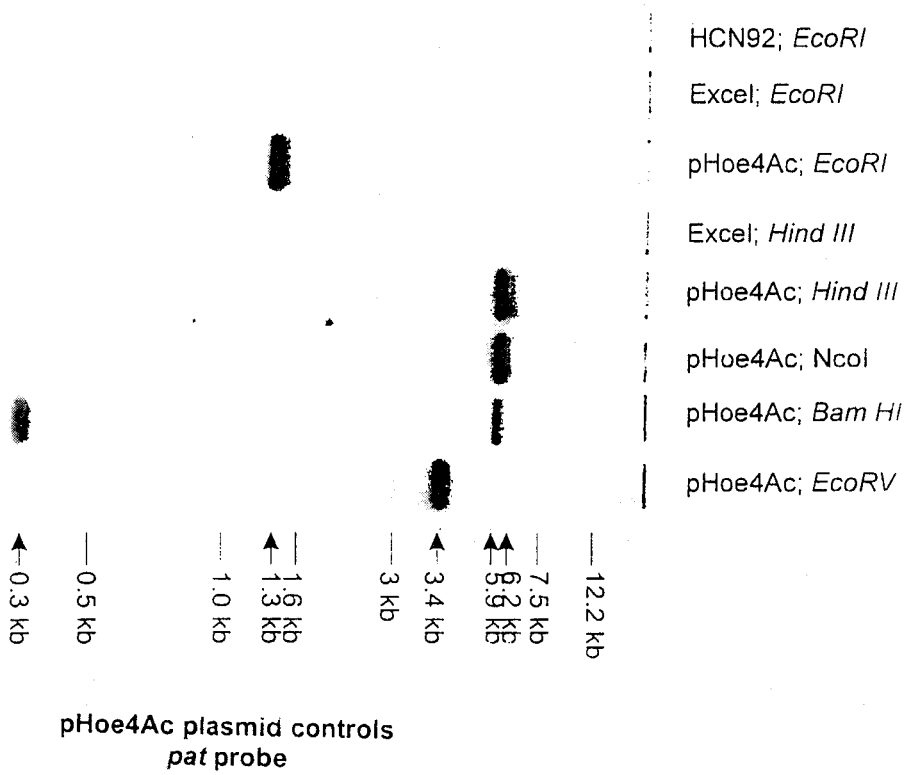
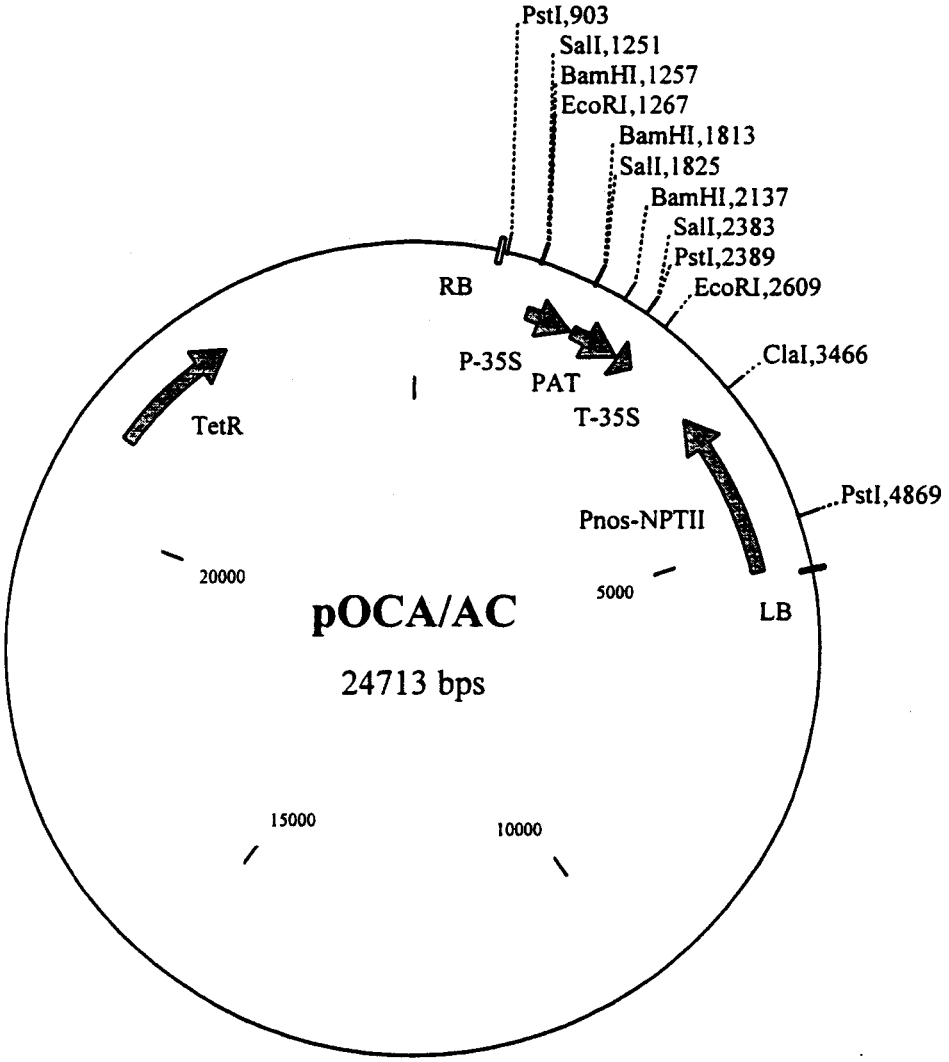


Figure Legend:
HCN92;EcoRI: Positive Control DNA, from transgenic HCN92 (INNOVATOR) canola, digested with EcoRI
EXCEL; EcoRI: nontransgenic canola (A.C. EXCEL) DNA digested with EcoRI
pHoe4Ac; EcoRI: transforming plasmid DNA digested with EcoRI
EXCEL; Hind III: nontransgenic canola (A.C. EXCEL) DNA digested with Hind III
pHoe4Ac; Hind III: transforming plasmid DNA digested with Hind III
EXCEL; Nco I: nontransgenic canola (A.C. EXCEL) DNA digested with Nco I
pHoe4Ac; Nco I: transforming plasmid DNA digested with Nco I
EXCEL; Bam HI: nontransgenic canola (A.C. EXCEL) DNA digested with Bam HI
pHoe4Ac; Bam HI: transforming plasmid DNA digested with Bam HI
EXCEL; EcoRV: nontransgenic canola (A.C. EXCEL) DNA digested with EcoRV
pHoe4Ac; EcoRV: transforming plasmid DNA digested with EcoRV

Figure 5: Plasmid Map of pOCA/AC



Glufosinate Tolerant Canola HCN92

Line Description

Cultivar Identification:	HCN92
Species name:	*Brassica napus* L.
Crop:	Canola
Transformation Method:	*B.napus* obtained through disarmed *Agrobacterium tumefaciens* mediated transformation
Vector :	pOCA/Ac
Trait 1:	tolerance to glufosinate ammonium
Gene 1:	phosphinothricin acetyltransferase (*pat*) gene
Donor 1:	*Streptomyces viridochromogenes*
Promoter 1/Donor:	35S gene promoter /Cauliflower Mosaic Virus (CaMV)
Terminator 1/Donor:	35S gene terminator /Cauliflower Mosaic Virus (CaMV)
Trait 2:	Tolerance to aminoglycosidic antibiotics
Gene 2:	Neomycin phosphotransferase II (NPT II)
Donor 2:	*Escherichia coli*
Promoter 2/Donor:	Nopaline synthase (*nos*)/*Agrobacterium tumefaciens*
Terminator 2/Donor :	Octopine synthase (*ocs*)/*Agrobacterium tumefaciens*

We have demonstrated that the incorporated DNA is limited to the T-DNA region. No additional coding sequences from the vector, other than the pat gene and the selectable marker, have been incorporated into the Brassica genome as part of the transformation process.

The original transformant Topas 19/2 was first crossed with the Agriculture Canada line ACSN-3. The R_1 was then crossed with the commercial line AC Excel. Initial crosses followed by several years of pedigree selection resulted in the production of the glufosinate tolerant line HCN92.

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

Plant Products Division
Food Production and Inspection Branch
Agriculture and Agri-Food Canada

Decision Document

DD95-01

Determination of Environmental Safety of Agrevo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits and its companion document Dir94-09 The Biology of *Brassica napus* L. (Canola/Rapeseed), and the proposed guidelines Pro94-04 Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed.

The Plant Biotechnology Office and the Feed Section of the Plant Products Division have evaluated information submitted by AgrEvo Canada Inc. regarding a glufosinate ammonium-tolerant and kanamycin-resistant canola line. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties and is considered substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

(publié aussi en français)

March 10, 1995

This bulletin is published by the Plant Products Division, Agriculture and Agri-Food Canada. For further information, please contact the Plant Biotechnology Office or the Feed Section at:

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Designation(s) of the PNT:	HCN92
Applicant:	AgrEvo Canada Inc.
Plant Species:	Canola (*Brassica napus* L.)
Novel Traits:	Glufosinate ammonium (herbicide) tolerance; kanamycin (antibiotic) resistance
Trait Introduction Method:	*Agrobacterium tumefaciens*-mediated transformation
Proposed Use of PNT:	Production of *B. napus* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo has developed a *Brassica napus* canola line tolerant to glufosinate ammonium, a broad spectrum non-residual herbicide. This *B. napus* line, referred to as HCN92 in the present document, will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides.

The development of HCN92 was based on recombinant DNA technology, by the introduction of two bacterial genes into a line of *B. napus*. A gene conferring tolerance to glufosinate ammonium was inserted, coding for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation. Another gene, conferring resistance to kanamycin, was also inserted; this gene is of no agronomic interest but was used to select modified plants from those that remained unmodified at the development stage.

HCN92 has been field tested in Canada under confined conditions in Saskatchewan (1990-94), Alberta (1991-94), Manitoba (1991-94), and Ontario (1993-94).

AgrEvo has provided data on the identity of HCN92, a detailed description of the modification method, data and information on the stability of the gene insertion, the role of the inserted genes in donor organisms and the role of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel proteins were identified and characterized, including their potential toxicity to livestock and non-target organisms, allergenicity, and levels of expression in the plant and feed. Numerous detailed scientific publications were also supplied.

Agronomic characteristics such as seed production, time to maturity, flowering period, and male and female fertility were compared to those of unmodified *B. napus* counterparts. Effects of HCN92 residues on growth and productivity of the following season's grain, forage, and pulse crops were assessed.

AgrEvo has also provided data on HNC92's survival adaptations: silique shattering potential, seed dormancy, seed dispersal mechanisms, vegetative vigor, reproductive characteristics, and the emergence in subsequent years of volunteer plants under mechanical or chemical fallow conditions. Stress adaptation was evaluated, including susceptibilities to various *B. napus* pests and pathogens, to abiotic stresses such as soil salinity and moisture regimes, and to herbicides other than glufosinate ammonium that are normally used on canola crops. Invasiveness studies were performed under disturbed, undisturbed, and agronomic conditions.

Data to support the efficacy of HCN92 as a livestock feed were provided. A proximate analysis to include crude protein, crude fat, crude fiber, ash and gross energy were supplied for the whole seed, processed meal and oil content.

Agriculture and Agri-Food Canada (AAFC) has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of the PNT to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

AAFC has also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Pro94-04:

- potential impact on livestock, and
- potential impact on livestock nutrition.

III. Description of the Novel Traits

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonium in susceptible plants within hours of application.

- The phosphinothricin tolerance gene engineered into HCN92 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- The gene is linked to a constitutive promoter, and protein expression was detected in roots, leaves, buds and seeds. However, it was not detected in stem tissue, protein extracts from the pollen, or unprocessed honey. Maximum expression was 0.001% of total plant protein.

- The expressed PAT enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post transcriptional modifications. Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed into feed ingredients.

- The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology the toxins or allergens entered in to GENEBANK DNA database.

2. Kanamycin Resistance:

- Kanamycin is an aminoglycosidic antibiotic that binds to bacterial ribosomes thus disrupting normal protein synthesis and killing the bacterial cell.

- The kanamycin-resistance gene codes for an enzyme that prevents kanamycin from binding to ribosomes, thereby rendering the cells resistant. The exact nature of the enzyme is considered Confidential Business Information by AgrEvo. The source of the gene was described, and the full nucleotide sequence was provided.

- The gene is linked to a weak constitutive promoter; expression was consistently stronger in root tissue, but was also observed in buds, leaves, and crude seed samples. The enzyme was not detected in unprocessed honey or pollen samples and was inactivated during processing of canola seed into feed ingredients.

- The expressed enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post-transcriptional modifications.

- The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENEBANK DNA database.

3. Development Method:

- *Brassica napus* cultivar Topas was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and replaced with genes coding for glufosinate ammonium tolerance and kanamycin resistance. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was shown to be the case in HCN92.

- The transformant was crossed with *B. napus* line ACSN3, then with AC Excel; HCN92 was derived from a bulk of single F_3 plants selected from the cross.

4. Stable Integration into the Plant's Genome:

- The provided data showed that there was no incorporation of any coding region from outside the T-DNA borders and that gene integration occurred at only one insertion site.

- HCN92 is several generations removed from the original transformant. Comparisons between the original transgenic plant and the HCN92 line show no difference in the presence and expression of both genes, nor in the insertion site.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to become a weed of agriculture or to be invasive of natural habitats

AAFC evaluated data submitted by AgrEvo on the reproductive and survival biology of HCN92, and determined that vegetative vigor, overwintering capacity, flowering period, time to maturity, seed production, and dormancy were within the normal range of expression of characteristics in unmodified *B. napus* counterparts. HCN92 has no specific added genes for cold tolerance or winter hibernation; no overwintered plants were observed by AgrEvo in post-harvest

years of field trials, and the number of volunteers in the year following a field trial were comparable between plots of HCN92 and counterpart *B. napus*. Seed morphology and average seed weight did not change, indicating that seed dispersal potential was not altered.

Based on the submitted data, AAFC has determined that HCN92 did not show any stress adaptation other than its resistance to glufosinate ammonium. Its resistance or susceptibility to major *B. napus* pests and pathogens (e.g., blackleg, sclerotinia, flea beetles) fall within the ranges currently displayed by commercial varieties. Moisture stress had a significant negative effect on both HCN92 and its counterparts.

The biology of *B. napus*, described in Dir94-09, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by AgrEvo, HCN92 was determined not to be different from its counterparts in this respect. Invasiveness was studied in disturbed and undisturbed habitats. Data showed that HCN92 was neither more invasive nor more persistent than commercial counterparts. No competitive advantage was conferred to glufosinate ammonium-tolerant plants, other than that conferred by tolerance to glufosinate ammonium.

Glufosinate ammonium is not used in normal crop rotation cycles, and resistance is therefore not an issue of concern in weed management control. Glufosinate-resistant *B. napus* volunteer plants can easily be managed by mechanical means and other available chemicals used to control *B. napus*.

The above considerations, together with the fact that the novel traits have no intended effect on weediness or invasiveness, led AAFC to conclude that HCN92 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

NOTE: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

Brassica napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa*, *B. juncea*, *B. carinata*, *B. nigra*, *Diplotaxis muralis*, *Raphanus raphanistrum*, and

Erucastrum gallicum (Dir 94-09). Studies show that introgression of the herbicide tolerance gene is most likely to occur with *B. rapa*, the other major canola species and an occasional weed of cultivated land especially in the eastern provinces of Canada.

If glufosinate ammonium-tolerant individuals arose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate ammonium. This would only occur in managed ecosystems where glufosinate ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate ammonium-tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would be easily controlled using mechanical and other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This, however, can be avoided by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from HCN92 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of both novel traits are unrelated to plant pest potential, and *Brassica napus* is not a plant pest in Canada (Dir94-09). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative composition of HCN92 were shown to be within the range of values displayed by currently commercialized *B. napus* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that HCN92 did not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

Data presenting the effect of plant residue from HCN92 on agronomic performance of succeeding crops were examined by AAFC for wheat, barley, lentils, peas, flax and alfalfa. No significant differences in either plant counts or grain yield between the HCN92 and counterpart canola plots were identified. This is an indirect indication that soil bacteria, involved in maintaining soil fertility, are not negatively affected by HCN92 plant residues.

PAT activity was not detected in pollen grains, neither was it detected in unprocessed honey collected from a bee colony which had foraged in the glufosinate-tolerant *B. napus* line. No negative impact on bees foraging in

HCN92 was observed, including brood development. Both enzymes are rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and
pH-mediated proteolysis. Neither of the two novel proteins contained potential glycosylation sites nor did they possess proteolytic or heat stability, indicating that neither protein is a likely allergen. A search of the GENEBANK DNA sequence database revealed no significant homology with the toxins or allergens entered in that database.

Based on the above, AAFC has determined that the unconfined release of HCN92 will not result in altered impacts on interacting organisms, including humans, compared with currently commercialized counterparts.

5. Potential Impact on Biodiversity

HCN92 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Since outcross species are only found in disturbed habitats, transfer of novel traits would not impact unmanaged environments. Studies have shown to AAFC that HCN92 is not invasive of natural habitats, and that it is no more competitive than its counterparts, both in natural and managed ecosystems.

AAFC has therefore concluded that the potential impact on biodiversity of HCN92 is equivalent to that of currently commercialized canola lines.

V. Assessment Criteria for Use as Livestock Feed

1. Anti-Nutritional Factors

Ninety-five percent confidence intervals were determined for glucosinolate and erucic acid content of the meal and oil produced from HCN92, grown under a variety of conditions. These confidence intervals demonstrated that the PNT contained levels of these anti-nutritional factors below the prescribed standards for both the meal and oil fractions, i.e., <30 micromoles glucosinolates per gram of dry meal and <2% erucic acid in the oil.

2. Nutritional Composition of PNT

No statistical differences in nutritional composition, i.e., crude protein, crude fat, crude fibre, ash and gross energy content, were noted between the whole seed, processed meal or oil of HCN92 and current commercial canola cultivars. These results collectively demonstrate that the introduction of this construct into *B. napus*, resulting in HCN92, did not likely result in any secondary effects impacting on the composition or nutritional quality of the cultivar. Accordingly, HCN92 was judged to be substantially equivalent to traditional canola varieties in terms of nutritional composition.

VI. Regulatory Decision

Based on the review of data and information submitted by AgrEvo Canada Inc., and through thorough comparisons of HCN92 with unmodified *B. napus* counterparts, AAFC has concluded that neither the novel genes, nor their resulting gene products and associated novel traits, confer any intended or unintended ecological advantage to HCN92. Should these traits be transferred through outcrossing to related plants, these would not result in any ecological advantage.

Based on the review of submitted data and information, AAFC has concluded that the novel genes and their corresponding traits do not in themselves raise any concerns regarding the safety or nutritional composition of this line. Canola oil and meal are currently described in Schedule IV of the *Feeds Regulations* and are therefore approved for use in livestock feeds in Canada. As HCN92 has been assessed and found to be substantially equivalent, HCN92 and its by-products are considered to meet the present definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

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Study Title

Southern Analysis of Two Lines of Transformation Event T45
to Determine T-DNA Copy Number

Author

C. F. Bennett

Report Date

September 25, 1995

Report Number

Canadian reference: ACI95-22

International reference: A56371

Study Submitted By

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Southern Analysis of Two Lines of Transformation Event T45 to Determine T-DNA Copy Number

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ACI95-22
September 25, 1995

Abstract

Brassica napus c.v. A.C. Excel protoplasts were used via *Agrobacterium tumafaciens* mediated transformation to produce the herbicide tolerant canola transformation event T45. The T45 transformant contains the construct pHoe4/Ac. Southern analysis of two lines of the T45 event HCN27 and HCN28 contain one copy of the T-DNA from pHoe4/Ac.

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Southern Analysis of Two Lines of Transformation Event T45 to Determine T-DNA Copy Number

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Southern Analysis of Two Lines of Transformation Event T45 to Determine T-DNA Copy Number

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ACI95-22
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Introduction

Agrobacterium tumafaciens [1] was used to transform *Brassica napus* protoplasts isolated from the cultivar A.C. Excel along with the vector pHoe4/Ac (figure 1) were utilized for the transformation process.

A specific transformation event T45 was selected on phosphinothricin containing media. The canola line HCN27 and HCN28 originate from the transformation event T45. Southern analysis, using the *pat* gene (figure 2) as a probe, was used to determine the number of T-DNA copies found within the transgenic line HCN27 and HCN28.

Materials and Methods

Plant DNA was extracted from HCN27 and HCN 28 using the Dellaporta DNA Miniprep[2] method. Innovator and Excel were used as positive and negative controls, respectively. The DNA was then digested with the restriction endonucleases Bam HI, Eco RV, Hind III and Nco I, then separated by gel electrophoresis[3]. Capillary transfer of the DNA onto Gene Screen Plus[3] membrane followed, using the protocol described by Sambrook[4]. Southern hybridizations were performed with the 550bp *pat* gene as a probe[5].

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Results

Figure 3 shows the Southern analysis of HCN27 and HCN 28 using the *pat* gene as a probe. Positive and negative controls are included (see Fig. 3).

The table below depicts the size of band observed for each of the restriction enzymes used (bands are marked by * in Fig. 3).

Restriction Enzyme	Size of Bands (kb)
Bam HI	10
Eco RV	3.8
Hind III	2.6
Nco I	4.0

Discussion

Four restriction digests were used to determine T-DNA copy number in HCN27 and HCN 28 using the *pat* gene as a probe.

When digesting with the enzyme Bam HI, which restricts at two locations within the T-DNA , two bands are expected (Figure 2). One band would be 333 bp in size, and the other would be of an unknown size but predicted to be at least 500 bp or greater. A band of approximately 300 bp is not detected due to its small size and the condition chosen in the experiment.

The enzymes Eco RV, Hind III and Nco I all restrict at one location within the T-DNA (Figure 2). The southern analysis revealed only one band when each of these restriction enzymes was used. This indicates that one copy of the T-DNA has been incorporated into the Brassica genome in the transformation event T45. Digestion with BamH I resulted in a single band of approximately 10 Kb.

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Conclusion

Southern analysis of HCN28 shows that there has been one copy of T-DNA from vector pHoe4/Ac inserted into the *Brassica napus* genome.

Note

HCN92 was used as a positive control in southern blots due in part to historical reasons. HCN92 was the first line developed for commercialization by AgrEvo with the *pat* gene in it. The use of HCN92, which contains the plasmid pOCA/AC, as a positive control demonstrates that even if a different plasmid is used the same insert (same molecular weight) shows up on the southern blot as for the pHoe4/AC plasmid.

See Page 10 for a plasmid map of pOCA/AC, the vector in line HCN92; Page 11 for a description of line HCN92, derived from transformation event Topas 19/2; and, Pages 12-17 for AAFC Decision Document DD95-01 regarding line HCN92.

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Figure 1. Plasmid Map of pHoe4/Ac

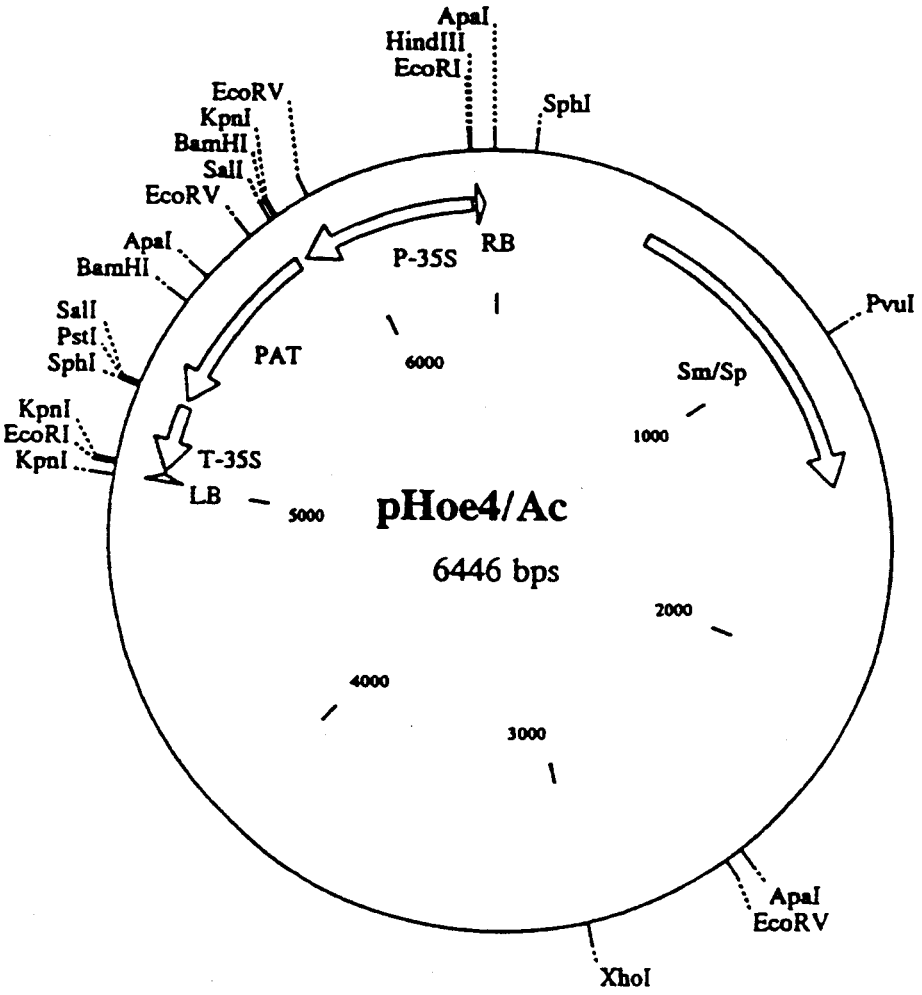

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Figure 2. Sequence of *pat* gene

```
  1  ATGTCTCCGG AGAGGAGACC AGTTGAGATT AGGCCAGCTA CAGCAGCTGA TATGGCCGCG
     TACAGAGGCC TCTCCTCTGG TCAACTCTAA TCCGGTCGAT GTCGTCGACT ATACCGGCGC

 61  GTTTGTGATA TCGTTAACCA TTACATTGAG ACGTCTACAG TGAACTTTAG GACAGAGCCA
     CAAACACTAT AGCAATTGGT AATGTAACTC TGCAGATGTC ACTTGAAATC CTGTCTCGGT

121  CAAACACCAC AAGAGTGGAT TGATGATCTA GAGAGGTTGC AAGATAGATA CCCTTGGTTG
     GTTTGTGGTG TTCTCACCTA ACTACTAGAT CTCTCCAACG TTCTATCTAT GGGAACCAAC

181  GTTGCTGAGG TTGAGGGTGT TGTGGCTGGT ATTGCTTACG CTGGGCCCTG GAAGGCTAGG
     CAACGACTCC AACTCCCACA ACACCGACCA TAACGAATGC GACCCGGGAC CTTCCGATCC

241  AACGCTTACG ATTGGACAGT TGAGAGTACT GTTTACGTGT CACATAGGCA TCAAAGGTTG
     TTGCGAATGC TAACCTGTCA ACTCTCATGA CAAATGCACA GTGTATCCGT AGTTTCCAAC

301  GGCCTAGGAT CCACATTGTA CACACATTTG CTTAAGTCTA TGGAGGCGCA AGGTTTTAAG
     CCGGATCCTA GGTGTAACAT GTGTGTAAAC GAATTCAGAT ACCTCCGCGT TCCAAAATTC

361  TCTGTGGTTG CTGTTATAGG CCTTCCAAAC GATCCATCTG TTAGGTTGCA TGAGGCTTTG
     AGACACCAAC GACAATATCC GGAAGGTTTG CTAGGTAGAC AATCCAACGT ACTCCGAAAC

421  GGATACACAG CCCGGGGTAC ATTGCGCGCA GCTGGATACA AGCATGGTGG ATGGCATGAT
     CCTATGTGTC GGGCCCCATG TAACGCGCGT CGACCTATGT TCGTACCACC TACCGTACTA

481  GTTGGTTTTT GGCAAAGGGA TTTTGAGTTG CCAGCTCCTC CAAGGCCAGT TAGGCCAGTT
     CAACCAAAAA CCGTTTCCCT AAAACTCAAC GGTCGAGGAG GTTCCGGTCA ATCCGGTCAA

541  ACCCAGATCT GA
     TGGGTCTAGA CT
```

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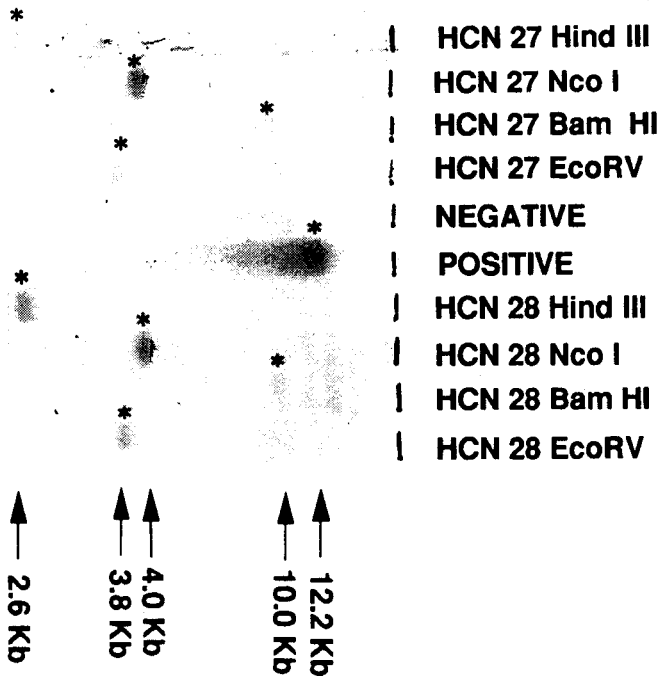
Figure 3: Southern Analysis of DNA either digested with one of the restriction enzymes Hind III, Nco I, Bam HI or EcoRV, respectively, or no restriction enzyme, prior to electrophoresis. The blot was hybridized with the *pat* coding region (see text). Hybridized bands are marked by *.



Vector pHoe4/AC
Probed with pat gene

Figure Legend:
HCN27 Hind III: DNA from canola line HCN27, derived from transformation event T45, digested with Hind III
HCN27 Nco I: DNA from canola line HCN27, derived from transformation event T45, digested with Nco I
HCN27 Bam HI: DNA from canola line HCN27, derived from transformation event T45, digested with Bam HI
HCN27 EcoRV: DNA from canola line HCN27, derived from transformation event T45, digested with EcoRV
Negative: unrestricted Negative Control DNA; nontransgenic canola (A.C. EXCEL)
Positive: unrestricted Positive Control DNA; HCN92 (INNOVATOR) derived from transformation event Topas 19/2
HCN28 Hind III: DNA from canola line HCN28, derived from transformation event T45, digested with Hind III
HCN28 Nco I: DNA from canola line HCN28, derived from transformation event T45, digested with Nco I
HCN28 Bam HI: DNA from canola line HCN28, derived from transformation event T45, digested with Bam HI
HCN28 EcoRV: DNA from canola line HCN28, derived from transformation event T45, digested with EcoRV

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Figure 4: Southern Analysis of DNA digested with one of the restriction enzymes EcoRI, Hind III, Nco I, Bam HI or EcoRV, respectively, prior to electrophoresis. The blot was hybridized with the *pat* coding region (see text).

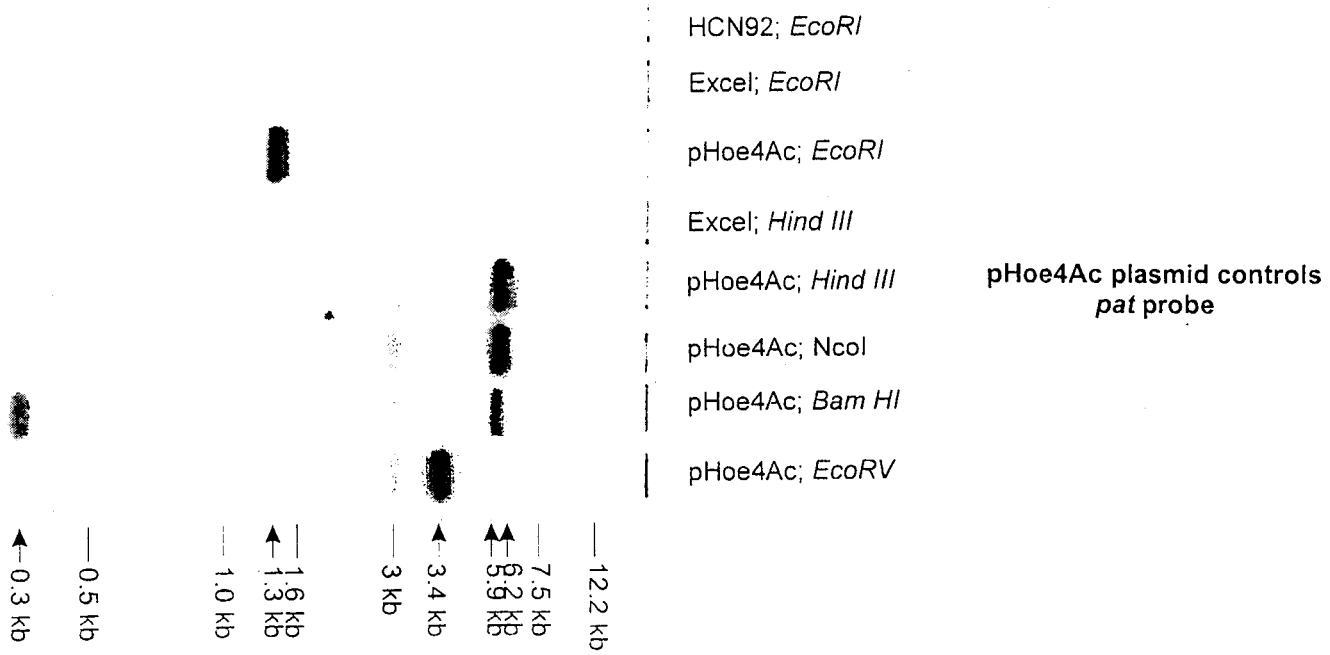
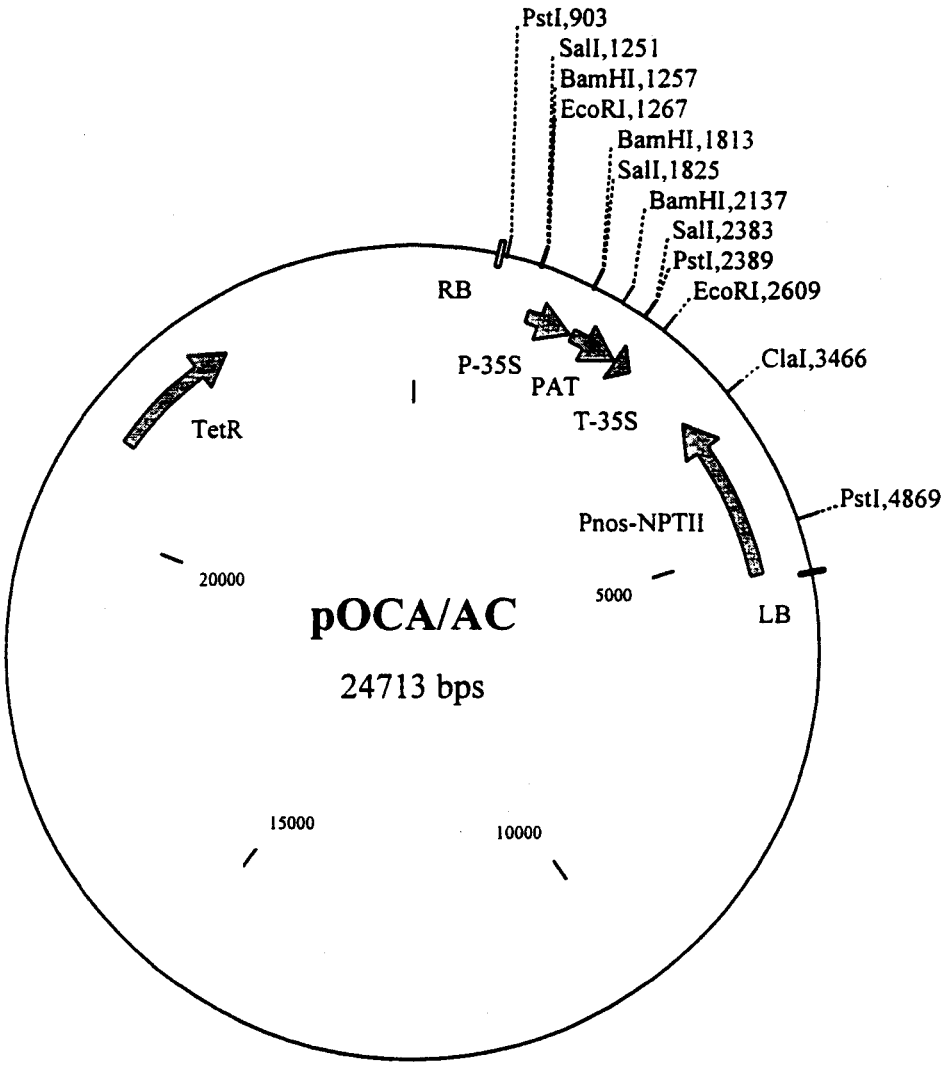


Figure Legend:
HCN92;EcoRI: Positive Control DNA, from transgenic HCN92 (INNOVATOR) canola, digested with EcoRI
EXCEL; EcoRI: nontransgenic canola (A.C. EXCEL) DNA digested with EcoRI
pHoe4Ac; EcoRI: transforming plasmid DNA digested with EcoRI
EXCEL; Hind III: nontransgenic canola (A.C. EXCEL) DNA digested with Hind III
pHoe4Ac; Hind III: transforming plasmid DNA digested with Hind III
EXCEL; Nco I: nontransgenic canola (A.C. EXCEL) DNA digested with Nco I
pHoe4Ac; Nco I: transforming plasmid DNA digested with Nco I
EXCEL; Bam HI: nontransgenic canola (A.C. EXCEL) DNA digested with Bam HI
pHoe4Ac; Bam HI: transforming plasmid DNA digested with Bam HI
EXCEL; EcoRV: nontransgenic canola (A.C. EXCEL) DNA digested with EcoRV
pHoe4Ac; EcoRV: transforming plasmid DNA digested with EcoRV

Figure 5: Plasmid Map of pOCA/AC



Glufosinate Tolerant Canola HCN92

Line Description

Cultivar Identification:	HCN92
Species name:	*Brassica napus* L.
Crop:	Canola
Transformation Method:	*B.napus* obtained through disarmed *Agrobacterium tumefaciens* mediated transformation
Vector :	pOCA/Ac
Trait 1:	tolerance to glufosinate ammonium
Gene 1:	phosphinothricin acetyltransferase (*pat*) gene
Donor 1:	*Streptomyces viridochromogenes*
Promoter 1/Donor:	35S gene promoter /Cauliflower Mosaic Virus (CaMV)
Terminator 1/Donor:	35S gene terminator /Cauliflower Mosaic Virus (CaMV)
Trait 2:	Tolerance to aminoglycosidic antibiotics
Gene 2:	Neomycin phosphotransferase II (NPT II)
Donor 2:	*Escherichia coli*
Promoter 2/Donor:	Nopaline synthase (*nos*)/*Agrobacterium tumefaciens*
Terminator 2/Donor :	Octopine synthase (*ocs*)/*Agrobacterium tumefaciens*

We have demonstrated that the incorporated DNA is limited to the T-DNA region. No additional coding sequences from the vector, other than the pat gene and the selectable marker, have been incorporated into the Brassica genome as part of the transformation process.

The original transformant Topas 19/2 was first crossed with the Agriculture Canada line ACSN-3. The R_1 was then crossed with the commercial line AC Excel. Initial crosses followed by several years of pedigree selection resulted in the production of the glufosinate tolerant line HCN92.

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

Plant Products Division
Food Production and Inspection Branch
Agriculture and Agri-Food Canada

Decision Document

DD95-01

Determination of Environmental Safety of Agrevo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits and its companion document Dir94-09 The Biology of *Brassica napus* L. (Canola/Rapeseed), and the proposed guidelines Pro94-04 Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed.

The Plant Biotechnology Office and the Feed Section of the Plant Products Division have evaluated information submitted by AgrEvo Canada Inc. regarding a glufosinate ammonium-tolerant and kanamycin-resistant canola line. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties and is considered substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

(publié aussi en français)

March 10, 1995

This bulletin is published by the Plant Products Division, Agriculture and Agri-Food Canada. For further information, please contact the Plant Biotechnology Office or the Feed Section at:

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I. Brief Identification of The Plant With Novel Traits (PNT)

Designation(s) of the PNT:	HCN92
Applicant:	AgrEvo Canada Inc.
Plant Species:	Canola (*Brassica napus* L.)
Novel Traits:	Glufosinate ammonium (herbicide) tolerance; kanamycin (antibiotic) resistance
Trait Introduction Method:	*Agrobacterium tumefaciens*-mediated transformation
Proposed Use of PNT:	Production of *B. napus* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo has developed a *Brassica napus* canola line tolerant to glufosinate ammonium, a broad spectrum non-residual herbicide. This *B. napus* line, referred to as HCN92 in the present document, will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides.

The development of HCN92 was based on recombinant DNA technology, by the introduction of two bacterial genes into a line of *B. napus*. A gene conferring tolerance to glufosinate ammonium was inserted, coding for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation. Another gene, conferring resistance to kanamycin, was also inserted; this gene is of no agronomic interest but was used to select modified plants from those that remained unmodified at the development stage.

HCN92 has been field tested in Canada under confined conditions in Saskatchewan (1990-94), Alberta (1991-94), Manitoba (1991-94), and Ontario (1993-94).

AgrEvo has provided data on the identity of HCN92, a detailed description of the modification method, data and information on the stability of the gene insertion, the role of the inserted genes in donor organisms and the role of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel proteins were identified and characterized, including their potential toxicity to livestock and non-target organisms, allergenicity, and levels of expression in the plant and feed. Numerous detailed scientific publications were also supplied.

Agronomic characteristics such as seed production, time to maturity, flowering period, and male and female fertility were compared to those of unmodified *B. napus* counterparts. Effects of HCN92 residues on growth and productivity of the following season's grain, forage, and pulse crops were assessed.

AgrEvo has also provided data on HNC92's survival adaptations: silique shattering potential, seed dormancy, seed dispersal mechanisms, vegetative vigor, reproductive characteristics, and the emergence in subsequent years of volunteer plants under mechanical or chemical fallow conditions. Stress adaptation was evaluated, including susceptibilities to various *B. napus* pests and pathogens, to abiotic stresses such as soil salinity and moisture regimes, and to herbicides other than glufosinate ammonium that are normally used on canola crops. Invasiveness studies were performed under disturbed, undisturbed, and agronomic conditions.

Data to support the efficacy of HCN92 as a livestock feed were provided. A proximate analysis to include crude protein, crude fat, crude fiber, ash and gross energy were supplied for the whole seed, processed meal and oil content.

Agriculture and Agri-Food Canada (AAFC) has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of the PNT to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

AAFC has also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Pro94-04:

- potential impact on livestock, and
- potential impact on livestock nutrition.

III. Description of the Novel Traits

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonium in susceptible plants within hours of application.

- The phosphinothricin tolerance gene engineered into HCN92 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- The gene is linked to a constitutive promoter, and protein expression was detected in roots, leaves, buds and seeds. However, it was not detected in stem tissue, protein extracts from the pollen, or unprocessed honey. Maximum expression was 0.001% of total plant protein.

- The expressed PAT enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post transcriptional modifications. Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed into feed ingredients.

- The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology the toxins or allergens entered in to GENEBANK DNA database.

2. Kanamycin Resistance:

- Kanamycin is an aminoglycosidic antibiotic that binds to bacterial ribosomes thus disrupting normal protein synthesis and killing the bacterial cell.

- The kanamycin-resistance gene codes for an enzyme that prevents kanamycin from binding to ribosomes, thereby rendering the cells resistant. The exact nature of the enzyme is considered Confidential Business Information by AgrEvo. The source of the gene was described, and the full nucleotide sequence was provided.

- The gene is linked to a weak constitutive promoter; expression was consistently stronger in root tissue, but was also observed in buds, leaves, and crude seed samples. The enzyme was not detected in unprocessed honey or pollen samples and was inactivated during processing of canola seed into feed ingredients.

- The expressed enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post-transcriptional modifications.

- The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENEBANK DNA database.

3. Development Method:

- *Brassica napus* cultivar Topas was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and replaced with genes coding for glufosinate ammonium tolerance and kanamycin resistance. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was shown to be the case in HCN92.

- The transformant was crossed with *B. napus* line ACSN3, then with AC Excel; HCN92 was derived from a bulk of single F_3 plants selected from the cross.

4. Stable Integration into the Plant's Genome:

- The provided data showed that there was no incorporation of any coding region from outside the T-DNA borders and that gene integration occurred at only one insertion site.

- HCN92 is several generations removed from the original transformant. Comparisons between the original transgenic plant and the HCN92 line show no difference in the presence and expression of both genes, nor in the insertion site.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to become a weed of agriculture or to be invasive of natural habitats

AAFC evaluated data submitted by AgrEvo on the reproductive and survival biology of HCN92, and determined that vegetative vigor, overwintering capacity, flowering period, time to maturity, seed production, and dormancy were within the normal range of expression of characteristics in unmodified *B. napus* counterparts. HCN92 has no specific added genes for cold tolerance or winter hibernation; no overwintered plants were observed by AgrEvo in post-harvest

years of field trials, and the number of volunteers in the year following a field trial were comparable between plots of HCN92 and counterpart *B. napus*. Seed morphology and average seed weight did not change, indicating that seed dispersal potential was not altered.

Based on the submitted data, AAFC has determined that HCN92 did not show any stress adaptation other than its resistance to glufosinate ammonium. Its resistance or susceptibility to major *B. napus* pests and pathogens (e.g., blackleg, sclerotinia, flea beetles) fall within the ranges currently displayed by commercial varieties. Moisture stress had a significant negative effect on both HCN92 and its counterparts.

The biology of *B. napus*, described in Dir94-09, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by AgrEvo, HCN92 was determined not to be different from its counterparts in this respect. Invasiveness was studied in disturbed and undisturbed habitats. Data showed that HCN92 was neither more invasive nor more persistent than commercial counterparts. No competitive advantage was conferred to glufosinate ammonium-tolerant plants, other than that conferred by tolerance to glufosinate ammonium.

Glufosinate ammonium is not used in normal crop rotation cycles, and resistance is therefore not an issue of concern in weed management control. Glufosinate-resistant *B. napus* volunteer plants can easily be managed by mechanical means and other available chemicals used to control *B. napus*.

The above considerations, together with the fact that the novel traits have no intended effect on weediness or invasiveness, led AAFC to conclude that HCN92 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

NOTE: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

Brassica napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa*, *B. juncea*, *B. carinata*, *B. nigra*, *Diplotaxis muralis*, *Raphanus raphanistrum*, and

Erucastrum gallicum (Dir 94-09). Studies show that introgression of the herbicide tolerance gene is most likely to occur with *B. rapa*, the other major canola species and an occasional weed of cultivated land especially in the eastern provinces of Canada.

If glufosinate ammonium-tolerant individuals arose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate ammonium. This would only occur in managed ecosystems where glufosinate ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate ammonium-tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would be easily controlled using mechanical and other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This, however, can be avoided by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from HCN92 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of both novel traits are unrelated to plant pest potential, and *Brassica napus* is not a plant pest in Canada (Dir94-09). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative composition of HCN92 were shown to be within the range of values displayed by currently commercialized *B. napus* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that HCN92 did not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

Data presenting the effect of plant residue from HCN92 on agronomic performance of succeeding crops were examined by AAFC for wheat, barley, lentils, peas, flax and alfalfa. No significant differences in either plant counts or grain yield between the HCN92 and counterpart canola plots were identified. This is an indirect indication that soil bacteria, involved in maintaining soil fertility, are not negatively affected by HCN92 plant residues.

PAT activity was not detected in pollen grains, neither was it detected in unprocessed honey collected from a bee colony which had foraged in the glufosinate-tolerant *B. napus* line. No negative impact on bees foraging in

HCN92 was observed, including brood development. Both enzymes are rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and
pH-mediated proteolysis. Neither of the two novel proteins contained potential glycosylation sites nor did they possess proteolytic or heat stability, indicating that neither protein is a likely allergen. A search of the GENEBANK DNA sequence database revealed no significant homology with the toxins or allergens entered in that database.

Based on the above, AAFC has determined that the unconfined release of HCN92 will not result in altered impacts on interacting organisms, including humans, compared with currently commercialized counterparts.

5. Potential Impact on Biodiversity

HCN92 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Since outcross species are only found in disturbed habitats, transfer of novel traits would not impact unmanaged environments. Studies have shown to AAFC that HCN92 is not invasive of natural habitats, and that it is no more competitive than its counterparts, both in natural and managed ecosystems.

AAFC has therefore concluded that the potential impact on biodiversity of HCN92 is equivalent to that of currently commercialized canola lines.

V. Assessment Criteria for Use as Livestock Feed

1. Anti-Nutritional Factors

Ninety-five percent confidence intervals were determined for glucosinolate and erucic acid content of the meal and oil produced from HCN92, grown under a variety of conditions. These confidence intervals demonstrated that the PNT contained levels of these anti-nutritional factors below the prescribed standards for both the meal and oil fractions, i.e., <30 micromoles glucosinolates per gram of dry meal and <2% erucic acid in the oil.

2. Nutritional Composition of PNT

No statistical differences in nutritional composition, i.e., crude protein, crude fat, crude fibre, ash and gross energy content, were noted between the whole seed, processed meal or oil of HCN92 and current commercial canola cultivars. These results collectively demonstrate that the introduction of this construct into *B. napus*, resulting in HCN92, did not likely result in any secondary effects impacting on the composition or nutritional quality of the cultivar. Accordingly, HCN92 was judged to be substantially equivalent to traditional canola varieties in terms of nutritional composition.

VI. Regulatory Decision

Based on the review of data and information submitted by AgrEvo Canada Inc., and through thorough comparisons of HCN92 with unmodified *B. napus* counterparts, AAFC has concluded that neither the novel genes, nor their resulting gene products and associated novel traits, confer any intended or unintended ecological advantage to HCN92. Should these traits be transferred through outcrossing to related plants, these would not result in any ecological advantage.

Based on the review of submitted data and information, AAFC has concluded that the novel genes and their corresponding traits do not in themselves raise any concerns regarding the safety or nutritional composition of this line. Canola oil and meal are currently described in Schedule IV of the *Feeds Regulations* and are therefore approved for use in livestock feeds in Canada. As HCN92 has been assessed and found to be substantially equivalent, HCN92 and its by-products are considered to meet the present definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

Title

Southern Analysis of Two Lines of Transformation Event T45 for the Presence of the Streptomycin/Spectinomycin Antibiotic Resistance Marker

Author

C. F. Bennett

Report No.

Canadian Reference ACI95-12

International Reference: A56368

Date

September 25, 1995

Study Submitted By

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Southern Analysis of two Breeding Lines of Transformation Event T45 for the Presence of the Streptomycin/Spectinomycin Antibiotic Resistance Marker

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ACI95-12
September 25, 1995

Abstract

Canola breeding lines HCN27 and HCN28 originate from the *Agrobacterium*-mediated transformation event T45. The T45 event was produced using the vector pHoe4/Ac.

Although *Agrobacterium* mediated transformation is widely employed, recent studies have suggested the possibility of vector born DNA located outside the T-DNA, being transferred and integrated into the plant genome during Agrobacterium-mediated transformation processes. Therefore it is necessary to determine if vector components have been incorporated into the *Brassica* genome.

This study demonstrates that the Streptomycin/Spectinomycin(Sm/Spc) antibiotic resistance marker gene representing pHoe4/Ac DNA outside the T-DNA borders is not present in the lines HCN27 or HCN28.

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Introduction

Protoplasts isolated from the cultivar *Brassica napus* cv *A. C. Excel* were used for transformation with the vector pHoe4/Ac (Figure 1). The transformation process was mediated by *Agrobacterium tumefaciens* (Ditta *et al*, 1980).

The transformation vector pHoe4/Ac contains an antibiotic resistance marker gene outside the borders of the T-DNA. This marker gene confers resistance in bacteria to the antibiotic *Streptomycin* and *Spectinomycin* (Sm/Spc).

In order to show that Sm/Spc gene has not been integrated into the HCN27 genome, Southern analysis was done using the Sm/Spc gene as a probe.

Objective

This study is designed to show that the Sm/Spc gene, located outside the right border of the T-DNA in the pHoe4/Ac vector, has not been incorporated into the HCN27 *Brassica* genome.

Materials and Methods

Plant DNA was extracted from several transgenic lines produced via *Agrobacterium* transformation with pHoe4/Ac, as well as an Excel negative control plant, using the Dellaporta DNA Miniprep method (Dellaporta *et al.*, 1983). *E. coli* plasmid pHP45Ω (figure 2), which contains the Sm/Spc antibiotic resistance marker gene, was used as a positive control (Prentki and Krisch, 1984).

Restriction digests, using the enzyme Hind III, was performed and the DNA separated by gel electrophoresis. Capillary transfer of the DNA onto Gene Screen Plus[4] followed, using the protocol previously described by Sambrook.

Southern hybridizations were done using the 2.1 Kb Sm/Spc gene, isolated from pHP45Ω, as a probe.

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Results

Figure 3 shows the Southern analysis of HCN27 and HCN28 including positive and negative controls. The positive undigested 4.3 Kb plasmid pHP45Ω showed the expected banding pattern whereas no bands were found in either the nontransformed plant DNA or any of the transformant lines.

Discussion

The results in Figure 3 indicate that there has been no integration on the Sm/Spc antibiotic resistance marker gene into the *Brassica napus* lines HCN27 or HCN28 genome.

Conclusion

Sequences of the pHoe4/Ac plasmid representing the Sm/Spc resistance gene have not been incorporated into the HCN27 or HCN28 *Brassica* genome. This provides evidence that border integrity has been maintained during the transformation process.

Note

HCN92 was used as a positive control in southern blots due in part to historical reasons. HCN92 was the first line developed for commercialization by AgrEvo with the *pat* gene in it. The use of HCN92, which contains the plasmid pOCA/AC, as a positive control demonstrates that even if a different plasmid is used the same insert (same molecular weight) shows up on the southern blot as for the pHoe4/AC plasmid.

See Page 10 for a plasmid map of pOCA/AC, the vector in line HCN92; Page 11 for a description of line HCN92, derived from transformation event Topas 19/2; and, Pages 12-17 for AAFC Decision Document DD95-01 regarding line HCN92.

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Southern Analysis of two Breeding Lines of Transformation Event T45 for the Presence of the Streptomycin/Spectinomycin Antibiotic Resistance Marker

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References:

Ditta, G.; Stanfield, S.; Corbin, D.; Helinski, D.; 1980. Broad Host Range DNA cloning system for gram negative bacteria: construction of a gene bank of *Rhizobium meliloti.* Proc. Natl. Acad. Sci. 77:7347

Dellaporta, S.; Wood,J.;Hicks,J. 1983 Plant Molecular Biology Report. Volume 1, p.19

Prentki, P.; Krisch, H.M., 1984 In vitro insertional mutagenesis with a selectable DNA fragment. Gene. 29:303-313

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Figure 1. Plasmid Map of pHoe4/Ac

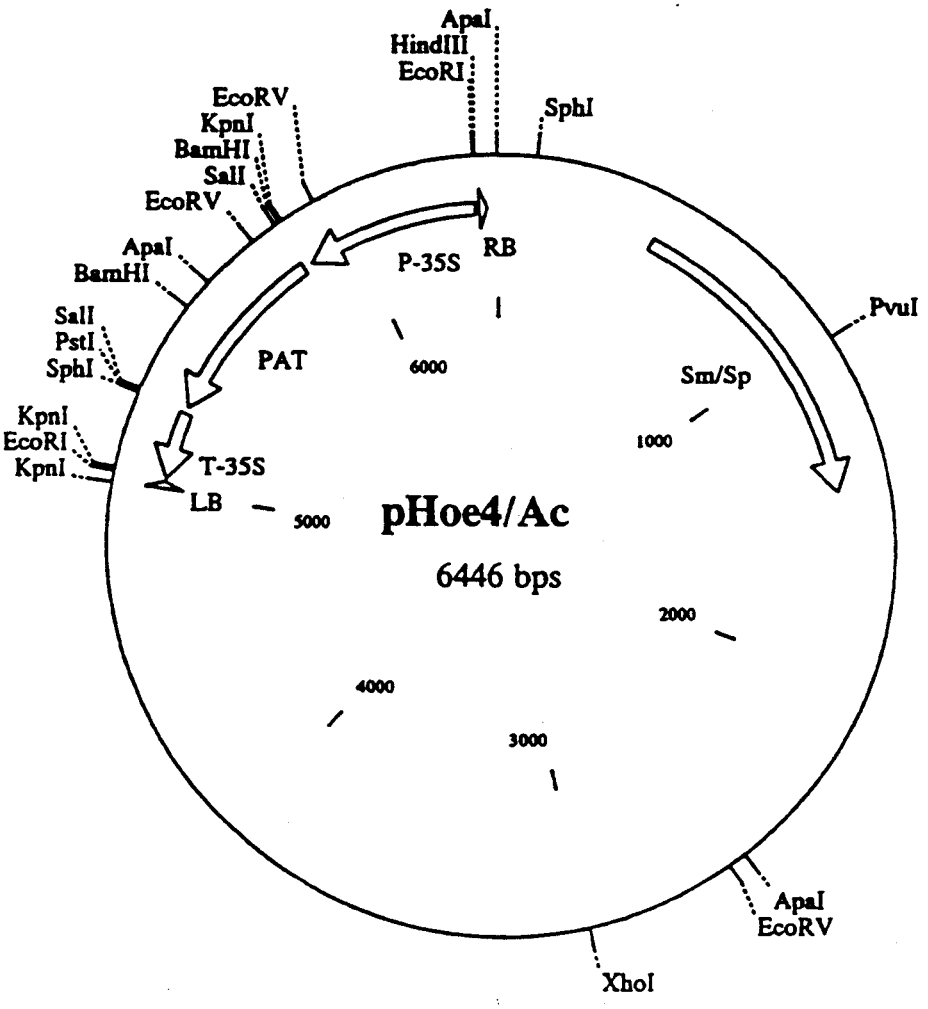

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Figure 2. Plasmid Map of pHP45W

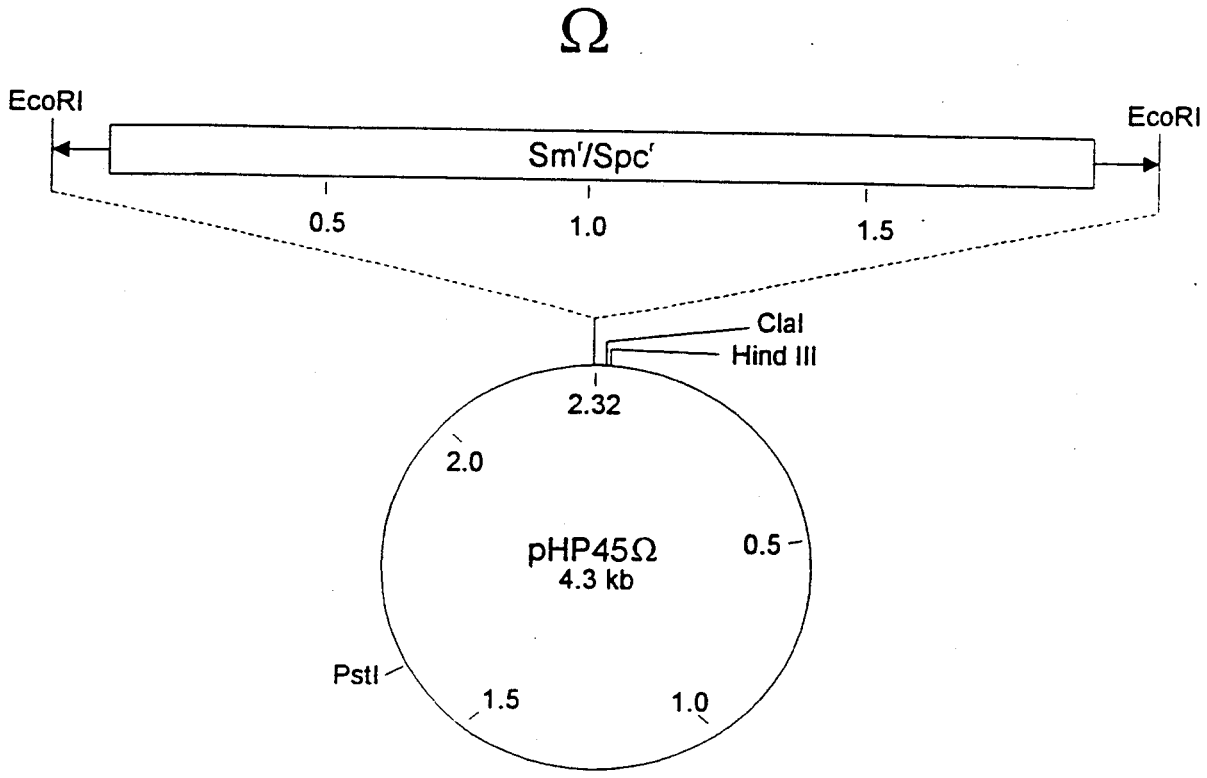

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Figure 3: Southern Analysis of DNA either digested with the restriction enzyme Hind III, or undigested (positive control), prior to electrophoresis. The blot was hybridized with the 2.1 kb *Streptomycin/Spectinomycin* resistance gene, isolated from pHP45Ω (see text).

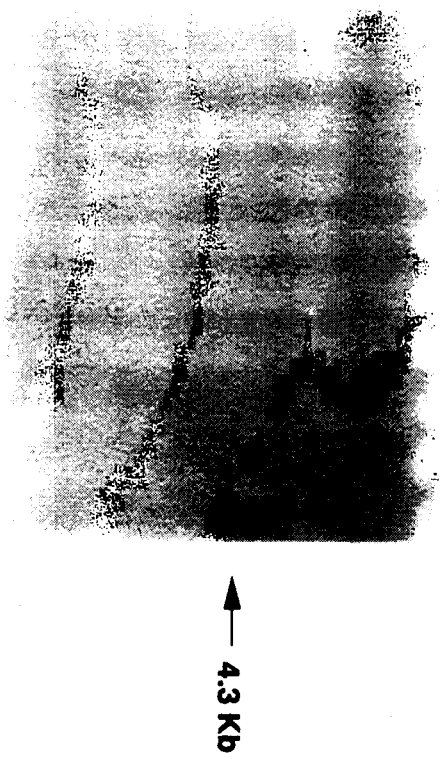
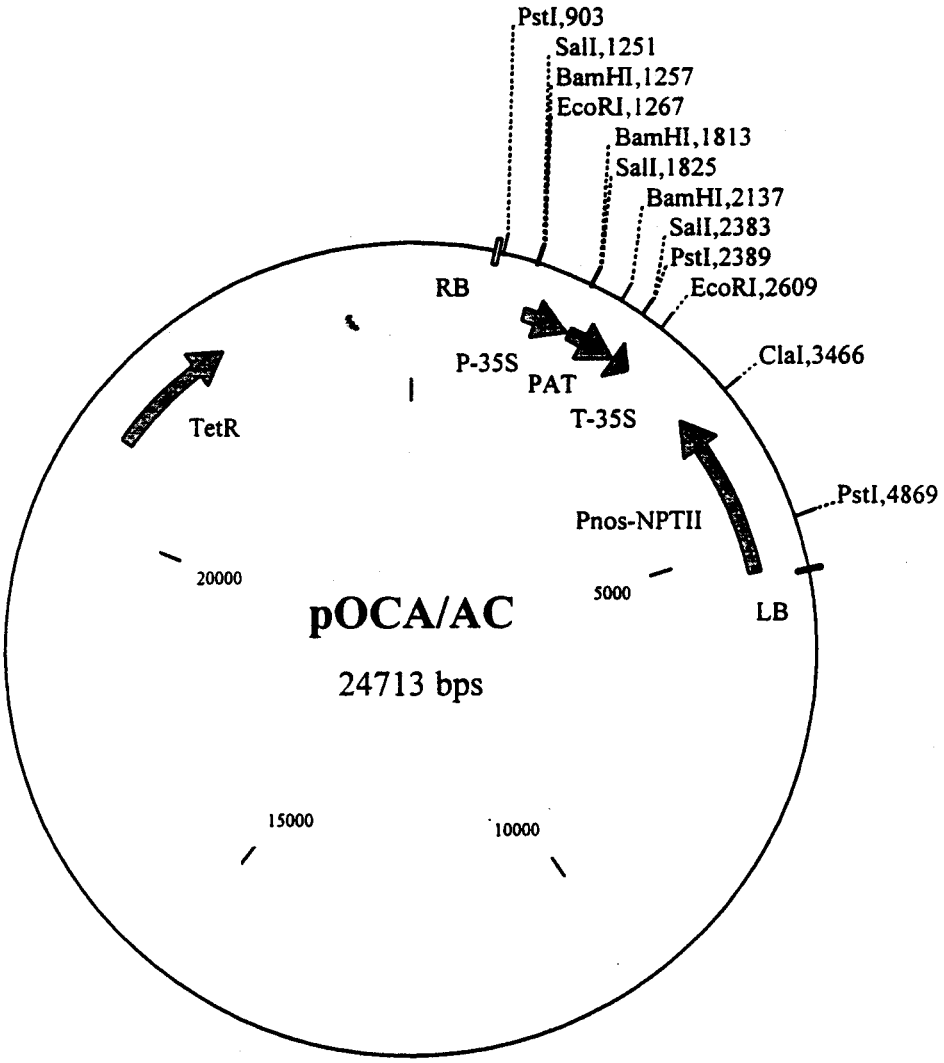


Figure Legend:

HCN27: DNA from canola line HCN27, derived from tranformation event T45
HCN28: DNA from canola line HCN28, derived from tranformation event T45
T74: DNA from a tranformation event not considered in this report
T177: DNA from a tranformation event not considered in this report
HCR1: DNA from a tranformation event not considered in this report
Negative Control: DNA from nontransgenic canola (A.C. EXCEL)
HCN92: Positive Control DNA, from transgenic HCN92 (INNOVATOR) canola
Positive Control: The plasmid pHP45Ω

Figure 5: Plasmid Map of pOCA/AC



Glufosinate Tolerant Canola HCN92

Line Description

Cultivar Identification:	HCN92
Species name:	*Brassica napus* L.
Crop:	Canola
Transformation Method:	*B.napus* obtained through disarmed *Agrobacterium tumefaciens* mediated transformation
Vector :	pOCA/Ac
Trait 1:	tolerance to glufosinate ammonium
Gene 1:	phosphinothricin acetyltransferase (*pat*) gene
Donor 1:	*Streptomyces viridochromogenes*
Promoter 1/Donor:	35S gene promoter /Cauliflower Mosaic Virus (CaMV)
Terminator 1/Donor:	35S gene terminator /Cauliflower Mosaic Virus (CaMV)
Trait 2:	Tolerance to aminoglycosidic antibiotics
Gene 2:	Neomycin phosphotransferase II (NPT II)
Donor 2:	*Escherichia coli*
Promoter 2/Donor:	Nopaline synthase (*nos*)/*Agrobacterium tumefaciens*
Terminator 2/Donor :	Octopine synthase (*ocs*)/*Agrobacterium tumefaciens*

We have demonstrated that the incorporated DNA is limited to the T-DNA region. No additional coding sequences from the vector, other than the pat gene and the selectable marker, have been incorporated into the Brassica genome as part of the transformation process.

The original transformant Topas 19/2 was first crossed with the Agriculture Canada line ACSN-3. The R_1 was then crossed with the commercial line AC Excel. Initial crosses followed by several years of pedigree selection resulted in the production of the glufosinate tolerant line HCN92.

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

Plant Products Division
Food Production and Inspection Branch
Agriculture and Agri-Food Canada

Decision Document

DD95-01

Determination of Environmental Safety of Agrevo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits and its companion document Dir94-09 The Biology of *Brassica napus* L. (Canola/Rapeseed), and the proposed guidelines Pro94-04 Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed.

The Plant Biotechnology Office and the Feed Section of the Plant Products Division have evaluated information submitted by AgrEvo Canada Inc. regarding a glufosinate ammonium-tolerant and kanamycin-resistant canola line. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties and is considered substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

(publié aussi en français)

March 10, 1995

This bulletin is published by the Plant Products Division, Agriculture and Agri-Food Canada. For further information, please contact the Plant Biotechnology Office or the Feed Section at:

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AgrEvo has provided data on the identity of HCN92, a detailed description of the modification method, data and information on the stability of the gene insertion, the role of the inserted genes in donor organisms and the role of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel proteins were identified and characterized, including their potential toxicity to livestock and non-target organisms, allergenicity, and levels of expression in the plant and feed. Numerous detailed scientific publications were also supplied.

Agronomic characteristics such as seed production, time to maturity, flowering period, and male and female fertility were compared to those of unmodified *B. napus* counterparts. Effects of HCN92 residues on growth and productivity of the following season's grain, forage, and pulse crops were assessed.

AgrEvo has also provided data on HNC92's survival adaptations: silique shattering potential, seed dormancy, seed dispersal mechanisms, vegetative vigor, reproductive characteristics, and the emergence in subsequent years of volunteer plants under mechanical or chemical fallow conditions. Stress adaptation was evaluated, including susceptibilities to various *B. napus* pests and pathogens, to abiotic stresses such as soil salinity and moisture regimes, and to herbicides other than glufosinate ammonium that are normally used on canola crops. Invasiveness studies were performed under disturbed, undisturbed, and agronomic conditions.

Data to support the efficacy of HCN92 as a livestock feed were provided. A proximate analysis to include crude protein, crude fat, crude fiber, ash and gross energy were supplied for the whole seed, processed meal and oil content.

Agriculture and Agri-Food Canada (AAFC) has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of the PNT to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

AAFC has also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Pro94-04:

- potential impact on livestock, and
- potential impact on livestock nutrition.

III. Description of the Novel Traits

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonium in susceptible plants within hours of application.

- The phosphinothricin tolerance gene engineered into HCN92 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- The gene is linked to a constitutive promoter, and protein expression was detected in roots, leaves, buds and seeds. However, it was not detected in stem tissue, protein extracts from the pollen, or unprocessed honey. Maximum expression was 0.001% of total plant protein.

- The expressed PAT enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post transcriptional modifications. Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed into feed ingredients.

- The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology the toxins or allergens entered in to GENEBANK DNA database.

2. Kanamycin Resistance:

- Kanamycin is an aminoglycosidic antibiotic that binds to bacterial ribosomes thus disrupting normal protein synthesis and killing the bacterial cell.

- The kanamycin-resistance gene codes for an enzyme that prevents kanamycin from binding to ribosomes, thereby rendering the cells resistant. The exact nature of the enzyme is considered Confidential Business Information by AgrEvo. The source of the gene was described, and the full nucleotide sequence was provided.

- The gene is linked to a weak constitutive promoter; expression was consistently stronger in root tissue, but was also observed in buds, leaves, and crude seed samples. The enzyme was not detected in unprocessed honey or pollen samples and was inactivated during processing of canola seed into feed ingredients.

- The expressed enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post-transcriptional modifications.

- The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENEBANK DNA database.

3. Development Method:

- *Brassica napus* cultivar Topas was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and replaced with genes coding for glufosinate ammonium tolerance and kanamycin resistance. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was shown to be the case in HCN92.

- The transformant was crossed with *B. napus* line ACSN3, then with AC Excel; HCN92 was derived from a bulk of single F_3 plants selected from the cross.

4. Stable Integration into the Plant's Genome:

- The provided data showed that there was no incorporation of any coding region from outside the T-DNA borders and that gene integration occurred at only one insertion site.

- HCN92 is several generations removed from the original transformant. Comparisons between the original transgenic plant and the HCN92 line show no difference in the presence and expression of both genes, nor in the insertion site.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to become a weed of agriculture or to be invasive of natural habitats

AAFC evaluated data submitted by AgrEvo on the reproductive and survival biology of HCN92, and determined that vegetative vigor, overwintering capacity, flowering period, time to maturity, seed production, and dormancy were within the normal range of expression of characteristics in unmodified *B. napus* counterparts. HCN92 has no specific added genes for cold tolerance or winter hibernation; no overwintered plants were observed by AgrEvo in post-harvest

years of field trials, and the number of volunteers in the year following a field trial were comparable between plots of HCN92 and counterpart *B. napus*. Seed morphology and average seed weight did not change, indicating that seed dispersal potential was not altered.

Based on the submitted data, AAFC has determined that HCN92 did not show any stress adaptation other than its resistance to glufosinate ammonium. Its resistance or susceptibility to major *B. napus* pests and pathogens (e.g., blackleg, sclerotinia, flea beetles) fall within the ranges currently displayed by commercial varieties. Moisture stress had a significant negative effect on both HCN92 and its counterparts.

The biology of *B. napus*, described in Dir94-09, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by AgrEvo, HCN92 was determined not to be different from its counterparts in this respect. Invasiveness was studied in disturbed and undisturbed habitats. Data showed that HCN92 was neither more invasive nor more persistent than commercial counterparts. No competitive advantage was conferred to glufosinate ammonium-tolerant plants, other than that conferred by tolerance to glufosinate ammonium.

Glufosinate ammonium is not used in normal crop rotation cycles, and resistance is therefore not an issue of concern in weed management control. Glufosinate-resistant *B. napus* volunteer plants can easily be managed by mechanical means and other available chemicals used to control *B. napus*.

The above considerations, together with the fact that the novel traits have no intended effect on weediness or invasiveness, led AAFC to conclude that HCN92 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

NOTE: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

Brassica napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa*, *B. juncea*, *B. carinata*, *B. nigra*, *Diplotaxis muralis*, *Raphanus raphanistrum*, and

Erucastrum gallicum (Dir 94-09). Studies show that introgression of the herbicide tolerance gene is most likely to occur with *B. rapa*, the other major canola species and an occasional weed of cultivated land especially in the eastern provinces of Canada.

If glufosinate ammonium-tolerant individuals arose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate ammonium. This would only occur in managed ecosystems where glufosinate ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate ammonium-tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would be easily controlled using mechanical and other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This, however, can be avoided by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from HCN92 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of both novel traits are unrelated to plant pest potential, and *Brassica napus* is not a plant pest in Canada (Dir94-09). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative composition of HCN92 were shown to be within the range of values displayed by currently commercialized *B. napus* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that HCN92 did not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

Data presenting the effect of plant residue from HCN92 on agronomic performance of succeeding crops were examined by AAFC for wheat, barley, lentils, peas, flax and alfalfa. No significant differences in either plant counts or grain yield between the HCN92 and counterpart canola plots were identified. This is an indirect indication that soil bacteria, involved in maintaining soil fertility, are not negatively affected by HCN92 plant residues.

PAT activity was not detected in pollen grains, neither was it detected in unprocessed honey collected from a bee colony which had foraged in the glufosinate-tolerant *B. napus* line. No negative impact on bees foraging in

HCN92 was observed, including brood development. Both enzymes are rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and
pH-mediated proteolysis. Neither of the two novel proteins contained potential glycosylation sites nor did they possess proteolytic or heat stability, indicating that neither protein is a likely allergen. A search of the GENEBANK DNA sequence database revealed no significant homology with the toxins or allergens entered in that database.

Based on the above, AAFC has determined that the unconfined release of HCN92 will not result in altered impacts on interacting organisms, including humans, compared with currently commercialized counterparts.

5. Potential Impact on Biodiversity

HCN92 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Since outcross species are only found in disturbed habitats, transfer of novel traits would not impact unmanaged environments. Studies have shown to AAFC that HCN92 is not invasive of natural habitats, and that it is no more competitive than its counterparts, both in natural and managed ecosystems.

AAFC has therefore concluded that the potential impact on biodiversity of HCN92 is equivalent to that of currently commercialized canola lines.

V. Assessment Criteria for Use as Livestock Feed

1. Anti-Nutritional Factors

Ninety-five percent confidence intervals were determined for glucosinolate and erucic acid content of the meal and oil produced from HCN92, grown under a variety of conditions. These confidence intervals demonstrated that the PNT contained levels of these anti-nutritional factors below the prescribed standards for both the meal and oil fractions, i.e., <30 micromoles glucosinolates per gram of dry meal and <2% erucic acid in the oil.

2. Nutritional Composition of PNT

No statistical differences in nutritional composition, i.e., crude protein, crude fat, crude fibre, ash and gross energy content, were noted between the whole seed, processed meal or oil of HCN92 and current commercial canola cultivars. These results collectively demonstrate that the introduction of this construct into *B. napus*, resulting in HCN92, did not likely result in any secondary effects impacting on the composition or nutritional quality of the cultivar. Accordingly, HCN92 was judged to be substantially equivalent to traditional canola varieties in terms of nutritional composition.

VI. Regulatory Decision

Based on the review of data and information submitted by AgrEvo Canada Inc., and through thorough comparisons of HCN92 with unmodified *B. napus* counterparts, AAFC has concluded that neither the novel genes, nor their resulting gene products and associated novel traits, confer any intended or unintended ecological advantage to HCN92. Should these traits be transferred through outcrossing to related plants, these would not result in any ecological advantage.

Based on the review of submitted data and information, AAFC has concluded that the novel genes and their corresponding traits do not in themselves raise any concerns regarding the safety or nutritional composition of this line. Canola oil and meal are currently described in Schedule IV of the *Feeds Regulations* and are therefore approved for use in livestock feeds in Canada. As HCN92 has been assessed and found to be substantially equivalent, HCN92 and its by-products are considered to meet the present definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

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Title

Invasive Potential of Transgenic *B. napus* (pHoe4) Under Disturbed and Undisturbed Field Conditions

Authors

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Report No.

Canadian Reference: ACI95-16

International Reference: A56369

Date

October, 1995

Study Submitted By

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I. INTRODUCTION

One of the identified environmental risks associated with transgenic crops is that the crop itself will become a weed (Rissler and Mellon, 1993). A weed is broadly defined as an unwanted plant which is objectionable or interferes with the activities or welfare of humans. While no plant can be said to be a weed, some characteristics are often associated with weediness. Some plants may possess those phenotypic characteristics which enable them to quickly adapt to a different or new habitat. This may result in a competitive advantage over desirable plants. A net replacement (invasiveness) potential compares the ecological performance of a population of plants to produce viable, fertile off-spring. Depending on the habitat, the net replacement of a particular phenotype can either increase or decrease over time.

II. OBJECTIVE

The objective of this study was to compare the invasiveness potential of transgenic *B. napus* variety HCN28 (pHoe4) with HCN92 (INNOVATOR) and three non-transgenic canola varieties in disturbed and undisturbed soils in two agricultural locations in western Canada.

**Invasive Potential of Transgenic *B. napus* (pHoe4)
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III. MATERIALS AND METHODS

In 1995, field experiments were established at Indian Head, SK. and Rosthern, SK. All fields were previously cropped in 1994. The field design consisted of 10 plots replicated 4 times in a randomized complete block design. Each plot was 1.5 m by 1.5 m with a minimum of 2 m buffer between all plots and replicated blocks. At both locations a 5 m buffer and a 10 m confinement border of non-transgenic canola surrounded the entire experiment.

One gram of seed for HCN28 (pHoe4), INNOVATOR (HCN92), EXCEL, LEGEND and CYCLONE canola varieties were individually packaged and hand scattered separately over the disturbed and undisturbed plots. Disturbed plots were prepared by roto-tilling the soil to a depth of 10 cm. Undisturbed plots received no cultivation prior to seeding. The selected transgenic and non-transgenic canola seeds were of high quality and grade to ensure good germination. All seeds were pre-treated with the fungicide Vitovax®. Seeding took place on May 10 and May 30, 1995 at Rosthern and Indian Head, respectively. No additional fertilizer or weed control applications were made to any variety.

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At Indian Head, only an early plant count was assessed during the growing season. Plant counts were measured twice (early and harvest) at Rosthern. Total plant counts were assessed on an entire plot basis. Seed yield from each plot was determined at both sites by selecting and hand thrashing those canola plants which produced seed pods. Seed weight was converted to seed number based on each varieties' 1000 seed weight. The 1000 seed weights for HCN28, INNOVATOR, EXCEL, LEGEND and CYCLONE were 3.2, 3.4, 3.3, 3.5 and 3.8, respectively (per comm. V. Ripley, AgrEvo Canada Inc.).

A net replacement rate was calculated for each canola variety grown on disturbed and undisturbed soil at Indian Head. The calculated rates were based on the following equation (Rissler and Mellon, 1993):

Net Replacement Rate = number of seeds collected ÷ number of seeds sown

All data was statistically analyzed by location using a multi-factorial analysis of variance with STATISTICA/W® software. Significant mean separation at a 5% level was determined by a Duncan's Multiple Range Test.

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IV. RESULTS

All raw data and statistical tables are summarized in Appendix I. A summary of mean counts, seed yield and net replacement for Indian Head and Rosthern are presented in Tables 1 and 2, respectively.

IV.I Indian Head, Saskatchewan

Statistical analysis of the early season plant counts at Indian Head indicated a significant difference between varieties and between disurbed and undisturbed plots; there was no interaction between variety and seedbed ($p<0.05$). Plots seeded with HCN28 the fewest number of plants emerge of all the canola varieties tested; however, HCN28 counts were not significantly different from LEGEND and CYCLONE on undisturbed plots (Table 1). With the exception of HCN28, there were no significant differences in the initial plant counts among INNOVATOR, LEGEND, CYCLONE and EXCEL when seeded onto a the disturbed

soil surface. Among all varieties tested, EXCEL had the highest counts on both disturbed and undisturbed plots.

Statistical analysis of the seed number indicated significant differences among canola varieties and their interaction with seedbed; seedbed alone did not result in significant differences in seed number ($p<0.05$). When seeded onto an undisturbed seedbed the net replacement value for HCN28 was 16.9. However, this value was not significantly different than CYCLONE and INNOVATOR varieties whose net replacement values were 10.9 and 5.8, respectively. EXCEL and LEGEND varieties produced the highest seed numbers on the undisturbed seedbed with net replacement values of 164.5 and 77.1, respectively. With the exception of HCN28, the net replacement values of all canola varieties tested on disturbed soil increased substantially compared with undisturbed soil; the net replacement values ranged from 45.7 to 137.4. HCN28 had a net replacement value of 9.8. In a similar study conducted in 1994 at Indian Head, the glufosinate tolerant canola variety INNOVATOR (HCN92) had net replacement values of 1.80 and 10.35 on undisturbed and disturbed plots, respectively (Belyk and MacDonald, 1994).

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IV.II Rosthern, Saskatchewan

Statistical analysis of early season counts indicated significantly differences among the canola varieties tested; there was no difference between disturbed and undisturbed plots and no interaction effect ($p<0.05$). Among all varieties tested, HCN28 had the fewest plants emerge in both the disturbed and undisturbed plots; however, HCN28 counts were not significantly different among all canola varieties tested on undisturbed plots. With the exception of LEGEND and CYCLONE, late season plant counts decreased substantially when compared to the early season counts in both disturbed and undisturbed plots. No HCN28 plants were observed in either the disturbed or undisturbed plots. The reduction in plant counts for HCN28, INNOVATOR and EXCEL was caused by heavy weed pressure which out-compete these canola plants for water, nutrients and sunlight.

As a result of the weed competition, seed yield was severely effected for all canola varieties seeded on both disturbed and undisturbed plots. LEGEND and EXCEL varieties did not result in higher seed numbers even with the higher plants per plot. Over, there were no significant differences in seed yield among all canola varieties regardless of the seedbed.

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The net replacement values ranged from 0-5.8 for all canola varieties tested on disturbed and undisturbed soil surfaces.

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Table 1. Mean plant counts, seed number and net replacement of various canola varieties grown on disturbed and undisturbed soil, Indian Head, SK, 1995.

Location	Seed bed[§]	Variety	Early Counts	Seed No.	Net Replace
Indian Head,SK	U	LEGEND	34.3 ab	22036 b	77.1 b
Indian Head,SK	U	HCN28 (pHoe4)	27.5 a	5281 a	16.9 a
Indian Head,SK	U	CYCLONE	26.8 a	2875 a	10.9 a
Indian Head,SK	U	INNOVATOR	67.8 bc	1699 a	5.8 a
Indian Head,SK	U	EXCEL	81.8 c	49856 c	164.5 c
Indian Head,SK	D	LEGEND	70.0 bc	39257 bc	137.4 bc
Indian Head,SK	D	HCN28 (pHoe4)	25.5 a	3063 a	9.8 a
Indian Head,SK	D	CYCLONE	93.5 c	30737 b	116.8 b
Indian Head,SK	D	INNOVATOR	78.5 c	28316 b	96.3 b
Indian Head,SK	D	EXCEL	104.8 c	13841 ab	45.7 ab

mean values followed by the same letter are not significantly different at a 5% level (Duncan's Multiple Range Test).

§ U indicates undisturbed; D indicates disturbed plots.

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Table 2. Mean plant counts, seed number and net replacement of various canola varieties grown on disturbed and undisturbed soil, Rosthern, SK, 1995.

Location	Seed bed[§]	Variety	Early Counts	Late Counts	Seed No.	Net Replace
Rosthern,SK	U	LEGEND	26.3 ab	1.3 a	300 a	1.1 a
Rosthern,SK	U	HCN28 (pHoe4)	17.5 ab	0 a	0 a	0 a
Rosthern,SK	U	CYCLONE	36.8 b	10.3 a	1119 a	4.3 a
Rosthern,SK	U	INNOVATOR	20.8 ab	2.0 a	1051 a	3.6 a
Rosthern,SK	U	EXCEL	34.8 ab	7.0 a	674 a	2.2 a
Rosthern,SK	D	LEGEND	30.0 ab	30.0 b	1400 a	4.9 a
Rosthern,SK	D	HCN28 (pHoe4)	8.3 a	0 a	0 a	0 a
Rosthern,SK	D	CYCLONE	65.0 c	61.0 c	572 a	2.2 a
Rosthern,SK	D	HCN92	36.3 b	7.3 a	853 a	2.9 a
Rosthern,SK	D	EXCEL	37.3 bc	3.5 a	1743 a	5.8 a

mean values followed by the same letter are not significantly different at a 5% level (Duncan's Multiple Range Test).

§ U indicates undisturbed; D indicates disturbed plots.

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V. DISCUSSION

HCN28 (pHoe4) had the lowest net replacement values (0-16.9) among all canola varieties tested at Indian Head and Rosthern, Saskatchewan. Ideally, commercially grown canola will yield a 300 fold increase in seed.

As a result of strong weed competition at Rosthern, both the transgenic canola plants and their non-transformed counterparts could not establish adequately to yield seed. The low net replacement values for all canola varieties grown at both Indian Head and Rosthern indicates a very poor invasiveness potential. Generally, canola is not identified as being invasive in natural habitats.

Regardless of variety, only a small fraction of the ~300 canola seeds (1 g) spread over both disturbed and undisturbed plots matured to produce seed. The early plant counts of the transgenic and non-transgenic canola varieties for both undisturbed and disturbed habitats was 8-35% and 4-21% at the Indian Head and Rosthern locations, respectively.

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At Rosthern, all canola varieties competed poorly for nutrients, water and sunlight against grassy and broadleaf weeds commonly found in the area.

Overall, the net replacement value calculated for HCN28 was lowest compared with the values calculated for the commercial varieties INNOVATOR, CYCLONE, EXCEL and LEGEND when grown on disturbed soil at Indian Head. There was not substantial difference in net replacement among all canola varieties when seeded onto disturbed and undisturbed seedbeds at Rosthern.

VI. CONCLUSION

The ecological performance of the glufosinate tolerant canola variety HCN28 (pHoe4) was not affected by the insertion of the phosphinothricin acetyl transferase (PAT) gene. Based on these findings, there was no evidence that HCN28 was more invasive or persistent in disturbed or undisturbed habitats compared with commercial canola varieties over one growing season. The net replacement potential of HCN928 was lowest among the canola varieties tested at Indian Head. There were no substantially differences among all canola varieties tested at Rosthern.

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VIII. APPENDIX I

Table 1. Raw data for Indian Head and Rosthern, SK. 1995.

Table 2. Statisitical Summary of All Effects. 1995.

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Table 1. Raw data for Indian Head and Rosthern, SK. 1995.

Location	Date	Variety	Seed bed*	Count	Harvest Date	Seed Wt g	1000 wt g	Seed No.
Indian Head, SK	07-Jul-95	Cyclone	U	31	10-Oct-95	0	3.8	0
Indian Head, SK	07-Jul-95	Cyclone	D	97	10-Oct-95	99.6	3.8	26211
Indian Head, SK	07-Jul-95	Cyclone	U	13	10-Oct-95	0	3.8	0
Indian Head, SK	07-Jul-95	Cyclone	D	124	10-Oct-95	131.7	3.8	34658
Indian Head, SK	07-Jul-95	Cyclone	D	92	10-Oct-95	161.1	3.8	42395
Indian Head, SK	07-Jul-95	Cyclone	U	45	10-Oct-95	43.7	3.8	11500
Indian Head, SK	07-Jul-95	Cyclone	D	61	10-Oct-95	74.8	3.8	19684
Indian Head, SK	07-Jul-95	Cyclone	U	18	10-Oct-95	0	3.8	0
Indian Head, SK	07-Jul-95	Excel	U	57	10-Oct-95	185.2	3.3	56121
Indian Head, SK	07-Jul-95	Excel	D	93	10-Oct-95	10.7	3.3	3242
Indian Head, SK	07-Jul-95	Excel	U	146	10-Oct-95	285.2	3.3	86424
Indian Head, SK	07-Jul-95	Excel	D	134	10-Oct-95	43.9	3.3	13303
Indian Head, SK	07-Jul-95	Excel	U	55	10-Oct-95	231.3	3.3	70091
Indian Head, SK	07-Jul-95	Excel	D	115	10-Oct-95	102.4	3.3	31030
Indian Head, SK	07-Jul-95	Excel	U	69	10-Oct-95	56.4	3.3	17091
Indian Head, SK	07-Jul-95	Excel	D	77	10-Oct-95	25.7	3.3	7788
Indian Head, SK	07-Jul-95	HCN92	U	62	10-Oct-95	16.6	3.4	4882
Indian Head, SK	07-Jul-95	HCN92	D	64	10-Oct-95	55.7	3.4	16382
Indian Head, SK	07-Jul-95	HCN92	U	74	10-Oct-95	6.5	3.4	1912
Indian Head, SK	07-Jul-95	HCN92	D	75	10-Oct-95	25.4	3.4	7471
Indian Head, SK	07-Jul-95	HCN92	D	63	10-Oct-95	115.3	3.4	33912
Indian Head, SK	07-Jul-95	HCN92	U	117	10-Oct-95	0	3.4	0
Indian Head, SK	07-Jul-95	HCN92	D	112	10-Oct-95	188.7	3.4	55500
Indian Head, SK	07-Jul-95	HCN92	U	18	10-Oct-95	0	3.4	0
Indian Head, SK	07-Jul-95	Legend	U	25	10-Oct-95	133.2	3.5	38057
Indian Head, SK	07-Jul-95	Legend	D	68	10-Oct-95	113.6	3.5	32457
Indian Head, SK	07-Jul-95	Legend	U	31	10-Oct-95	134.1	3.5	38314
Indian Head, SK	07-Jul-95	Legend	D	65	10-Oct-95	126.8	3.5	36229
Indian Head, SK	07-Jul-95	Legend	D	50	10-Oct-95	187	3.5	53429
Indian Head, SK	07-Jul-95	Legend	U	19	10-Oct-95	18.1	3.5	5171
Indian Head, SK	07-Jul-95	Legend	D	97	10-Oct-95	122.2	3.5	34914
Indian Head, SK	07-Jul-95	Legend	U	62	10-Oct-95	23.1	3.5	6600
Indian Head, SK	07-Jul-95	pHOE4	D	11	10-Oct-95	23.3	3.2	7281
Indian Head, SK	07-Jul-95	pHOE4	U	18	10-Oct-95	16	3.2	5000
Indian Head, SK	07-Jul-95	pHOE4	U	53	10-Oct-95	0	3.2	0
Indian Head, SK	07-Jul-95	pHOE4	D	23	10-Oct-95	0	3.2	0
Indian Head, SK	07-Jul-95	pHOE4	D	26	10-Oct-95	0	3.2	0
Indian Head, SK	07-Jul-95	pHOE4	U	5	10-Oct-95	1.7	3.2	531
Indian Head, SK	07-Jul-95	pHOE4	D	42	10-Oct-95	15.9	3.2	4969
Indian Head, SK	07-Jul-95	pHOE4	U	34	10-Oct-95	49.9	3.2	15594

Location	Date	Variety	seed	Early	Harvest	Seed	Late	1000 wt	Seed

Invasive Potential of Transgenic *B. napus* (pHoe4)
Under Disturbed and Undisturbed Field Conditions

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			bed*	Count	Date	Wt g	Count	g	No.
Rosthern, SK	19-Jul-95	Cyclone	U	51	17-Aug-95	9.2	8	3.8	2421
Rosthern, SK	19-Jul-95	Cyclone	D	96	17-Aug-95	0	89	3.8	0
Rosthern, SK	19-Jul-95	Cyclone	U	41	17-Aug-95	0	0	3.8	0
Rosthern, SK	19-Jul-95	Cyclone	D	74	17-Aug-95	4.5	59	3.8	1184
Rosthern, SK	19-Jul-95	Cyclone	D	18	17-Aug-95	4.2	11	3.8	1105
Rosthern, SK	19-Jul-95	Cyclone	U	21	17-Aug-95	3.5	17	3.8	921
Rosthern, SK	19-Jul-95	Cyclone	D	72	17-Aug-95	0	85	3.8	0
Rosthern, SK	19-Jul-95	Cyclone	U	34	17-Aug-95	4.3	16	3.8	1132
Rosthern, SK	19-Jul-95	Excel	U	45	17-Aug-95	5	11	3.3	1515
Rosthern, SK	19-Jul-95	Excel	D	15	17-Aug-95	6.3	10	3.3	1909
Rosthern, SK	19-Jul-95	Excel	U	45	17-Aug-95	3.1	7	3.3	939
Rosthern, SK	19-Jul-95	Excel	D	40	17-Aug-95	0	0	3.3	0
Rosthern, SK	19-Jul-95	Excel	U	14	17-Aug-95	0.5	8	3.3	152
Rosthern, SK	19-Jul-95	Excel	D	42	17-Aug-95	16.7	4	3.3	5061
Rosthern, SK	19-Jul-95	Excel	U	35	17-Aug-95	0.3	2	3.3	91
Rosthern, SK	19-Jul-95	Excel	D	52	17-Aug-95	0	0	3.3	0
Rosthern, SK	19-Jul-95	HCN92	U	5	17-Aug-95	8.8	5	3.4	2588
Rosthern, SK	19-Jul-95	HCN92	D	28	17-Aug-95	0	0	3.4	0
Rosthern, SK	19-Jul-95	HCN92	U	46	17-Aug-95	0.1	1	3.4	29
Rosthern, SK	19-Jul-95	HCN92	D	49	17-Aug-95	0	12	3.4	0
Rosthern, SK	19-Jul-95	HCN92	D	11	17-Aug-95	11.6	17	3.4	3412
Rosthern, SK	19-Jul-95	HCN92	U	25	17-Aug-95	5.4	2	3.4	1588
Rosthern, SK	19-Jul-95	HCN92	D	57	17-Aug-95	0	0	3.4	0
Rosthern, SK	19-Jul-95	HCN92	U	7	17-Aug-95	0	0	3.4	0
Rosthern, SK	19-Jul-95	Legend	U	26	17-Aug-95	0	0	3.5	0
Rosthern, SK	19-Jul-95	Legend	D	69	17-Aug-95	0	76	3.5	0
Rosthern, SK	19-Jul-95	Legend	U	25	17-Aug-95	0	0	3.5	0
Rosthern, SK	19-Jul-95	Legend	D	2	17-Aug-95	0	0	3.5	0
Rosthern, SK	19-Jul-95	Legend	D	30	17-Aug-95	0	25	3.5	0
Rosthern, SK	19-Jul-95	Legend	U	46	17-Aug-95	4.2	5	3.5	1200
Rosthern, SK	19-Jul-95	Legend	D	19	17-Aug-95	19.6	17	3.5	5600
Rosthern, SK	19-Jul-95	Legend	U	8	17-Aug-95	0	0	3.5	0
Rosthern, SK	19-Jul-95	pHOE4	D	12	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	U	1	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	U	16	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	D	4	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	D	9	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	U	25	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	D	8	17-Aug-95	0	0	3.2	0
Rosthern, SK	19-Jul-95	pHOE4	U	28	17-Aug-95	0	0	3.2	0

* U and D indicate undisturbed and disturbed, respectively.

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Table 2. Statisitical Summary of All Effects. 1995.

Indian Head Early Counts

Effect	F	p - level
Variety	7.12210*	.000372*
Seedbed	10.44929*	.002978*
Variety x Seedbed	2.01295	.117962

Indian Head Seed Number

Effect	F	p - level
Variety	6.125325*	.000999*
Seedbed	2.546075	.121051
Variety x Seedbed	8.124715*	.000146*

Indian Head Net Replacement

Effect	F	p - level
Variety	6.061854*	.001066*
Seedbed	3.335115	.077783
Variety x Seedbed	8.286783*	.000130*

Rosthern Early Counts

Effect	F	p - level
Variety	4.094408*	.009148*
Seedbed	1.782955	.191831
Variety x Seedbed	1.089912	.379290

Rosthern Late Counts

Effect	F	p - level
Variety	6.39893*	.000757*
Seedbed	10.26019*	.003212*
Variety x Seedbed	4.14881*	.008589*

* indicates a significant effect (P<0.05)

Table 2. continued...

Rosthern Seed Numbers

Effect	F	p - level
Variety	.817596	.524121
Seedbed	.399202	.532287
Variety x Seedbed	.562114	.691923

Rosthern Net Replacement

Effect	F	p - level
Variety	.796302	.537025
Seedbed	.355852	.555294
Variety x Seedbed	.576667	.681732

* indicates a significant effect (P<0.05)

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Title

Invasive Potential of Transgenic pHOE4 *B. napus* (HCN28) Under Agronomic Conditions

Authors

Murray Belyk and Robert MacDonald

Report No.

Canadian reference: ACI95-18

International reference: A56370

Date

October, 1995

Study Submitted By

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Invasive Potential of Transgenic pHOE4
***B. napus* (HCN28) Under Agronomic Conditions**

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ACI95-18
October, 1995

Summary

A glufosinate tolerant transgenic canola line HCN28 (pHoe4) and three commercial canola cultivars (Excel, Legend and Cyclone) were investigated in a replacement series experiment under field conditions at three locations in western Canada. Above-ground biomass, collected just prior to bolting, was used to evaluate the competitive ability and aggressivity of HCN28 with its non-transgenic counterparts. Results from this study demonstrated that the presence of the gene coding for phosphinothricin acetyl-transferase (PAT) does not enhance or inhibit the competitive ability of canola under agronomic conditions. Calculated aggressivity values indicated HCN28 was not significantly different when seeded with the commercial canola varieties. Mean aggressivity values calculated across all planting densities for all three locations were 0.12, 0.06 and 0.04 for Excel, Cyclone and Legend, respectively. Therefore, it is not anticipated that the glufosinate tolerant canola line HCN28 has an increased invasive potential over commercial varieties, even in fields which were not treated with Liberty™ (glufosinate ammonium).

Invasive Potential of Transgenic pHOE4
***B. napus* (HCN28) Under Agronomic Conditions**

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I. INTRODUCTION

The replacement series design has been used widely to study interactions between two species of plants. The design maintains a constant total plant density while varying the relative proportions of the two species. Some researchers have criticized the series design as it does not address the contributions made by intra and interspecific competition. However, the present study design is ideal because only one species is evaluated in the series and all comparisons are between different cultivars. The presence of the glufosinate tolerant gene, phosphinothricin acetyl transferase (PAT) in the *Brassica napus* line HCN28 (pHoe4) serves as a useful marker for distinguishing plant from one another in the field study. Without the presence of the PAT gene such a study of intraspecific competition would be impossible under field conditions.

The results of the replacement series can be used to define and contrast any differences in the competitiveness and aggressivity of HCN28 with commercial cultivars.

Invasive Potential of Transgenic pHOE4
***B. napus* (HCN28) Under Agronomic Conditions**

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II. OBJECTIVE

The objective of this research was to compare the vegetative growth (above-ground biomass) as a means to evaluate the competitive ability and aggressivity of the glufosinate tolerant canola line HCN28 with standard commercial varieties.

III. MATERIALS AND METHODS

The transgenic canola line HCN28 was grown in monoculture and in mixed populations with one of three standard commercially canola cultivars (Legend, Excel, Cyclone). Each series consisted of the two monoculture and three mixtures; 25/75, 50/50, and 75/25 planting ratios. For both monoculture and mixtures plots were seeded at 100 seeds per m^2. All seed used in the study was treated with Vitavax Plus. Plots were replicated four times in a randomized complete block design. A precision seeder was used to seed an area of 1.25 m x 7 m. The trial was conducted at three locations in western Canada (Indian Head, SK, Rosthern, SK and High Bluff, MB) where canola is typically grown.

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All plots were treated with 800 g ai/ha of glufosinate ammonium (Liberty™) when the canola plants had approximately 5 leafs and had not yet bolted. The phytotoxic effect of glufosinate would allow for easy identification of non-resistant plants. Plant counts were collected from within a 1 m^2 quadrat. By 20 - 24 hrs after herbicide application, the resistant plants were separated from the non-resistant based on visual herbicide symptoms. Biomass collected from each plot was harvested from a 1 m^2 quadrat by clipping shoots at soil level. Plants were dried at 80 °C for no less than 48 hours and total shoot biomass was determined for each species.

Relative yield total values were determined by the following formula:

Relative Yield $r_a = x_{ab}/x_{aa}$
$r_b = x_{ba}/x_{bb}$

Relative Yield Total $RYT = r_a + r_b$

where r_a and r_b are relative yields of cultivar a and b, respectively; x_{ab} is the yield of cultivar 'a' grown in the mixture with cultivar 'b'; x_{aa} and x_{bb} are the yields of the cultivars a and b grown in monoculture.

Aggressivity values were calculated by the following formula:

$$A = (r_a - r_b)/RYT$$

where r_a and r_b values represent the relative yields of opposite seeding mixtures (ie. 75%Cyclone:25%HCN28 vs 75%HCN28:25%Cyclone). Aggressivity values were statistically analyzed across all locations using a one-way analysis of variance ($p<0.05$) with STATISTICA/W® software.

Replacement diagrams, according to Dewit (1960), were constructed from the average above-ground biomass for various seed mixtures across all locations. These diagrams are used to illustrate the competitive ability of HCN28 against its commercial counterparts.

IV. RESULTS AND DISCUSSION

The relative yield total (RYT) values may be used to describe how the cultivar pair utilizes resources. RYT values that are approximately 1.0 indicate that the pair are competing for the same limiting resources (Harper, 1977).

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Relative yield values calculated over several different seeding ratios demonstrate the competitive ability between two canola varieties. Two straight lines indicate that the ability of the two species to compete is equivalent, where as concave and convex lines indicate that one species is more competitive than the other and gains resources at the others expense (Harper, 1977).

Above ground biomass is a more direct measure than gain of the limiting resources among plants. Relative yield (RY) of the transgenic line HCN28 did not differ from linearity when compared to the pooled results of the RY from Excel, Legend and Cyclone (Figure 1). With the exception of Excel, this linearity was also observed on an individual variety basis. Seed mixtures containing a higher ratio of Excel (50-75%) caused a slight depression in relative yield. Overall, the competitive ability of HCN28 was not substantially different from the three standard commercial varieties evaluated.

Values of aggressivity were determined to provide a measurement of competitiveness between HCN28 and Legend, Excel or Cyclone varieties. Aggressivity values near zero (0.0) indicate a similar competitive ability between the transgenic and non-transgenic

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varieties. A positive (+) value would indicate a higher competitive ability; conversely a negative (-) value would indicate a reduced competitiveness.

Average aggressivity values for Cyclone, Excel and Legend versus HCN28 are presented in Figure 5. These average values were obtained from all seeding ratios for all three locations. An analysis of variance across all locations indicated no significant difference ($F=1.0252$; $p=0.3738$) in aggressivity between HCN28 and the three commercial varieties tested. Aggressivity indices for Excel vs HCN28, Cyclone vs HCN28 and Legend vs HCN28 were +0.12, +0.06 and +0.04 respectively.

Overall, the aggressivity values are consistent with the replacement diagrams and indicate that HCN28 has a similar competitive ability to its non-transgenic counterparts. The results of this study confirm the findings of a similar field study which determined no significant difference between the glufosinate tolerant line HCN92 (Innovator) and standard commercial canola varieties (MacDonald, 1994).

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V. CONCLUSION

This field study determined that the competitive ability of the line HCN28 was equivalent to the standard commercial lines currently available to producers. Results from this study demonstrated that the presence of the gene coding for phosphinothrin acetyl-transferase (PAT) does not enhance or inhibit the competative ability of canola under agronomic conditions. Calculated aggressivity values indicated HCN28 was not substantially different when seeded with the commercial canola standards Cyclone, Excel or Legend. Therefore, it is not anticipated that HCN28 has an increased invasive potential over commercial varieties, even in fields which were not treated with glufosinate ammonium.

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VI. REFERENCES

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2. Harper, J.L. 1977. Mixtures of Species I. Space and Proportions. Pages 237-276 in Population Biology of Plants. Academic Press, New York.

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Invasive Potential of Transgenic pHOE4
B. napus (HCN28) Under Agronomic Conditions

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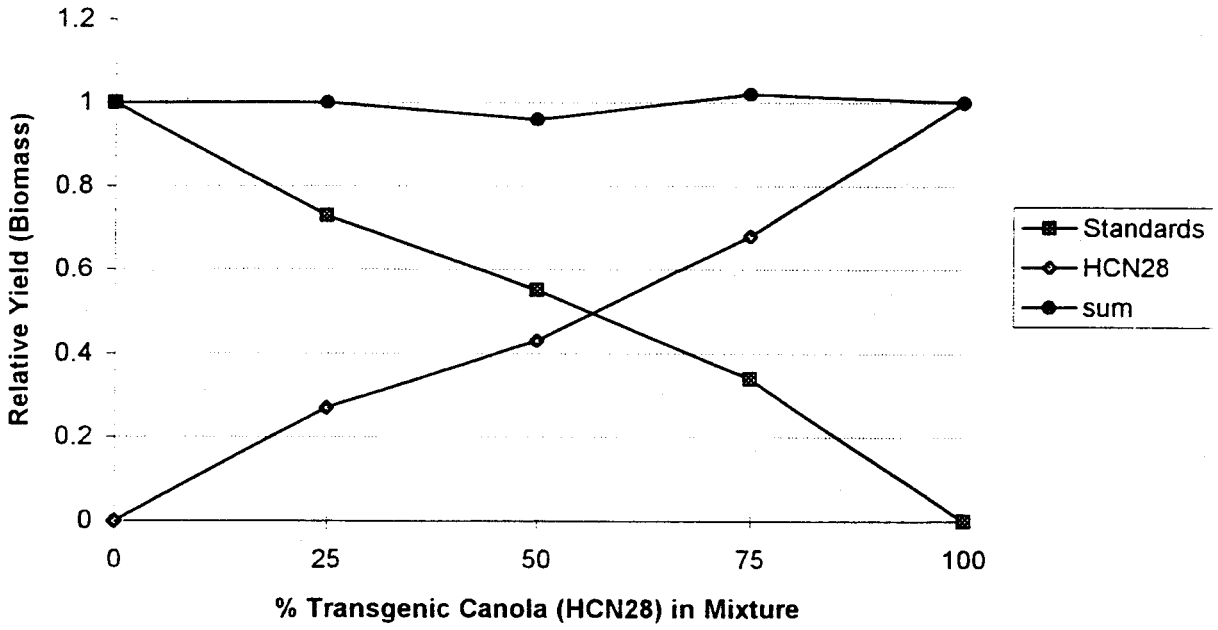

Figure 1. HCN28 vs Standard Commercial Lines - Plot of Means

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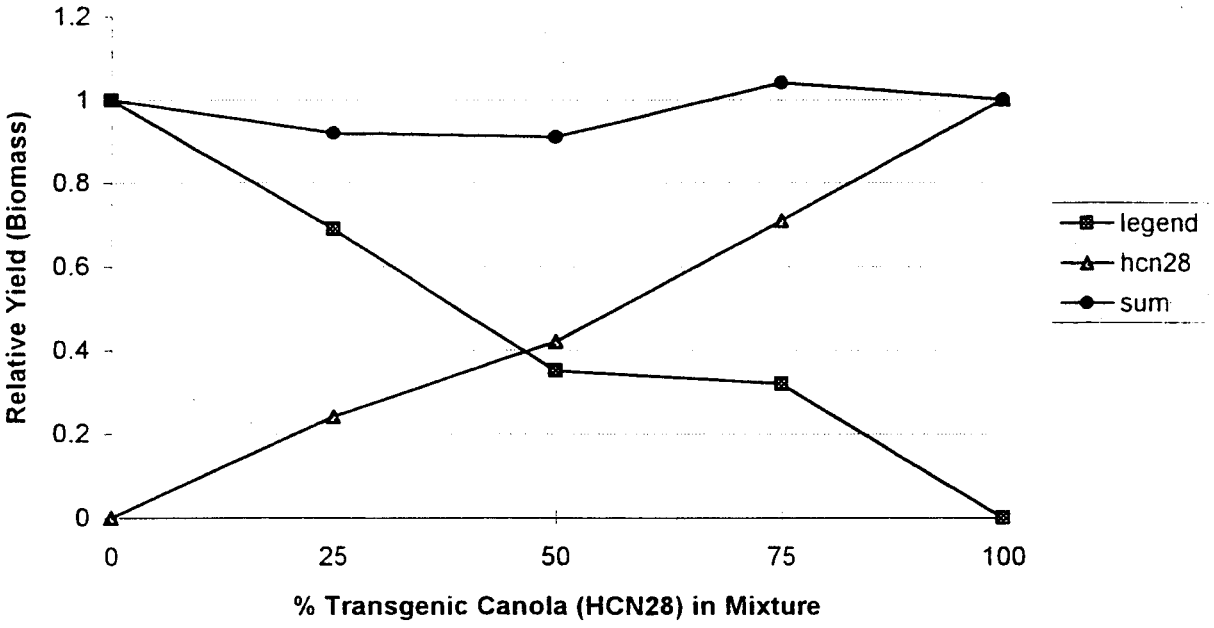

Figure 2. HCN28 vs LEGEND - Plot of Means

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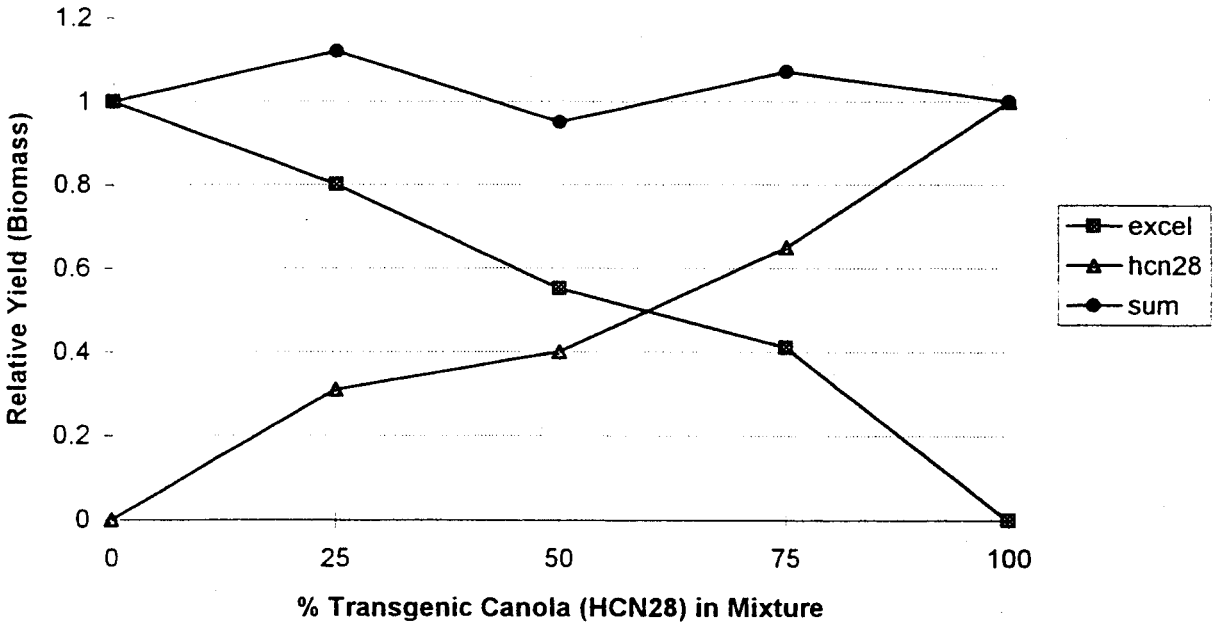

Figure 3. HCN28 vs EXCEL - Plot of Means

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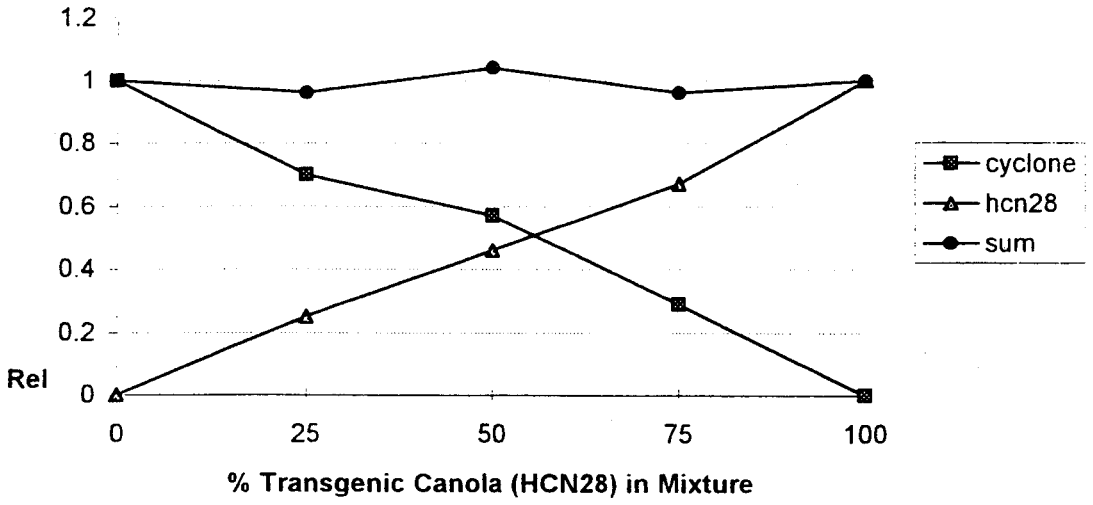

Figure 4. HCN28 vs CYCLONE - Plot of Means

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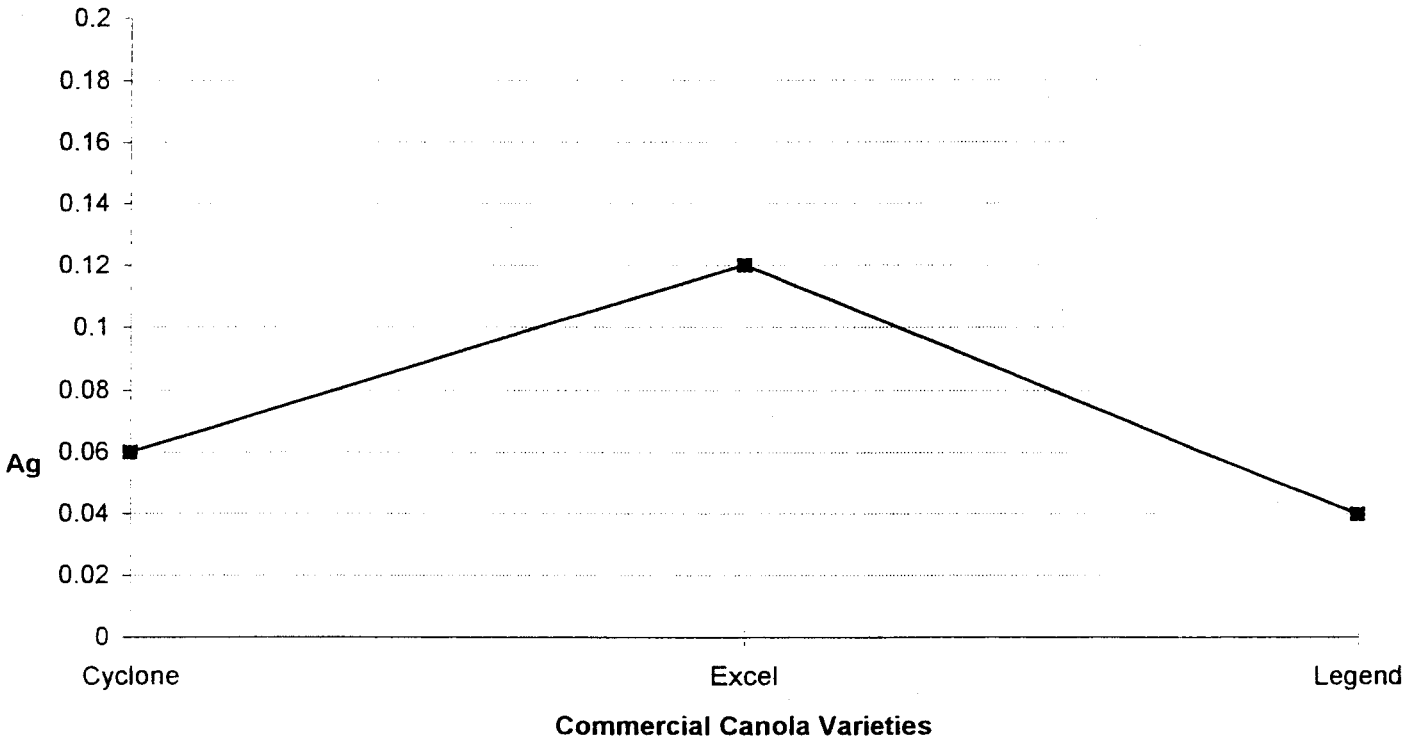

Figure 5. Aggressivity Indices for Canola Lines vs HCN28 - Plot of Means

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VIII. APPENDIX I

Table 1. Raw Data for Indian Head, SK., Rosthern, SK. and High Bluff, MB. 1995.

Location	% trans	Variety	trans count #/m2	non count #/m2	trans-bio g	non-bio g	total count #/m2	mean non-bio	mean trans-bio	Ra[1]	Rb[2]	RYT[3]	Aggressive[4]
High Bluff	0	Cyclone	0	49	0.00	62.80	49	87	0	1	0	1	1
High Bluff	0	Cyclone	0	115	0.00	97.80	115						
High Bluff	0	Cyclone	0	96	0.00	100.30	96						
High Bluff	0	Cyclone	0	149	0.00	100.60	149						
High Bluff	25	Cyclone	17	64	32.40	81.60	81	56	22	0.64	0.23	0.876	0.101
High Bluff	25	Cyclone	23	75	32.20	62.40	98						
High Bluff	25	Cyclone	3	19	6.60	23.60	22						
High Bluff	25	Cyclone	17	92	17.90	53.60	109						
High Bluff	50	Cyclone	19	19	53.20	35.40	38	43	52	0.50	0.54	1.039	-0.043
High Bluff	50	Cyclone	22	47	64.00	50.50	69						
High Bluff	50	Cyclone	23	36	37.30	43.80	59						
High Bluff	75	Cyclone	36	13	58.40	22.90	49	20	53	0.22	0.55	0.778	-0.012
High Bluff	75	Cyclone	31	6	49.10	10.20	37						
High Bluff	75	Cyclone	23	19	41.30	27.00	42						
High Bluff	75	Cyclone	72	13	61.70	18.10	85						
High Bluff	0	Excel	0	128	0.00	131.70	128	125	0	1	0	1	1
High Bluff	0	Excel	0	158	0.00	127.10	158						
High Bluff	0	Excel	0	153	0.00	94.90	153						
High Bluff	0	Excel	0	135	0.00	147.40	135						
High Bluff	25	Excel	16	35	19.90	45.50	51	87	22	0.69	0.23	0.926	0.135
High Bluff	25	Excel	25	62	38.80	117.60	87						
High Bluff	25	Excel	7	79	11.00	82.10	86						
High Bluff	25	Excel	26	102	19.20	101.80	128						
High Bluff	50	Excel	6	14	10.00	16.70	20	51	29	0.40	0.31	0.713	0.133
High Bluff	50	Excel	30	117	40.40	59.10	147						
High Bluff	50	Excel	20	65	23.10	57.50	85						
High Bluff	50	Excel	45	69	44.00	69.30	114						
High Bluff	75	Excel	36	13	54.80	48.90	49	42	54	0.33	0.57	0.901	0.112
High Bluff	75	Excel	38	22	76.70	40.10	60						
High Bluff	75	Excel	32	52	38.00	47.90	84						
High Bluff	75	Excel	30	29	46.00	30.50	59						

[1] indicates Relative Yield of non-transgenic cultivars; [2] indicates Relative Yield of transgenic cultivar;
[3] indicates Relative Yield Total of non-transgenic cultivar and transgenic cultivar;
[4] indicates Aggressivity of non-transgenic cultivar to transgenic cultivar.

Invasive Potential of Transgenic pHOE4
***B. napus* (HCN28) Under Agronomic Conditions**

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Location	% trans	Variety	trans count #/m2	non count #/m2	trans-bio g	non-bio g	total count #/m2	mean non-bio	mean trans-bio	Ra	Rb	RYT	Aggre-ssive
High Bluff	0	Legend	0	56	0.00	74.90	56	73	0	1	0	1	1
High Bluff	0	Legend	0	136	0.00	123.60	136						
High Bluff	0	Legend	0	53	0.00	46.70	53						
High Bluff	0	Legend	0	29	0.00	47.90	29						
High Bluff	25	Legend	2	45	5.00	69.70	47	51	11	0.70	0.11	0.807	-0.182
High Bluff	25	Legend	20	39	15.40	53.70	59						
High Bluff	25	Legend	1	30	2.50	24.00	31						
High Bluff	25	Legend	15	56	19.20	56.90	71						
High Bluff	50	Legend	20	29	30.90	42.60	49	39	29	0.54	0.30	0.839	0.283
High Bluff	50	Legend	32	29	34.10	28.40	61						
High Bluff	50	Legend	9	36	13.70	48.30	45						
High Bluff	50	Legend	22	31	35.70	38.40	53						
High Bluff	75	Legend	59	15	104.20	19.00	74	28	80	0.38	0.84	1.222	0.219
High Bluff	75	Legend	38	22	60.20	29.10	60						
High Bluff	75	Legend	76	32	76.20	35.00	108						
High Bluff	100	HCN28	84	0	125.10	0.00	84	2	95	0	1	1	-1
High Bluff	100	HCN28	40	6	97.30	11.50	46						
High Bluff	100	HCN28	51	2	63.90	6.60	53						
High Bluff	100	HCN28	108	0	136.90	0.00	108						
High Bluff	100	HCN28	119	0	103.30	0.00	119						
High Bluff	100	HCN28	57	0	67.10	0.00	57						
High Bluff	100	HCN28	51	10	99.10	14.40	61						
High Bluff	100	HCN28	53	0	67.50	0.00	53						
I. Head	0	Cyclone	0	182	0.00	94.20	182	108	0	1	0	1	1
I. Head	0	Cyclone	0	194	0.00	110.80	194						
I. Head	0	Cyclone	0	214	0.00	119.80	214						
I. Head	25	Cyclone	56	161	31.30	79.80	217	87	31	0.80	0.32	1.128	0.002
I. Head	25	Cyclone	52	189	34.90	88.50	241						
I. Head	25	Cyclone	32	110	31.50	97.90	142						
I. Head	25	Cyclone	29	151	25.60	82.00	180						
I. Head	50	Cyclone	63	93	44.00	77.80	156	72	43	0.67	0.45	1.119	0.195
I. Head	50	Cyclone	62	117	49.20	74.00	179						
I. Head	50	Cyclone	34	73	35.20	65.50	107						
I. Head	75	Cyclone	107	43	88.30	32.00	150	37	76	0.34	0.80	1.141	0.013
I. Head	75	Cyclone	53	32	64.00	41.50	85						

Invasive Potential of Transgenic pHOE4
***B. napus* (HCN28) Under Agronomic Conditions**

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ACI95-18
October, 1995

Location	% trans	Variety	trans count #/m2	non count #/m2	trans-bio g	non-bio g	total count #/m2	mean non-bio	mean trans-bio	Ra	Rb	RYT	Aggre-ssive
I. Head	0	Excel	0	259	0.00	126.30	259	101	0	1	0	1	1
I. Head	0	Excel	0	219	0.00	91.10	219						
I. Head	0	Excel	0	141	0.00	86.60	141						
I. Head	25	Excel	50	159	25.10	69.70	209	85	26	0.83	0.27	1.105	0.056
I. Head	25	Excel	20	116	22.30	101.30	136						
I. Head	25	Excel	52	126	36.60	89.80	178						
I. Head	25	Excel	21	92	18.90	77.30	113						
I. Head	50	Excel	34	76	37.40	68.10	110	74	41	0.73	0.43	1.161	0.263
I. Head	50	Excel	57	151	36.00	86.90	208						
I. Head	50	Excel	61	161	36.60	76.70	222						
I. Head	50	Excel	58	44	52.60	65.70	102						
I. Head	75	Excel	53	40	62.70	58.40	93	59	73	0.58	0.77	1.356	0.231
I. Head	75	Excel	86	67	78.50	57.40	153						
I. Head	75	Excel	82	79	78.80	61.90	161						
I. Head	0	Legend	0	112	0.00	79.00	112	108	0	1	0	1	1
I. Head	0	Legend	0	132	0.00	118.90	132						
I. Head	0	Legend	0	188	0.00	112.80	188						
I. Head	0	Legend	0	108	0.00	120.80	108						
I. Head	25	Legend	44	118	31.50	86.80	162	87	30	0.81	0.32	1.126	0.148
I. Head	25	Legend	47	122	27.50	68.60	169						
I. Head	25	Legend	66	138	43.90	72.50	204						
I. Head	25	Legend	14	97	16.80	122.00	111						
I. Head	50	Legend	53	57	47.60	48.90	110	51	46	0.47	0.49	0.957	-0.017
I. Head	50	Legend	63	53	56.40	45.60	116						
I. Head	50	Legend	53	74	53.00	58.80	127						
I. Head	50	Legend	23	47	27.90	49.80	70						
I. Head	75	Legend	43	40	58.60	44.90	83	34	61	0.31	0.64	0.954	-0.005
I. Head	75	Legend	62	31	54.10	29.80	93						
I. Head	75	Legend	20	22	47.90	30.20	42						
I. Head	75	Legend	62	29	83.90	29.20	91						
I. Head	100	HCN28	81	5	95.50	7.20	86	6	95	0	1	1	-1
I. Head	100	HCN28	150	5	142.60	8.10	155						
I. Head	100	HCN28	67	9	70.00	8.40	76						
I. Head	100	HCN28	123	7	117.00	5.40	130						
I. Head	100	HCN28	113	5	92.40	7.20	118						
I. Head	100	HCN28	87	5	94.70	9.70	92						

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Location	% trans	Variety	trans count #/m2	non count #/m2	trans-bio g	non-bio g	total count #/m2	mean non-bio	mean trans-bio	Ra	Rb	RYT	Aggre-ssive
I. Head	100	HCN28	114	2	94.50	4.00	116						
I. Head	100	HCN28	148	2	94.10	2.50	150						
I. Head	100	HCN28	102	2	76.80	4.00	104						
I. Head	100	HCN28	123	1	120.60	2.10	124						
I. Head	100	HCN28	54	2	70.70	3.40	56						
I. Head	100	HCN28	52	6	70.40	6.40	58						
Rosthern	0	Cyclone	0	82	0.00	23.30	82	26	0	1	0	1	1
Rosthern	0	Cyclone	0	71	0.00	35.60	71						
Rosthern	0	Cyclone	0	59	0.00	21.85	59						
Rosthern	0	Cyclone	0	51	0.00	22.30	51						
Rosthern	25	Cyclone	10	46	4.55	21.90	56	18	4	0.68	0.19	0.865	0
Rosthern	25	Cyclone	4	41	2.50	19.65	44						
Rosthern	25	Cyclone	11	59	5.45	16.90	70						
Rosthern	25	Cyclone	7	38	4.10	11.60	44						
Rosthern	50	Cyclone	17	31	13.50	15.85	48	14	9	0.55	0.40	0.946	0.165
Rosthern	50	Cyclone	11	29	7.80	13.25	40						
Rosthern	50	Cyclone	10	26	4.05	7.60	36						
Rosthern	50	Cyclone	13	34	9.90	20.15	46						
Rosthern	75	Cyclone	22	13	15.05	7.80	35	8	15	0.30	0.66	0.960	0.139
Rosthern	75	Cyclone	17	15	14.50	7.55	31						
Rosthern	0	Excel	0	93	0.00	50.85	93	33	0	1	0	1	1
Rosthern	0	Excel	0	51	0.00	20.55	51						
Rosthern	0	Excel	0	68	0.00	27.70	68						
Rosthern	25	Excel	33	46	20.00	26.65	78	29	10	0.88	0.45	1.331	0.186
Rosthern	25	Excel	10	59	5.25	31.00	68						
Rosthern	25	Excel	11	57	7.20	24.85	68						
Rosthern	25	Excel	10	65	7.80	33.75	75						
Rosthern	50	Excel	20	35	14.00	19.35	55	17	10	0.53	0.45	0.982	0.076
Rosthern	50	Excel	12	26	8.30	13.20	38						
Rosthern	50	Excel	14	35	6.90	16.55	48						
Rosthern	50	Excel	12	39	11.25	20.75	51						
Rosthern	75	Excel	22	17	13.20	8.60	39	11	14	0.32	0.63	0.956	-0.133
Rosthern	75	Excel	19	19	13.00	10.60	37						
Rosthern	75	Excel	18	13	11.70	7.75	31						
Rosthern	75	Excel	26	23	18.50	15.90	49						

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ACI95-18
October, 1995

Location	% trans	Variety	trans count #/m2	non count #/m2	trans-bio g	non-bio g	total count #/m2	mean non-bio	mean trans-bio	Ra	Rb	RYT	Aggre-ssive
Rosthern	0	Legend	0	51	0.00	34.80	51	28	0	1	0	1	1
Rosthern	0	Legend	0	39	0.00	14.05	39						
Rosthern	0	Legend	0	58	0.00	35.15	58						
Rosthern	0	Legend	0	50	0.00	28.55	50						
Rosthern	25	Legend	19	50	14.45	26.65	69	16	6	0.55	0.28	0.834	-0.115
Rosthern	25	Legend	6	22	4.20	10.95	28						
Rosthern	25	Legend	7	34	3.50	17.20	40						
Rosthern	25	Legend	6	29	3.10	7.30	35						
Rosthern	50	Legend	32	31	15.40	17.40	63	13	10	0.46	0.46	0.918	0.003
Rosthern	50	Legend	10	23	5.30	11.75	33						
Rosthern	50	Legend	15	19	9.60	9.15	34						
Rosthern	50	Legend	13	23	10.55	13.55	36						
Rosthern	75	Legend	22	21	19.20	12.15	43	8	14	0.29	0.64	0.937	0.007
Rosthern	75	Legend	20	10	15.05	6.65	30						
Rosthern	75	Legend	20	12	9.10	5.65	32						
Rosthern	100	HCN28	46	0	22.80	0.00	46	1	22		1	1	
Rosthern	100	HCN28	32	4	19.90	1.55	36						
Rosthern	100	HCN28	30	2	25.75	28.00	32						
Rosthern	100	HCN28	29	0	17.40	0.00	29						
Rosthern	100	HCN28	36	0	27.95	0.00	36						
Rosthern	100	HCN28	33	3	22.35	2.05	36						
Rosthern	100	HCN28	36	0	17.15	0.00	36						
Rosthern	100	HCN28	24	0	18.10	0.00	24						
Rosthern	100	HCN28	20	0	13.60	0.00	20						
Rosthern	100	HCN28	34	0	28.50	0.00	34						
Rosthern	100	HCN28	41	0	31.75	0.00	41						

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Title

Agronomic Characteristics (mean and coefficient of variance) of *Brassica napus* varieties grown at two Saskatchewan Locations in 1995

Author

Murray Belyk

Report No.

Canadian Reference: ACI96-09
International Reference: A55198

Date

March 6, 1996

Agronomic Characteristics (mean and coefficient varience)
of *Brassica napus* varieties at Two Saskatchewan Location in 1995

ACI96-09
March 6, 1996

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The following tables represent a summary of the agronomic characteristics determined from transgenic (Innovator, HCN28) and non-transgenic (Cyclone, Excel) canola plots grown at two locations in 1995 in RCBD trials with 3 relicate of all treatments.

Table 1. Agronomic Characteristics (mean and coefficient of variance) of *Brassica napus* varieties grown at Outlook, Saskatchewan, 1995.

Trait	Innovator		HCN28		Cyclone		Excel	
	mean	c.v.	mean	c.v.	mean	c.v.	mean	c.v.
Cotyledon width (mm)	9.20	4.0	9.26	5.4	10.33	2.4	10.66	3.0
Days to 50% Flowering	51.50	1.1	57.25	1.7	52.50	1.9	52.75	0.9
Days to Finish Flowering	71.75	2.9	78.50	0.7	72.50	2.9	73.00	3.0
Days to Maturity	92.50	0.6	100.8	1.0	97.50	2.0	97.25	1.5
Plant Height (cm)	102.5	7.7	119.0	11.2	112.0	12.1	115.0	12.4
Lodging Score (0-5)*	0.00	0	1.00	115	0.50	200	0.25	200
Thousand Seed Weight (g)	2.80	13.4	2.79	6.4	3.14	5.4	2.83	10.6
Yield (g/m^2)	183	13.1	241.7	10.7	266.1	9.8	214.6	15.0
Leaf Width (cm)	9.05	2.3	12.70	7.8	12.10	3.5	11.45	11.7
Leaf Length (cm)	19.40	4.4	27.60	3.6	27.15	1.3	24.70	8.6
Pedicel Length (cm)	2.20	32.1	2.05	17.2	2.10	0	1.80	0
Silique Length (cm)	4.50	34.6	6.45	1.1	6.10	4.6	6.15	1.1
Beak Length (mm)	1.20	23.6	1.50	28.3	1.20	0	1.35	5.2
Pod Width (mm)	0.50	0	0.45	15.7	0.50	0	0.50	0
Protein Content (%)	47.55		47.35		48.75		48.75	
Oil Content (%)	47.65		46.75		45.25		47.10	

* 0 = no lodging, 5 = flat

Agronomic Characteristics (mean and coefficient varience)
of *Brassica napus* varieties at Two Saskatchewan Location in 1995

ACI96-09
March 6, 1996

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Table 2. Agronomic Characteristics (mean and coefficient of variance) of *Brassica napus* varieties grown at Rosthern, Saskatchewan, 1995.

Trait	Innovator		HCN28		Cyclone		Excel	
	mean	c.v.	mean	c.v.	mean	c.v.	mean	c.v.
Cotyledon width (mm)	11.35	4.4	10.45	0.7	11.00	2.6	11.83	8.1
Days to 50% Flowering	50.0	0	54.5	1.3	51.5	4.1	51.0	0
Days to Finish Flowering	74.0	0	79.5	2.7	74.0	1.9	74.0	1.9
Days to Maturity	ND		ND		ND		ND	
Plant Height (cm)	105.5	7.4	111.0	2.5	115.5	3.1	105.0	0
Lodging Score (0-5)*	ND		ND		ND		ND	
Thousand Seed Weight (g)	3.87	16.6	3.80	16.0	4.40	9.1	3.83	13.4
Yield (g/m^2)	222	10.1	202	47	258	8.5	196	20.0
Leaf Width (cm)	9.25	23.7	12.7	6.7	11.25	1.9	10.2	6.9
Leaf Length (cm)	20.15	22.8	25.95	6.8	23.00	2.5	21.30	4.0
Pedicel Length (cm)	2.55	8.3	2.35	9.0	2.35	3.0	1.95	3.6
Silique Length (cm)	6.50	6.5	6.95	15.3	6.35	3.3	5.90	2.4
Beak Length (mm)	1.05	6.7	1.05	6.7	1.15	6.1	1.00	14.1
Pod Width (mm)	0.45	15.7	0.40	0	0.45	15.7	0.40	0
Protein Content (%)	47.55	0.1	47.35	1.3	48.75	1.9	48.75	1.9
Oil Content (%)	47.65	0.7	46.75	0.8	45.25	2.0	47.10	0.9

* 0 = no lodging, 5 = flat
ND = not determined

Title

Comparison of Standard Canola to Glufosinate-Tolerant Canola Survival Adaptations - Seed Dormancy

Author

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Report No.

HCI93-21

Date

November 24, 1993

Comparison of Standard Canola to Glufosinate-Tolerant Canola: Survival Adaptations - Seed Dormancy

Hoechst Canada Inc.
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November 24, 1993

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**Hoechst Canada Inc.
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Comparison of Standard Canola to Glufosinate-Tolerant Canola: Survival Adaptations - Seed Dormancy

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I. INTRODUCTION

A seed can be defined as being dormant when it fails to germinate due to internal conditions, even though external conditions are suitable. *Dormancy* should then be differentiated from *quiescence*, the stage a seed undergoes when external conditions are not suitable for germination (1).

There are 2 types of dormancy which can be expressed by seeds. Primary dormancy exists genetically in seeds at the time of harvest. Secondary dormancy may also occur, triggered by environmental effects which may occur after harvest. Cool temperatures (ie 5OC temperatures and wet, possibly anaerobic conditions) have been implicated in the triggering of secondary dormancy (2).

Canola (*Brassica napus*) is considered to have very little primary dormancy, and the issues surrounding dormancy in *B. napus* have been described (3). The weed Wild Mustard (*Sinapis arvensis*) on the other hand, maintains dormancy by a specific growth-inhibiting substance that is produced at low oxygen concentrations in the embryo (4). This dormancy factor, which will allow Wild Mustard seeds to remain dormant and viable in the soil for up to 60 years (5), has contributed to the virulence of this species as a weed in Western Canada.

Recent advances in tissue culture and transformation technologies for *B. napus* have allowed breeders to introduce novel traits to cultivars for plant improvement. These

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advances have allowed for the development of a *B. napus* line that is resistant to the non-selective herbicide glufosinate ammonium (Harvest®).

The application of this new technology has raised concerns regarding the safety of environmental releases of transgenic plants. In this case, questions have been raised as to the dormancy characteristics exhibited by these varieties, primarily in comparison to those of standard *B. napus* varieties.

Volunteer Canola (*B. napus*) is not considered to be a serious weed of crops in Eastern or Western Canada, as it is not included in any public weed control or description documents, such as the Province of Ontario Government Publication 505 (Ontario Weeds) (6), or Agriculture Canada's Budd's Flora of the Canadian Prairie Provinces(7). This is likely due in part to it's inability to trigger dormancy within its seed population.

A *B. napus* line which possessed seed dormancy characteristics would be both agronomically undesireable and would have enhanced weediness.

II. OBJECTIVE

To assess the level of primary and secondary dormancy exhibited by glufosinate-tolerant canola seed.

Comparison of Standard Canola to Glufosinate-Tolerant Canola: Survival Adaptations - Seed Dormancy

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III. RESULTS AND DISCUSSION

Data has been generated by Hoechst Canada Inc. over the past year which has described the ecological characteristics of HCN-92, a variety of Canola which has been transformed to be resistant to Glufosinate-ammonium. Much of that information (provided under separate cover) supports the contention that HCN-92 and standard Canola varieties express the same dormancy characteristics.

For example, "Assessment of Volunteer Glufosinate-Tolerant Canola Under Chemical Fallow Conditions", Report HCI93-04, describes the germination in 1993 of plots of HCN-92 and standard Canola varieties which were cultivated and harvested in 1992.

Comparison of Standard Canola to Glufosinate-Tolerant Canola: Survival Adaptations - Seed Dormancy

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Table 1 Plant counts (#/m^2) prior to and following a 1993 application of glufosinate- ammonium and glyphosate/2,4-D Amine for Standard Canola, Treated and Untreated Transgenic Canola.

Treatment (1993)	Standard Canola	Transgenic Canola (untreated)	Transgenic Canola (Treated)
Pre-Spray	212	235	257
Post-Glufosinate	19* (91%)	157 (33%)	161 (36%)
Post-Glyphosate/2,4-D	0.7 (0.3%)	0.2 (0.1)	0.1 (0.03%)

*Indicates significant difference where $p < 0.05$

Plant counts collected prior to the 1993 application of glufosinate ammonium were not significantly different among the transgenic and non-transgenic plots and ranged between 212- 257 per m^2. This indicates that volunteer plant populations were equivalent when transgenic and non-transgenic plots were compared.

This suggests that any dormancy differences (if dormancy was expressed at all), between the standard Canola varieties and the transgenic (HCN-92) variety, both sprayed and unsprayed, were similar.

With regards to the evaluation of secondary dormancy in Canola, a study is underway which promises to provide further information. Dr. A.I. Hsiao, at Agriculture Canada, Regina

Comparison of Standard Canola to Glufosinate-Tolerant Canola: Survival Adaptations - Seed Dormancy

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has undertaken a study whereby seed of HCN-92, both sprayed and unsprayed, will be compared to standard Canola cultivars including Excel, Westar, Profit, and Garrison. The study compares a standard germination test of samples of these varieties before and after a treatment to induce secondary dormancy if it exists in the genetic potential of the seed populations. Results from this study will be available in the first quarter, 1994, with preliminary results likely available in early February. The proposal is described below. The resulting report will be forwarded as a supplement to the existing one.

Proposal for the Evaluation of Primary and Secondary Dormancy Characteristics of HCN-92 in Comparison with Standard Canola Varieties.

Varieties to be included in study:
HCN-92 (seed from plants sprayed in 1993 with glufosinate-ammonium)
HCN-92 (seed from unsprayed plants)
Excel
Westar
Profit
Garrison

Seed of the above mentioned varieties, which has been stored under refrigerated conditions (5 +/-1°C) since harvest in the fall of 1993, will be subjected to standard germination tests at 20°/20° day/night temperatures. Seed which has been stored at room temperature will also be included. At the same time, subsamples of the seed in question will be subjected to burial in cool, wet soil, which has been known to induce secondary

dormancy if it exists in the genetic potential of seeds. After 1 week of this treatment, standard germination tests will be carried out on the rescued seed.

The tests will be carried out by Dr. A. Hsiao, Agriculture Canada, Regina, Research Scientist, Weed and Herbicide Physiology and Seed Dormancy

- Relevant publications from Dr. Hsiao include:

Hsiao, A.I. 1980. The effect of sodium hypochlorite, gibberellic acid and light on seed dormancy and germination of stinkweed and wild mustard. Can. J. Plant Sci. 60: 643-649

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IV. CONCLUSIONS

Dormancy characteristics play a role in the weediness of species. It is unlikely that HCN-92 as a volunteer weed will be any different in nature or characteristic than other *B. napus* varieties which are commercially available. Germination of HCN-92 seed in the year following cultivation is similar to that of standard varieties.

Studies are presently underway to confirm that there is no difference in secondary or induced dormancy between HCN-92 and standard Canola varieties.

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V. REFERENCES

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Title

Assessment of Outcross Frequency of Glufosinate-Tolerant *Brassica napus* cv. HCN-92

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HCI93-01

Date

October 27, 1993

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I. INTRODUCTION

This protocol was conducted as part of a series of greenhouse and field studies to determine the potential for outcrossing of the phosphinothricin acetyl transferase (PAT) gene from a transformed canola line (HCN-92) into other plant species. The presence of this gene is readily identifiable as it imparts tolerance to the herbicide glufosinate ammonium (GA). Although *B. napus* is self compatible, cross pollination has been shown to account for as much as 33% of fertilization (1).

II. OBJECTIVE

The objective of this study was to provide an estimate of the occurrence of outcrossing between a GA tolerant canola line (HCN-92) and two commercially available varieties (Legend and Global). In addition, the influence of distance and orientation from the pollen source on outcross frequency was examined. Outcross frequency was determined by spraying the F1 progeny with glufosinate ammonium to remove any non-tolerant plants from the population.

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III. MATERIALS AND METHODS

A) 1991: Field Collection of Outcross Seed

Approximately 500 grams of mature seed was collected randomly from within each of the sampling areas plots located within the 12 m isolation borders which surrounded the transgenic canola trials conducted in 1991 (Figure A). Seed was hand harvested from mature plants at three distances (0 - 4 m, 4 - 8 m, and 8 - 12 m) away from the edge of the transgenic plot area (Figure A). Seed was collected from the north and east isolation borders. Seed was first cleaned an then stored dry at room temperature during the winter season. The canola variety Legend and Global were grown in the isolation border at Rosthern, SK and Irricanna, AB, respectively. These isolation borders were established in compliance with Agriculture Canada regulations to serve as a barrier to the movement of transgenic pollen beyond the test site. The isolation border flowered synchronously with the transgenic canola. Pollen reached the border via wind dissemination and/or by foraging insects (e.g., honey bees). This was representative of naturally occurring conditions where outcrosses might occur.

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B) 1992: F1 Generation

Seed collected in 1991 was sown into a series of trials conducted at Edgely, SK, Homewood, MB, and Innisfail, AB. At each of the three sites the level of outcrossing to the border canola from Rosthern and Irricanna was evaluated. The treatments are seed subsamples taken from the three sampling distances as identified in the 1991 Irricanna and Rosthern canola borders.

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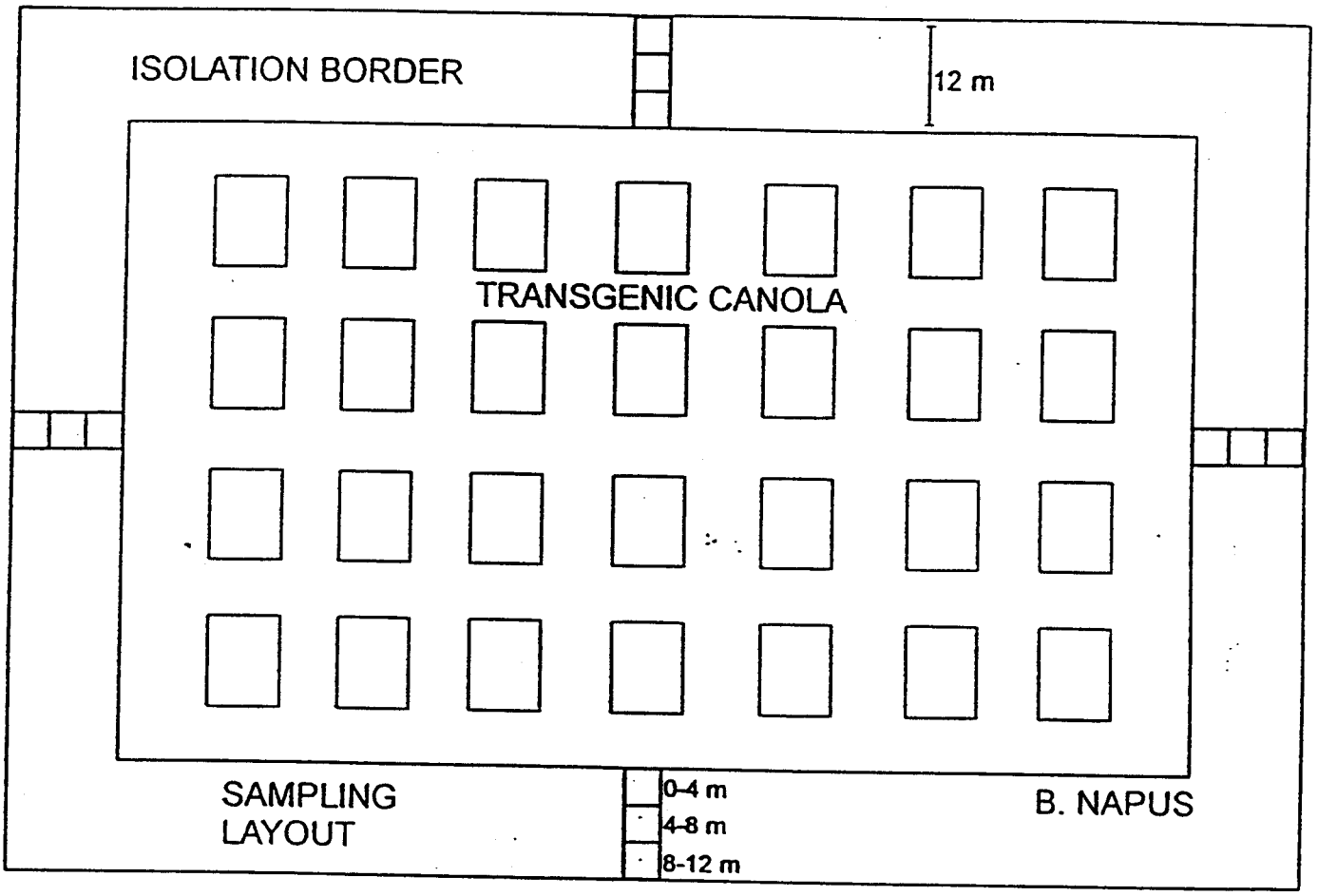
Figure A Transgenic (HCN92) Plot Layout



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Seed samples were treated with Vitavax Plus prior to planting with a precision seeder. Individual plot size was 1.5 m x 7 m. The trial design was a randomized complete block design with four replicates. The trial design and randomization is summarized in Table 1.

Seed was selected from the North and East plot borders of the 1991 trials in Rosthern and Irricana. These orientations were used for screening as the prevailing wind direction during flowering is typically southwesterly. Plots were seeded at a rate of 250 seeds per m^2. Total plant populations were approximately 2100 plants per plot.

All plots in 1992 located at Innisfail and Homewood received two applications of 1000 g ai/ha of glufosinate ammonium. Due to staggered emergence of the population a third application was required at the Edgely site to remove all non-tolerant plants.

Assessments included germination rating (0 - 9; where 0 = no germination and 9 = all seeds germinated), a visual tolerance estimate (%) and a stem count of the remaining plots at plant maturity..

IV. RESULTS

The trial results are summarized in tabular form (Appendix 1) and were analyzed using a multifactorial analysis of variance and regression analysis using Statistica W\4.2 software.

On the rating scale of 0 - 9, a germination score of 8 was the most common. Such a high rating represents healthy germination rating. There was no significant difference ($p > 0.05$) in the germination scores for each of the trials seeded in 1992 (Table 2). The mean and standard deviation of the germination rating for the outcross seed collected from Rosthern and Irricanna averaged across all 3 locations in 1992 was 7.6 +/- 0.6 and 7.7 +/- 0.6, respectively.

The direction, and the distance from the pollen source had no significant effect ($p > 0.05$) on seed germination in 1992. (Table 2)

V. OUTCROSS FREQUENCY

The frequency of outcrossing between the transgenic plants and the commercial varieties in the isolation border was estimated by determining the number of plants in each plot which survived the application of the herbicide glufosinate ammonium. The outcross frequency averaged across all trails conducted in 1992 was observed to be significantly different ($p < 0.05$) between the two seed sources, Irricanna and Rosthern (Table 3). The equation used to calculate the outcross frequency is:

$$\text{Outcross frequency} = \frac{\text{plant survival count} \times 100}{\text{plot size (ha)} \times \text{seeding rate (g/ha)} \times \text{germination rate(\%)}}$$

The averages of the plant counts across all replicates from all three sites for each seed source were used to calculate outcross frequency. Germination rate was based on the

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average rating for germination for each of the trials. An 85% germination rate was used in the calculation based on an average rating of 8. A greater number of outcrosses occurred at the Irricanna site than at the Rosthern site. The calculated outcross frequency for Rosthern and Irricanna seed sources are 0.03% and 0.1%, respectively. The average outcross frequency observed across all sites and plots was 0.06 %.

The seed source (Irricanna and Rosthern) did have a significant effect ($p < 0.05$) on the surviving plant count at all sites in 1992. A greater number of plants survived the herbicide application seed collected from the Irricanna site than the Rosthern site. Distance from and orientation relative to the plots did not influence outcross frequency among seed collected at the Rosthern location across all sites in 1992 (Table 4).

Seed collected from the east plot border at Irricanna indicated an influence of distance on the frequency ($p < 0.1$, Table 5). As anticipated, the number of tolerant progeny decreased as a function of distance from the transgenic plants. Figure B depicts the distance (in metres) from the pollen source against the tolerant plants per plot. Included are the 95% confidence bands and the equation for the linear relationship between the two variables.

With the exception of the Edgely site, there was a very clear distinction between tolerant and non-tolerant plants. The popualtion fo volunteer plants emerged through much of the growng season, consequently, many plants emerged after the herbicide application. In addition to the mature plants at harvest, there were a number of immature plants present at Edgely, these plants did not produce seed at the end of the growing season.. The

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source of the seed, the direction and the distance of the seed planted from the pollen source had no significant effect on the immature plant count. (Table 6) It is assumed that they were volunteer plants which emerged after spray treatment. Immature plants were not included in the count as they had not produced seed.

VI. DISCUSSION

Seed harvested from the isolation borders from both Rosthern and Irricanna was viable and grew vigorously. Germination of the seed was comparable from both sources. The application of glufosinate ammonium herbicide to the trial area resulted in rapid necrosis to the canola foliage. Plant death occurred within 14 days of application. A greater number of plants at all locations survived the herbicide application from the Irricanna seed source as compared to the Rosthern seed source. Factors contributing to the different level of outcrossing observed between the two sites would include meteorological conditions and honey bee activity. Outcross seed from the Irricanna site indicated that the greater the distance from the pollen source, the fewer the number of tolerant outcrosses.

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VII. CONCLUSION

The experimental conditions addressed a situation where a traditional canola crop was grown adjacent to a transgenic crop (within 4 m). The observed outcross frequency was very low (avg.= 0.06%). This study demonstrates that the likelihood of the outcrossing into an adjacent traditional canola crop is very low. Furthermore, an inverse correlation between distance and outcross frequency was established with the seed collected from the Irricanna site.

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VII. TABLES AND FIGURES Table 1 Trial Design and Randomization

Treatment	Rep 1	Rep 2	Rep 3	Rep 4
1. N Direction/1st Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	101	204	309	404
2. N Direction/2nd Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	102	210	312	409
3. N Direction/3rd Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	103	212	310	403
4. E Direction/1st Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	104	206	303	408
5. E Direction/2nd Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	105	205	306	412
6. E Direction/3rd Distance/Rosthern - glufosinate 150 SN @ 1.0 kg/ha	106	211	307	405
7. N Direction/1st Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	107	203	304	410
8. N Direction/2nd Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	108	209	301	402
9. N Direction/3rd Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	109	201	311	407
10. E Direction/1st Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	110	207	308	411
11. E Direction/2nd Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	111	202	305	406
12. E Direction/3rd Distance/Irricanna - glufosinate 150 SN @ 1.0 kg/ha	112	208	302	401

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Figure B Scatterplot of Irricanna Main Effect of Distance on Surviving Plants

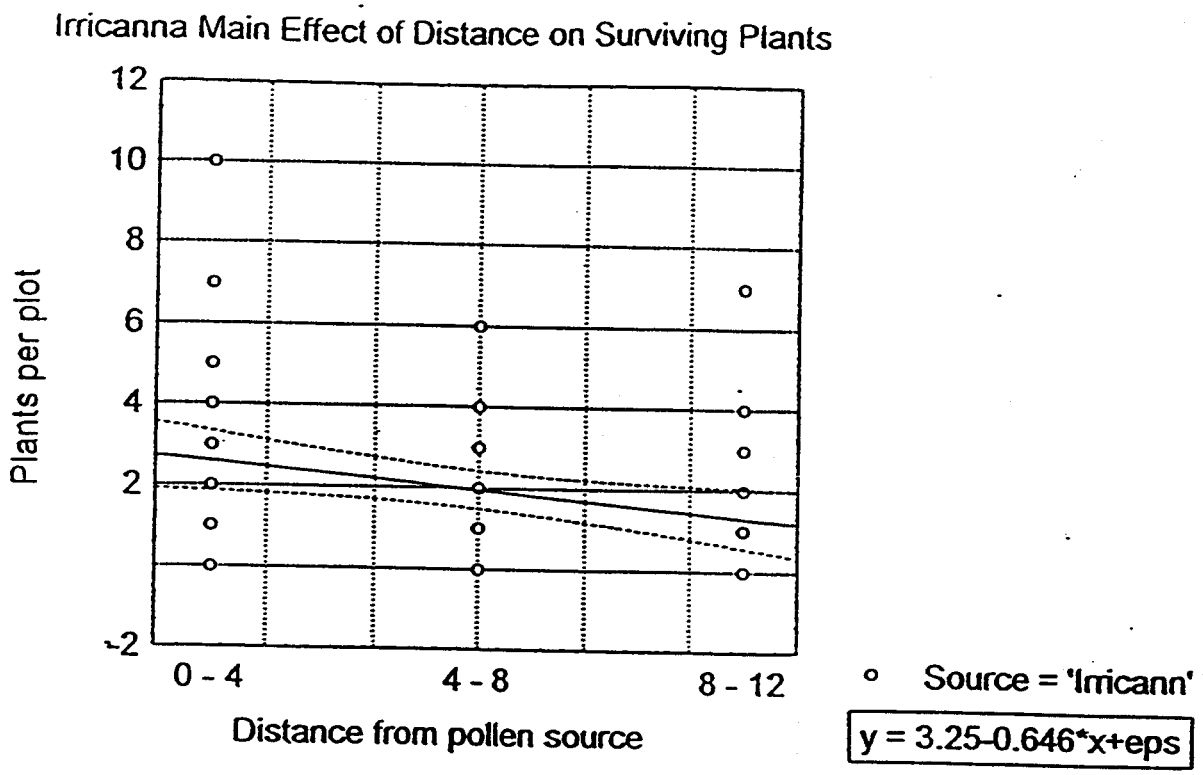

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Table 2 ANOVA of Seed Germination

CASE SELECTION CONDITIONS:
Exclude if:
loc = 'innisfai'

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
11	GERM	8.3	-9999	GERMINATION/VIGOUR
4	DIRE	8.3	-9999	DIRECTION
5	DIS	8.3	-9999	DISTANCE
6	SOURCE	8.3	-9999	SEED SOURCE

INDEPENDENT VARIABLES (between groups factors):

DIRE	Number of Levels:	2	Codes:	level	1:	100-NORTH
				level	2:	101-EAST
DIS	Number of Levels:	3	Codes:	level	1:	1
				level	2:	2
				level	3:	3
SOURCE	Number of Levels:	2	Codes:	level	1:	100-ROSTHERN
				level	2:	101-IRRICANN

DESIGN: 3 - way ANOVA, fixed effects
DEPENDENT: 1 variable: GERM
BETWEEN: 1-DIRE (2): NORTH EAST
2-DIS (3): 1 2 3
3-SOURCE (2): ROSTHERN IRRICANN
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (outsort.bak) 1-DIRE, 2-DIS, 3-SOURCE					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	.008876	83	.364404	.024358	.876357
2	2	.191958	83	.364404	.526772	.592469
3	1	.170641	83	.364404	.468273	.495689
12	2	.875461	83	.364404	2.402445	.096764
13	1	.158876	83	.364404	.435989	.510893
23	2	.114196	83	.364404	.313378	.731835
123	2	.017903	83	.364404	.049128	.952087

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Table 3 ANOVA of Plant Count

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
10	PLANTS	8.3	-9999	SURVIVING PLANT COUNT
4	DIRE	8.3	-9999	DIRECTION
5	DIS	8.3	-9999	DISTANCE
6	SOURCE	8.3	-9999	SEED SOURCE

INDEPENDENT VARIABLES (between groups factors):

DIRE	Number of Levels:	2	Codes:	level	1:	100-NORTH
				level	2:	101-EAST
DIS	Number of Levels:	3	Codes:	level	1:	1
				level	2:	2
				level	3:	3
SOURCE	Number of Levels:	2	Codes:	level	1:	100-ROSTHERN
				level	2:	101-IRRICANN

DESIGN: 3 - way ANOVA, fixed effects
DEPENDENT: 1 variable: PLANTS
BETWEEN: 1-DIRE (2): NORTH EAST
2-DIS (3): 1 2 3
3-SOURCE (2): ROSTHERN IRRICANN
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (outsort.bak) 1-DIRE, 2-DIS, 3-SOURCE					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	3.22648	131	3.543604	.91051	.341737
2	2	2.43942	131	3.543604	.68840	.504187
3	1*	68.42197*	131*	3.543604*	19.30858*	.000023*
12	2	2.05571	131	3.543604	.58012	.561264
13	1	4.09114	131	3.543604	1.15451	.284582
23	2	9.82001	131	3.543604	2.77119	.066260
123	2	2.82001	131	3.543604	.79580	.453388

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Table 4 ANOVA of Rosthern Plant Count

CASE SELECTION CONDITIONS:
Include if:
source = 'rosthern'

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
10	PLANTS	8.3	-9999	SURVIVING PLANT COUNT
4	DIRE	8.3	-9999	DIRECTION
5	DIS	8.3	-9999	DISTANCE

INDEPENDENT VARIABLES (between groups factors):

DIRE Number of Levels: 2 Codes: level 1: 100-NORTH
level 2: 101-EAST
DIS Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 2 - way ANOVA, fixed effects
DEPENDENT: 1 variable: PLANTS
BETWEEN: 1-DIRE (2): NORTH EAST
2-DIS (3): 1 2 3
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects (Type III SS); design: (outsort.bak) 1-DIRE, 2-DIS					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	.025441	65	2.999417	.008482	.926904
2	2	2.093694	65	2.999417	.698034	.501254
12	2	1.077763	65	2.999417	.359324	.699526

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Table 5 ANOVA of Irricanna Plant Count

CASE SELECTION CONDITIONS:
Include if:
source = 'irricann'

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
10	PLANTS	8.3	-9999	SURVIVING PLANT COUNT
4	DIRE	8.3	-9999	DIRECTION
5	DIS	8.3	-9999	DISTANCE

INDEPENDENT VARIABLES (between groups factors):

DIRE Number of Levels: 2 Codes: level 1: 100-NORTH
level 2: 101-EAST
DIS Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 2 - way ANOVA, fixed effects
DEPENDENT: 1 variable: PLANTS
BETWEEN: 1-DIRE (2): NORTH EAST
2-DIS (3): 1 2 3
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (outsort.bak) 1-DIRE, 2-DIS					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	7.34722	66	4.079545	1.800990	.184191
2	2	10.29167	66	4.079545	2.522748	.087952
12	2	3.84722	66	4.079545	.943052	.394622

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Table 6 ANOVA of Immature Plant Count

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
12	IMMAT	8.3	-9999	IMMATURE PLANTS
4	DIRE	8.3	-9999	DIRECTION
5	DIS	8.3	-9999	DISTANCE
6	SOURCE	8.3	-9999	SEED SOURCE

INDEPENDENT VARIABLES (between groups factors):

DIRE	Number of Levels:	2	Codes:	level	1:	100-NORTH
				level	2:	101-EAST
DIS	Number of Levels:	3	Codes:	level	1:	1
				level	2:	2
				level	3:	3
SOURCE	Number of Levels:	2	Codes:	level	1:	100-ROSTHERN
				level	2:	101-IRRICANN

DESIGN: 3 - way ANOVA, fixed effects
DEPENDENT: 1 variable: IMMAT
BETWEEN: 1-DIRE (2): NORTH EAST
2-DIS (3): 1 2 3
3-SOURCE (2): ROSTHERN IRRICANN
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (outcomb.bak) 1-DIRE, 2-DIS, 3-SOURCE					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	620.7770	35	1172.293	.529541	.471640
2	2	382.9153	35	1172.293	.326638	.723520
3	1	52.7230	35	1172.293	.044974	.833282
12	2	921.3758	35	1172.293	.785960	.463559
13	1	868.5608	35	1172.293	.740908	.395229
23	2	341.9416	35	1172.293	.291686	.748801
123	2	195.6324	35	1172.293	.166880	.846970

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VIII. APPENDIX

Raw Data

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
1	DIRE	8.3	-9999	Direction from the transgenic plot.
2	DIS	8.0	-9999	Distance from the transgenic plot.
3	SOURCE	8.3	-9999	Source of seed from 1991.
4	LOC	8.3	-9999	Experiment location in 1992.
5	PLANTS	8.0	-9999	Surviving plant count after herbicide application..
6	GERM	8.0	-9999	Germination rating on a scale of 0 - 9.
7	IMMAT	8.3	-9999	Immature plant count at harvest.
8	MAT	8.3	-9999	Mature plant count at harvest.

STATISTICA: DATA MANAGEMENT

data file: OUTCOMB.BAK [143 cases with 8 variables]

	1 DIRE	2 DIS	3 SOURCE	4 LOC	5 PLANTS	6 GERM	7 IMMAT	8 MAT
1	NORTH	4	ROSTHERN	INNISFAI	0			
2	NORTH	8	ROSTHERN	INNISFAI	0			
3	NORTH	12	ROSTHERN	INNISFAI	1			
4	EAST	4	ROSTHERN	INNISFAI	0			
5	EAST	8	ROSTHERN	INNISFAI	0			
6	EAST	12	ROSTHERN	INNISFAI	0			
7	NORTH	4	IRRICANN	INNISFAI	1			
8	NORTH	8	IRRICANN	INNISFAI	4			
9	NORTH	12	IRRICANN	INNISFAI	1			
10	EAST	4	IRRICANN	INNISFAI	1			
11	EAST	8	IRRICANN	INNISFAI	3			
12	EAST	12	IRRICANN	INNISFAI	1			
13	NORTH	12	IRRICANN	INNISFAI	0			
14	EAST	8	IRRICANN	INNISFAI	2			
15	NORTH	4	IRRICANN	INNISFAI	1			
16	NORTH	4	ROSTHERN	INNISFAI	0			
17	EAST	8	ROSTHERN	INNISFAI	0			
18	EAST	4	ROSTHERN	INNISFAI	0			
19	EAST	4	IRRICANN	INNISFAI	1			
20	EAST	12	IRRICANN	INNISFAI	0			
21	NORTH	8	IRRICANN	INNISFAI	0			
22	NORTH	8	ROSTHERN	INNISFAI	0			
23	EAST	12	ROSTHERN	INNISFAI	0			
24	NORTH	12	ROSTHERN	INNISFAI	0			
25	NORTH	8	IRRICANN	INNISFAI	0			
26	EAST	12	IRRICANN	INNISFAI	0			
27	EAST	4	ROSTHERN	INNISFAI	0			
28	NORTH	4	IRRICANN	INNISFAI	1			
29	EAST	8	IRRICANN	INNISFAI	1			
30	EAST	8	ROSTHERN	INNISFAI	0			
31	EAST	12	ROSTHERN	INNISFAI	0			
32	EAST	4	IRRICANN	INNISFAI	1			
33	NORTH	4	ROSTHERN	INNISFAI	0			
34	NORTH	12	ROSTHERN	INNISFAI	0			
35	NORTH	12	IRRICANN	INNISFAI	0			
36	NORTH	8	ROSTHERN	INNISFAI	0			
37	EAST	12	IRRICANN	INNISFAI	0			
38	NORTH	8	IRRICANN	INNISFAI	0			
39	NORTH	12	ROSTHERN	INNISFAI	0			
40	NORTH	4	ROSTHERN	INNISFAI	0			
41	EAST	12	ROSTHERN	INNISFAI	0			
42	EAST	8	IRRICANN	INNISFAI	1			
43	NORTH	12	IRRICANN	INNISFAI	3			
44	EAST	4	ROSTHERN	INNISFAI	0			
45	NORTH	8	ROSTHERN	INNISFAI	0			
46	NORTH	4	IRRICANN	INNISFAI	1			
47	EAST	4	IRRICANN	INNISFAI	2			
48	EAST	8	ROSTHERN	INNISFAI	0			
49	NORTH	4	ROSTHERN	HOMEWOOD	0	7		
50	NORTH	8	ROSTHERN	HOMEWOOD	0	8		
51	NORTH	12	ROSTHERN	HOMEWOOD	0	8		
52	EAST	4	ROSTHERN	HOMEWOOD	0	8		
53	EAST	8	ROSTHERN	HOMEWOOD	1	7		
54	EAST	12	ROSTHERN	HOMEWOOD	0	8		
55	NORTH	4	IRRICANN	HOMEWOOD	1	8		
56	NORTH	8	IRRICANN	HOMEWOOD	3	7		
57	NORTH	12	IRRICANN	HOMEWOOD	2	8		

	1 DIRE	2 DIS	3 SOURCE	4 LOC	5 PLANTS	6 GERM	7 IMMAT	8 MAT
58	EAST	4	IRRICANN	HOMEWOOD	3	8		
59	EAST	8	IRRICANN	HOMEWOOD	0	7		
60	EAST	12	IRRICANN	HOMEWOOD	0	7		
61	NORTH	4	ROSTHERN	HOMEWOOD	1	6		
62	NORTH	8	ROSTHERN	HOMEWOOD	2	7		
63	NORTH	12	ROSTHERN	HOMEWOOD	1	7		
64	EAST	4	ROSTHERN	HOMEWOOD	0	7		
65	EAST	8	ROSTHERN	HOMEWOOD	1	7		
66	EAST	12	ROSTHERN	HOMEWOOD	0	7		
67	NORTH	4	IRRICANN	HOMEWOOD	1	6		
68	NORTH	8	IRRICANN	HOMEWOOD	3	7		
69	NORTH	12	IRRICANN	HOMEWOOD	2	7		
70	EAST	4	IRRICANN	HOMEWOOD	10	7		
71	EAST	8	IRRICANN	HOMEWOOD	3	6		
72	EAST	3	IRRICANN	HOMEWOOD	1	7.		
73	NORTH	4	ROSTHERN	HOMEWOOD	1	7		
74	NORTH	8	ROSTHERN	HOMEWOOD	1	8		
75	NORTH	12	ROSTHERN	HOMEWOOD	0	7		
76	EAST	4	ROSTHERN	HOMEWOOD	1	7		
77	EAST	8	ROSTHERN	HOMEWOOD	0	7		
78	EAST	12	ROSTHERN	HOMEWOOD	0	7		
79	NORTH	4	IRRICANN	HOMEWOOD	0	7		
80	NORTH	8	IRRICANN	HOMEWOOD	4	8		
81	NORTH	12	IRRICANN	HOMEWOOD	1	8		
82	EAST	4	IRRICANN	HOMEWOOD	7	8		
83	EAST	8	IRRICANN	HOMEWOOD	3	7		
84	EAST	12	IRRICANN	HOMEWOOD	1	8		
85	NORTH	4	ROSTHERN	HOMEWOOD	0	8		
86	NORTH	8	ROSTHERN	HOMEWOOD	0	8		
87	NORTH	12	ROSTHERN	HOMEWOOD	0	7		
88	EAST	4	ROSTHERN	HOMEWOOD	0	7		
89	EAST	8	ROSTHERN	HOMEWOOD	0	7		
90	EAST	12	ROSTHERN	HOMEWOOD	0	8		
91	NORTH	4	IRRICANN	HOMEWOOD	5	8.		
92	NORTH	8	IRRICANN	HOMEWOOD	0	8		
93	NORTH	12	IRRICANN	HOMEWOOD	4	7		
94	EAST	4	IRRICANN	HOMEWOOD	2	8.		
95	EAST	8	IRRICANN	HOMEWOOD	4	8		
96	EAST	12	IRRICANN	HOMEWOOD	0	8		
97	NORTH	8	ROSTHERN	EDGELY	0	8	94.000	0.000
98	NORTH	12	ROSTHERN	EDGELY	0	8	81.000	0.000
99	EAST	4	ROSTHERN	EDGELY	1	8	61.000	1.000
100	EAST	8	ROSTHERN	EDGELY	0	8	47.000	0.000
101	EAST	12	ROSTHERN	EDGELY	0	8	62.000	0.000
102	NORTH	4	IRRICANN	EDGELY	1	8	55.000	0.000
103	NORTH	8	IRRICANN	EDGELY	4	8	53.000	3.000
104	NORTH	12	IRRICANN	EDGELY	0	8	37.000	0.000
105	EAST	4	IRRICANN	EDGELY	1	8	148.000	0.000
106	EAST	8	IRRICANN	EDGELY	6	8	77.000	3.000
107	EAST	12	IRRICANN	EDGELY	3	8	113.000	1.000
108	NORTH	12	IRRICANN	EDGELY	1	8	77.000	0.000
109	EAST	8	IRRICANN	EDGELY	2	8	92.000	2.000
110	NORTH	4	IRRICANN	EDGELY	3	8	58.000	1.000
111	NORTH	4	ROSTHERN	EDGELY	1	8	96.000	0.000
112	EAST	8	ROSTHERN	EDGELY	0	7	57.000	0.000
113	EAST	4	ROSTHERN	EDGELY	0	8	48.000	1.000
114	EAST	4	IRRICANN	EDGELY	4	8	35.000	2.000
.15	EAST	12	IRRICANN	EDGELY	7	9	66.000	1.000
116	NORTH	8	IRRICANN	EDGELY	1	8	32.000	1.000
117	NORTH	8	ROSTHERN	EDGELY	1	8	29.000	0.000
118	EAST	12	ROSTHERN	EDGELY	0	8	121.000	0.000
119	NORTH	12	ROSTHERN	EDGELY	2	8	90.000	0.000

STATISTICA: DATA MANAGEMENT

	1 DIRE	2 DIS	3 SOURCE	4 LOC	5 PLANTS	6 GERM	7 IMMAT	8 MAT
120	NORTH	8	IRRICANN	EDGELY	0	8	77.000	0.000
121	EAST	12	IRRICANN	EDGELY	2	8	58.000	1.000
122	EAST	4	ROSTHERN	EDGELY	0	8	129.000	0.000
123	NORTH	4	IRRICANN	EDGELY	1	8	49.000	0.000
124	EAST	8	IRRICANN	EDGELY	0	8	69.000	0.000
125	EAST	8	ROSTHERN	EDGELY	0	8	59.000	0.000
126	EAST	12	ROSTHERN	EDGELY	13	8	IMMATURE	2.000
127	EAST	4	IRRICANN	EDGELY	5	9	48.000	2.000
128	NORTH	4	ROSTHERN	EDGELY	0	8	13.000	0.000
129	NORTH	12	ROSTHERN	EDGELY	4	9	72.000	3.000
130	NORTH	12	IRRICANN	EDGELY	2	8	33.000	0.000
131	NORTH	8	ROSTHERN	EDGELY	3	8	127.000	0.000
132	EAST	12	IRRICANN	EDGELY	1	8	76.000	0.000
133	NORTH	8	IRRICANN	EDGELY	0	8	90.000	0.000
134	NORTH	12	ROSTHERN	EDGELY	1	8	14.000	0.000
135	NORTH	4	ROSTHERN	EDGELY	1	8	80.000	1.000
136	EAST	12	ROSTHERN	EDGELY	0	8	48.000	0.000
137	EAST	8	IRRICANN	EDGELY	0	8	44.000	0.000
138	NORTH	12	IRRICANN	EDGELY	1	8	70.000	0.000
139	EAST	4	ROSTHERN	EDGELY	3	8	25.000	1.000
140	NORTH	8	ROSTHERN	EDGELY	1	8	33.000	0.000
141	NORTH	4	IRRICANN	EDGELY	7	8	32.000	3.000
142	EAST	4	IRRICANN	EDGELY	4	8	28.000	4.000
143	EAST	8	ROSTHERN	EDGELY	0	8	19.000	0.000

Assessment of Outcross Frequency of Glufosinate Tolerant Brassica napus cv. HCN-92

Hoechst

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HCI93-01
October 27, 1993

IX. REFERENCES

Olsson, G. (1960) "Self - incompatibility and outcrossing in rape and white mustard." Hereditas 46, 241 - 252

Hoechst

Title

Estimation of the Influence of Distance on the Outcrossing Frequency of Glufosinate-Tolerant Canola with Tame Mustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

Authors

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Report No.

HCI93-03

Date

November 1, 1993

Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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HCI93-03
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I. INTRODUCTION

The recent development of genetically engineered (transgenic) crops has raised a number of questions as to the impact of the introduction of these organisms on the environment.

An assessment of the impact of the release of these genetically modified organisms into the surrounding environment is required. The potential for the transfer of genetic information to wild or cultivated species which are related to *B. napus* must be addressed.

Outcross frequency can be examined by either artificially inducing crosses under controlled conditions or by measuring the frequency of outcrosses occurring under field conditions. This study examines the outcross frequency between genetically modified *B. napus*, tame mustard, wild mustard and a commercially available *B. napus* variety (Legend). The transgenic canola used in this study contains a gene which encodes for the protein phosphinothricin acetyl transferase. Any cross which carries this dominant gene will express tolerance to the herbicide glufosinate ammonium. This serves as an excellent marker as all non-tolerant plants can be removed with a selective application of glufosinate ammonium. This allows for the rapid screening of a large number of progeny under greenhouse conditions.

Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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II. OBJECTIVE

The objective of the study was to evaluate the influence of distance and orientation on the outcross frequency of Ignite® tolerant canola with tame mustard, wild mustard, and non-transgenic *B. napus* under field conditions.

III. MATERIALS AND METHODS

Field Trial

The trial test site was conducted at Indian Head, Saskatchewan.

The trial was located greater then 200 m from adjacent canola fields. This buffer zone was necessary to comply with trial guidelines for the field testing of genetically modified organisms outlined by Agriculture Canada. An isolation border could not be substituted as it would have conflicted with the experimental design.

Site Preparation

Fertilizer was broadcast at a rate of approximately 25 kg/ha of 11-51-0, also applied was 50 kg/ha of sulphur. The seed bed was prepared according to local agronomic procedure. Total site dimensions were 128 m by 128 m. The entire plot area was seeded to winter wheat at a rate of 100 kg/ha. The central 16 m by 16 m of the plot area was seeded to HCN-92 (transgenic canola) at a rate of 250 seeds per m^2, (8 Kg/ha), with

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a precision seeder. The tame mustard, wild mustard and standard *B. napus* were seeded at 6 kg/ha in two drill passes across the length and the width of the plot using a precision cone seeder (Plate 1). The first pass with the drill occurred on the same day as the transgenic canola planting. The second pass occurred 10 days after the initial seeding. After all plants had emerged, the winter wheat was removed from the seeded areas with an application of Poast® at 2 L/ha.

Each of the three species were seeded at a distance of 0, 8, 16, 32 and 64 m from the centre plot. These distances were repeated in each of the four orientations from the plot centre (Plate 2). The seeded areas were 3 m by 2 m (l x w).

Seed was harvested from the plot areas by first swathing and combining 10 days later with a Wintersteiger small plot combine. A randomly selected subsample of seed was cleaned with the Clipper Seed Cleaner and then was further divided into 20 g subsamples. Harvested seed was stored at 3°C for 3 - 4 weeks prior to greenhouse screening.

Greenhouse Screening

Greenhouse flats were filled with soil-less Redi-earth and saturated with water in preparation for planting of the samples. Sixteen randomly chosen seeds from each test plot were planted approximately 0.5 - 1.0 cm deep in the prepared flats. The experimental design was completely random with four replicates of four plants per

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harvested plot.

After planting, the remaining wild mustard seed samples were put into a freezer and stored at -20°C. A second planting of wild mustard was conducted 14 days later.

The greenhouse conditions remained constant throughout the duration of the study. The temperature was maintained at 25°C and the relative humidity at 80%. The flats were watered every three days and received a photoperiod of 18 light and 6 dark hours.

At the 3 - 5 leaf stage, F1 plants were sprayed with the herbicide glufosinate ammonium applied at a rate of 750 ai/ha. Mortality assessments were taken 7 days after treatment by comparison to controls.

IV. RESULTS AND DISCUSSION

Legend and tame mustard seed produced healthy vigorous plants (Plate 3). Plants reached the 3 - 5 leaf stage approximately 18 days after seeding. The first planting of wild mustard seed failed to germinate. The lack of germination was attributed to seed dormancy. However, after the seed was frozen for 2 weeks, seed germination increased markedly.

All seed collected from both *S. arvensis* and tame mustard plots was highly susceptible to the glufosinate treatment.Therefore herbicide tolerance was not observed

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to be present in the subsamples of the wild mustard and tame mustard populations evaluated.

A number of outcross seeds from the *B. napus* (Garrison) plots were observed to be tolerant to glufosinate ammonium. All non -tolerant, susceptible plants showed chlorosis within 48 hours after treatment, and were completely necrotic within 96 hours ((Plate 4). Tolerant plants displayed minimal injury from the herbicide treatment, damage was limited to chlorosis of leaf margins.

Approximately 35% of the seed from Garrison plots grown within 8 m of the transgenic canola was observed to be tolerant. As anticipated, the outcross frequency between Garrison and the transgenic canola decreased markedly beyond the 8 m plots. The outcross frequency for 16, 32, and 64 m was 3, 8, and 9%, respectively. The number of tolerant plants resulting from the two planting times was equal. The effect of the orientations of the plots to the centally seeds area did not influence the outcross frequency of any of the species evaluated..

V. CONCLUSION

These results indicate that the transfer of glufosinate tolerance from genetically modified canola to tame mustard and wild mustard is negligible regardless of distance. Clearly, *B. napus* is able to outcross interspecifically over limited distances. The marked decrease in outcrossing beyond 8 m indicates limited movement of pollen by wind or insects.

Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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VI. FIGURES

Plate 1 Cross pollination subplot (64 m).

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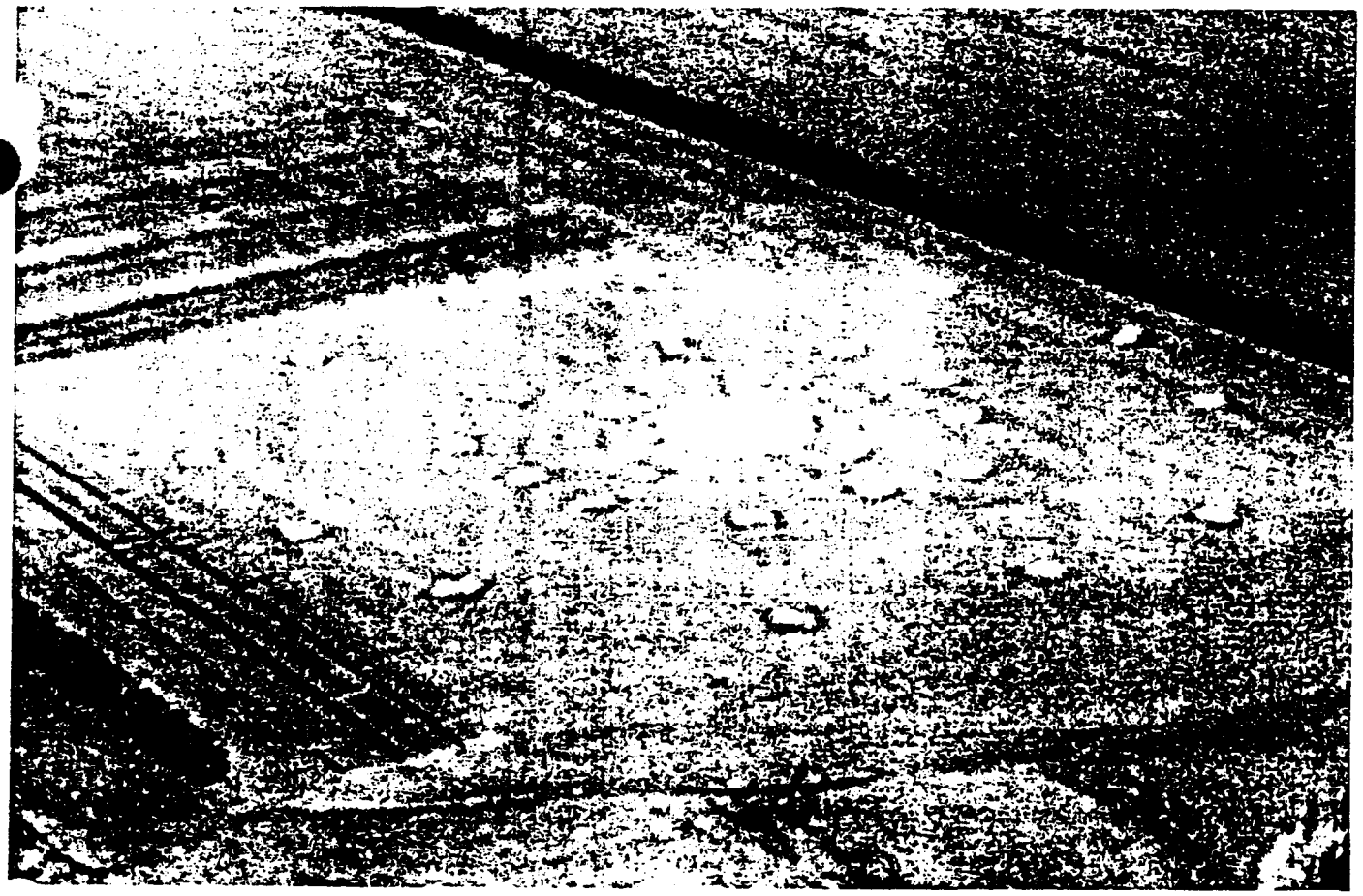
Plate 2 Aerial view of cross pollination trial (128 m diameter).



Estimation of the Influence of Distance on the Outcrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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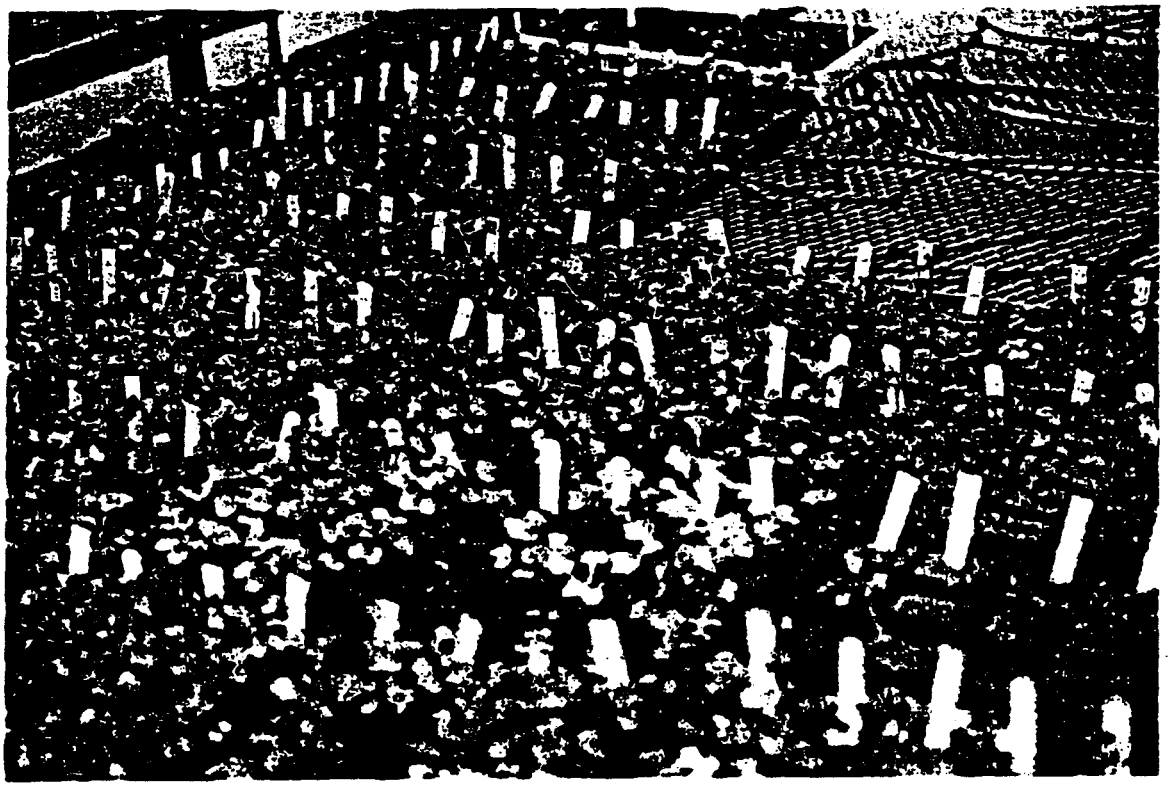
Plate 3 **F_1 tame mustard from cross pollination trial at 3 - 5 leaf stage prior to herbicide application.**



Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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Plate 4 **3 - 5 leaf stage seedlings from cross pollination trial 7 days after treatment with 750 g/ha glufosinate ammonium.**

Estimation of the Influence of Distance on the Outrossing Frequency of Glufosinate-Tolerant Canola with TameMustard, Wild Mustard, and *B. napus* Canola Cultivated Under Field Conditions

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VI. APPENDIX

STATISTICA: DATA MANAGEMENT

data file: CROSSPOL.STA [281 cases with 227 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
1	PLOT	8.3	-9999	Plot Code Identification
2	NEWVAR	8.3	-9999	
3	DISTANCE	8.3	-9999	Distance (m) from central area
4	GERMINAT	8.3	-9999	Germinated Plants
5	SURVIVOR	8.3	-9999	Plants which survived herbicide treatment
6	PERCENT	8.3	-9999	= survivor/germinat*100

	1 PLOT	2 NEWVAR	3 DISTANCE	4 GERMINAT	5 SURVIVOR	6 PERCENT
1						
2	GA13	G	8.000	4.000	4.000	100.000
3	GA13	G	8.000	4.000	4.000	100.000
4	GA13	G	8.000	4.000	4.000	100.000
5	GA4	G	8.000	1.000	1.000	100.000
6	GA1	G	64.000	4.000	0.000	0.000
7	GA1	G	64.000	4.000	0.000	0.000
8	GA1	G	64.000	4.000	0.000	0.000
9	GA2	G	32.000	4.000	0.000	0.000
10	GA2	G	32.000	4.000	0.000	0.000
11	GA2	G	32.000	4.000	0.000	0.000
12	GA3	G	16.000	4.000	0.000	0.000
13	GA3	G	16.000	4.000	0.000	0.000
14	GA4	G	8.000	4.000	0.000	0.000
15	GA4	G	8.000	4.000	0.000	0.000
16	GA4	G	8.000	3.000	0.000	0.000
17	GA5	G	8.000	3.000	0.000	0.000
18	GA5	G	8.000	4.000	0.000	0.000
19	GA5	G	8.000	4.000	0.000	0.000
20	GA6	G	16.000	4.000	0.000	0.000
21	GA6	G	16.000	4.000	0.000	0.000
22	GA6	G	16.000	4.000	0.000	0.000
23	GA7	G	32.000	4.000	0.000	0.000
24	GA7	G	32.000	4.000	0.000	0.000
25	GA8	G	64.000	4.000	0.000	0.000
26	GA8	G	64.000	4.000	0.000	0.000
27	GA8	G	64.000	4.000	0.000	0.000
28	GA9	G	64.000	4.000	0.000	0.000
29	GA9	G	64.000	4.000	0.000	0.000
30	GA10	G	32.000	3.000	0.000	0.000
31	GA10	G	32.000	4.000	0.000	0.000
32	GA10	G	32.000	4.000	0.000	0.000
33	GA11	G	16.000	4.000	0.000	0.000
34	GA11	G	16.000	4.000	0.000	0.000
35	GA11	G	16.000	4.000	0.000	0.000
36	GA12	G	8.000	3.000	0.000	0.000
37	GA12	G	8.000	4.000	0.000	0.000
38	GA12	G	8.000	4.000	0.000	0.000
39	GA14	G	16.000	4.000	0.000	0.000
40	GA14	G	16.000	4.000	0.000	0.000
41	GA14	G	16.000	4.000	0.000	0.000
42	GA15	G	32.000	3.000	0.000	0.000
43	GA15	G	32.000	4.000	0.000	0.000
44	GA15	G	32.000	2.000	0.000	0.000
45	GA16	G	64.000	4.000	0.000	0.000

STATISTICA: DATA MANAGEMENT

	1 PLOT	2 NEWVAR	3 DISTANCE	4 GERMINAT	5 SURVIVOR	6 PERCENT
46	GA16	G	64.000	4.000	0.000	0.000
47	GA16	G	64.000	2.000	0.000	0.000
48	GB1	G	64.000	2.000	0.000	0.000
49	GB1	G	64.000	2.000	0.000	0.000
50	GB2	G	32.000	4.000	0.000	0.000
51	GB2	G	32.000	4.000	0.000	0.000
52	GB2	G	32.000	4.000	0.000	0.000
53	GB3	G	16.000	3.000	0.000	0.000
54	GB3	G	16.000	3.000	0.000	0.000
55	GB3	G	16.000	4.000	0.000	0.000
56	GB4	G	8.000	2.000	0.000	0.000
57	GB4	G	8.000	2.000	0.000	0.000
58	GB4	G	8.000	3.000	0.000	0.000
59	GB6	G	16.000	3.000	0.000	0.000
60	GB7	G	32.000	3.000	0.000	0.000
61	GB7	G	32.000	4.000	0.000	0.000
62	GB7	G	32.000	4.000	0.000	0.000
63	GB8	G	64.000	4.000	0.000	0.000
64	GB8	G	64.000	4.000	0.000	0.000
65	GB8	G	64.000	4.000	0.000	0.000
66	GB9	G	64.000	4.000	0.000	0.000
67	GB9	G	64.000	4.000	0.000	0.000
68	GB9	G	64.000	4.000	0.000	0.000
69	GB10	G	32.000	4.000	0.000	0.000
70	GB10	G	32.000	4.000	0.000	0.000
71	GB10	G	32.000	4.000	0.000	0.000
72	GB11	G	16.000	4.000	0.000	0.000
73	GB11	G	16.000	4.000	0.000	0.000
74	GB11	G	16.000	4.000	0.000	0.000
75	GB13	G	8.000	4.000	0.000	0.000
76	GB13	G	8.000	4.000	0.000	0.000
77	GB13	G	8.000	4.000	0.000	0.000
78	GB14	G	16.000	4.000	0.000	0.000
79	GB14	G	16.000	4.000	0.000	0.000
80	GB14	G	16.000	4.000	0.000	0.000
81	GB16	G	64.000	3.000	0.000	0.000
82	TA1	T	64.000	4.000	0.000	0.000
83	TA1	T	64.000	4.000	0.000	0.000
84	TA1	T	64.000	4.000	0.000	0.000
85	TB1	T	64.000	4.000	0.000	0.000
86	TB1	T	64.000	4.000	0.000	0.000
87	TB1	T	64.000	4.000	0.000	0.000
88	TB2	T	32.000	4.000	0.000	0.000
89	TB2	T	32.000	4.000	0.000	0.000
90	TA2	T	32.000	4.000	0.000	0.000
91	TA2	T	32.000	4.000	0.000	0.000
92	TA2	T	32.000	4.000	0.000	0.000
93	TA3	T	16.000	4.000	0.000	0.000
94	TA3	T	16.000	4.000	0.000	0.000
95	TB3	T	16.000	4.000	0.000	0.000
96	TB3	T	16.000	4.000	0.000	0.000
97	TB3	T	16.000	4.000	0.000	0.000
98	TA4	T	8.000	4.000	0.000	0.000
99	TA4	T	8.000	4.000	0.000	0.000
100	TA4	T	8.000	4.000	0.000	0.000
101	TB4	T	8.000	4.000	0.000	0.000
102	TB4	T	8.000	4.000	0.000	0.000
103	TB4	T	8.000	4.000	0.000	0.000
104	TA5	T	8.000	4.000	0.000	0.000
105	TA5	T	8.000	4.000	0.000	0.000
106	TA5	T	8.000	4.000	0.000	0.000
107	TB5	T	8.000	4.000	0.000	0.000

STATISTICA: DATA MANAGEMENT

	1 PLOT	2 NEWVAR	3 DISTANCE	4 GERMINAT	5 SURVIVOR	6 PERCENT
108	TB5	T	8.000	4.000	0.000	0.000
109	TB5	T	8.000	4.000	0.000	0.000
110	TA6	T	16.000	4.000	0.000	0.000
111	TA6	T	16.000	4.000	0.000	0.000
112	TA6	T	16.000	4.000	0.000	0.000
113	TB6	T	16.000	4.000	0.000	0.000
114	TB6	T	16.000	4.000	0.000	0.000
115	TB6	T	16.000	4.000	0.000	0.000
116	TA7	T	32.000	4.000	0.000	0.000
117	TA7	T	32.000	4.000	0.000	0.000
118	TA7	T	32.000	4.000	0.000	0.000
119	TB7	T	32.000	4.000	0.000	0.000
120	TB7	T	32.000	4.000	0.000	0.000
121	TB7	T	32.000	4.000	0.000	0.000
122	TA8	T	64.000	4.000	0.000	0.000
123	TA8	T	64.000	4.000	0.000	0.000
124	TB8	T	64.000	4.000	0.000	0.000
125	TB8	T	64.000	4.000	0.000	0.000
126	TB8	T	64.000	4.000	0.000	0.000
127	TA12	T	8.000	4.000	0.000	0.000
128	TA12	T	8.000	4.000	0.000	0.000
129	TB12	T	8.000	4.000	0.000	0.000
130	TB12	T	8.000	4.000	0.000	0.000
131	TB12	T	8.000	4.000	0.000	0.000
132	TA11	T	16.000	4.000	0.000	0.000
133	TA11	T	16.000	4.000	0.000	0.000
134	TB11	T	16.000	4.000	0.000	0.000
135	TB11	T	16.000	4.000	0.000	0.000
136	TB11	T	16.000	4.000	0.000	0.000
137	TA10	T	32.000	4.000	0.000	0.000
138	TB10	T	32.000	4.000	0.000	0.000
139	TB10	T	32.000	4.000	0.000	0.000
140	TA9	T	64.000	4.000	0.000	0.000
141	TA9	T	64.000	4.000	0.000	0.000
142	TB9	T	64.000	4.000	0.000	0.000
143	TB9	T	64.000	4.000	0.000	0.000
144	TA13	T	8.000	4.000	0.000	0.000
145	TA13	T	8.000	4.000	0.000	0.000
146	TA13	T	8.000	4.000	0.000	0.000
147	TB13	T	8.000	4.000	0.000	0.000
148	TB13	T	8.000	4.000	0.000	0.000
149	TB13	T	8.000	4.000	0.000	0.000
150	TA14	T	16.000	4.000	0.000	0.000
151	TA14	T	16.000	4.000	0.000	0.000
152	TA14	T	16.000	4.000	0.000	0.000
153	TB14	T	16.000	4.000	0.000	0.000
154	TB14	T	16.000	4.000	0.000	0.000
155	TA15	T	32.000	4.000	0.000	0.000
156	TA15	T	32.000	4.000	0.000	0.000
157	TB15	T	32.000	4.000	0.000	0.000
158	TB15	T	32.000	4.000	0.000	0.000
159	TA16	T	64.000	4.000	0.000	0.000
160	TA16	T	64.000	4.000	0.000	0.000
161	TB16	T	64.000	4.000	0.000	0.000
162	TB16	T	64.000	4.000	0.000	0.000
163	TB16	T	64.000	4.000	0.000	0.000
164	GA13	G	8.000	1.000	0.000	0.000
165	GA13	G	8.000	1.000	0.000	0.000
166	GB13	G	8.000	1.000	1.000	100.000
167	GB13	G	8.000	1.000	1.000	100.000
168	GB5	G	8.000	2.000	2.000	100.000
169	GB5	G	8.000	4.000	4.000	100.000

STATISTICA: DATA MANAGEMENT

	1 PLOT	2 NEWVAR	3 DISTANCE	4 GERMINAT	5 SURVIVOR	6 PERCENT
170	GB5	G	8.000	4.000	4.000	100.000
171	TA12	T	8.000	1.000	0.000	0.000
172	WA12	W	8.000	4.000	0.000	0.000
173	WA12	W	8.000	3.000	0.000	0.000
174	WA13	W	8.000	4.000	0.000	0.000
175	WA13	W	8.000	3.000	0.000	0.000
176	WA13	W	8.000	4.000	0.000	0.000
177	WA4	W	8.000	3.000	0.000	0.000
178	WA4	W	8.000	4.000	0.000	0.000
179	WA4	W	8.000	4.000	0.000	0.000
180	WA5	W	8.000	3.000	0.000	0.000
181	WA5	W	8.000	4.000	0.000	0.000
182	WA5	W	8.000	4.000	0.000	0.000
183	WB12	W	8.000	4.000	0.000	0.000
184	WB12	W	8.000	4.000	0.000	0.000
185	WB12	W	8.000	4.000	0.000	0.000
186	WB13	W	8.000	4.000	0.000	0.000
187	WB13	W	8.000	3.000	0.000	0.000
188	WB13	W	8.000	3.000	0.000	0.000
189	WB4	W	8.000	4.000	0.000	0.000
190	WB4	W	8.000	4.000	0.000	0.000
191	WB4	W	8.000	4.000	0.000	0.000
192	WB5	W	8.000	4.000	0.000	0.000
193	WB5	W	8.000	4.000	0.000	0.000
194	WB5	W	8.000	4.000	0.000	0.000
195	GA3	G	16.000	2.000	1.000	50.000
196	GB6	G	16.000	4.000	0.000	0.000
197	GB6	G	16.000	4.000	0.000	0.000
198	TA11	T	16.000	1.000	0.000	0.000
199	TA3	T	16.000	1.000	0.000	0.000
200	TB14	T	16.000	1.000	0.000	0.000
201	WA11	W	16.000	4.000	0.000	0.000
202	WA11	W	16.000	4.000	0.000	0.000
203	WA11	W	16.000	4.000	0.000	0.000
204	WA14	W	16.000	4.000	0.000	0.000
205	WA14	W	16.000	3.000	0.000	0.000
206	WA14	W	16.000	3.000	0.000	0.000
207	WA3	W	16.000	4.000	0.000	0.000
208	WA3	W	16.000	3.000	0.000	0.000
209	WA3	W	16.000	4.000	0.000	0.000
210	WA6	W	16.000	4.000	0.000	0.000
211	WA6	W	16.000	2.000	0.000	0.000
212	WA6	W	16.000	3.000	0.000	0.000
213	WB11	W	16.000	4.000	0.000	0.000
214	WB11	W	16.000	3.000	0.000	0.000
215	WB14	W	16.000	4.000	0.000	0.000
216	WB14	W	16.000	3.000	0.000	0.000
217	WB3	W	16.000	4.000	0.000	0.000
218	WB3	W	16.000	4.000	0.000	0.000
219	WB3	W	16.000	3.000	0.000	0.000
220	GA7	W	32.000	4.000	0.000	0.000
221	GB15	G	32.000	4.000	0.000	0.000
222	GB15	G	32.000	4.000	0.000	0.000
223	GB15	G	32.000	1.000	0.000	0.000
224	GB15	G	32.000	1.000	1.000	100.000
225	GB2	G	32.000	1.000	1.000	100.000
226	TA10	T	32.000	1.000	0.000	0.000
227	TA10	T	32.000	1.000	0.000	0.000
228	TA15	T	32.000	1.000	0.000	0.000
229	TB10	T	32.000	1.000	0.000	0.000
230	TB15	T	32.000	1.000	0.000	0.000
231	TB2	T	32.000	1.000	0.000	0.000

STATISTICA: DATA MANAGEMENT

	1 PLOT	2 NEWVAR	3 DISTANCE	4 GERMINAT	5 SURVIVOR	6 PERCENT
232	WA10	W	32.000	3.000	0.000	0.000
233	WA10	W	32.000	3.000	0.000	0.000
234	WA15	W	32.000	4.000	0.000	0.000
235	WA15	W	32.000	4.000	0.000	0.000
236	WA15	W	32.000	4.000	0.000	0.000
237	WA16	W	32.000	4.000	0.000	0.000
238	WA2	W	32.000	3.000	0.000	0.000
239	WA2	W	32.000	4.000	0.000	0.000
240	WA2	W	32.000	3.000	0.000	0.000
241	WA7	W	32.000	4.000	0.000	0.000
242	WA7	W	32.000	4.000	0.000	0.000
243	WB10	W	32.000	4.000	0.000	0.000
244	WB10	W	32.000	3.000	0.000	0.000
245	WB10	W	32.000	3.000	0.000	0.000
246	WB15	W	32.000	4.000	0.000	0.000
247	WB15	W	32.000	4.000	0.000	0.000
248	WB15	W	32.000	4.000	0.000	0.000
249	WB2	W	32.000	3.000	0.000	0.000
250	WB2	W	32.000	3.000	0.000	0.000
251	WB2	W	32.000	3.000	0.000	0.000
252	GA1	G	64.000	4.000	0.000	0.000
253	GB1	G	64.000	1.000	1.000	100.000
254	GB16	G	64.000	1.000	1.000	100.000
255	TA16	T	64.000	1.000	0.000	0.000
256	TA8	T	64.000	1.000	0.000	0.000
257	TA9	T	64.000	1.000	0.000	0.000
258	TB9	T	64.000	1.000	0.000	0.000
259	WA1	W	64.000	4.000	0.000	0.000
260	WA1	W	64.000	3.000	0.000	0.000
261	WA1	W	64.000	4.000	0.000	0.000
262	WA1	W	64.000	4.000	0.000	0.000
263	WA16	W	64.000	3.000	0.000	0.000
264	WA16	W	64.000	3.000	0.000	0.000
265	WA16	W	64.000	3.000	0.000	0.000
266	WA8	W	64.000	3.000	0.000	0.000
267	WA8	W	64.000	4.000	0.000	0.000
268	WA8	W	64.000	2.000	0.000	0.000
269	WA9	W	64.000	4.000	0.000	0.000
270	WA9	W	64.000	4.000	0.000	0.000
271	WB1	W	64.000	4.000	0.000	0.000
272	WB1	W	64.000	4.000	0.000	0.000
273	WB1	W	64.000	3.000	0.000	0.000
274	WB16	W	64.000	1.000	0.000	0.000
275	WB16	W	64.000	1.000	0.000	0.000
276	WB8	W	64.000	3.000	0.000	0.000
277	WB8	W	64.000	3.000	0.000	0.000
278	WB8	W	64.000	3.000	0.000	0.000
279	WB9	W	64.000	4.000	0.000	0.000
280	WB9	W	64.000	4.000	0.000	0.000
281	WB9	W	64.000	3.000	0.000	0.000

STATISTICA: GENERAL ANOVA/MANOVA 12-02-93 16:_____ _____ __

data file: CROSSPOL.STA [281 cases with 227 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
6	PERCENT	8.3	-9999	= survivor/germinat*100
2	NEWVAR	8.3	-9999	
3	DISTANCE	8.3	-9999	Distance (m) from central area

INDEPENDENT VARIABLES (between groups factors):

NEWVAR Number of Levels: 3 Codes: level 1: 100-G
level 2: 102-T
level 3: 103-W

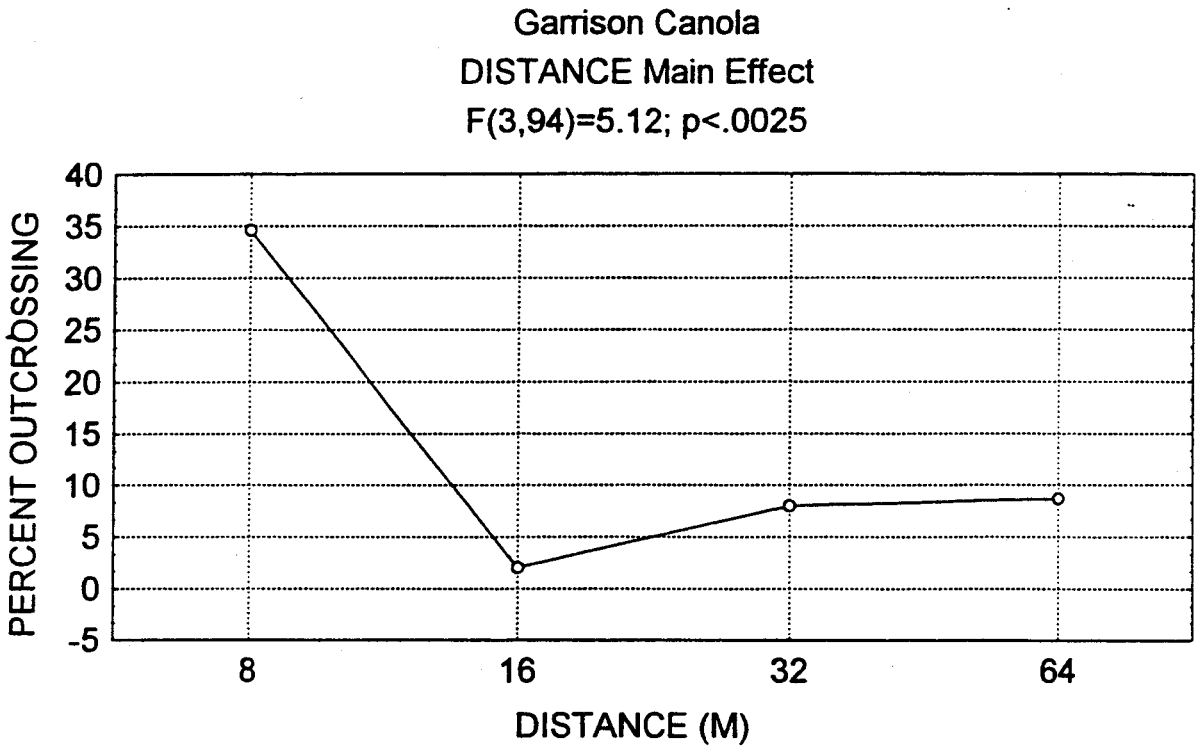
DISTANCE Number of Levels: 4 Codes: level 1: 8
level 2: 16
level 3: 32
level 4: 64

DESIGN: 2 - way ANOVA, fixed effects
DEPENDENT: 1 variable: PERCENT
BETWEEN: 1-NEWVAR (3): G T W
2-DISTANCE(4): 8 16 32 64
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (crosspol.sta) 1-NEWVAR, 2-DISTANCE					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2*	5661.678*	268*	365.3092*	15.49832*	.000000*
2	3*	1659.194*	268*	365.3092*	4.54189*	.004002*
12	6*	1717.961*	268*	365.3092*	4.70276*	.000143*

STAT. GENERAL MANOVA		Means (crosspol.sta) F(2,268)=15.50; p<.0000
NEWVAR	DISTANCE	PERCENT
G	....	13.34859
T	....	0.00000
W	....	0.00000

STAT. GENERAL MANOVA	Summary of all Effects; design: (crosspol.sta) 1-DISTANCE					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	3*	5333.401*	94*	1041.520*	5.120788*	.002520*


Garrison Canola
DISTANCE Main Effect
F(3,94)=5.12; p<.0025
PERCENT OUTCROSSING
40
35
30
25
20
15
10
5
0
-5
8
16
32
64
DISTANCE (M)

Hoechst

Title

Assessment of Outcrossing of Glufosinate-Tolerant Canola to Related Species

Author

Robert L. MacDonald
Hoechst Canada Inc.
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Regina, Saskatchewan
S4N 6C2

Report No.

HCI93-18

Date

November 22, 1993

Assessment of Outcrossing of Glufosinate-Tolerant Canola to Related Species

Hoechst

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I. INTRODUCTION

The production of genetically engineered plants requires an assessment of the risk of movement of genetic material into related species. This is particularily important where *Brassica* species related to canola exist as weeds in cultivated and non-cultivated areas. If such a transfer were possible, it could result in glufosinate-tolerant weed populations. Although *B. napus* is self compatible, cross pollination has been shown to account for anywhere from 5 to 95% of fertilization (1).

II. OBJECTIVE

The objective of this study was to determine the cross compatibility between glufosinate-tolerant *B. campestris, Sinapsis arvensis* L. and *B. napus* under controlled conditions to evaluate the possibility of "gene escapes" from glufosinate-tolerant *Brassica napus* into a relayed crop and one its weedy relatives.

III. MATERIALS AND METHODS

III.I Plant Materials

B. napus (n=19 AACC)* Glufosinate-tolerant spring canola line (HCN-92) developed by Hoechst Canada Inc. in Saskatoon, Saskatchewan, contains the phosphinothricin acetyl transferase (PAT) gene responsible for the degradation of the

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herbicide glufosinate ammonium.

Sinapsis arvensis L.(n=9 SS) Naturally occuring seed collected from 1991 field trialsconducted near Indian Head, Saskatchewan.

Brassica Campestris (n=10 AA) Commercially available spring turnip rape variety (Parkland) obtained from Saskatoon Research Station.

* n refers to chromosome number; A, C and S refer to genomic classification

III.II Growth of Plants

All species were seeded to a depth of one inch in greenhouse pots containing a soil-less media (Redi-Earth). Pots were saturated with water proir to planting. Drip irrigation was used twice daily for six minutes durations. Liquid NPK-fertilizer was supplied twice daily with the irrigation solution. Fertilizer was further supplemented with the addition of approximately 50 grams of 15-15-5 granular fertilizer. The greenhouse was maintained at 25/21^{o}C with an 18 hour photoperiod. Natural sunlight was supplemented by high pressure sodium lamps.

III.III Cross Combinations

There were in total, 108 crosses among (54) *B. napus* (HCN-92) x *S. arvensis* ,and reciprocal (27) *B.napus* (HCN-92) x *B. napus* (Legend), and (27) *B. napus* (HCN-92) x *B. campestris*. HCN-92 was used as the pollen donor for all crosses except

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with *S. arvensis* where the reciprocal was also examined. At flowering, the sepals and petals were removed from buds which were nearing maturity but had not opened. The buds were emasculated using sterilized tweezers. The stigma was immediately hand pollinated using a fresh flower from the male plant. The bud was then tagged and covered with a bag to exclude foreign pollen. Seed was harvested at maturity.

III.IV Determination of Gene Transfer

Plants from handcrossed seed was planted and grown under greenhouse conditions as described above. The 3 - 5 leaf stage (approximately 21 days after seeding) plants were treated with 750 g ai/ha of glufosinate ammonium Harvest 150 SN. Treated plants were evaluated as either living or dead 14 days after treatment.

IV. RESULTS AND DISCUSSION

Two Types of Species Crossed	Bud Pollinated	Planted	Herbicide Tolerant Progeny
B. napus (HCN-92) x *S. arvensis*	27	12	0
S. arvensis x *B. napus*(HCN-92)	27	4	0

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B. napus x *B. napus*(HCN-92)	27	36	36
B. campestris x *B. napus*(HCN-92)	27	27	0

IV.I Controlled crosses between *B. napus*(T) and *S. arvensis*

From the 54 bud pollinated stigmas of *B. napus* and *S. arvensis* crosses, a total of 16 seeds were obtained. Of the seeds sown, only one F1 plant was produced and it was completely desiccated with a 750 g ai/ha application of glufosinate ammonium indicating that the PAT gene was not transferred. The F1 plant's morphology was typical of *S. arvensis* the maternal parent

IV.II Controlled Crosses Between *B. napus* (T) and *B. napus* (Legend)

A total of 27 buds were pollinated using *B. napus* (T), transgenic as the male. Seed set was very high. All seed produced plants which were tolerant to the herbicide application. Injury from the application was limited to chlorosis on leaf margins. The high level of tolerance displayed by the F1 generation demonstrates the dominant characteristic of the herbicide tolerance trait.

IV.III Controlled Crosses Between *B. napus* and *B. campestris*

A total of 27 buds of *B. campestris* were pollinated with the pollen of *B. napus* (Transgenic). Greater than 200 seeds were produced. Germination of the seed was

quite poor with only 30% of seeds producing plants. Plants were not vigorous in their growth and were completely dessicated by the application of 750 g ai/ha of glufosinate ammonium. The morphology of the reared plants was indicative of *B. campestris*.

Therefore, in our study *B. campestris* and *B. napus* were not highly cross compatible as no tolerant crosses were observed. Our findings are not in agreement with the findings of Mackay (1) who determined the cross compatibilities of the two species to be as high as 75%. The low compatibility observed in the present study may be attributed to the selection of buds which were too advanced and the stigmas were no longer receptive to foreign pollen. Emasculation of the fertilized flowers may have interfered with seed development resulting in poor quality seed.

V. CONCLUSION

The probability of transfer of the glufosinate tolerance gene to other cultivars of *B. napus* is possible as it is expressed as a dominant trait. All artificial crosses between Legend and HCN-92 resulted in herbicide tolerant progeny. The risk of transfer of glufosinate tolerance to the related weedy species *S. arvensis* without the use of embryo rescue is negligible as no crosses between the two species resulted in herbicide tolerant progeny. Although our results suggest that the risk of outcrossing to *B. campestris* is limited, the literature suggests that the two species are cross compatible.

Assessment of Outcrossing of Glufosinate-Tolerant Canola to Related Species

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VI. REFERENCES

Mackay, G.R, 1977, The Introgression of S. alles into forage rape *Brassica napus*L from turnip *Brassica campestris* L.ssp. *rapefera* Euphytica 21:71 - 77

Hoechst

Title

Assessment of Volunteer Glufosinate-Tolerant Canola Under Chemical Fallow Conditions

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Report No.

HCI93-04

Date

November 4, 1993

Assessment of Volunteer Glufosinate-Tolerant Canola Under Chemical Fallow Conditions

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Assessment of Volunteer Glufosinate-Tolerant Canola Under Chemical Fallow Conditions

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I. INTRODUCTION

Canola acreages have expanded dramatically in recent years as a result of an increased world demand for seed, oil and meal. In 1993, canola acreages in Canada grew to over 4 million hectares. (1).

Weeds are one of the most limiting factors in canola production. Canola is not a strong competitor especially if weeds emerge before the crop. In Western Canada, the estimated loss from potential canola production due to weeds range from 10 to 13 % (2). Some weeds are very difficult to control because they are closely related to canola or because no effective herbicide is available.

Recent advances in tissue culture and transformation technologies for *Brassica napus* have allowed breeders to introduce novel traits to cultivars for plant improvement. These advances have allowed for the development of a *B. napus* line (HCN92) that is tolerant to the non-selective herbicide glufosinate ammonium. This highly effective herbicide is rapidly biodegraded to a non-toxic metabolite in tolerant plants.

II. OBJECTIVE

A recurring question regarding engineered crops is, do transgenic plants suffer any undesired effects as a consequence of the genetic modification? Concern has been raised as to the potential risk for a glufosinate-tolerant crop becoming a weedy pest. The objective of this study was to both assess the weediness of glufosinate-tolerant canola, and to determine it's susceptibility to other commercial herbicides under

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chemical fallow conditions. The behaviour of glufosinate tolerant canola HCN92 was contrasted to commercial non-transgenic varieties.

III. MATERIALS AND METHODS

In 1993, a field study was established at Indian Head, Saskatchewan on the exact area where the 1992 Pre-Coop Transgenic study planted to HCN92 was previously located. This site had not been cultivated in the fall of 1992 so the precise location of each plot was easily established. The same randomization scheme was maintained from the previous season (Table 1.). Wooden plot stakes were positioned in the left corner of each of the plots and marked with the plot number in indelible marker. The site was neither cultivated nor fertilized in the spring of 1993.

Herbicide applications were made using a water volume of 110 L/ha with a CO_2 backpack sprayer pressurized at 275 kPa via 110° flat fan nozzles. The 2 m boom was maintained at approximately 50 cm above the soil surface.

On June 3, plots were treated with a single application of glufosinate-ammonium at 750 g ai/ha to ensure the removal of any emerged weeds and non-transgenic canola. Plant counts (2 x ¼ m^2) were collected prior to and 15 days after the application.

On June 28, the entire site received a chemical-fallow treatment of ROUNDUP (glyphosate) at 356 g/ha tank mixed with 2,4-D amine at 600 g/ha. Plant counts were collected 10 days later.

Assessment of Volunteer Glufosinate-Tolerant
Canola Under Chemical Fallow Conditions

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A randomized complete block design was used in this study. An analysis of variance was conducted on all count data (Statistica/W ver. 4.2). Statistical significance was determined between the standard canola plots, untreated transgenic canola (HCN92) plots and transgenic canola (HCN92) plots sprayed with glufosinate ammonium in 1992. All effects were tested with a probability of $P < 0.05$.

Table 1. Treatment list for the 1992 Pre-Coop Sites.

TREATMENTS	APPLICATION	REPLICATES			
		1	2	3	4
Guard Plot B. napus	Non Sprayed	100	200	300	400
Westar - B. napus	Non Sprayed	101	205	306	404
Transgenic - HCN92	Non Sprayed	102	203	302	408
Transgenic - HCN92	Glufosinate @ 750 g	103	201	301	410
Legend - B. napus	Non Sprayed	104	213	304	415
Transgenic - HCN92	Non Sprayed	105	211	303	405
Transgenic - HCN92	Glufosinate @ 750 g	106	217	305	401
Delta - B. napus	Non Sprayed	107	207	312	417
Transgenic - HCN92	Non Sprayed	108	216	308	411
Transgenic - HCN92	Glufosinate @ 750 g	109	210	313	414
Profit - B. napus	Non Sprayed	110	218	315	413
Transgenic - HCN92	Non Sprayed	111	208	314	418
Transgenic - HCN92	Glufosinate @ 750 g	112	214	317	409
Excel - B. napus	Non Sprayed	113	215	316	416

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TREATMENTS	APPLICATION	REPLICATES			
		1	2	3	4
Transgenic - HCN92	Non Sprayed	114	212	309	406
Transgenic - HCN92	Glufosinate @ 750 g	115	204	318	402
Cyclone - B. napus	Non Sprayed	116	206	307	407
Tristar - TTC napus	Non Sprayed	117	209	311	412
Tristar - TTC napus	Sprayed Poast/Bladex	118	202	310	403
Guard Plot - Standard Napus	Non Sprayed	119	219	319	419

Note: All plots received a pre-emergent treatment of trifluralin at recommended rates in the spring of 1992.

IV. RESULTS AND DISCUSSION

All field data and statistical analysis are summarized in Appendix I. Mean plant counts has been transformed to a m^2 basis (Table 2).

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Table 2. Mean plant counts (#/m²) prior to and following a 1993 application of glufosinate-ammonium and glyphosate/2,4-D Amine for Standard Canola, Treated and Untreated Transgenic Canola.

Treatment (1993)	Standard Canola	Transgenic Canola (untreated)	Transgenic Canola (Treated)
Pre-Spray	212	235	257
Post-Glufosinate	19* (91%)	157 (33%)	161 (36%)
Post-Glyphosate/2,4-D	0.7 (0.3%)	0.2 (0.1)	0.1 (0.03%)

* Indicates significant difference where $p < 0.05$

Plant counts collected prior to the 1993 application of glufosinate ammonium were not significantly different among the transgenic and non-transgenic plots and ranged between 212- 257 per m². This indicates that volunteer plant populations were equivalent when transgenic and non-transgenic plots were compared.

Non-transgenic canola plants were very susceptible to glufosinate ammonium and were controlled (90%) in the study area after the 1993 application of glufosinate ammonium. Mean plant counts collected 15 DAT were 157, 161 and 19 per m² for untreated HCN92, treated HCN92 and standard canola plots, respectively (Plate 1.). As a result, volunteer transgenic and non-transgenic plants were associated with their respective plots.

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Tolerance to glufosinate ammonium was expressed in a high number of plants that emerged in the following year. Visual observations indicated that these volunteer, transgenic canola plants were healthy and vigorously growing (Plate 2.). However, not all canola plants in transgenic plots were tolerant to glufosinate ammonium. Plant counts in the transgenic canola plots were reduced by approximately 33% compared with the pre-spray counts.

The treatment of glyphosate/2,4-D amine provided excellent (99-100%) weed control. Plant counts collected 10 days after the chemical fallow treatment were below 1 per m^2 for all three treatments. This indicates that glufosinate-tolerant canola plants were controlled by the traditional chemical fallow treatment.

The survival of less than 1% of the population could likely be attributed to a lack of spray coverage on the target or on the emergence of the plants after the herbicide application as their is no residual activity.

V. CONCLUSION

Tolerance to the herbicide glufosinate ammonium can be transferred and expressed in volunteer, transgenic-canola plants that emerge in the following growing season. An application of glyphosate and 2,4-D amine, at recommended rates as a chemical fallow application, provided excellent control of volunteer, glufosinate-tolerant canola plants. This study confirms that glufosinate-tolerant canola plants are susceptible to other herbicidal active ingredients with different modes of action. Therefore, glufosinate-tolerant canola plants do not exhibit characteristics which would lead them

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to become a weedy pest under chemical fallow.

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VI. FIGURES

Plate 1 **Transgenic and non-transgenic canola plots after appliction of glufosinate ammonium at 750 g ai/ha.**

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Plate 2 **HCN92 canola stand 15 days after application of glufosinate ammonium at 750 g ai/ha.**

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VII. APPENDIX

STATISTICA: DATA MANAGEMENT

data file: IHEADCNT.STA [160 cases with 6 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
1	VARIETY	8.0	0	The variety of crop planted.
2	TREAT	8.0	0	1=transgenic;untreat, 2=Transgenic;treat, 3=Std.;untreat
3	PLOT	8.0	0	Plot label.
4	COUNT_1	8.0	-1	Plant count taken on chemfallow, May 26, 1993
5	COUNT_2	8.0	-1	Plant count taken on chemfallow, June 18, 1993.
6	COUNT_3	8.0	-1	Plant count taken on chemfallow , July 8, 1993.

STATISTICA: GENERAL ANOVA/MANOVA

data file: IHEADCNT.STA [160 cases with 6 variables]

0 CASE	1 VARIETY	2 TREAT	3 PLOT	4 COUNT_1	5 COUNT_2	6 COUNT_3
1	STANDARD	3	100	71	4	0
2	STANDARD	3	100	46	1	0
3	STANDARD	3	101	92	2	0
4	STANDARD	3	101	56	3	0
5	TRANSGEN	1	102	115	68	0
6	TRANSGEN	1	102	80	51	0
7	TRANSGEN	2	103	69	40	0
8	TRANSGEN	2	103	64	56	0
9	STANDARD	3	104	75	1	0
10	STANDARD	3	104	84	6	0
11	TRANSGEN	1	105	75	69	0
12	TRANSGEN	1	105	112	77	0
13	TRANSGEN	2	106	88	48	0
14	TRANSGEN	2	106	130	56	0
15	STANDARD	3	107	67	11	0
16	STANDARD	3	107	60	8	0
17	TRANSGEN	1	108	107	37	0
18	TRANSGEN	1	108	54	49	0
19	TRANSGEN	2	109	86	63	0
20	TRANSGEN	2	109	94	40	0
21	STANDARD	3	110	58	6	0
22	STANDARD	3	110	94	3	0
23	TRANSGEN	1	111	63	27	0
24	TRANSGEN	1	111	50	33	0
25	TRANSGEN	2	112	40	46	0
26	TRANSGEN	2	112	69	69	0
27	STANDARD	3	113	51	3	0
28	STANDARD	3	113	85	7	0
29	TRANSGEN	1	114	53	58	0
30	TRANSGEN	1	114	85	31	0
3	TRANSGEN	2	115	0	1	0
32	TRANSGEN	2	115	0	1	0
33	STANDARD	3	116	2	4	0
34	STANDARD	3	116	1	0	0
35	STANDARD	3	117	17	3	0
36	STANDARD	3	117	6	1	0
37	STANDARD	3	118	6	0	0
38	STANDARD	3	118	14	0	0
39	STANDARD	3	119	37	3	0
40	STANDARD	3	119	120	4	0
41	STANDARD	3	200	101	6	0
42	STANDARD	3	200	96	3	0
43	TRANSGEN	2	201	110	35	0
44	TRANSGEN	2	201	74	49	0
45	STANDARD	3	202	60	3	0
46	STANDARD	3	202	10	1	0
47	TRANSGEN	1	203	69	41	0
48	TRANSGEN	1	203	27	69	0
49	TRANSGEN	2	204	78	38	0
50	TRANSGEN	2	204	53	37	0
51	STANDARD	3	205	48	7	0
52	STANDARD	3	205	71	6	0
.	STANDARD	3	206	45	3	0
54	STANDARD	3	206	49	3	0
55	STANDARD	3	207	26	9	0
56	STANDARD	3	207	78	6	0
57	TRANSGEN	1	208	61	42	0

STATISTICA: GENERAL ANOVA/MANOVA

0 CASE	1 VARIETY	2 TREAT	3 PLOT	4 COUNT_1	5 COUNT_2	6 COUNT_3
58	TRANSGEN	1	208	58	29	0
59	STANDARD	3	209	57	12	0
60	STANDARD	3	209	7	7	0
61	TRANSGEN	2	210	62	48	0
62	TRANSGEN	2	210	68	38	0
63	TRANSGEN	1	211	79	46	2
64	TRANSGEN	1	211	45	34	0
65	TRANSGEN	1	212	60	27	0
66	TRANSGEN	1	212	79	20	1
67	STANDARD	3	213	53	6	0
68	STANDARD	3	213	54	4	0
69	TRANSGEN	2	214	68	52	0
70	TRANSGEN	2	214	62	28	0
71	STANDARD	3	215	75	11	0
72	STANDARD	3	215	66	2	0
73	TRANSGEN	1	216	56	66	0
74	TRANSGEN	1	216	25	30	0
75	TRANSGEN	2	217	85	28	2
76	TRANSGEN	2	217	76	36	0
77	STANDARD	3	218	97	7	0
78	STANDARD	3	218	84	4	0
79	STANDARD	3	219	108	5	0
80	STANDARD	3	219	134	2	0
81	STANDARD	3	300	111	3	0
82	STANDARD	3	300	65	3	0
83	TRANSGEN	2	301	88	36	0
84	TRANSGEN	2	301	86	31	0
85	TRANSGEN	1	302	59	31	0
86	TRANSGEN	1	302	33	40	0
87	TRANSGEN	1	303	68	39	0
88	TRANSGEN	1	303	52	29	0
89	STANDARD	3	304	51	9	1
90	STANDARD	3	304	30	4	0
91	TRANSGEN	2	305	63	45	0
92	TRANSGEN	2	305	23	25	0
93	STANDARD	3	306	29	3	0
94	STANDARD	3	306	4	2	0
95	STANDARD	3	307	47	3	0
96	STANDARD	3	307	13	3	0
97	TRANSGEN	1	308	45	29	0
98	TRANSGEN	1	308	30	33	0
99	TRANSGEN	1	309	20	20	0
100	TRANSGEN	1	309	43	27	0
101	STANDARD	3	310	21	2	0
102	STANDARD	3	310	11	2	0
103	STANDARD	3	311	19	9	0
104	STANDARD	3	311	18	2	0
105	STANDARD	3	312	43	4	0
106	STANDARD	3	312	27	8	0
107	TRANSGEN	2	313	27	29	0
108	TRANSGEN	2	313	60	37	0
019	TRANSGEN	1	314	45	32	0
110	TRANSGEN	1	314	91	46	0
111	STANDARD	3	315	51	2	0
112	STANDARD	3	315	67	7	0
113	STANDARD	3	316	59	18	0
114	STANDARD	3	316	45	22	0
15	TRANSGEN	2	317	44	40	0
116	TRANSGEN	2	317	58	31	0
117	TRANSGEN	2	318	26	20	0
118	TRANSGEN	2	318	58	19	0
119	STANDARD	3	319	103	1	0

STATISTICA: GENERAL ANOVA/MANOVA

0 CASE	1 VARIETY	2 TREAT	3 PLOT	4 COUNT_1	5 COUNT_2	6 COUNT_3
120	STANDARD	3	319	82	3	0
121	STANDARD	3	400	82	1	1
122	STANDARD	3	400	24	3	0
123	TRANSGEN	2	401	101	47	0
124	TRANSGEN	2	401	69	70	0
125	TRANSGEN	2	402	100	56	0
126	TRANSGEN	2	402	67	35	0
127	STANDARD	3	403	39	4	0
128	STANDARD	3	403	43	2	0
129	STANDARD	3	404	77	12	0
130	STANDARD	3	404	33	5	0
131	TRANSGEN	1	405	64	42	0
132	TRANSGEN	1	405	77	48	0
133	TRANSGEN	1	406	29	31	0
134	TRANSGEN	1	406	92	39	0
135	STANDARD	3	407	78	1	0
136	STANDARD	3	407	49	6	0
137	TRANSGEN	1	408	8	22	4
138	TRANSGEN	1	408	23	24	0
139	TRANSGEN	2	409	72	32	0
140	TRANSGEN	2	409	33	57	0
141	TRANSGEN	2	410	57	43	0
142	TRANSGEN	2	410	37	40	0
143	TRANSGEN	1	411	15	29	0
144	TRANSGEN	1	411	34	22	0
145	STANDARD	3	412	41	4	0
146	STANDARD	3	412	10	4	0
147	STANDARD	3	413	57	4	0
148	STANDARD	3	413	18	6	0
149	TRANSGEN	2	414	66	46	0
150	TRANSGEN	2	414	59	59	0
151	STANDARD	3	415	55	10	0
152	STANDARD	3	415	91	5	0
153	STANDARD	3	416	74	5	0
154	STANDARD	3	416	79	3	0
155	STANDARD	3	417	69	14	0
156	STANDARD	3	417	38	4	0
157	TRANSGEN	1	418	71	27	0
158	TRANSGEN	1	418	97	52	0
159	STANDARD	3	419	20	1	0
160	STANDARD	3	419	14	1	0

STATISTICA: GENERAL ANOVA/MANOVA

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
4	COUNT_1	8.0	-9999	First plant count taken on the Chemfallow study at Indian
5	COUNT_2	8.0	-9999	Second plant count taken on the Chemfallow st udy at Indi
2	TREAT	8.0	-9999	The treatment the plot received.

INDEPENDENT VARIABLES (between groups factors):

TREAT Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 1 - way MANOVA, fixed effects
DEPENDENT: 2 variables: COUNT_1 COUNT_2
BETWEEN: 1-TREAT (3): 1 2 3
WITHIN: none

data file: IHEADCNT.STA [160 cases with 6 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
4	COUNT_1	8.0	-9999	First plant count taken on the Chemfallow study at Indian
2	TREAT	8.0	-9999	The treatment the plot received.

INDEPENDENT VARIABLES (between groups factors):

TREAT Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 1 - way ANOVA, fixed effects
DEPENDENT: 1 variable: COUNT_1
BETWEEN: 1-TREAT (3): 1 2 3
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (iheadcnt.sta) 1-TREAT					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	1712.259	157	857.2909	1.997291	.139136

STATISTICA: GENERAL ANOVA/MANOVA

STAT. GENERAL MANOVA	Means (iheadcnt.sta) F(2,157)=2.00; p<.1391
TREAT	COUNT_1
1	58.72500
2	64.25000
3	53.10000

STATISTICA: GENERAL ANOVA/MANOVA

data file: IHEADCNT.STA [160 cases with 6 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
5	COUNT_2	8.0	-9999	Second plant count taken on the Chemfallow st udy at Indi
2	TREAT	8.0	-9999	The treatment the plot received.

INDEPENDENT VARIABLES (between groups factors):

TREAT Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 1 - way ANOVA, fixed effects
DEPENDENT: 1 variable: COUNT_2
BETWEEN: 1-TREAT (3): 1 2 3
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (iheadcnt.sta) 1-TREAT					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2*	24353.26*	157*	120.7823*	201.6293*	0.00*

STATISTICA: GENERAL ANOVA/MANOVA

STAT. GENERAL MANOVA	Means (iheadcnt.sta) F(2,157)=201.63; p<0.000
TREAT	COUNT_2
1	39.15000
2	40.17500
3	4.77500

STATISTICA: GENERAL ANOVA/MANOVA

data file: IHEADCNT.STA [160 cases with 6 variables]

VARIABLE SPECIFICATIONS:

No	Name	Format	MD Code	Long Label
6	COUNT_3	8.0	-9999	The third plant count taken for the Chemfallow study ot I
2	TREAT	8.0	-9999	The treatment the plot received.

INDEPENDENT VARIABLES (between groups factors):

TREAT Number of Levels: 3 Codes: level 1: 1
level 2: 2
level 3: 3

DESIGN: 1 - way ANOVA, fixed effects
DEPENDENT: 1 variable: COUNT_3
BETWEEN: 1-TREAT (3): 1 2 3
WITHIN: none

STAT. GENERAL MANOVA	Summary of all Effects; design: (iheadcnt.sta) 1-TREAT					
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	.309375	157	.163217	1.895488	.153667

STATISTICA: GENERAL ANOVA/MANOVA

STAT. GENERAL MANOVA	Means (iheadcnt.sta) F(2,157)=1.90; p<.1537
TREAT	COUNT_3
1	.175000
2	.050000
3	.025000

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Hoechst

Title

Residual Effects of Glufosinate-Tolerant Canola on the Growth and Productivity of Grain, Forage and Pulse Crops

Authors

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Report No.

HCI93-02

Date

November 30, 1993

Residual Effects of Glufosinate Tolerant Canola on the Growth and Productivity of Grain, Forage, and Pulse Crops

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Residual Effects of Glufosinate Tolerant Canola on the Growth and Productivity of Grain, Forage, and Pulse Crops

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Abstract

Residual effects of glufosinate-tolerant canola (HCN92) on soil productivity was assessed by comparing the performance of a number of typical rotational crops (ie. grains, forage and pulse) at locations where transgenic and non-transgenic plants were previuosly grown (HCI93-02). Crop vigour, growth and yeild were used as indicators of the productivity of the soils.

The effect of transgenic canola residue on agronomic performance was examined for wheat, barley, lentils, peas, flax and alfalfa. In 1993, plant counts, mid-season biomass and yeild were measured at Edgeley and Rosthern, Saskatchewan and Rosebank, Manitoba. Although some differences were statistically significant on an individual location basis, these effects were not consistent across the three study locations. Performance differences in Manitoba were attributed to the cool, wet growing season; in Saskatchewan, excessive weed pressure at early stages of development, particularly in the less competitive crops (ie. flax), reduced productivity. Therefore, results from these trials indicate no residual effect of transgenic canola on rotational crops grown in the following year.

Residual Effects of Glufosinate Tolerant Canola on the Growth and Productivity of Grain, Forage, and Pulse Crops

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HCI93-02
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I. INTRODUCTION

Canola acreage has expanded dramatically in recent years as a result of an increased world demand for its seed, oil and meal. In 1993, canola acreage in Canada grew to over 4 million hectares. (1).

Weeds are one of the most limiting factors in canola production. Canola is not a strong competitor if weeds emerge before the crop. In Western Canada, the estimated loss from potential canola production due to weeds range from 10 to 13 % (2). Weeds that are closely related to canola are particularly difficult to control in canola because no effective herbicide is available.

Recent advances in tissue culture and transformation technologies for *Brassica napus* have allowed breeders to introduce novel traits to cultivars for plant improvement. These advances have allowed for the development of a *B. napus* line (HCN-92) that is tolerant to the non-selective herbicide glufosinate ammonium. This herbicide is rapidly biodegraded to a non-toxic metabolite in tolerant plants.

The application of this new technology has raised questions regarding the impact of environmental releases of transgenic plants. Residual effects of transgenic plants on soil productivity can be addressed by comparing a number of typical rotational crops (ie. grains, forage and pulses) at locations where transgenic and non-transgenic plants were grown.

Residual Effects of Glufosinate Tolerant Canola on the Growth and Productivity of Grain, Forage, and Pulse Crops

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Canada S4N 6C2

HCI93-02
October 28, 1993

II. OBJECTIVE

The objective of this study was to determine the residual effects of glufosinate-tolerant canola (HCN-92) on typical rotational crops grown in the following year. Plant performance was evaluated in the plots which previously grew transgenic canola and non-transgenic canola varieties. Crop vigour, growth and yield were used as indicators of the productivity of the soils.

III. MATERIALS AND METHODS

Trials were conducted in 1993 on the 1992 Pre-Coop and Weed-Control sites at the following locations:

A. Edgeley, Saskatchewan
B. Rosthern, Saskatchewan
C. Rosebank, Manitoba

The Pre-coop and weed control sites contained plots of both transgenic (HCN92) and non-transgenic canola varieties. The locations of these plots were randomized across the trial area. Plots were identified using the same randomization scheme as the previous season (Figures A and B). Stakes were located in front left corner of each of the plots and marked with the plot number in indelible marker. Prior to seeding, each site was cultivated in one direction along the length of the plots. A press or double disc seed drill was used to plant each of the rotational crops. All crops were seeded separately at recommended rates across each plot. Wheat, barley and flax were seeded as a 1.2 m wide strip, whereas the

lentils, peas and alfalfa were seeded as a 2.4 m wide strip. The crops were seeded across each of the replicates of the trials (Figure A and B).

Table 1 Treatment list for the 1992 Pre-Coop Sites

TREATMENTS	APPLICATION	REPLICATES			
		1	2	3	4
Guard Plot B. napus	Non Sprayed	100	200	300	400
Westar - B. napus	Non Sprayed	101	205	306	404
Transgenic - B. napus	Non Sprayed	102	203	302	408
Transgenic - B.napus	Glufosinate @ 750 g	103	201	301	410
Legend - B. napus	Non Sprayed	104	213	304	415
Transgenic - B. napus	Non Sprayed	105	211	303	405
Transgenic - B. napus	Glufosinate @ 750 g	106	217	305	401
Delta - B. napus	Non Sprayed	107	207	312	417
Transgenic - B. napus	Non Sprayed	108	216	308	411
Transgenic - B. napus	Glufosinate @ 750 g	109	210	313	414
Profit - B. napus	Non Sprayed	110	218	315	413
Transgenic - B. napus	Non Sprayed	111	208	314	418
Transgenic - B. napus	Glufosinate @ 750 g	112	214	317	409
Excel - B. napus	Non Sprayed	113	215	316	416
Transgenic - B. napus	Non Sprayed	114	212	309	406
Transgenic - B. napus	Glufosinate @ 750 g	115	204	318	402
Cyclone - B. napus	Non Sprayed	116	206	307	407
Tristar - TTC napus	Non Sprayed	117	209	311	412
Tristar - TTC napus	Sprayed Poast/Bladex	118	202	310	403
Guard Plot - Standard Napus	Non Sprayed	119	219	319	419

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Note: All plots received a pre-emergent treatment of trifluralin at recommended rates in the spring of 1992.

Table 2 Treatment list for the 1992 Weed Control Sites.

TREATMENTS	APPLICATION	REPLICATES			
		1	2	3	4
Guard Plot B. napus	Non Sprayed	100	200	300	400
Standard - B. napus	Non Sprayed	101	210	306	407
Transgenic - B. napus	Trifluralin	102	207	316	404
Transgenic - B.napus	Glufosinate @ 200 g X1	103	211	314	409
Standard - B. napus	Glufosinate @ 200 g X2	104	215	313	403
Transgenic - B. napus	Glufosinate @ 300 g X1	105	216	310	405
Transgenic - B. napus	Glufosinate @ 300 g X2	106	206	309	410
Transgenic- B. napus	Glufosinate @ 400 g X1	107	203	304	411
Transgenic - B. napus	Glufosinate @ 400 g X2	108	214	301	408
Transgenic - B. napus	Non Sprayed	109	202	307	412
Transgenic - B. napus	Glufosinate @ 500 g X2	110	201	308	413
Transgenic - B. napus	Glufosinate @ 600 g X1	111	209	303	413
Transgenic - B. napus	Glufosinate @ 700 g X1	112	204	302	416
Transgenic - B. napus	Glufosinate @ 800 g X1	113	205	315	402
Transgenic - B. napus	Glufosinate @ 1000 g X1	114	213	312	414
Transgenic - B. napus	Glufosinate @ 2000 g X1	115	212	305	415
Tristar - TTC napus	Sprayed Poast/Bladex	116	208	311	401
Guard Standard napus	Non Sprayed	117	217	317	417

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III.I Edgeley, Saskatchewan

On May 13, 1993, both the Pre-Coop and Weed Control sites were pre-worked and seeded to Katepwa wheat (@ 90 kg/ha), Harrington barley (@ 80 kg/ha), Vimy flax (@ 30 kg/ha) and Laird lentils (@ 60 kg/ha) with a double-disc press drill. Monoammonium phosphate (11-51-0) was seed placed at 80 kg/ha. Alfalfa (Canada No.1) was broadcast at 8 kg/ha and harrowed into the surface layer on June 24, 1993. On June 3, a variety of herbicides were used to control both grassy and broadleaf weeds at the suggested leaf stages. Recommended rates of Achieve Extra® (1 kg/ha + 560 g/ha) was applied to the wheat, barley and flax crops; and, a split application of Poast® and Sencor® (184 g/ha + 275 g/ha) was applied to lentils. Broadleaf weeds had to be hand-rogued out of the lentil plots due to poor performance of the broadleaf herbicide. On September 9, 1993, both the lentils and alfalfa were sprayed with Reglone at the recommended rate (400 g/ha) to enhance maturity.

On July 20, plant counts and above-ground biomass were collected from 2 - ¼ m^2 quadrats per plot. Dry matter weights were determined by placing the biomass samples in a drying room at 60 °C for a minimum of 72 hours until samples reached a constant weight. On September 30, a small plot combine was used to collect wheat, barley, flax and lentil yields. On October 14, an alfalfa yield measurement was determined from the above-ground biomass collected from inside a 1 m^2 quadrat from each plot.

III.II Rosthern, Saskatchewan

On May 13, 1993, both the Pre-Coop and Weed Control sites were pre-worked and seeded to Katepwa wheat (@ 90 kg/ha), Harrington barley (@ 80 kg/ha), Vimy flax (@ 30 kg/ha), Tipu peas (@ 130 kg/ha) and Laird lentils (@ 60 kg/ha) with a double-disc press drill. Monoammonium phosphate (11-51-0) was seed placed at 80 kg/ha. A variety of herbicides were used to control both grassy and broadleaf weeds at the suggested leaf stages. The recommended rate of Achieve Extra® (1 kg/ha + 560 g/ha) was applied to wheat and barley; a tank mixture of Poast® + Buctril® M (184 g/ha + 560 g/ha) was applied to flax; and, a split application of Poast® and Sencor® (184 g/ha + 275 g/ha) was applied to peas and lentils.

On July 27, plant counts and above-ground biomass were collected from 2 - ¼ m^2 quadrats per plot. Dry matter weights were determined by placing the biomass samples in a drying room at 60 °C for a minimum of 72 hours. On September 22, a small plot combine was used to collect wheat and barley yields. Flax, lentil or pea yields could not be collected due to logistic difficulties.

III.III Rosebank, Manitoba

On May 11, 1993, both the Pre-Coop and Weed Control sites were pre-worked with a cultivator/mulcher and seeded to Katepwa wheat (@ 90 kg/ha), Bedford barley (@ 80 kg/ha), Norlin flax (@ 38 kg/ha), Titan peas (@ 130 kg/ha) and Eston lentils (@ 55 kg/ha) with a double-disc press drill. Monoammonium phosphate (11-51-0) was seed placed at 100 kg/ha. On June 3, a variety of herbicides were used to control both grassy and

broadleaf weeds at the suggested leaf stages. Recommended rates of Achieve Extra® (1 kg/ha + 560 g/ha) was applied to wheat, barley and flax crops; a split application of Poast® and Sencor® (184 g/ha + 275 g/ha) was applied to peas and lentils. Broadleaf weeds had to be hand-rogued due to the poor performance of the broadleaf herbicide.

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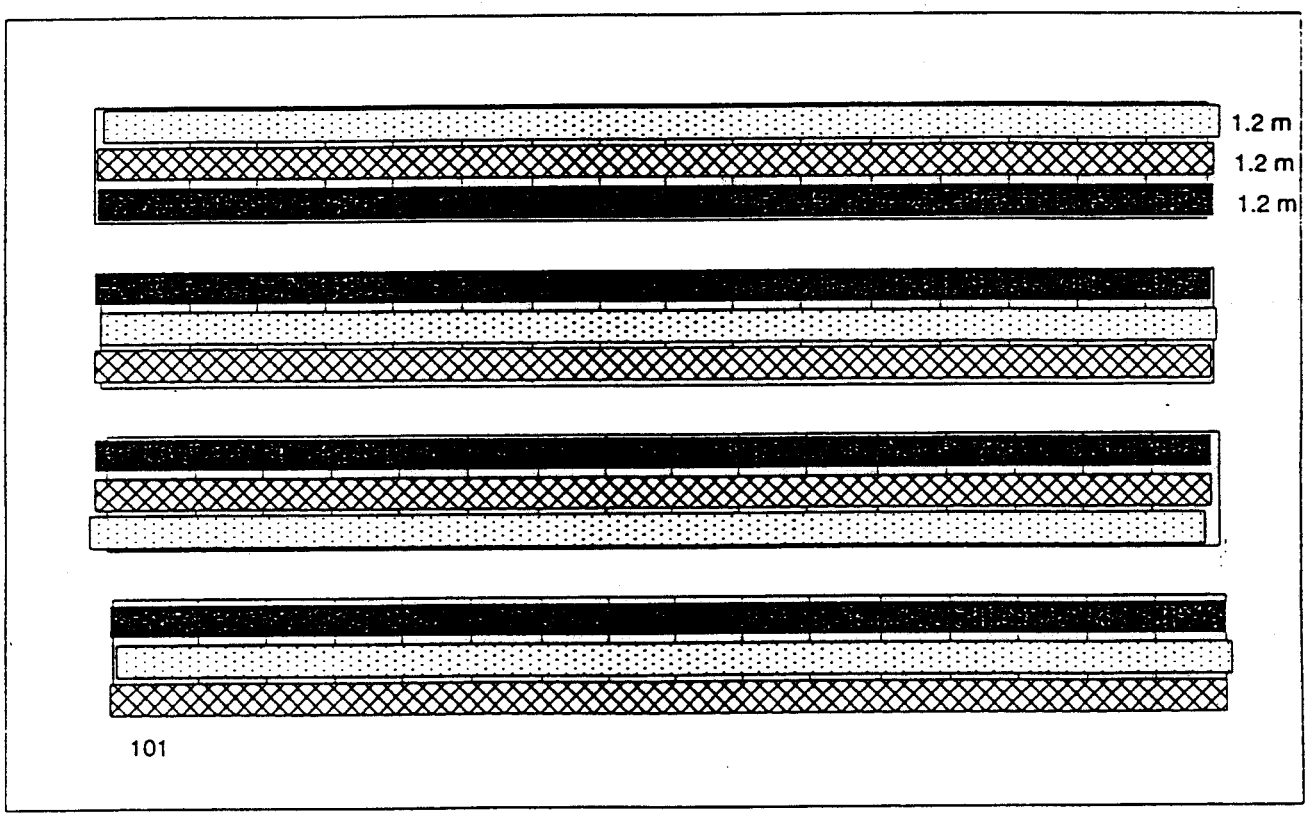
Figure A

1993 Recropping Evaluation



Flax Wheat Barley

Site of 1992 PreCoop Trials

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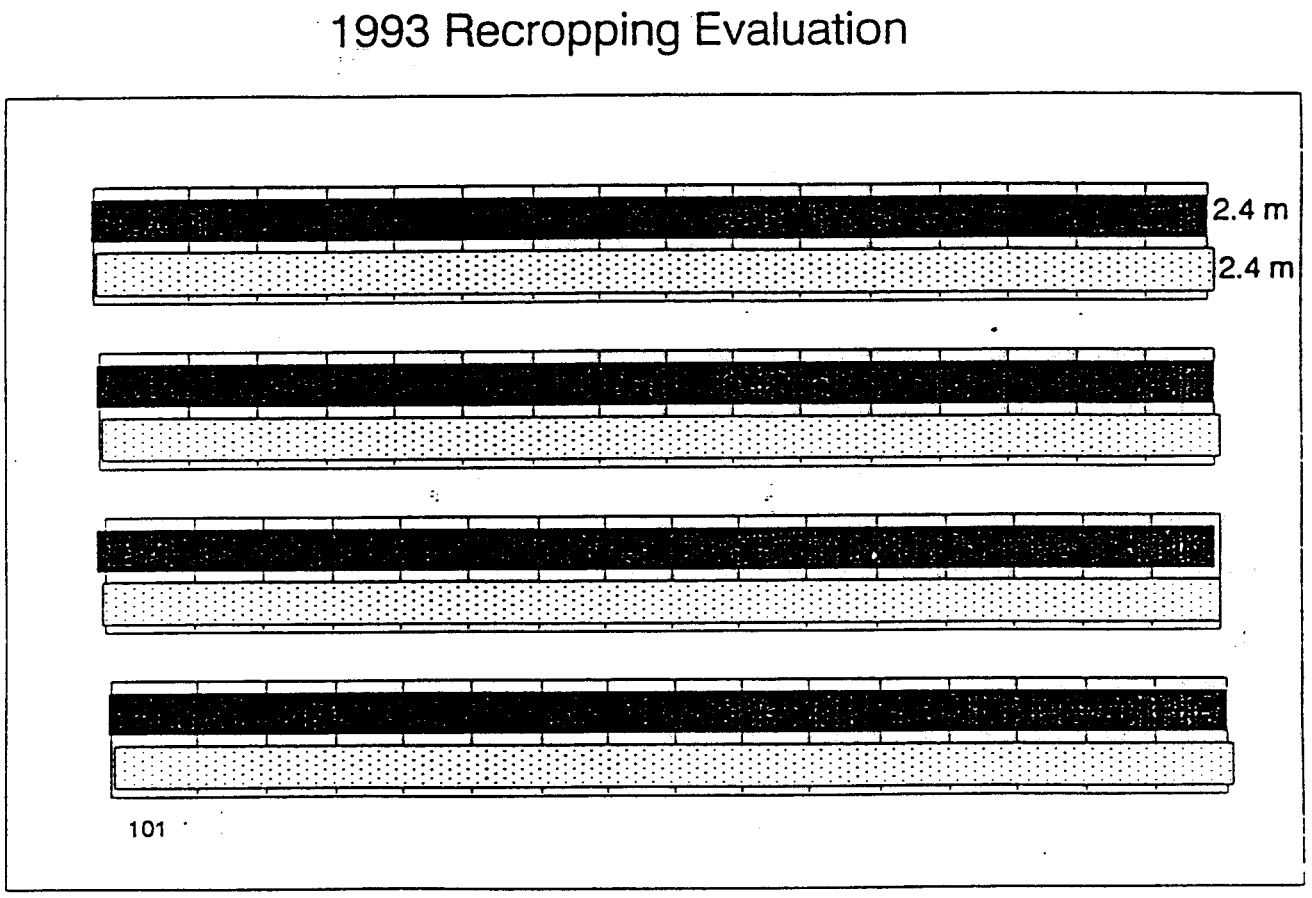
Figure B



Site of 1992 Weed Control Trials

On July 15, plant counts and above-ground biomass were collected from 2 - ¼ m^2 quadrats per plot. Dry matter weights were determined by placing the biomass samples in a drying room at 60 °C for a minimum of 72 hours. On September 28, a small plot combine was used to collect wheat, barley, flax, pea and lentil yields.

Population counts, dry weight measurements, and yield data were collected from each of the three study sites. The collected data was analyzed using a combination of multifactorial analysis with Statistica/W software. The confidence level 95% was used for all statistical comparisons.

IV. RESULTS AND DISCUSSION

Data is presented both in summary form (Table 3) and in raw data tables (Appendix I). Analysis of variance (ANOVA) tables and associated main effect means are presented in Appendix II.

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Table 3 Table of Means

Crop	Edgely		Rosthern		Rosebank	
Wheat	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	71.15	72.05	97.13	100.44	141.75	140.75
Dry weight	149.50	161.06	177.63	205.19	242.38	235.88
Yield	313.93	319.19	407.95	376.51	388.86	400.61
Barley	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	70.89	71.43	83.62	84.44	77.19	75.00
Dry weight	153.88	169.12	172.75	183.00	207.31	213.61
Yield	362.27	323.00	466.11	415.74	315.29	314.29
Flax	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	73.06	74.37	20.61	21.95	155.44	155.78
Dry weight	*91.88	*106.88	*80.13	*86.60	161.74	159.19
Yield	50.69	56.73	-	-	263.44	280.00
Lentils	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	39.00	36.75	20.75	12.50	79.50	76.25
Dry weight	107.88	112.43	97.75	100.75	196.45	195.78
Yield	276.80	301.98	-	-	29.88	35.08
Alfalfa	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	107.50	129.13	-	-	-	-
Dry weight	91.88	99.81	-	-	-	-
Yield	254.65	265.86	-	-	-	-
Peas	**S**	**T**	**S**	**T**	**S**	**T**
Plant count	-	-	*11.25	*6.33	13.75	13.38
Dry weight	-	-	101.88	104.31	205.78	216.64
Yield	-	-	-	-	131.08	122.69

* signifies a significant difference at 5% confidence level between the standard and transgenic canola
S = Standard Canola Variety ; T = Transgenic (HCN92) Canola

IV.I Edgeley, Saskatchewan

Each crop was examined and analyzed separately. There were no significant differences in counts, dry weights or grain yield for wheat, barley, lentils or alfalfa when grown on either transgenic or non-transgenic residue.

With the exception of flax dry weight, there were no significant differences in either plant counts or grain yield between the transgenic and non-transgenic plots. Flax dry weights were 91.9 and 106.9 g for non-transgenic and transgenic plots, respectively. The calculated p-level was 0.0041. Of the five crops, flax is the least competitive, particularly at early stages of development. Consequently its yield is most easily influenced by weed pressure. The small enhancement in yield associated with the transgenic plot may be attributed to superior weed control the previous season.

IV.II Rosthern, Saskatchewan

Results for each rotational crop were examined and analyzed separately. There were no significant differences ($p < 0.05$) in plant counts, dry weight or grain yield for wheat, barley and lentils (yields not taken) when grown on transgenic and non-transgenic residue.

Although no significant difference in flax counts was found, there was a significant difference in dry weights between transgenic and non-transgenic plots. However, less than ten percent in difference was observed.

A significant difference was found in pea counts between the transgenic ($25/m^2$) and non-transgenic plots ($45/m^2$). Although population counts were lower in the transgenic plots, biomass harvest was not significantly different between transgenic and non-transgenic plots.

IV.III Rosebank, Manitoba

When each crop was examined separately, there were no significant differences in plant counts, dry weights or yields for the wheat, barley, lentils, flax, or pea crops when grown on either transgenic or non-transgenic residue. Yields for all rotational crops at Rosebank were well below average. Manitoba received excessive moisture and below average temperatures for much of the growing season, consequently crops were highly stressed.

V. CONCLUSION

The effect of transgenic canola residue on agronomic performance was examined for wheat, barley, lentils, peas, flax and alfalfa. Although some differences were statistically significant, these effects were transient and were not consistently displayed across the three study areas. Therefore, results from these trials indicate no residual effects of transgenic canola on rotational crops the following season.

VII. APPENDIX I

Appendix I is available upon request. It is too voluminous to be included here.

VIII. APPENDIX II

Appendix II is available upon request. It is too voluminous to be included here.

VI. REFERENCES

1. Criterion. 1993. Canadian Farmers' Herbicide Use Study: Western Crop Herbicide Use Tabular Reports. Criterion Research Corp. Winnipeg, Manitoba, Canada.

2. Swanton, C.J., Harker, K.N. and Anderson, R.L. 1993. Crop losses due to weeds in Canada. Weed Technol. Vol. 7: 537-542.

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Title

Behaviour of Honey Bees Foraging on Transgenic Canola (Brassica napus L.)

Author

Murray Belyk and Robert MacDonald
Hoechst Canada Inc.
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Regina, Saskatchewan
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Report No.

HCI93-14

Date

November 16, 1993

Honey Bee Behaviour Study in Reference
to the Pollination of Transgenic Canola

Hoechst

Hoechst Canada Inc.
Agriculture Division
295 Henderson Drive
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I. INTRODUCTION

The recent development of transgenic canola has raised a number of questions as to the impact of the introduction of this plant on other organisms. Canola flowers produce an abundant supply of nectar for insect pollinators. Cross pollination of flowers often occurs when bees are searching for nectar. Because a genetic modification may elicit a biochemical change in plants, it is important to evaluate the impact of the transgenic on the crop on the behaviour of honey bees.

II. OBJECTIVE

The objective of the study is to evaluate the impact of transgenic canola on the behaviour of honey bees (Apis mellifera) under field conditions

III. MATERIALS AND METHODS

Study Site

The glufosinate tolerant canola line HCN92 was seeded in a 1.0 hectare (100 X 100 m) area near Indian Head, Saskatchewan. The seed was treated with Vitavax Plus prior to planting. The canola was seeded on May 11, 1993 with a double disc drill at a rate of 6 Kg/ha with 25 Kg/ha of 11-51-0-10 fertilizer. The site was treated with trifluralin at a rate

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of 1500 g/ha for preemergent weed control on May 5, 1993.

Honey Bees

An overwintered colony (approximately 100,000 bees) was split into two colonies each with a fresh queen on May 12, 1993. The colonies were transported from the apiary of Mr. Garnet Hall, Stoughton, Saskatchewan to Indian Head, Saskatchewan on June 25, 1993.

Both colonies were treated with terramycin for protection against foul brood just prior to transport. The hive's food supply was supplemented with 50:50 sugar-water mix using a hive entrance feeder. The sugar water was removed at the onset of flowering of the canola. The hive was positioned beside a Carigana hedge adjacent (within 10 m of the transgenic canola) to the 1.0 hectare stand of HCN92 canola. A pollen trap was installed at the base of the hive to collect pollen to ascertain the source of foraging bees food supply. The trap consisted of a fine wire mesh which allowed the bees to enter but would scrape the pollen pellets from thier legs. The pollen trap was removed after one week.

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Nectar Flow

Fresh supers were introduced at the onset of canola flowering. These supers were inspected during flowering on a regular basis.

May 12 - requeen
June 25 - fresh supers
July 30 - pollen trap
August 6 - pollen collection
August 13 - collected honey

The supers were removed after canola flowering was completed. The honey was immediately extracted with a centrefuge and stored refrigerated at 4°C. A 10 Kg subsample was stored frozen at 5°C. The brood chambers were inspected at the time of harvest for brood development, pollen stores, pattern of egg laying, queen activity, and honey stores as an indication of colony health. Two fresh honey supers were then introduced to the colony.

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Overwintering of Colony

After the fall Nectar flow was completed, the colony was transported back to Stoughton, Saskatchewan for both inspection and overwintering under the supervision of a local bee keeper, Mr. Garnet Hall.

IV. RESULTS AND DISCUSSION

Table 1 Critical Events

May 12	Requeened split colony
June 25	Transported colony to Indian Head
	Treated hive with Terramycin
July 15	Canola flowering commenced
	Introduced two fresh supers to colony
July 30	Installed pollen trap board to bottom of hive
August 6	Removed pollen trap from hive entrance

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August 13 Flowering of canola terminated
Removed honey supers and extract honey
Introduced fresh supers
Inspected colony

October 10 Removed honey supers

October 15 Transported colony to Stoughton

October 22 Final inspection of colony completed

After transporting the hive to Indian Head, Saskatchewan, the first inspection took place when fresh honey supers were introduced to the colony when canola flowering commenced. Inspection of brood chamber revealed the queen actively laying with both eggs and larvae present in abundance with minimal honey stores in the brood chambers. The bees were flying on the day of inspection and were observed to be actively foraging in the transgenic crop.

Colony was again inspected approximately two weeks after the commencement of canola flowering (Plate B). The lower honey super was approximately 75% filled, while the upper super was 25% filled (Plate D). Cells were partially filled with a very light honey characteristic of canola. Many of the frames were fully capped. Frames towards the

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center of supers were fully capped with less capping evident on the outer frames. Bee population in the honey supers as well as larvae in the brood chamber had increased markedly from the commencement of canola flowering. The presence of drones near the hive entrance was noted. The queen was observed to be actively laying and both eggs and developing brood were observed.

The majority of pollen collected was bright yellow with a minor component being orange. The identity of these pollens is being determined and will be included in a supplementary report. A large quantity of pollen was collected over the seven day period such that the hive entrance was partially blocked, approximately 200 grams was collected in total.

The honey supers were removed after the canola flowering had terminated. The lower super was completely filled and the upper super was approximatley 80% capped. Approximately 25 Kg of honey was extracted from the two supers in total. The honey extracted was light coloured and highly viscous. Honey yield was anticipated to be low due to both the smaller population of worker bees in the first year of a split colony and due to the cool and wet weather during the canola flowering period (Appendix III). Considering the weather conditions limiting the number of flying days a 25 kg yield from a split colony is acceptable Fresh supers were then introduced and left on the colony until October 10. During this period, the hive filled the two fresh supers. Honey colour was distinctly darker than the initial honey which was taken off. Brood chamber was not inspected at this time due to cool outside temperatures which could be damaging to the

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hive.

Final inspection of the hive was conducted after the bees were transported to Stoughton, Saskatchewan. The hive was rated to in "above average". It was noted that pollen stores were low. This can be attributed to the placement of the pollen trap during a period of peak hive activity. Honey stores were rated as very good with greater than 100 lbs of stored honey. The harvested canola crop yielded approximately 35 bushels per acre. This is considered a high yield for the Indian Head region indicating a high percentage of seed set.

V. CONCLUSION

The results from this study indicate that bees will actively forage on glufosinate tolerant canola variety HCN-92 and produce a light coloured honey crop. Hive development was observed to be normal during and subsequent to the flowering of the glufosinate tolerant canola. Prior to overwintering the hive health was rated in above average condition.

Plate A

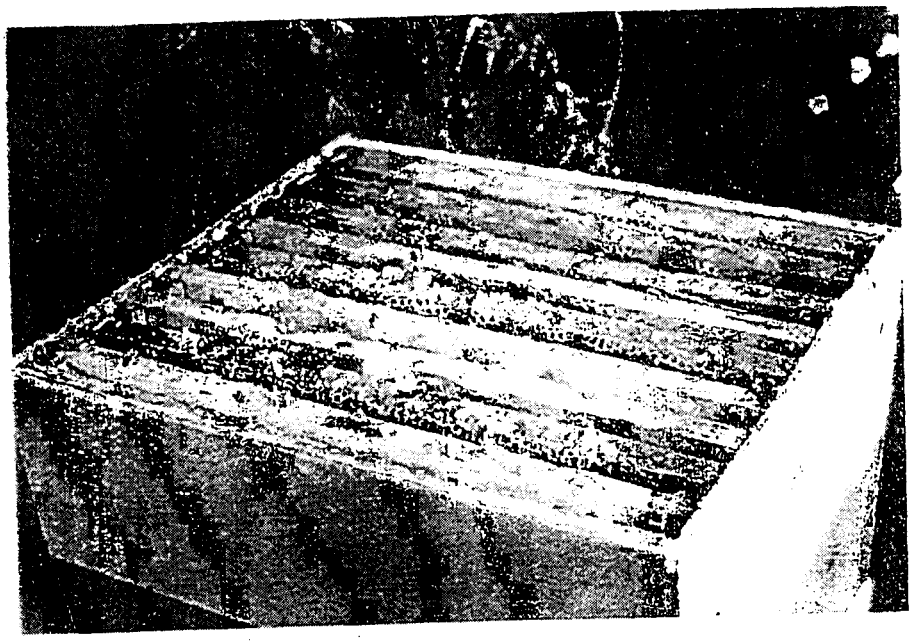

Plate C

Plate B

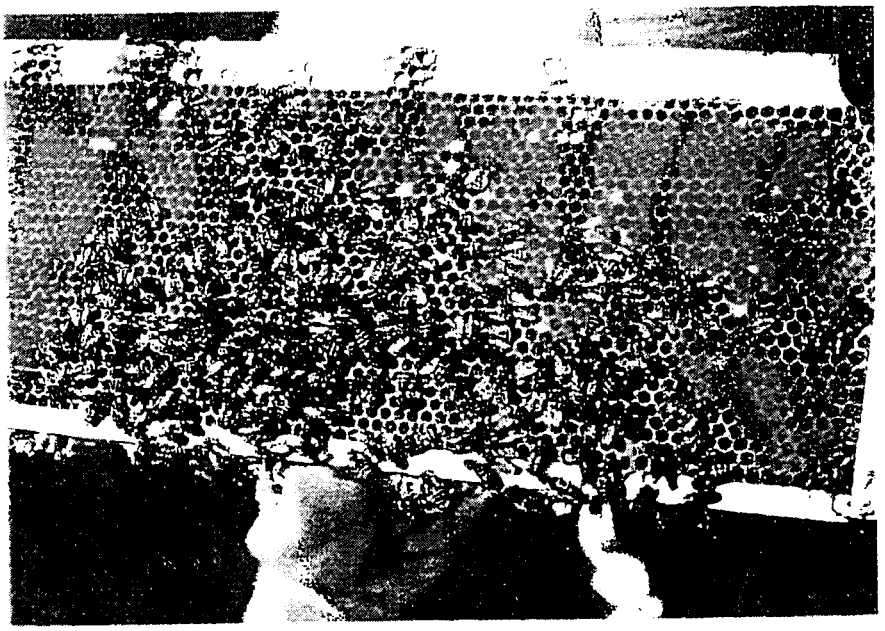

Plate D

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VI. APPENDIX I

Protocol Number 93H-01

PROTOCOL NUMBER: 93H-01

Principal Field Investigator: R.H. Donald

Nearest Town: **Indian Head, Sask.** Legal Land Description:

Landowner Name, Mailing Address & Phone Number:

Hoechst Canada Inc.
295 Henderson Dr.
Regina, SK
S4N - 6C2

Site History: list crops grown on site for previous three growing seasons & any pesticides used (include rates) and source of information.

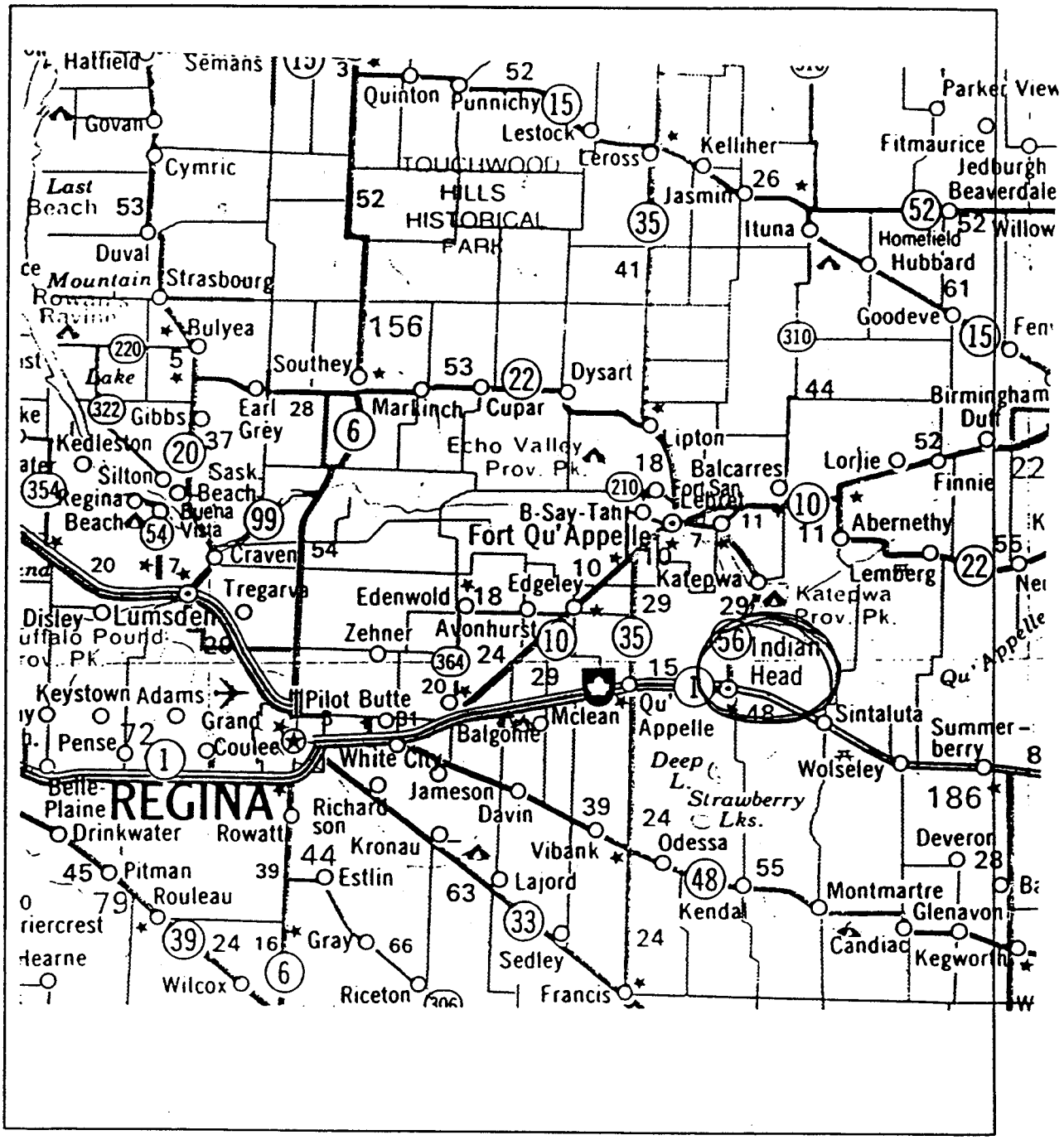
1992 - Mechanical Fallow
1991 - Durum Wheat
1990 - Durum Wheat

PROTOCOL NUMBER: 93H-01

GEOGRAPHIC LOCATION OF FIELD TEST SITE

INSTRUCTIONS: - Attach municipal road map or equivalent with a legible circle identifying the location of the field test site.

Indian Head, Sask.



Signature: _____ Date: June 25/93

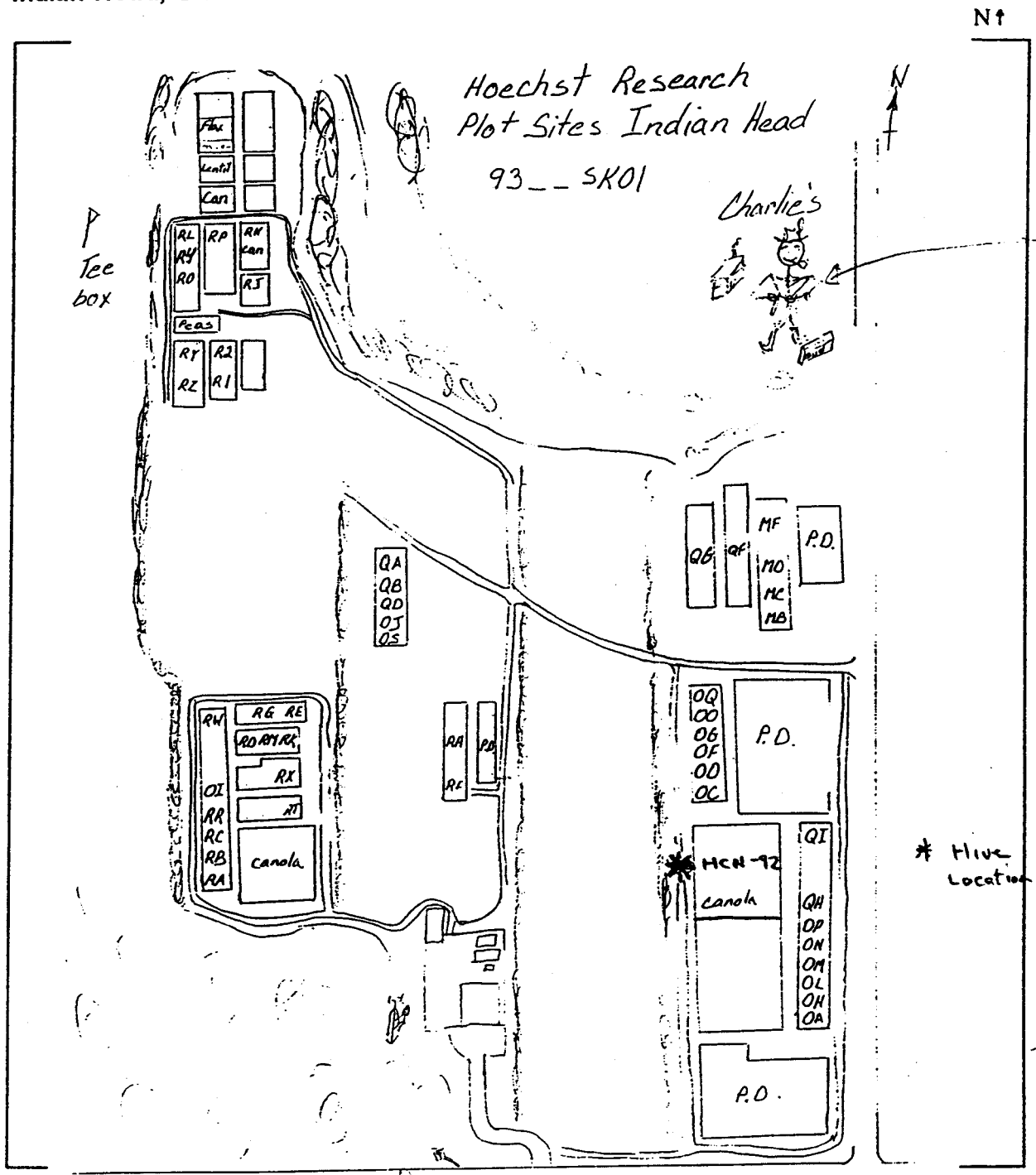
093011

PROTOCOL NUMBER: 93H-01

DETAILED MAP OF FIELD TEST SITE

INSTRUCTIONS: - Shall include relative location of nearest town
- identify roads leading to test site
- relative location of trial area to the nearest service road

Indian Head, Sask.



Verified by: _____ Date: _____

000012

WHITE COPY: REGINA OFFICE YELLOW COPY: REGIONAL OFFICE

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VII. APPENDIX II

Appraisal of Indian Head Hive.

Oct 18. 1993

Appendix 13

The following is my appraisal of the Hive marked Indian Head.

POPULATION : above average

QUEEN : present & looked normal.

EGGS OR LARVE : not present, normal for time of year

BROOD : about 8 sq. in., normal for time of year

HONEY STORES : very good, about 100LB enough for wintering.

POLLEN : very little, below normal

OVER ALL CONDITION : above average

COMMENTS : I have medicated and feed the Hive sugar syrup. It should winter well but will need some pollen substitute in spring to make up for low pollen stores.

Janet Hall

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VIII. APPENDIX III

Meteorological Data, Indian Head, Saskatchewan, 1993

Table 1. Meteorological Data, Indian Head, Saskatchewan, 1993

Date	Time	Air Max °C	Air Min °C	Ave Air	Ave RH	Wind Speed	Wind Direction	Rainfall mm
159	0	16.9	11.06	14.06	50.88	11.95	210.2	1.524
160	0	23.46	8.15	15.99	46.11	14.68	181.6	0
161	0	25.22	13.62	20.43	53.93	3.857	7.98	0.254
162	0	26.84	11.22	19.41	53.16	8.44	54.56	0
163	0	21.7	11.77	15.7	80.8	4.55	270.4	3.81
164	0	21.6	11.6	15.56	85.8	9.49	5.097	4.318
165	0	17.33	6.291	10.88	88	9.11	94.1	2.54
166	0	21.43	6.377	13.5	66.83	7.24	133.7	0
167	0	19.92	10.22	15.57	59.03	10.91	351.6	0
168	0	15.57	7.13	11.76	76.7	8.6	192.7	0
169	0	21.46	2.507	13.88	55.52	4.362	28.72	0
170	0	22.48	9.27	16.47	53.74	12.67	9.43	0
171	0	27.81	12.51	20.77	51.9	20.79	6.314	0
172	0	28.98	14.07	22.72	53.04	11.61	82.7	0
173	0	30.21	8.58	21.42	50.46	10.68	291.7	0
174	0	25.07	12.66	18.25	75.2	13.8	351.2	0
175	0	18.12	6.108	12.18	59.18	15.89	16.72	0
176	0	16.29	6.142	10.22	85.7	11.78	63.64	7.37
177	0	10.52	6.335	8.42	96.7	11.69	115.2	10.41
178	0	11.69	4.99	7.87	89.8	8.7	121.9	0
179	0	15.63	4.288	9.17	84.1	7.71	209.6	0
180	0	16.15	5.556	10.96	84	11.28	267.3	0
181	0	12.83	8.44	10.49	98	10.44	269.9	19.3
182	0	18.82	11.27	14.77	83.8	14.1	23.23	0
183	0	21.62	8.58	15.29	78.9	11.2	43.15	0
184	0	24.41	10.74	17.98	69.17	7.17	46.02	0
185	0	18.23	11.52	14.04	95.3	11.05	217.3	37.08
186	0	14.95	11.71	13.59	98.4	17.1	186.5	23.88
187	0	14.27	10.93	11.85	97.8	9.92	139.1	19.3
200	1200	19.14	18.54	18.76	63.6	7.58	92.6	0
200	1300	19.97	18.54	19.11	62.8	7.42	94.5	0
200	1400	20.87	19.51	20.08	60.25	7.89	95.3	0
200	1500	21.01	20.1	20.55	57.53	8.27	93.8	0
200	1600	21.4	17.05	18.8	63.23	7.91	91.4	0
200	1700	20.97	18.65	20.36	59.8	6.393	90.1	0
200	1800	21.12	18.21	19.68	58.83	7.2	150.9	0

200	1900	21.04	19.04	19.95	58.06	6.57	176.6	0
200	2000	20.11	19.04	19.58	59.4	2.911	183.6	0
200	2100	19.07	15.96	17.59	71	0.893	288.8	0
200	2200	15.9	13.18	14.49	82.3	0.882	320.7	0
200	2300	13.18	10.68	12.19	88.9	2.002	330.4	0
201	0	10.61	9.46	9.8	94.2	0.092	22.99	0
201	100	10.1	8.63	9.45	95.7	1.82	196.9	0
201	200	9.18	8.32	8.76	97.7	0	354.7	0
201	300	8.32	7.02	7.59	98.5	0.244	39.71	0
201	400	7.18	6.067	6.794	98.4	0.119	335.3	0
201	500	6.048	4.855	5.505	98.4	0.033	343.1	0
201	600	7.44	4.68	5.642	98.7	0.559	282.8	0
201	700	9.96	7.24	8.61	96.8	0.769	231	0
201	800	13.14	10.05	11.71	94.5	3.368	252.3	0
201	900	16.54	13.19	14.82	88.6	5.55	262	0
201	1000	19.28	16.64	18.22	74.8	5.816	294.6	0
201	1100	19.64	18.62	18.91	68.72	8.56	340.1	0
201	1200	20.99	19.28	20.05	65.68	8.92	322.8	0
201	1300	21.53	20.79	21.2	57.69	9.09	316	0
201	1400	22.21	21.38	21.81	54.49	8.93	291.7	0
201	1500	22.86	22.01	22.5	50.95	10.25	295.9	0
201	1600	23	22.06	22.61	49.96	10.75	301.5	0
201	1700	23.16	22.23	22.6	54.48	10.86	293.2	0
201	1800	22.34	21.43	21.88	58.57	12.26	282.3	0
201	1900	21.78	20.82	21.29	60.57	13.51	275.8	0
201	2000	20.76	18.96	20.07	63.35	12.87	275.4	0
201	2100	18.93	16.74	17.75	73	9.77	275.3	0
201	2200	16.7	15.56	16.13	79.6	9.4	273.3	0
201	2300	15.55	14.42	15.02	84.4	9.6	268.3	0
202	0	14.37	13.7	13.96	89.8	9.44	273.7	0
202	100	13.83	13.35	13.63	92.2	8.67	266.1	0
202	200	13.36	13.16	13.25	93.9	10.38	263.3	0
202	300	13.52	13.35	13.44	94	12.83	264.2	0
202	400	14.38	13.49	13.98	93.2	17.06	269	0
202	500	14.34	13.65	14.02	93.3	11.08	296.4	0
202	600	15.06	13.76	14.75	93.2	20.64	332.9	0
202	700	14.92	14.66	14.78	93.4	26.48	334	0
202	800	15.81	14.95	15.43	92.6	28.74	348.3	0

202	900	16.11	15.73	15.87	92	22.16	347.6	0
202	1000	16.16	15.75	15.97	91.9	17.12	342.7	0
202	1100	16.2	15.93	16.05	91.8	18.83	341.4	0
202	1200	17.34	16.18	16.84	90.9	18.03	331.4	0
202	1300	17.72	16.77	17.08	91.1	17.57	329.9	0
202	1400	19.35	17.7	18.68	88.8	16.43	332.8	0
202	1500	19.4	18.61	19.05	87.5	17.22	328.9	0
202	1600	19.64	19.14	19.45	86.4	14.75	333.5	0
202	1700	19.8	19.33	19.56	85.5	14.75	328.3	0
202	1800	20.12	19.8	19.98	84.6	15.54	320.6	0
202	1900	19.99	19.78	19.86	84.9	16.91	325.1	0
202	2000	19.92	18.81	19.32	85.7	13.33	313.7	0
202	2100	18.79	17.94	18.42	88.1	14.8	328.9	0
202	2200	17.92	17.31	17.56	92.3	13.93	321.3	0
202	2300	17.47	17.33	17.39	93	12.09	308.1	0
203	0	17.6	17.26	17.41	93.2	12.17	314	0
203	100	17.62	16.35	17.2	93.8	5.899	255.8	0
203	200	16.32	15.81	16.12	96.8	8.05	261.1	8.38
203	300	16.02	15.8	15.87	98.8	6.793	237.9	3.81
203	400	15.84	15.6	15.69	99.1	8.69	224.1	0.508
203	500	15.72	15.56	15.62	99	7.64	232.2	3.556
203	600	15.76	15.54	15.62	99	5.765	227.9	1.524
203	700	15.96	15.76	15.88	98.8	2.326	197	0.254
203	800	16.19	15.83	16.01	98.6	3.44	38.89	0
203	900	16.34	16.1	16.21	98.1	12.44	36.49	0.254
203	1000	17.2	16.33	16.76	95.5	20.26	50.6	0
203	1100	17.08	16.92	17	94.2	13.46	53.95	0
203	1200	18.5	17.05	17.91	92.2	12.52	53.01	0
203	1300	18.84	17.7	18.27	90	10.19	52.57	0
203	1400	19.08	18.38	18.65	86.9	12.7	33.97	0
203	1500	19.95	18.42	19.18	83	11.87	39.36	0
203	1600	20	15.75	18.8	80.3	15.04	36.73	0
203	1700	20.73	15.43	18.49	79.7	13	35.89	0
203	1800	20.7	20.28	20.52	76.8	13.46	30.63	0
203	1900	20.73	20.31	20.55	72.9	9.39	34.87	0
203	2000	20.28	18.68	19.48	77.7	6.436	15.61	0
203	2100	18.64	16.07	17.24	86.3	6.578	13.68	0
203	2200	16.02	15.19	15.62	92	8.18	20.8	0

203	2300	15.37	14.5	15.09	94.9	6.544	28.13	0
204	0	14.55	13.47	13.9	95.1	5.888	13.31	0
204	100	14.13	13.39	13.71	95.7	7.98	17.74	0
204	200	14.12	12.99	13.55	95.4	7.08	15.96	0
204	300	13.06	12.76	12.96	96	6.544	22.24	0
204	400	12.86	12.05	12.68	96.3	6.824	37.15	0
204	500	12.24	11.2	11.49	97.7	0.993	40.33	0
204	600	11.47	11.17	11.33	98.1	2.878	348.9	0
204	700	14.12	11.37	12.62	97.4	1.003	12.13	0
204	800	16.79	14.14	15.36	94.7	2.022	55.18	0
204	900	19.91	16.8	17.96	87.5	1.021	151.2	0
204	1000	20.51	19.46	20.07	76.4	1.382	150.4	0
204	1100	21.5	20.26	20.73	72.7	2.376	103.5	0
204	1200	22.21	21.13	21.64	69.39	3.178	110.8	0
204	1300	22.97	21.87	22.41	65.72	4.093	84.7	0
204	1400	23.45	22.87	23.11	64.26	3.295	129.9	0
204	1500	23.89	23.15	23.47	62.42	3.998	96.8	0
204	1600	24.62	23.19	23.64	61.36	3.055	139.9	0
204	1700	24.87	23.76	24.33	56.84	3.729	93.9	0
204	1800	25.5	24.36	24.97	55.31	1.907	155.1	0
204	1900	25.33	24.53	24.81	54.54	1.627	251.8	0
204	2000	24.91	20.86	24.32	56.11	2.206	355.6	0
204	2100	20.63	17.06	18.29	84.9	9.43	1.863	0
204	2200	17.04	15.82	16.44	91	11.35	15.52	0
204	2300	15.84	15.12	15.47	92.9	10.22	12.76	0
205	0	15.1	14.42	14.76	93.8	9.5	18.57	0
205	100	14.73	14.3	14.58	95.5	10.69	25.22	0
205	200	14.27	13.78	14.1	95.9	9.6	20.1	0
205	300	13.84	13.48	13.66	96.9	8.44	29.08	0
205	400	13.56	13.11	13.25	97.3	4.195	2.7	0
205	500	13.2	11.86	12.67	97.2	0.802	69.67	0
205	600	11.86	11.34	11.6	98.4	0.06	227.3	0
205	700	14	11.65	12.61	97.7	0.777	19.98	0
205	800	16.9	14.05	15.45	94.6	2.167	43.86	0
205	900	19.79	16.96	18.36	89	1.015	90.5	0
205	1000	21.76	19.82	20.62	83.6	1.176	146.1	0
205	1100	23.17	21.61	22.31	75.1	1.984	278.8	0
205	1200	24.36	22.83	23.74	64.61	1.35	26.12	0

205	1300	25.35	23.7	24.41	61.15	3.135	54.2	0
205	1400	25.37	24.45	24.96	57.59	2.624	132.9	0
205	1500	25.67	23.19	24.38	60.34	3.052	146.2	0
205	1600	24.55	23.39	23.92	62.72	3.016	88	0
205	1700	25.09	24.21	24.59	57.23	2.051	99.7	0
205	1800	25.58	24.33	25.07	55.72	2.067	252.2	0
205	1900	24.51	21.32	22.03	60.51	9.36	179.8	0
205	2000	22.17	21.68	21.96	62.55	0.716	208.9	0
205	2100	22.02	20.18	21.29	69.53	0	90	0
205	2200	20.15	17.19	18.56	83	0	90	0
205	2300	17.25	15.97	16.39	91.3	0	90	0
206	0	15.97	14.94	15.43	93.1	0.009	24.39	0
206	100	14.92	14.57	14.7	94.1	0.266	72	0
206	200	14.63	14.02	14.29	94.2	0.105	82.8	0
206	300	14.06	13.56	13.73	94.8	0.1	84.7	0
206	400	13.79	13.44	13.66	95.1	0.217	89.7	0
206	500	13.76	13.35	13.6	96.5	0.385	81	0
206	600	13.35	12.68	13.05	97.4	0.154	90.7	0
206	700	14.78	12.69	13.66	97.4	0.737	111.2	0
206	800	16.83	14.79	15.82	95.8	1.031	115.7	0
206	900	19.11	16.79	17.71	93	1.834	89.3	0
206	1000	21.44	19.2	20.41	86.4	3.187	136	0
206	1100	22.93	21.46	22.29	78.1	6.027	146.2	0
206	1200	23.59	22.85	23.32	69.76	7.78	155.6	0
206	1300	23.88	23.39	23.63	65.59	9.15	168.6	0
206	1400	24.16	23.7	23.9	63.76	8.76	172	0
206	1500	24.54	24.12	24.37	62.88	5.78	154.2	0
206	1600	24.6	24.1	24.33	61.95	4.341	151.3	0
206	1700	24.57	24.16	24.35	63.16	2.797	125.1	0
206	1800	24.12	23.35	23.72	67.88	1.312	131.7	0
206	1900	23.35	21.99	22.86	73.1	0.415	145.9	0
206	2000	21.95	20.68	21.27	76.9	0.329	161.8	0
206	2100	20.64	19.57	20.09	81.1	0.77	163.8	0
206	2200	19.53	17.9	18.68	88.1	0.039	113.9	0
206	2300	17.9	17.2	17.51	92	0.142	82.7	0
207	0	17.17	15.77	16.49	93.9	0.009	228.5	0
207	100	16.07	15.27	15.66	95.9	1.678	149.2	0
207	200	16.12	15.44	15.86	93.9	0.748	156.7	0

207	300	15.41	14.47	14.81	94.8	0.417	49.72	0
207	400	15.2	14.27	14.67	92.9	0.199	42.8	0
207	500	14.98	14	14.39	92.1	0.018	80.9	0
207	600	14.01	12.97	13.34	93.4	0.067	11.18	0
207	700	14.73	13.13	14.03	94.5	0.023	146.9	0
207	800	17.76	14.4	15.62	94.9	0.247	179.8	0
207	900	19.37	17.77	18.74	87.5	4.865	221.6	0
207	1000	21.04	19.25	20.08	83.2	6.454	230.8	0
207	1100	22.16	20.76	21.49	77	7.27	234.3	0
207	1200	23.1	22.04	22.53	70.1	6.753	228.3	0
207	1300	23.78	22.83	23.21	65.45	6.692	227.3	0
207	1400	24.34	23.39	23.79	63.45	7.65	240.1	0
207	1500	24.7	23.48	24.05	61.8	8.13	220.3	0
207	1600	25.03	23.13	23.99	61.6	9.09	220.1	0
207	1700	24.24	23.51	23.94	62.25	9.58	234.9	0
207	1800	23.82	22.82	23.25	63.13	8.64	226.4	0
207	1900	23.07	21.08	21.93	66.25	7.16	231.9	0
207	2000	21.31	19.09	20.32	71	5.056	228.7	0
207	2100	19.05	17.01	18.03	79.8	1.924	230.4	0
207	2200	16.99	14.89	15.86	85.7	1.801	242.3	0
207	2300	15.08	13.9	14.68	89.2	3.55	182.1	0
208	0	13.84	13.11	13.44	91.6	1.756	200.4	0
208	100	14.33	13.38	13.91	94.6	2.228	208.3	0
208	200	14.34	13.56	14.08	95.7	3.268	199.4	0
208	300	13.52	12.51	13.11	97.6	2.29	200.7	0
208	400	14	12.02	12.5	98.9	3.596	195.5	0
208	500	15.29	14.04	14.82	98.9	6.384	233.5	0
208	600	15.44	14.83	15.12	98.2	5.767	212.2	0
208	700	15.79	15.46	15.69	97.7	8.13	195.6	0
208	800	15.63	15.49	15.55	97.2	9.47	194.6	0
208	900	15.56	15.02	15.27	97.3	9.57	192.1	2.286
208	1000	16.76	15.08	15.95	96.8	8.2	203	0
208	1100	18.08	16.76	17.27	93.5	9.38	221	0
208	1200	19.06	17.96	18.54	89.2	11.32	219.1	0
208	1300	20.08	18.91	19.53	84.4	12.01	209.5	0
208	1400	20.14	19.58	19.85	81.2	9.95	220.7	0
208	1500	20.39	19.93	20.11	77.6	9.85	223.4	0
208	1600	21.76	20.18	20.95	74.4	8.41	211	0

208	1700	21.19	20.54	20.87	69.55	8.2	203.1	0
208	1800	22	20.81	21.36	68.78	8.67	175.6	0
208	1900	20.97	19.47	20.46	70.3	5.365	172.6	0
208	2000	19.46	18.95	19.19	81.4	0.928	172.3	0
208	2100	18.87	17.28	17.87	85.8	2.453	210.6	0
208	2200	17.28	16.17	16.86	90	0	90	0
208	2300	16.13	14.3	15.19	94.7	0.103	69.43	0
209	0	14.3	13.34	13.73	97	0.192	40.29	0
209	100	13.39	12.53	12.99	97.6	0.615	46.79	0
209	200	12.64	12.34	12.54	97.3	1.204	58.25	0
209	300	12.26	11.89	12.05	97	2.239	46.75	0
209	400	12.1	11.75	11.86	97.1	0.863	48.15	0
209	500	11.82	9.54	10.36	97.9	1.364	233.6	0
209	600	11.31	9.41	10.3	99.2	0.046	26.36	0
209	700	13.45	11.23	12.15	96.6	0.908	246.5	0
209	800	17.57	13.53	15.59	93.1	0.114	271.1	0
209	900	20.36	17.63	19.35	84.7	0.338	18.61	0
209	1000	21.64	20.11	20.91	78.1	1.969	40.81	0
209	1100	23.38	21.44	22.63	67.19	2.455	41.36	0
209	1200	23.63	22.77	23.11	63.86	4.672	49.08	0
209	1300	24.04	22.89	23.47	61.74	6.714	34.68	0
209	1400	24.85	23.65	24.27	58.45	9.31	53.71	0
209	1500	24.67	23.56	24.16	57.98	6.435	65.97	0
209	1600	25.19	23.38	24.06	58.55	6.25	70.5	0
209	1700	25.8	24.62	25.33	55.78	6.004	73.2	0
209	1800	25.67	25.08	25.41	54.81	6.142	71.5	0
209	1900	26.05	24.6	25.28	55.77	1.702	84.5	0
209	2000	26.08	25.02	25.46	57.34	0.152	78.9	0
209	2100	25.26	21.48	23.31	62.45	0.657	315.7	0
209	2200	21.37	18.54	19.79	76.8	3.226	312.2	0
209	2300	18.53	15.28	16.89	87	5.164	312	0
210	0	15.29	14.96	15.12	93.1	5.16	316.5	0
210	100	15.39	14.92	15.24	92.5	6.784	322.5	0
210	200	15.6	14.85	15.2	92.3	6.712	329.6	0
210	300	15.76	15.25	15.55	91.5	7.34	336.5	0
210	400	16.52	15.57	16.07	91.3	6.94	2.478	0
210	500	16.23	15.85	15.99	91.1	8.8	5.651	0
210	600	16.71	16.25	16.54	90.5	11.11	2.757	0

210	700	18.61	16.73	17.57	88.9	11.3	6.603	0
210	800	20.4	18.66	19.64	83.6	11.95	6.155	0
210	900	23.06	20.42	21.67	77.7	12.06	6.904	0
210	1000	25.22	23.08	24.17	70.2	11.64	355.7	0
210	1100	26.11	25.14	25.76	64.29	13.57	354.5	0
210	1200	27.06	26.08	26.6	60.13	16.76	353.6	0
210	1300	27.89	27.05	27.39	55.7	17.83	348.2	0
210	1400	28.24	27.64	27.85	54.05	17.72	341.2	0
210	1500	28.44	27.98	28.18	53.15	17.88	333.1	0
210	1600	28.48	28.1	28.25	51.97	18.72	334.1	0
210	1700	28.42	28.05	28.22	52.46	18.73	329.8	0
210	1800	28.21	27.46	27.83	54.24	17.83	327.9	0
210	1900	27.48	26.53	26.99	58.24	16.63	332.8	0
210	2000	26.49	24.92	25.78	64.6	12.2	328.6	0
210	2100	24.85	22.63	23.56	75	8.31	317.3	0
210	2200	22.58	21.69	21.98	81.1	9.48	311.7	0
210	2300	21.73	21.06	21.41	83.8	11.71	312.9	0
211	0	21.35	21.06	21.19	83.6	12.49	321	0
211	100	21.08	20.77	20.91	83.5	13.37	324.4	0
211	200	21.18	20.91	21.07	84.3	14.09	326.9	0
211	300	20.97	20.03	20.41	87.1	14.97	330.3	0
211	400	20.1	19.86	20.01	89.7	6.95	336.1	0
211	500	19.87	18.85	19.46	92.4	4.264	309.1	0
211	600	18.88	18.58	18.75	95.2	5.534	309.8	0
211	700	19.28	18.41	18.73	95.6	5.58	5.219	0
211	800	19.89	19.29	19.62	94.9	6.025	7.75	0
211	900	20.82	19.92	20.53	94.2	3.991	42.95	0
211	1000	22.79	20.88	22.04	92.4	10.84	73.4	0
211	1100	25.01	22.67	24.06	80.9	13.6	79.3	0
211	1200	25.14	24.47	24.84	53.49	16.28	81.7	0
211	1300	25.39	24.65	25.01	46.9	17.45	75.8	0
211	1400	25.57	24.91	25.26	41.6	20.79	71.9	0
211	1500	25.53	24.9	25.21	39.97	24.17	69.46	0
211	1600	25.86	24.53	25.14	37.74	24.01	68.41	0
211	1700	24.62	23.51	23.95	36.7	25.49	68.77	0
211	1800	23.6	21.59	22.9	36.08	26.95	68	0
211	1900	21.58	20.33	20.93	40.21	24.97	69.67	0
211	2000	20.38	18.84	19.77	41.6	17.79	66.13	0

211	2100	18.68	14.95	16.91	54.09	5.606	64.07	0
211	2200	14.82	12.12	13.25	74.1	2.749	47.38	0
211	2300	12.24	11.12	11.69	83.6	6.914	42.43	0
212	0	12.15	10.69	11.31	86.1	5.762	48.08	0
212	100	10.92	10.3	10.64	87.8	9.32	39.02	0
212	200	10.55	10.14	10.27	88.1	10.77	33.45	0
212	300	11.43	10.6	11.08	84.8	11.67	21.7	0
212	400	10.92	10.37	10.63	86.3	11.9	25.64	0
212	500	10.79	10.46	10.62	85.7	14.32	24.56	0
212	600	11.13	10.6	10.88	84.6	14.95	28.89	0
212	700	12.44	11.12	11.53	81.5	15.51	35.59	0
212	800	15.55	12.48	14.29	71.3	14.67	51.23	0
212	900	17.06	15.58	16.38	64.41	17.59	51.16	0
212	1000	18.41	17.02	17.77	63.35	17.62	49.02	0
212	1100	19.98	18.34	19	60.94	15.97	64.37	0
212	1200	21.35	19.92	20.69	50.18	16.7	67.19	0
212	1300	22.04	21.07	21.49	44.64	19.54	73.5	0
212	1400	22.52	21.63	22.1	41.14	23.93	67.01	0
212	1500	22.78	21.29	22.22	39.6	27.5	71.9	0
212	1600	22.17	20.81	21.51	41.05	24.9	75.2	0
212	1700	22.04	20.43	21	42.98	24.59	78.7	0
212	1800	20.55	19.39	19.99	41.69	21.58	84.3	0
212	1900	19.67	18.95	19.4	43.04	17.06	83.1	0
212	2000	18.83	17.59	18.02	47.27	15.81	71.4	0
212	2100	17.61	17.22	17.41	51.39	14.62	75.6	0
212	2200	17.52	16.16	16.97	54.01	16.08	81	0
212	2300	16.14	14.71	15.25	63.39	11.66	71.8	0
213	0	15.83	14.73	15.4	67.68	16.5	65.22	0
213	100	15.54	15.1	15.34	68.38	17.29	69.94	0
213	200	15.12	14.48	14.73	71.8	14.91	69.82	0
213	300	14.92	14.26	14.69	73.7	11.81	82.7	0
213	400	14.21	12.68	13.42	80.8	7.9	90.3	0
213	500	12.65	12.28	12.44	87.3	11.7	82.5	0
213	600	12.5	11.42	12.09	85.9	10.26	84.5	0
213	700	12.8	11.41	12	89.2	13.03	82.5	0
213	800	13.4	12.85	13.2	82.8	13.62	93.8	0
213	900	15.04	13.44	14.38	77.2	14.08	102.5	0
213	1000	16.32	14.94	15.62	68.71	15.01	110.9	0

213	1100	17.33	15.88	16.62	64.29	15.34	110.3	0
213	1200	18.2	16.93	17.65	62.3	15.03	110.4	0
213	1300	19.81	18.08	18.9	60.06	14.01	108.7	0
213	1400	21.38	19.43	20.25	57.51	14.78	101.4	0
213	1500	21.26	20.16	20.72	57.32	16.4	104.8	0
213	1600	20.98	19.82	20.42	57.86	14.05	105.2	0
213	1700	21.49	19.77	20.7	57.6	13.8	120	0
213	1800	20.24	19.46	19.83	61.19	11.71	122.5	0
213	1900	19.97	18.67	19.48	61.44	10.41	124.8	0
213	2000	19.13	17.43	18.49	63.09	6.706	119.8	0
213	2100	17.39	15.25	16	73.5	2.58	125.4	0
213	2200	15.39	13.29	14.09	78.2	2.149	98.8	0
213	2300	13.27	11.98	12.54	86.1	2.111	80.3	0
214	0	12.49	12	12.16	90.4	3.991	79.9	0
214	100	13.01	12.53	12.88	90.7	4.239	88.8	0
214	200	13.08	12.76	12.9	94.3	4.865	98	0.508
214	300	12.84	11.83	12.52	96.7	3.631	104.4	0
214	400	11.8	11.31	11.52	97.9	2.852	90	0
214	500	11.95	11.48	11.77	98.1	4.424	101.1	0
214	600	11.85	11.51	11.72	97.2	5.512	117.3	0
214	700	11.52	11.26	11.36	96	6.319	124	0
214	800	12.52	11.47	11.78	95.9	5.736	109.1	0
214	900	13.57	12.23	12.63	93.5	7.65	136.1	0
214	1000	14.6	12.52	13.44	87.6	9.23	138.9	0
214	1100	14.35	13.33	13.85	79.2	10.44	144.4	0
214	1200	15.02	13.66	14.26	72	12.63	148	0
214	1300	14.89	13.97	14.37	69	12.29	139	0
214	1400	15.18	13.68	14.67	69.25	18.93	155.4	0
214	1500	15.12	13.74	14.29	70.3	13.54	136.8	0
214	1600	14.64	13	13.78	72.4	16.36	155.3	0
214	1700	14.69	13.61	14.23	72.5	14.98	154.2	0
214	1800	13.91	11.2	12.78	79.4	19.16	171.4	0
214	1900	11.7	11.2	11.58	90.6	15.72	171.4	0
214	2000	11.61	11.34	11.52	87.5	15.3	177.3	0
214	2100	11.33	10.5	10.97	86.9	16.81	181	0
214	2200	10.5	9.55	10.01	86	15.85	185.3	0
214	2300	9.54	9.21	9.32	87.1	4.186	176.1	0
215	0	9.89	9.29	9.54	89.7	3.18	156.7	0

215	100	10.12	9.86	9.95	89.5	4.815	154.4	0
215	200	10.13	9.83	9.94	90.2	2.419	142.6	0
215	300	9.82	9.23	9.5	91.5	1.079	100.3	0
215	400	9.26	8.93	9.15	92.9	0.977	121.5	0
215	500	8.99	8.8	8.86	94.8	1.667	100.2	0
215	600	8.82	8.27	8.64	95.9	0.874	102.7	0
215	700	8.68	8.12	8.24	97.2	1.116	103.3	0
215	800	10.64	8.73	9.9	95.5	3.116	105.7	0
215	900	11.62	10.64	11.31	90.2	4.023	105.5	0
215	1000	13.54	11.52	12.95	81.9	3.612	109.9	0
215	1100	15.37	13.48	14.45	71.2	4.027	77.8	0
215	1200	15.86	14.38	15.09	63.51	6.364	77.8	0
215	1300	16.31	14.8	15.37	62.77	6.773	84	0
215	1400	15.67	15.1	15.41	63.21	6.437	83.2	0
215	1500	15.53	15.03	15.3	63.05	7.8	78.3	0
215	1600	15.59	15.05	15.28	63.58	5.925	89.2	0
215	1700	15.71	15.22	15.46	63.77	5.945	76.5	0
215	1800	16.48	15.47	15.76	63.23	4.168	100.3	0
215	1900	16.8	16.25	16.58	62.25	2.921	87.4	0
215	2000	16.33	14.67	15.72	66.67	0.211	62.31	0
215	2100	14.61	11.11	12.67	78.6	0.42	26.39	0
215	2200	11.49	10.59	11.04	84.2	3.816	23.93	0
215	2300	10.72	9.11	9.73	89.8	5.347	31.52	0
216	0	9.12	8.65	8.79	92.5	6.748	32.21	0
216	100	8.7	8.24	8.5	92.6	6.9	33.08	0
216	200	8.3	7.85	8.05	93.4	8.09	29.36	0
216	300	8.04	7.84	7.98	94.2	8.27	31.95	0
216	400	8.03	7.78	7.94	94.5	8.5	36.4	0
216	500	7.8	7.66	7.71	94.7	8.02	37.89	0
216	600	8.07	7.52	7.69	95	7.28	37.29	0
216	700	9.62	8.08	8.73	94.4	8.01	32.29	0
216	800	11.65	9.63	10.65	92.3	7.29	33.18	0
216	900	14.84	11.72	13.32	86.8	6.101	44.26	0
216	1000	16.91	14.81	15.88	77.5	5.599	46.6	0
216	1100	18.38	16.88	17.47	70.8	2.313	74.1	0
216	1200	18.47	17.67	18.06	69.45	3.659	171.7	0
216	1300	17.47	15.27	15.97	78.8	9	192.3	0
216	1400	15.31	14.42	14.82	86	6.913	216.1	0

216	1500	15.09	14.45	14.74	86.4	6.162	229.3	0
216	1600	15.47	14.67	14.98	85.5	3.589	231.7	0
216	1700	16.29	15.5	15.88	82.3	0.874	239.5	0
216	1800	18.14	16.24	17.32	77.6	1.131	97.2	0
216	1900	18.55	17.84	18.21	72.9	1.784	132	0
216	2000	17.94	16.39	17.44	77	0.271	129.7	0
216	2100	16.34	13.4	15.17	83.2	0	90	0
216	2200	13.37	12.29	12.8	91.5	0.003	65.87	0
216	2300	12.76	12.02	12.27	94.6	1.316	177.3	0
217	0	13.1	12.61	12.91	96.2	4.975	182.1	0
217	100	12.97	11.78	12.54	97.1	4.773	193.8	0
217	200	11.8	10.65	11.07	98.9	0.848	172.5	0
217	300	11.17	9.5	10.52	99.5	0.754	199.8	0
217	400	10.45	9.56	10.08	99.9	0.57	182.5	0
217	500	9.78	6.373	7.19	100.2	0.173	160.9	0
217	600	7.2	6.654	6.924	100.6	0	90	0
217	700	8.04	6.79	7.48	100.6	0.003	86.9	0
217	800	9.86	7.99	8.9	100.2	0.679	76.3	0
217	900	12.88	9.88	11.42	99.4	0.878	159.4	0
217	1000	17.05	12.95	14.92	90.1	1.513	198.4	0
217	1100	18.9	17.04	17.95	65.54	3.489	189.4	0
217	1200	19.71	18.05	18.87	57.9	4.387	199.9	0
217	1300	20.42	18.26	19.22	56.99	3.9	144.8	0
217	1400	21.29	19.41	20.36	50.89	4.799	213.5	0
217	1500	21.18	19.82	20.37	49.54	4.568	193.7	0
217	1600	22.42	20.55	21.5	47.3	4.988	249.4	0
217	1700	22.8	21.52	22.1	44.49	4.195	199.6	0
217	1800	22.96	21.37	22.25	43.64	2.224	192.6	0
217	1900	23.04	21.67	22.36	43.4	1.138	55.05	0
217	2000	21.95	20.4	21.51	45.8	0.063	79.4	0
217	2100	20.32	15.74	17.96	57.31	0.694	299.7	0
217	2200	15.68	12.61	14.19	69.99	4.617	317.2	0
217	2300	12.58	10.59	11.87	78	6.568	321.3	0
218	0	10.69	10.13	10.42	83.7	6.641	322.4	0
218	100	11.79	10.13	10.84	85.2	8.14	334.9	0
218	200	12.51	11.72	12.26	81.8	9.31	356.4	0
218	300	12.41	11.95	12.16	81.3	9.69	7.1	0
218	400	12.26	11.57	11.88	82.1	10.41	350.7	0

218	500	11.68	10.25	11.02	84.5	7.94	6.125	0
218	600	10.28	9.86	10.07	88	8.6	11.73	0
218	700	11.96	10.07	10.78	88	8.21	8.11	0
218	800	14.86	12.02	13.5	81.9	8.89	12.82	0
218	900	17.1	14.96	15.95	74.6	11.55	22.62	0
218	1000	18.67	17.11	17.89	68.63	12.18	23.03	0
218	1100	20.13	18.61	19.34	61.72	11.54	22.27	0
218	1200	21.02	20	20.52	55.28	12.47	4.252	0
218	1300	21.67	20.79	21.3	51.28	12.39	356.8	0
218	1400	22.66	21.71	22.09	47	11.76	355.4	0
218	1500	23.09	22.31	22.69	45.14	12.26	5.192	0
218	1600	23.43	22.83	23.13	43.99	12.39	0.398	0
218	1700	23.21	22.89	23.06	42.98	14.38	359.7	0
218	1800	23.18	22.81	22.98	43.06	12.65	355.5	0
218	1900	22.96	22.2	22.6	43.83	10.3	358.9	0
218	2000	22.23	19.64	21.12	50.03	6.795	343	0
218	2100	19.54	14.99	17.21	66.69	4.379	317.1	0
218	2200	14.91	12.63	13.5	82.1	4.678	315.5	0
218	2300	14.08	12.62	13.33	84.4	8.26	330.2	0
219	0	14.67	14.12	14.54	78.9	10.1	347.7	0
219	100	15.27	14.54	14.92	77.1	12.53	352.1	0
219	200	15.38	15.17	15.28	78.7	13.97	357.4	0
219	300	15.17	14.26	14.66	81.5	15.25	355.6	0
219	400	14.26	13.42	13.8	86.7	13.67	355.7	0
219	500	13.4	12.95	13.14	91.1	11.06	2.689	0
219	600	13.11	12.79	12.9	93.6	9.69	0.3	0
219	700	14.38	13.13	13.72	92.8	11.11	354	0
219	800	16.34	14.4	15.37	89.2	13.51	352.3	0
219	900	18.34	16.38	17.41	80.5	13.29	356.1	0
219	1000	19.82	18.41	19.08	73.4	12.12	354.9	0
219	1100	21.52	19.81	20.64	69.78	12.52	340	0
219	1200	22.84	21.51	22.26	64.92	14.38	333.4	0
219	1300	23.91	22.82	23.17	61.91	17.19	344.1	0
219	1400	24.26	23.47	23.8	60.69	19.07	345.8	0
219	1500	25.06	24.08	24.57	59.24	18.99	346	0
219	1600	25.18	24.38	24.83	57.85	17.72	338.3	0
219	1700	25.11	24.26	24.78	57.44	17.18	338.9	0
219	1800	25.06	24.39	24.83	58.26	15.82	330.9	0

219	1900	24.4	23.2	24.01	60.16	17.79	332.5	0
219	2000	23.13	21.09	22.05	65.97	11.58	330.3	0
219	2100	21.05	19.1	20.08	73	7.55	321.1	0
219	2200	19.41	18.9	19.06	78.1	9.45	327.9	0
219	2300	19.12	18.53	18.9	79.8	13.64	329.7	0
220	0	18.56	18.41	18.49	82.8	17.27	335.8	0
220	100	18.48	18	18.17	84.9	20.27	345.3	0
220	200	18.05	17.44	17.71	87.1	18.41	340.7	0
220	300	17.79	17.35	17.52	89.4	14.92	336.8	0
220	400	17.76	17.31	17.52	90.4	16.45	347.9	0
220	500	17.54	17.03	17.22	91.4	14.5	340.8	0
220	600	17.38	16.83	16.98	91.9	8.97	334.7	0
220	700	17.45	16.57	16.95	92.9	5.645	2.927	0
220	800	16.72	16.37	16.54	95.3	8.15	358.9	0
220	900	17.69	16.6	17.06	94.8	10.91	348.2	0
220	1000	19.44	17.68	18.72	90.8	6.54	28.19	0
220	1100	22.23	19.36	20.53	84.1	6.187	359.2	0
220	1200	23.59	22.25	22.93	73.6	10.55	27.34	0
220	1300	25.19	23.76	24.39	66.61	13.5	47.31	0
220	1400	26.3	24.4	25.15	63.5	11.08	65.52	0
220	1500	26.39	24.69	25.41	59.41	10.78	74.5	0
220	1600	26.27	25	25.65	54.55	11.77	75.7	0
220	1700	26.32	25.52	25.87	48.07	14.34	69.37	0
220	1800	25.7	24.83	25.26	47	13.74	79.3	0
220	1900	24.74	23.08	23.97	49.25	12.39	79.9	0
220	2000	23.17	20.15	21.79	58.31	5.461	91.3	0
220	2100	20.12	16.67	18.15	74.8	2.04	77.3	0
220	2200	16.62	15.52	16.04	83.5	0.715	68.19	0
220	2300	16.13	14.41	15.27	84	0.944	50.58	0
221	0	15.1	14	14.46	85.6	0.116	350.5	0
221	100	14.12	12.24	13.53	87.6	0.523	351.5	0
221	200	12.55	12	12.22	92.6	3.922	347.4	0
221	300	12	11.44	11.59	94.1	4.43	320.6	0
221	400	12.02	11.06	11.48	95.1	5.072	0.295	0
221	500	12.26	11.95	12.07	95.4	7.29	23.45	0
221	600	12.6	12.05	12.22	95.3	9.49	20.9	0
221	700	14.77	12.62	13.47	94	9.52	18.93	0
221	800	18.7	14.82	16.82	86.5	9.18	6.883	0

221	900	21.84	18.78	20.51	73	7.34	354.7	0
221	1000	24.3	21.86	23	63.59	3.972	340.7	0
221	1100	25.14	23.52	24.42	59.71	3.445	29.15	0
221	1200	26.74	24.88	25.71	56.54	3.223	47.88	0
221	1300	27.61	25.98	26.69	52.63	5.062	145.4	0
221	1400	27.97	26.71	27.35	48.29	7.22	69.13	0
221	1500	28.76	27.61	28.13	42.6	7.73	81.8	0
221	1600	29.08	28.06	28.55	39.87	7.93	71.1	0
221	1700	29.49	28.86	29.2	38.93	3.862	116.8	0
221	1800	29.78	29.05	29.39	38.18	2.947	146.3	0
221	1900	29.69	28.61	29.17	38.18	2.343	176.5	0
221	2000	28.65	25.02	26.85	50.56	1.46	244.1	0
221	2100	25	20.89	23.03	57.71	1.48	266.6	0
221	2200	20.81	18.23	19.4	70.5	3.271	316.9	0
221	2300	18.18	17.08	17.46	76.6	5.887	330.4	0
222	0	17.84	15.97	17.2	77.2	3.857	311.2	0
222	100	15.91	14.82	15.36	85.2	0.235	222.7	0
222	200	15.62	14.23	14.81	87.6	1.121	229.3	0
222	300	15.59	13.05	13.85	88.6	1.655	240.2	0
222	400	13.52	12.81	13.08	90.7	1.989	258.5	0
222	500	12.9	12.64	12.78	93.3	1.404	258.2	0
222	600	12.96	12.12	12.61	94	1.393	280.5	0
222	700	14.31	12.3	13.16	92.8	1.017	264.5	0
222	800	19.06	14.34	16.42	88.2	4.718	256.5	0
222	900	22.51	19.12	21.49	65.41	7.04	9.89	0
222	1000	24.5	22.5	23.51	58.55	10.21	38.88	0
222	1100	26.87	24.49	25.43	55.18	11.22	48.92	0
222	1200	29.07	26.93	27.98	41.32	11.09	64.95	0
222	1300	30.13	28.67	29.36	32.88	11.78	69.4	0
222	1400	30.72	29.44	30	29.74	9.82	69.09	0
222	1500	30.65	29.32	30.06	27.22	11.55	65.92	0
222	1600	30.78	30.1	30.36	24.69	12.76	64.86	0
222	1700	30.47	29.92	30.16	25.46	10.45	72.8	0
222	1800	30.15	29.15	29.72	31.53	5.148	90.3	0
222	1900	29.1	28.07	28.6	39.08	2.188	114.4	0
222	2000	28	26.57	27.25	45.42	0.238	118.1	0
222	2100	26.47	20.69	23.46	60.27	0.16	300.1	0
222	2200	20.68	17.64	18.95	72.6	0.532	301.5	0

222	2300	18.15	16.19	16.95	76.6	2.536	310.1	0
223	0	16.29	14.42	14.99	83.8	0.027	325.7	0
223	100	17.36	13.86	16.05	89.4	6.911	164.8	0
223	200	17.11	15.2	16.35	92.1	3.738	190	0
223	300	15.18	14.64	14.81	95.8	2.602	266.7	0
223	400	14.85	14.5	14.67	96.6	4.71	282.4	0
223	500	14.85	14.41	14.66	97	3.478	158.4	0
223	600	14.82	14.48	14.6	97.2	9.15	181.5	0
223	700	15.99	14.57	15.22	96.5	7.05	221.8	0
223	800	16.77	16.03	16.46	93	10.22	172.8	0
223	900	17.53	16.77	17.13	90.9	13.95	187.5	0
223	1000	18.02	17.15	17.61	89.3	18.73	183.7	0
223	1100	19.78	18.11	19.12	79.3	18.15	181.4	0
223	1200	20.11	18.66	19.52	77.3	14.05	164.9	0
223	1300	18.57	17.58	18.17	81.3	12.77	143	0
223	1400	17.86	16.71	17.29	82.9	16.63	152	0
223	1500	17.25	15.93	16.84	82.1	14.79	146.5	0
223	1600	15.86	13.88	14.58	91	7.03	131.8	0
223	1700	14.38	13.59	14.01	96.4	6.543	131	0
223	1800	13.63	12.57	13.13	96.2	7.21	107.4	0
223	1900	12.79	12.32	12.52	96.1	7.08	116.3	0
223	2000	12.39	12.27	12.36	95.3	5.79	107.6	0
223	2100	12.27	10.43	11.62	94.9	2.975	104.5	0
223	2200	10.41	9.62	9.9	97.3	1.138	103.7	0
223	2300	9.6	9.05	9.27	98.1	1.796	83	0
224	0	9.15	8.15	8.67	98.6	3.255	88.7	0
224	100	8.14	7.62	7.86	98.4	2.814	83.5	0
224	200	8.46	7.9	8.29	98	5.581	78.3	0
224	300	8.05	7.13	7.49	98.2	3.257	85.7	0
224	400	7.14	6.563	6.785	98.7	1.504	103.1	0
224	500	6.647	5.985	6.26	98.7	2.073	75.8	0
224	600	6.294	5.697	6.024	98.4	1.882	77.4	0
224	700	7.46	5.774	6.414	98	1.246	72.2	0
224	800	9.88	7.49	8.66	96.5	3.987	78.9	0
224	900	12.24	9.88	11.16	91.7	3.773	81.1	0
224	1000	14.89	12.29	13.59	81.1	4.753	68.31	0
224	1100	15.92	14.85	15.37	56.57	7.44	69.69	0
224	1200	17.36	15.96	16.47	50.43	7.55	75.2	0

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Title

The Response of Transgenic Canola (*Brassica napus* L.cv. HCN-92) to Soil Salinity Under Growth Chamber Conditions

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I. INTRODUCTION

In 1988, a *Brassica napus* L. plant was genetically transformed to become tolerant to the broad spectrum herbicide, glufosinate ammonium (IGNITE, HARVEST, BASTA, Hoechst AG), the formulated form of phosphinothricin (PPT). PPT inhibits glutamine synthetase which is involved in the assimilation of ammonia in plants (Manderscheid and Wild 1986). The rapid accumulation of ammonia results in the death of plant cells. The resistant gene was originally isolated from a streptomyces microorganism expressing the enzyme phosphinothricin acetyltransferase (PAT). The expression of this detoxifying enzyme has been demonstrated in tobacco, tomato and potato plants (De Block et al. 1987).

II. OBJECTIVE

Some environmental factors that can adversely affect plant growth are currently being investigated to ensure that herbicide tolerant plants will not show unintended genetic changes in hardiness that could lead to altered weediness when compared to their unmodified counterparts. The object of this study was to compare a glufosinate ammonium tolerant canola variety *(Brassica* napus L. cv. HCN92) to a commercial standard canola variety *(Brassica napus* L. cv. Legend) when grown in soils with a wide range of salinities.

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III. MATERIALS AND METHODS

Soil samples were collected from several sites near Porter Lake, a saline slough located 29 km east of Saskatoon, Sask., and transported to the laboratory for salinity measurement. Electrical conductivities of the saturation extracts (EC) at five of these sites fell in the range of 0.5 to 12 dS m^{-1}. About 350 to 400 kg of bulk soil was collected from each of the sites with the desired salinities, spread evenly on plastic sheets and dried at 60°C. Plant roots were removed, and the soil was ground, sieved and mixed. Three replicate samples were taken from each soil for salinity measurement in order to confirm the desired salinities. The EC values for the five soil treatments were 0.8, 2.4, 4.0, 6.2, and 11.5 dS m^{-1}. The EC 2.4 treatment had to be obtained by mixing non-saline soil with EC 6.2 soil. A sub-sample from each soil was sent to the Saskatchewan Soil Testing Laboratory, University of Saskatchewan, for detailed salinity and nutrient analyses (Table I). Field capacity (0.01 MPa) was determined for the five soils using a 0.5-MPa pressure plate apparatus (Soil Moisture Equipment Co.).

One hundred 3-liter pots were filled with 2400 g of dry soil (5 salinity treatments x 2 canola varieties x 10 replicate pots). The soil was compacted to obtain an uniform bulk density (1.2 g cm^{-3}) throughout the pot. Deionized water was added to each pot until field capacity was reached.

Ten seeds of HCN92 or Legend canola were planted 1 cm deep in each pot. All pots

were positioned randomly in a growth chamber with a 14 hour photoperiod and day/night temperatures of 22/15°C and relative humidities of 50/80%. Photon irradiance was maintained at approximately 350 µmol m^{-2} s^{-1} at the top of the plant canopy throughout the experiment.

The number of emerged seedlings was recorded every day during the first five days after planting and then every two days until 11 days after planting. Seedling emergence rate was calculated following Maguire (1962). Seedlings were thinned to two seedlings per pot after no more seedlings had emerged. A thin layer of small styrofoam prills was added to the soil surface to minimize evaporation. Plastic bags were placed around each pot to trap any drainage. Sufficient deionized water was added to each pot every three or four days to raise the water content to 85% of field capacity. Water content during the experiment fluctuated between 65 and 85% of field capacity. The amount of water added provided a measure of evapotranspiration.

Plant measurements included:

I) date and rate of emergence,

2) plant height between bolting and harvest,

3) number of leaves,at bolting,

4) plant vigour and health,

5) aboveground biomass (fresh and dry weights),

6) root biomass (dry weight),

7) leaf area. At the end of the experiment, one soil sample was collected from each pot to confirm that the electrical conductivities had not changed during the experimental period (Table 1).

The experiment was arranged in a completely randomized factorial design, and analysis of variance was carried out for all parametric data using Minitab. Significant effects were considered to be those with a probability $P < 0.01$.

IV. RESULTS

IV.I Seedling Emergence

Salinity had a significant effect on both total seedling emergence (%) and emergence rate (%/day) in both varieties. The interacting effect of variety and salinity level on total seedling emergence also was significant. The maximum seedling emergence of HCN92 was above 80% until salinity reached the very severely-saline level, when emergence dropped to 30% (Table 2). Legend had lower seedling emergence than HCN92 under non-saline to moderately saline conditions, but higher emergence under slightly-saline and severely-saline conditions. Seedling emergence rate in both varieties was reduced at higher salinity levels, but there was no significant difference in the emergence rate between the two varieties.

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IV.II Plant Development

Plants of both varieties showed vigorous growth from emergence until 25 days after planting, when older leaves of some plants developed bronze to yellow leaf colouration, probably due to phosphorus deficiency. No fertilizer was used in this study in order not to alleviate nutrient stress arising from the salinity treatment. The plants were harvested at 32 days after planting, shortly after deficiency symptoms developed.

HCN92 plants were significantly taller than those of Legend at 32 days after planting (Table 2). Salinity reduced plant height about the same extent in both varieties.

Number of leaves at harvesting decreased significantly with increasing salinity in both varieties. HCN92 had 0.7 more leaves per plant than Legend at harvesting. Salinity significantly reduced leaf area at harvesting, but the difference in leaf area between the two varieties was not significant

Salt content of the soil affected the time at which bolting was initiated. Under non-saline and slightly-saline conditions, bolting started at 29 to 32 days after planting. About 30% of HCN92 plants in the moderately-saline treatment also bolted, but none of the Legend plants bolted at this salinity level. Higher salinity values prevented bolting in either variety during the experimental period.

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IV.III Shoot and Root Biomass

Shoot biomass of 32-day old plants was significantly reduced by salinity, except that shoot weights in the slightly-saline treatment were similar to the controls (Table 2). Shoot biomass in HCN92 was greater than in Legend under all salinity conditions.

Root biomass in both varieties also was significantly reduced by salinity (Table 2). The highest root biomass in both varieties was observed under non-saline and slightly-saline conditions. The difference of root biomass between two varieties was not significant. It is unclear why both varieties had less biomass under moderately saline than severely saline conditions.

IV.IV Evapotranspiration

Salinity treatments significantly reduced cumulative evapotranspiration from over 2.4 kg pot^{-1} in the control to about 0.75 kg pot^{-1} in the highest salt treatment (Table 2). Plants under low salinities had high leaf areas, which probably is the main factor explaining the higher transpiration rates. The two varieties did not differ in evapotranspiration.

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V. DISCUSSION

Reduced total emergence and emergence rates of both canola varieties under the very severely saline (11.5 dS m^{-1}) treatment could have resulted from osmotic effects, ion toxicity, nutrient limitations in the soil, or a combination of these factors. Francois (1984) found that salinities up to 11.6 dS m^{-1} only delayed seed germination of *Brassica rapa* L, but did not significantly reduce final germination percentage. The greater sensitivity of HCN92 and Legend could be because emergence, rather than germination, was measured. Emergence is affected by soil properties such as texture, water content and aeation, as well as salinity. Despite this, emergence of canola in this study was shown to be less sensitive to salt concentration than were other plant growth parameters.

HCN92 plants were taller and had more leaves than Legend under saline conditions in this study, suggesting that HCN92 may be slightly more salt tolerant. Furthermore, the early bolting in HCN92 plants under slight and moderate salinities could lead to higher seed yield, assuming that early flowering is beneficial. However, additional testing will be needed to establish this relationship.

Holm (1983) reported that Regent rapeseed was the most salt sensitive of nine Saskatchewan crops, based on a regression analysis using percentage of normal yield versus electrical conductivity. The regression equation for relative yield (Y) in relation to salinity (EC) was: $Y = -8.1EC + 100$ (Holm 1983). The equivalent relationship for HCN92

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and Legend (pooled) was similar: Y = -9.8EC + 107. Mengel and Kirkby (1982) compared the salt tolerance of several important field crops using the conductivity at which yield is reduced by 25%. For HCN92 and Legend, this value was about 3 dS m^{-1}, intermediate between published values for flax (4.8 dS m^{-1}) and bean (2.5 dS m^{-1}). Canola is salt-sensitive, compared with sunflower (11.3 dS m^{-1}) and barley (15.8 dS m^{-1}).

Other published literature suggests that canola is somewhat tolerant to salinity. For example, canola was rated as a moderately salt-tolerant crop (tolerant to EC in the range of 4-8 dS m^{-1}) in a Saskatchewan survey (Henry et al. 1987). In another study, *Brassica napus* L. cv. ACaacc was judged the most salt-tolerant of several *Brassica* species, because the reduction of its total dry matter relative to the control was only 52% at 12 dS m^{-1} (He and Cramer 1992). Ashraf and McNeilly (1990) also reported that *Brassica napus* L. cv. DGL is relatively tolerant to NaCl. A linear correlation between plant biomass and salinity also was found in turnip *(Brassica* rapa L.)(Francois 1984).

HCN92 and Legend canola are more salt tolerant at seedling emergence than at later stages of development. Moderate and severe salinity levels did not reduce total seedling emergence, but significantly reduced plant growth parameters. HCN92 had higher shoot weight, greater plant height, and larger number of leaves than Legend, but in general the two varieties responded similarly to soil salinity stress. We conclude that vegetative growth of the genetically transformed variety, HCN92 is substantially equivalent to that of variety Legend under salt stress conditions.

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VI. TABLES

Table 1. Analyses of salinity and nutrients of five field collected soils used to test the response canola to salinity.

Salinity	EC^1 (dS m^{-1})	EC^2 (dS m^{-1})	EC^3 (dS m^{-1})	%Sat	pH	Na^+ (mg L^{-1})	Ca^{++} (mg L^{-1})	Mg^{++} (mg L^{-1})	K^+ (mg L^{-1})	Cl^- (mg L^{-1})	$SO_4^=$ (mg L^{-1})	S.A.R.	HCO_3 (mg L^{-1})
Non-saline	1.2	0.8	0.5	63	7.7	17	155	35	34	11	141	0.3	311
Slight	4.0	2.4	2.3	44	7.0	218	490	190	75	40	1640	2.1	328
Moderate	4.2	4.0	3.5	60	7.7	347	464	202	42	150	2184	3.4	249
Severe	6.6	6.2	5.4	52	7.9	818	458	411	50	222	3553	6.7	260
V. Severe	13.8	11.5	9.7	48	8.0	2118	621	828	42	1142	6834	13.1	252

Note: EC^1: soil conductivities tested in the Soil Testing Laboratory.
EC^2: soil conductivity measurements before planting.
EC^3: soil conductivity measurements after harvesting.

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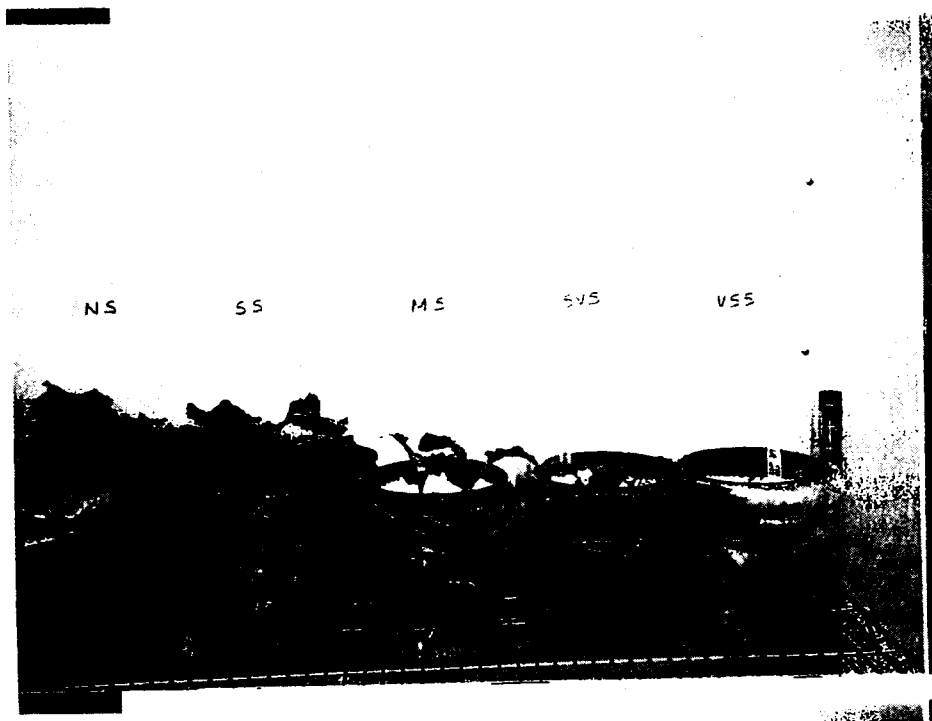
Table 2. Measurements of seedling emergence and plant growth of two canola varieties under salinity conditions in growth chamber.

Salinity levels	Seedling emergence (%)	Emergence rate (% day^{-1})	Shoot weight (g pot^{-1})	Root weight (g pot^{-1})	Leaf area (cm^2 pot^{-1})	Number of leaves (pot^{-1})	Height (cm)	Evapo-transpiration kg pot^{-1}
HCN92								
Non-saline	91	25	6.5	5.0	573	23	15.8	2.40
Slight	94	26	7.7	5.3	666	24	17.8	2.28
Moderate	95	26	1.8	2.5	169	16	9.9	1.41
Severe	81	23	2.4	2.8	382	19	13.7	1.55
V. Severe	30	19	0.2	0.04	29	11	3.5	0.73
LEGEND								
Non-saline	71	26	6.4	4.5	641	23	15.5	2.43
Slight	88	25	6.9	4.5	652	21	15.3	2.20
Moderate	76	24	1.7	2.9	197	15	9.6	1.45
Severe	93	22	2.3	2.3	388	17	13.3	1.57
V. Severe	43	15	0.1	0.03	22	10	2.7	0.77
$LSD_{0.01}$	11.90	2.40	0.47	1.42	42.97	1.62	1.11	2.44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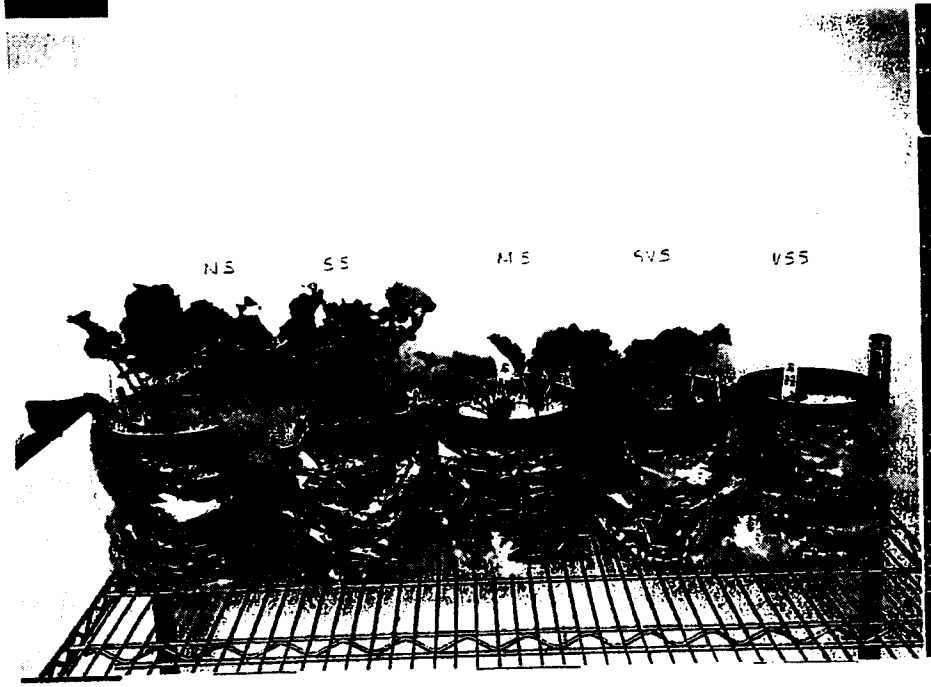
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SS
MS
SVS
VSS

Description of Pictures

1. Canola plants under non-saline, slightly-saline, moderately-saline, severely-saline, and very-severely saline conditions at 25 days after planting.

2. Plants of HCN92 and Legend canola in the growth chamber at 25 days after planting.

3. Canola plants under salinity conditions ranging from non-saline to very-severely saline at 32 days after planting.

4. Plants of HCN92 and Legend canola in the growth chamber at 32 days after planting.

5. HCN92 and Legend canola plants under non-saline condition at 32 days after planting.

6. Legend canola plants under moderately and severely-saline conditions at 32 days after planting.

7. Bronzed and yellow leaves at 32 days after planting, probably indicating phosphorus deficiency.

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Title

Susceptibility Glufosinate-Tolerant Canola to Flea Beetles

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Susceptibilty Glufosinate-Tolerant
Canola to Flea Beetles

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Canola to Flea Beetles

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Hoechst Canada Inc.
Agriculture Division
295 Henderson Drive
Regina, Saskatchewan
Canada S4N 6C2

HCI93-12
November 4, 1993

List of Tables

Susceptibilty Glufosinate-Tolerant
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I. INTRODUCTION

Flea beetles are pests to canola, mustard, flixweed and other cruciferous weeds (1). The most serious damage is caused by over-wintering adults which feed on the cotyledons and first true leaves. The "shot-holes" are an early sign of damage. Seedlings that are severely damaged may die, while less serious damage can result in yield loss. Once a plant gets beyond the seedling stage, serious damage does not usually occur because the plant material has increased many-fold and the adult flea beetle population has often begun to decline.

Flea beetles have one generation per year in Western Canada. The overwintered beetles mate and lay their eggs during May and June, and the adult population die off by the end of June.

II. OBJECTIVE

Numerous flea beetles in a field can act as a biological control agent for cruciferous species. Therefore, any tolerance to flea beetle damage can enhance the invasiveness of an adaptive weed. The objective of this study was to compare the susceptibility of flea beetle damage on glufosinate tolerant canola with commercial varieties of canola.

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II. MATERIALS AND METHODS

The trial was established near Minto, Manitoba on May 20, 1993. The experimental design was a randomized complete block with 4 replications. Individual plot dimensions were 1.5 m x 7.5 m. The canola varieties evaluated in this study were 3 non-transgenic (Legend, Excel and Cyclone) and one glufosinate-tolerant line (HCN-92). Site and seeding information is summarized in Appendix 1.

The area was cultivated twice in October, 1992. The first cultivation incorporated Edge herbicide at the recommended rate of 11.3 kg/ha. The second cultivation included anhydrous at 60 kg N/ha. The area was also cultivated, at a rate of 7 kg/ha with a double disc seeder. Row spacing was 15 cm. Phosphate was applied with the seed at 20 kg/ha.

The trial was surrounded by a 10 m border of *B. napus*. A blend of Alto, Cyclone, Delta, Excel, Global, Legend and Westar seed was planted on May 20 to ensure that the flowering of the border would be synchronous with the test plots. trial guidelines for the filed testing of genetically modified organisms, regulated by Agriculture Canada, were followed.

Grassy and broadleaf weeds were controlled by a tank-mixed application of Lontrel/Poast/Muster = Merge on June 22. The plots were monitored and rogued for wild

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mustard throughout the growing season, as required by Agriculture Canada guidelines.

No insecticides were sprayed within drift distance of these plots.

The plots were assessed for visual emergence, plant counts per m^2, visual vigour, flowering dates, flea beetle damage, maturity and yield.

Plant populations were estimated as percent germination on June 3 - June 11 and counted as plants/m^2 on June 28.

Vigour was rated on the following 0 - 10 scale:

10 - very vigorous

5 - poor

0 - dead

Vigour was assessed 30 DAE.

First flower dates were recorded when 5% of the plants per plot had their first open flower. End of flower dates were taken when 50% of the plants per plot had their last 5 open flowers left on the main raceme. Flower durations were calculated from these dates.

Feeding damage ratings for the natural population of flea beetles were assessed on June

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6 at the cotyledon stage, an on June 18 at the 2 - 3 leaf stage. The leaf damage rating scale is as follows:

0 - no damage

1 - up to 25% of leaf area damaged

2 - up to 50% of leaf area damaged

3 - up to 75% of leaf area damaged

4 - plant mortality

These ratings were an average damage estimate of the entire plot.

Flea beetle bioassays were conducted using plastic cages, approximately 11 cm in diameter an 23 cm in height. Each cage had 5 ventilation holes, one on top and 4 around the circumference at 90 degree intervals. These ventilation holes allowed air movement and prevented excessive interior temperatures. Each cage was equipped with an entry hole on the side which was plugged with a rubber stopper to prevent the beetles from escaping.

Flea beetle bioassays were conducted on June 3, 14 days after seeding, at the cotyledon stage and on June 15, 26 days after seeding, at the 2 leaf stage. Caged damage ratings were taken 3 days after beetle introduction.

Prior to the bioassays, flea beetles were caught in non-contaminated sweep nets and transferred to unused clear plastic bags. Test beetles were caught away from the plots

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in areas which had not been exposed to any insecticides. The beetles were stored in the fridge or cooler except when being transferred via pipettes to containment vials. Each vial held 5 beetles. The test beetles were not stored longer than 2 days.

Each cage in the first bioassay covered 3 seedlings. The cages were set out in the following manner. A cluster of 3 seedlings was isolated and the ground around them was smoothed away. Fine, light-coloured silica sand was spread around the seedlings to a depth of not less than 2 cm. Very dry sand was used to prevent the formation of cracks around the base of the cage as base and securely anchored with a wire restraint which crossed over the top of the cage and was pushed into the ground. Soil was banked up around the outside of the cage walls to complete the seal of the flea beetle environment. Three cages were established in each plot. A total of ten beetles were introduced to each cage. After 3 days, feeding damage ratings inside the cages were taken on the 0 - 4 scale previously described.

The cages were moved to fresh plants for bioassay #2 and the same methodology as explained above was used, with the exception that only 2 seedlings were covered per cage.

Maturity was assessed as percent seed turn on September 14, immediately after a killing frost. Reps C&D were harvested on September 17, and reps A&B on September 24, with a Wintersteiger small plot combine. There was no lodging in these plots at any time.

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Yields were adjusted to 9% moisture, and the data was analyzed with Duncan's MRT at 5%.

III. RESULTS AND DISCUSSION

All raw data is summarized in Appendix I.

For all canola varieties (Excel, Legend, Cyclone and HCN92), induced flea-beetles caused greater plant damage compared with the natural occurring flea-beetle population. At the cotyledon to 1 leaf stage (June 6), an injury rating representing up to 75% and 25% damage were observed for caged and natural flea-beetles, respectively (Table 1a). It was assumed that greater injury occurred with caged flee-beetles as a result of the limited number of plants available to feed upon. Flea-beetle damage decreased rapidly as the canola plants grew. By June 18, very little damage (0.5 rating) was observed by the 3-4 leaf stage (Table 1a.).

HCN92 exhibited similar characteristics to the other commercial varieties with respect to emergence, plant counts, plant vigor, number of days to first and last flower, maturity and grain yield. There was no significant difference in emergence, plant counts, plant vigor and yield for all canola varieties tested (Table 1b.). There was a significant difference in the number of days to first flowering (3 day difference), last flowering (2 day difference), duration of flowering (1 day difference) and maturity (17 day difference)

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between all varieties. HCN-92 was observed to be intermediate among the lines evaluated in terms of the number of days to first flower and last flower. While HCN-92 displayed the highest percentage of seed turn at maturity. These differences are attributable to the lineage of HCN-92 which have been selected for using traditional plant breeding methodologies.

Table 1a. Summary of the Flea Beetle impact on Transgenic and Conventional Canola.

Variety	Caged Beetle Damage 0-4 06-06-93	Natural Beetle Damage 0-4 06-06-93	Caged Beetle Damage 0-4 06-18-93	Natural Beetle Damage 0-4 06-18-93	Emerg-ence Percent 06-11-93	Plant Counts per m2 06-28-93
Excel	2.9 a	1.0 a	1.7 a	1.0 a	53 ab	117 a
Cyclone	2.8 a	1.0 a	1.5 a	1.0 a	65 a	119 a
Legend	3.1 a	1.0 a	1.6 a	1.0 a	50 b	96 a
HCN92	3.2 a	1.0 a	2.0 a	1.0 a	55 ab	148 a
LSD (.05)	1	0	0.4	0	14	52

Treatment means followed by the same letter are not significantly different ($p < .05$).

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Table 1b. Summary of the Flea Beetle impact on Transgenic and Conventional Canola.

Variety	Visual Vigor 0-10 06-22-93 30 DAE	# days to first flower	# days to last flower	Duration of flowering	Maturity Seedturn Percent 09-14-93 Maturity	Grain Yield kg/ha 09-17-93 Maturity
Excel	7.3 ab	55 a	85 a	31 ab	49 b	1911.6 a
Cyclone	8.3 a	54 b	83 c	31 b	41 c	2363.4 a
Legend	7.0 b	52 c	83 c	32 a	50 b	1986.6 a
HCN92	7.3 ab	53 b	84 b	32 a	58 a	2078.8 a
LSD (.05)	1.1	1	1	1	7	423.4

Treatment means followed by the same letter are not significantly different ($p<.05$).

IV. CONCLUSIONS

There was no difference between the glufosinate tolerant canola line (HCN-92) and the standard commercial canola lines (Legend, Excel and Cyclone) in thier susceptibility and reaction to flea beetle feeding.

Susceptibilty Glufosinate-Tolerant
Canola to Flea Beetles

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VI. APPENDIX.

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Flea Beetle Feeding Impact Study

– Transgenic vs. Conventional Cultivars –

1993

DAVID R.S ROURKE. P.Ag., B.S.A., M.Sc.

10-29-93 (H320.DD)

Ag-Quest, Inc.
Flea Beetle Feeding Impact Study
-- Transgenics vs. Conventional Cultivars --

Project Code:H320
Cooperator :Hoechst Canada, Ltd.
Location :Minto, Manitoba
By:David R.S. Rourke

Experimental Management

Date Planted : May 20
Variety : various
Row Width : 15 cm.
Design : RCB
No. Reps. : 4 Plot Size : 1.5 x 7.5 m.
Field Preparation and Plot Maintenance : Fall and spring cultivation. Fall applied anhydrous @ 60 kg N/ha; 20 kg P205/ha with seed. No insecticide.

Site Description

Season Moisture : see weather appendix
Soil Texture : clay loam
% Sand : 27 % Silt : 48 % Clay : 25
Soil Series : Ryerson
% OM : 6 pH : 7.8 CEC : 23

Application Information

	A	B	C	D	E	F
Pest Name, Stage & Density						

Application Equipment

Sprayer Type	Speed MPH	Nozzle Type	Nozzle Size	Nozzle Height	Nozzle Spacing	Boom Width	GPA	Carrier	PSI

Comments

There were no significant differences between the standard cultivars and the transgenic for flea beetle damage, either naturally occurring or induced.
[illegible]N-92 showed good yield potential, exceding Excel and Legend (NSD).
[illegible]N-92 matured significantly earlier than any of the standards.

10-29-93 (H320.DAT)

Ag-Quest, Inc.
Flea Beetle Feeding Impact Study
-- Transgenics vs. Conventional Cultivars --
Location :Minto, Manitoba
Project Code:H320
Cooperator :Hoechst Canada, Ltd. By:David R.S. Rourke

Trt No	Treatment Name	Grain Yield	Caged Beetle Damage	Natural Btle Damage	Caged Beetle Damage	Natural Btle Damage	Emergence Plot	Emergence Plot
Character rated								
Rating data type		Kg/ha	0-4	0-4	0-4	0-4	Per Cent	Per Cent
Rating unit								
Rating date		09-17-93	06-06-93	06-06-93	06-18-93	06-18-93	06-03-93	06-11-93
Trt-Eval Interval			GS. 1	GS. 1	GS.2.2/3	GS.2.2/3	GS. 1	GS.1-2.2
PRM Data Type								
1	Excel	1911.6 a	2.9 a	1.0 a	1.7 a	1.0 a	6 b	53 ab
2	Cyclone	2363.4 a	2.8 a	1.0 a	1.5 a	1.0 a	10 a	65 a
3	Legend	1986.6 a	3.1 a	1.0 a	1.6 a	1.0 a	4 b	50 b
4	HCN - 92	2078.8 a	3.2 a	1.0 a	2.0 a	1.0 a	6 b	55 ab
LSD (.05) =		423.4	1.0	0	0.4	0	3	14
Standard Dev.=		264.682	.634705	0	.270801	0	2.10983	8.53913
CV =		12.69	21.11	0	15.93	0	33.42	15.35
:k F		0.589	0.213	0.000	0.114	0.000	2.448	6.486
b..ck Prob(F)		0.6372	0.8852	1.0000	0.9499	1.0000	0.1305	0.0125
Treatment F		2.232	0.254	0.000	1.977	0.000	5.743	2.371
Treatment Prob(F)		0.1539	0.8567	1.0000	0.1880	1.0000	0.0178	0.1382

Means followed by same letter do not significantly differ (P=.05, Duncan's MRT)

11-10-93 (H320.DAT) SUMMARY Page

Ag-Quest, Inc.
Flea Beetle Feeding Impact Study
-- Transgenics vs. Conventional Cultivars --

Project Code:H320 Location :Minto, Manitoba
Cooperator :Hoechst Canada, Ltd. By:David R.S. Rourke

Character rated	Plant	Visual	# Days	# Days	Duration	Maturity
Rating data type	Count	Vigor	To	To	of	SeedTurn
Rating unit	per m2	0-10	1st Flower	Last Flowr	Flowering	Per Cent
Rating date	06-28-93	06-22-93				09-14-93
Trt-Eval Interval	GS.2.4/5	30 DAE				Maturity
PRM Data Type						
Trt No Treatment Name						
1 Excel	117 a	7.3 ab	55 a	85 a	31 ab	49 b
2 Cyclone	119 a	8.3 a	54 b	83 c	31 b	41 c
3 Legend	96 a	7.0 b	52 c	83 c	32 a	50 b
4 HCN - 92	148 a	7.3 ab	53 b	84 b	32 a	58 a
LSD (.05) =	52	1.1	1	1	1	7
Standard Dev.=	32.4689	.712001	0.5	.416667	.583334	4.48764
CV =	27.13	9.57	0.94	0.50	1.86	9.09
k F	4.795	1.110	8.333	33.000	4.592	27.000
Prob(F)	0.0291	0.3949	0.0058	0.0001	0.0326	0.0001
Treatment F	1.728	2.425	20.333	22.440	6.061	8.793
Treatment Prob(F)	0.2305	0.1328	0.0002	0.0002	0.0153	0.0049

Means followed by same letter do not significantly differ (P=.05, Duncan's MRT)

DATA SUMMARY COMMENTS

Flea beetle damage was assessed on a 0-4 scale:
0 - no damage
1 - up to 25% damage
2 - up to 50% damage
3 - up to 75% damage
4 - plant mortality

Caged beetle damage ratings were obtained by placing 3 cages in each plot, each cage housing 10 beetles. Each cage covered 3 plants for the June 6 bioassay and 2 plants for the June 18 bio. Damage inside the cages was assessed 3 days after beetle introduction. Vigor was rated on a 0-10 scale, with 10 being the best. First flower dates were recorded when 5% of the plants per plot had their first open flower. Last flower dates were recorded when 50% of the plants per plot had <5 open flowers left on the main raceme.
Maturity was assessed as % seed turn on September 14, immediately after a killing frost.
Reps C&D were harvested on September 17, reps A&B on September 24, with a Wintersteiger plot combine. Yields were adjusted to 9% moisture, and the data analyzed with Duncan's MRT at 5%.
June 8, @ cotyledon to 1st leaf, and on June 14, @ 2 leaf, all plots had natural flea beetle damage of 1.0. On June 21, @ 3-4 leaf, all plots were 0.5.

- End of Report -

10-29-93 (H320.DAT)

Ag-Quest, Inc.
Flea Beetle Feeding Impact Study
-- Transgenics vs. Conventional Cultivars --

Project Code:H320
Cooperator :Hoechst Canada, Ltd.
Location :Minto, Manitoba
By:David R.S. Rourke

Trt No	Treatment Name	Plot No.	Grain Yield Kg/ha	Caged Beetle Damage 0-4	Natural Btle Damage 0-4	Caged Beetle Damage 0-4	Natural Btle Damage 0-4	Emergence Plot Per Cent	Emergence Plot Per Cent
	Character rated								
	Rating data type								
	Rating unit								
	Rating date		09-17-93	06-06-93	06-06-93	06-18-93	06-18-93	06-03-93	06-11-93
	Trt-Eval Interval			GS. 1	GS. 1	GS.2.2/3	GS.2.2/3	GS. 1	GS.1-2.2
	PRM Data Type								
1	Excel	101	1577.2	2.0	1.0	1.7	1.0	2	30
		203	2024.5	3.3	1.0	2.0	1.0	5	40
		304	2266.7	2.7	1.0	1.5	1.0	5	70
		402	1778.1	3.7	1.0	1.7	1.0	10	70
		Mean =	1911.6	2.9	1.0	1.7	1.0	6	53
2	Cyclone	102	2567.6	3.3	1.0	1.7	1.0	10	70
		201	2566.0	3.3	1.0	1.5	1.0	10	50
		303	1957.3	2.7	1.0	1.3	1.0	10	70
		404	2362.5	2.0	1.0	1.5	1.0	10	70
		Mean =	2363.4	2.8	1.0	1.5	1.0	10	65
3	Legend	103	2130.1	3.7	1.0	1.3	1.0	2	40
		204	2027.9	3.0	1.0	1.7	1.0	5	40
		302	1877.8	3.0	1.0	2.0	1.0	5	60
		401	1910.8	2.7	1.0	1.5	1.0	5	60
		Mean =	1986.6	3.1	1.0	1.6	1.0	4	50
4	HCN - 92	104	2191.5	3.7	1.0	2.0	1.0	2	50
		202	2102.1	2.7	1.0	1.7	1.0	10	50
		301	2307.2	3.0	1.0	1.8	1.0	5	60
		403	1714.2	3.3	1.0	2.3	1.0	5	60
		Mean =	2078.8	3.2	1.0	2.0	1.0	6	55

10-29-93 (H320.DAT)

Ag-Quest, Inc.
Flea Beetle Feeding Impact Study
-- Transgenics vs. Conventional Cultivars --

Project Code:H320
Cooperator :Hoechst Canada, Ltd.

Location :Minto, Manitoba
By:David R.S. Rourke

Trt No	Treatment Name	Plot No.	Plant Count per m2 06-28-93 GS.2.4/5	Visual Vigor 0-10 06-22-93 30 DAE	# Days To 1st Flower	# Days To Last Flowr	Duration of Flowering	Maturity SeedTurn Per Cent 09-14-93 Maturity
1	Excel	101	39	6.0	56	86	31	30
		203	129	7.0	55	86	32	40
		304	125	8.0	54	83	30	60
		402	175	8.0	54	85	32	65
		Mean =	117	7.3	55	85	31	49
2	Cyclone	102	103	9.0	54	84	31	35
		201	138	8.0	54	84	31	30
		303	101	7.0	53	82	30	50
		404	133	9.0	53	82	30	50
		Mean =	119	8.3	54	83	31	41
3	Legend	103	68	7.0	53	84	32	40
		204	112	7.0	53	84	32	40
		302	77	7.0	51	81	31	60
		401	125	7.0	51	83	33	60
		Mean =	96	7.0	52	83	32	50
4	HCN - 92	104	77	7.0	53	85	33	50
		202	227	7.0	54	85	32	50
		301	155	7.0	53	83	31	60
		403	131	8.0	53	84	32	70
		Mean =	148	7.3	53	84	32	58

1993 WEATHER DATA FOR MINTO, MANITOBA

Date April	Daily Temperature(°C) High	Low	Precip. (mm)
1	3	-10	
2	6	-5	
3	6	-2	
4	10	-3	
5	12	-2	
6	10	2	
7	5	2	
8	5	2	
9	3	-2	
10	3	2	1
11	8	2	
12	8	2	
13	10	2	
14	12	-1	
15	12	-2	
16	11	2	
17	15	3	
18	3	-1	
19	5	-3	
20	10	-4	
21	16	-3	
22	18	0	
23	15	-1	
24	8	0	
25	5	-5	
26	18	7	2
27	9	1	1.8
28	5	0	
29	13	-1	
30	8	-1	

Temperature	
Mean High	8.7
Mean Low	-0.7
Mean	4.0
100 yr. av.	3.2
Difference	+0.8
Total Precip.	4.8
100 yr. av.	30.1
% of Average	16%

1993 WEATHER DATA FOR MINTO, MANITOBA

Date May	Daily Temperature (°C) High	Low	Precip. (mm)
1	12	2	
2	16	4	
3	21	7	
4	23	7	
5	27	10	
6	25	12	
7	15	11	1.8
8	16	8	
9	17	5	8.5
10	26	10	
11	29	11	
12	29	11	
13	28	11	
14	14	6	
15	13	5	
16	15	3	
17	11	4	
18	11	5	
19	13	3	
20	16	6	
21	23	11	
22	25	10	
23	13	8	trace
24	15	6	
25	14	7	1
26	17	3	
27	8	5	3
28	17	4	
29	14	9	8
30	16	6	7
31	13	3	

Temperature	
Mean High	18.8
Mean Low	8.2
Mean	10.2
100 year av.	10.5
Difference	-0.3
Total Precip.	29.3
100 year av.	48.6
% of Average	60%

1993 WEATHER DATA FOR MINTO, MANITOBA

Date June	Daily Temperature (°C) High	Low	Precip. (mm)
1	17	3	
2	14	5	
3	17	8	
4	19	3	
5	21	6	1
6	18	7	
7	17	7	18
8	12	9	
9	23	10	
10	26	15	
11	28	12	
12	28	15	12+hail
13	17	11	3
14	17	8	
15	20	-	
16	11	8	8.3
17	18	8	
18	22	6	
19	23	7	
20	28	12	
21	27	16	
22	26	15	5.3
23	18	10	9.7
24	15	10	1.7
25	14	8	
26	13	8	
27	13	7	
28	14	7	2.5
29	14	8	21
30	20	11	trace

Temperature	
Mean High	19.0
Mean Low	8.9
Mean	14.0
30 year av.	15.3
Difference	-1.3
Total Precip.	82.5
30 year av.	78.4
% of Average	105%

1993 WEATHER DATA FOR MINTO, MANITOBA

Date July	Daily Temperature(°C) High	Low	Precip. (mm)
1	20	12	
2	24	10	
3	16	14	25
4	18	15	7
5	15	15	4.3
6	12	10	
7	20	8	
8	20	9	0.7
9	21	12	
10	18	9	
11	17	9	
12	18	7	
13	18	8	2.5
14	20	7	
15	16	10	9
16	18	10	6.6
17	20	12	
18	17	12	20
19	18	12	
20	18	9	
21	16	11	
22	18	15	13.3
23	20	14	3
24	20	11	_
25	19	18	1
26	22	16	
27	17	17	13.5
28	22	14	
29	24	18	
30	23	16	
31	22	12	

Temperature	
Mean High	18.9
Mean Low	12.0
Mean	15.5
100 year av.	18.7
Difference	-3.2
Total Precip.	105.8
100 year av.	70.4
% of Average	150%

1993 WEATHER DATA FOR MINTO, MANITOBA

Date August	Daily Temperature(°C) High	Low	Precip. (mm)
1	17	12	1
2	15	9	
3	17	8	
4	19	8	
5	17	8	
6	20	10	
7	21	15	
8	23	13	
9	25	15	
10	28	12	
11	27	9	25.8
12	16	10	
13	18	8	
14	19	12	11.5
15	15	11	
16	20	13	
17	20	15	4.2
18	19	13	0.8
19	19	12	
20	21	13	
21	26	14	
22	20	15	11.5
23	22	15	
24	26	11	
25	22	14	1.2
26	21	13	
27	20	11	2.0
28	18	12	3.0
29	13	11	4.8
30	16	8	
31			

Temperature	
Mean High	20.1
Mean Low	11.9
Mean	16.0
30 year av.	17.3
Difference	-1.3
Total Precip.	65.8
30 year av.	64.7
% of Average	102%

1993 WEATHER DATA FOR MINTO, MANITOBA

Date September	Daily Temperature(°C) High	Low	Precip. (mm)
1	18	11	
2	15	9	1.5
3	22	7	
4	13	7	4.0
5	15	5	trace
6	16	3	
7	18	4	
8	25	10	trace
9	16	9	2.0
10	17	4	
11	24	8	
12	15	3	6.0
13	5	3	
14	11	-1	
15	15	2	
16	13	6	trace
17	17	1	
18	19	2	
19	18	3	
20	20	5	
21	9	5	4.0
22	12	3	
23	18	4	
24	22	5	
25	20	4	
26	9	4	
27	17	3	
28	10	1	
29	13	0	
30	17	3	

Temperature	
Mean High	16.0
Mean Low	4.4
Mean	10.2
100 yr. av.	11.5
Difference	-1.3
Total Precip.	17.5
100 yr. av.	46.8
% of Average	41 %

SUMMARY OF GROWTH STAGES IN CANOLA

Stage	Description of Main Raceme
0	Pre-emergence
1	Seedling
2	Rosette 2.1 First true leaf expanded 2.2 Second true leaf expanded 2.3 etc. for each additional leaf
3	Bud 3.1 Flower cluster visible at centre of rosette 3.2 Flower cluster raised above level of rosette 3.3 Lower buds yellowing
4	Flower 4.1 First flower open 4.2 Many flowers opened, lower pods elongating 4.3 Lower pods starting to fill 4.4 Flowering complete, seed enlarging in lower pods
5	Ripening 5.1 Seeds in lower pods full size, translucent 5.2 Seeds in lower pods green 5.3 Seeds in lower pods green-brown or green-yellow, mottled 5.4 Seeds in lower pods yellow or brown 5.5 Seeds in all pods brown, plant dead.

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VII. REFERENCES

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