

# Safety Assessment of Roundup Ready® Canola Event GT73

## Executive Summary

Using modern biotechnology, Monsanto Company has developed Roundup Ready® canola plants (*Brassica napus*) that are tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. Glyphosate is an inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a well-known enzyme of the shikimate pathway for aromatic amino acid biosynthesis that is ubiquitous in plants, fungi, and bacteria. Plants, including weeds, exposed to glyphosate are unable to produce aromatic amino acids and hence die. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic animals. This explains the selective activity in plants and contributes to the low risk to human health and the environment from the use of glyphosate according to label directions. Two genes were introduced into the canola genome to produce this product: the *cp4 epsps* gene, derived from the common soil bacterium *Agrobacterium* strain CP4, which encodes for the production of the CP4 EPSPS enzyme, and the *gox* gene from *Ochrobactrum anthropi* strain LBAA, which encodes for the production of the enzyme glyphosate oxidase (GOX). Both gene products, the CP4 EPSPS and GOX proteins, are expressed constitutively in the plant, and together they are responsible for conferring tolerance to glyphosate. Because CP4 EPSPS has a naturally high tolerance to inhibition by glyphosate, Roundup Ready canola plants continue to produce aromatic amino acids even after treatment with glyphosate. In addition, the GOX protein catalyzes the breakdown of glyphosate into glyoxylic acid and aminomethylphosphonic acid (AMPA).

The glyphosate tolerance of Roundup Ready canola has been demonstrated in field tests starting in 1992 and in additional field tests conducted throughout growing regions in the United States, Canada, Europe and Australia. Roundup Ready canola was first planted commercially in 1996 on 50,000 acres in Canada. In the 2000 growing season, approximately 5.4 million acres (2.2 million hectares) of Roundup Ready canola were planted in Canada and the USA. Growers planting Roundup Ready canola have reduced the number and amount of herbicides used to control economically destructive weeds that grow in their fields, especially cruciferous weeds that are difficult to control with conventional weed control methods in canola, and have thereby realized a savings in weed control costs. This reduction in herbicide use has benefited the environment and also allows growers to implement integrated weed management practices that are generally not possible when pre-plant or pre-emergent herbicides are used.

The following summary provides information on the methods used to develop Roundup Ready canola, and an overview of the food, feed and environmental safety studies that have been conducted. These include molecular characterization of the DNA inserted into Roundup Ready canola, an assessment of the safety of the introduced proteins, compositional analyses of the food and feed components to establish substantial equivalence to conventional canola varieties, feeding studies in broiler, rat, fish and quail, and an assessment of the environmental safety of this product.

These studies establish the food, feed and environmental safety of Roundup Ready canola by demonstrating the safety of the CP4 EPSPS and GOX proteins to humans and animals, establishing equivalent nutritional composition and wholesomeness of Roundup Ready canola compared to conventional canola varieties, and confirming that the potential impact of Roundup Ready canola on the environment is no different than conventional canola varieties.

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## Introduction

Monsanto Company has developed Roundup Ready canola (*Brassica napus*) to enable farmers to effectively control weeds during the growing season by using Roundup agricultural herbicides. The primary mode of action of glyphosate, the active ingredient in Roundup agricultural herbicides, is the competitive inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Padgett *et al.*, 1996a; Franz *et al.*, 1997). This enzyme is critical for the production of aromatic amino acids in the shikimate plant pathway (Steinrucken and Amrhein 1980). When conventional plants, including weeds, are treated with glyphosate, the plants cannot produce the aromatic amino acids and other compounds derived from this pathway which are needed to survive.

Roundup Ready canola plants produce the CP4 5-enolpyruvylshikimate-3-phosphate synthase protein (CP4 EPSPS) derived from *Agrobacterium* strain CP4. The CP4 EPSPS is naturally less sensitive to inhibition by glyphosate and has been shown to impart tolerance to glyphosate in several crops (Padgett *et al.*, 1996b). In addition, Roundup Ready canola produces the glyphosate oxidoreductase (GOX) protein, derived from the bacteria *Ochrobactrum anthropi* strain LBAA, as a second mechanism to impart tolerance to glyphosate. The GOX protein breaks glyphosate down into the non-toxic compounds aminomethylphosphonic acid (AMPA), the primary metabolite of glyphosate in plants, and glyoxylic acid (Padgett *et al.*, 1996a). Together, CP4 EPSPS and GOX confer glyphosate tolerance to canola plants containing these proteins.

Weed management is a critical step in maximizing canola yields while retaining a high-quality grain harvest that is free of weed seeds. For effective weed control, the farmer typically selects a herbicide based on several factors: weed spectrum, lack of crop injury, cost, and environmental characteristics. Few herbicides available prior to the introduction of Roundup Ready canola delivered optimal performance in all of these areas. Several classes of herbicides were effective for broad-spectrum weed control, but many were either non-selective and kill crop plants or significantly injured some crops at the application rates required for effective weed control. Roundup agricultural herbicides are used as foliar-applied, non-selective herbicides. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Furthermore, glyphosate has no pre-emergence or residual soil activity (Franz *et al.*, 1997), is not prone to leaching, degrades in soil over time, and will not cause unreasonable adverse effects to human health or the environment under normal use conditions (US EPA, 1993; WHO, 1994; Geisy *et al.*, 2000; Williams *et al.*, 2000).

Roundup Ready canola positively impacts current agronomic practices in canola by providing improved weed control. Weeds are a significant constraint in the production of canola worldwide and their control is commonly one of the highest growing costs in *Brassica* oilseeds (Orson, 1995). Canola cannot compete effectively with weeds in its early growth stages, so weeds must be controlled to maximize yield potential. Conventional management systems interweave cultural and mechanical practices with herbicides to overcome the competition from weeds (Orson, 1995). In a recent survey by the Canola Council of Canada (2001), growers cited more efficient weed control was a key factor in their decision to adopt herbicide tolerant canola among growers, in addition to the economic benefits derived from this technology. The survey results also conclude that biotechnology-based systems, including Roundup Ready canola GT73, result in a 10% yield advantage over conventional systems, thus contributing to an overall economic value and increase in canola production. Additional benefits reported included easier herbicide management to prevent weed resistance, more flexible crop rotation and the ability to seed earlier in the spring or the fall, thus benefiting from soil moisture conservation.

Other benefits were harvest management, use of less toxic chemicals or those that leave less soil residue and approximately 6000 tons reduction in herbicides used per year by 2000 by the introduction of Roundup Ready and Basta tolerant canola. In addition, approximately 2.6 million acres in canola rotations in western Canada have been positively impacted by increased conservation tillage practice (Canola Council of Canada, 2001).

## Molecular Development and Characterization of Roundup Ready Canola

Roundup Ready canola line GT73 was developed by transforming the well-known Westar canola variety (*Brassica napus* L.) (Downey and Röbbelen, 1989). Since 1982, this variety has had widespread use in the commercial production and breeding of canola. In 1985, Westar was used to plant over 80% of all *B. napus* (canola) acres in Canada, and was used as a standard in the Western Canadian Cooperative Rapeseed Test (Co-Op Test) until 1994 (Klassen *et al.*, 1987). Due to the development of varieties that are higher yielding and more tolerant to blackleg disease (*Phoma lingam*), the Westar variety has steadily lost market share to less than 1% in 1993. The Roundup Ready trait has since been transferred into over 38 commercial canola varieties by traditional breeding techniques.

The disarmed *Agrobacterium tumefaciens* plant transformation system was used to produce Roundup Ready canola line GT73 (White, 1989; Howard *et al.*, 1990). This delivery system is well documented to transfer and stably integrate T-DNA into a plant nuclear genome (White, 1989; Howard *et al.*, 1990). The plant transformation vector used to produce Roundup Ready canola line GT73 was plasmid PV-BNGT04 (Figure 1), a double-border plasmid vector. The following elements between the right and left borders were shown to be incorporated into the Westar genome:

- The Figwort Mosaic Virus (FMV) 35S promoter (Gowda *et al.*, 1989; Richins *et al.*, 1987) used to regulate expression of the *cp4 epsps* gene.
- The Arab-SSU1A-CTP1 sequence, designated CTP1, fused to the *gox* gene is a chloroplast transit peptide derived from the small subunit of ribulose biphosphate carboxylase of *Arabidopsis thaliana* (Timko *et al.*, 1988). The CTP sequence facilitates import of the translated protein into the chloroplast, the site of the shikimate pathway.
- A variant of the glyphosate oxidoreductase (GOX) gene designated *goxv247*.
- The E9 3' region from pea ribulose biphosphate carboxylase gene (*rbcS*) to direct transcriptional termination and polyadenylation of the *goxv247* gene (Corruzi *et al.*, 1984; Morelli *et al.*, 1985).
- The *epsps/ctp2* sequence, designated *ctp2*, fused to the *cp4 epsps* gene is a chloroplast transit peptide from *Arabidopsis thaliana epsps* gene (Gowda *et al.*, 1989; Klee *et al.*, 1987).
- The synthetic 5-enolpyruvylshikimate-3-phosphate synthase *cp4 epsps* gene based on the sequence from *Agrobacterium* sp. strain CP4.
- The E9 3' region from pea ribulose biphosphate carboxylase gene (*rbcSA*) to direct transcriptional termination and polyadenylation of the *cp4 epsps* gene (Corruzi *et al.*, 1984; Morelli *et al.*, 1985).

Only the DNA required to confer the glyphosate-tolerance phenotype was transferred and inserted at a single locus in the canola genome. No genetic elements from outside of the right and left borders of the plasmid were transferred into or are present in the genomic DNA of Roundup Ready canola. The insert was characterized using Southern blot and PCR analysis. A single chromosomal copy of the inserted DNA is present in event GT73 and is stably inherited across generations. Moreover, the

consistent commercial performance of Roundup Ready canola over the past five years further supports the stability of the inserted DNA and functioning of the CP4 EPSPS and GOX proteins.

### *Genetic Stability*

Both *cp4 epsps* and *gox* genes responsible for conferring glyphosate tolerance in Roundup Ready canola containing event GT73 have been demonstrated to be stably integrated into the chromosome. This conclusion is based on molecular analyses, data on phenotypic expression, and inheritance patterns. The results of these studies may be summarized as follows:

- Molecular analyses of plants from the R3 and R5 generations of Roundup Ready canola GT73 establish that the introduced genes are maintained in the same chromosomal location;
- Analyses of seed obtained from multi-site trials using generations R<sub>2</sub> and R<sub>5</sub> showed no marked change in expression of the glyphosate-tolerance proteins;
- The level of glyphosate tolerance has been maintained for at least six generations and over five years of commercial production on 5.4 millions of acres (in 2000) with no reports of loss of glyphosate tolerance;
- Glyphosate tolerance has been confirmed under different environmental conditions, and in many genetic backgrounds;
- Single gene Mendelian inheritance of the glyphosate tolerance trait was observed after self-pollination or backcrossing with other canola varieties;
- Seed quality of Roundup Ready canola was maintained after transfer of the glyphosate tolerance genes into different genetic backgrounds.

In summary, it is concluded that the inserted genes in Roundup Ready canola are stably integrated and the event is phenotypically and genetically stable across generations and in various environments. The genetic stability has been confirmed with the successful commercialization of Roundup Ready canola over the past five years. Based on this information, the likelihood of instability is considered to be negligible.

### **CP4 EPSPS and GOX Protein Levels in Roundup Ready Canola Plants**

To thoroughly characterize Roundup Ready canola event GT73, the levels of CP4 EPSPS and GOX proteins were measured in leaf and seed tissue from three Canadian field sites in 1992, in seed from four Canadian field sites in 1993, and in leaf and seed tissue from six European field sites in 1995. Production of the CP4 EPSPS and GOX proteins is constitutive, with both proteins being detectable at relatively low levels in the tissues analyzed. Expression levels of CP4 EPSPS and GOX proteins in canola plant tissues were measured by a validated enzyme linked immunosorbent assay (ELISA).

Analyses of CP4 EPSPS and GOX protein levels in Roundup Ready canola event GT73 were conducted in 1992, 1993 and 1995. Mean expression levels in leaf tissue from 1992 to 1995 ranged between 0.027-0.034 µg/mg tissue for CP4 EPSPS and 0.108-0.133 µg/mg tissue for GOX on a fresh weight basis. Mean expression levels measured in seed from 1992 to 1995 ranged between 0.028-0.049 µg/mg tissue for CP4 EPSPS and 0.154-0.211 µg/mg tissue for GOX on a fresh weight basis (Table 1). These expression levels are relatively low, with the CP4 EPSPS and GOX proteins accounting for less than 0.02% and 0.07% of the total protein in the seed, respectively.

## Safety Assessment of CP4 EPSPS and GOX Proteins in Roundup Ready Canola

The safety assessment of the CP4 EPSPS and GOX proteins in Roundup Ready canola event GT73 consisted of several key components including protein characterization, the history of safe consumption, digestion in simulated gastric and intestinal fluids, acute oral toxicity tests in mice, and amino acid comparisons to known toxins and allergens for both expressed proteins (OECD, 1997).

### *CP4 EPSPS and GOX Protein Characterization and History of Safe Consumption*

The CP4 EPSPS protein expressed in Roundup Ready canola is functionally similar to a diverse set of EPSPS proteins typically present in food and feed derived from plant and microbial sources (Levin and Sprinson, 1964; Harrison, *et al.*, 1996). The EPSPS proteins are required for the production of aromatic amino acids in plants and microbes. Genes for numerous EPSPS proteins have been cloned (Padgett *et al.*, 1988, 1991, and references therein), and active site domains are conserved among the known EPSPS proteins (Padgett *et al.*, 1988; 1991). Bacterial EPSPS proteins have been well characterized with respect to the three-dimensional X-ray crystal structure (Stallings *et al.*, 1991) and the detailed kinetic and chemical reaction mechanisms have been determined (Anderson and Johnson, 1990). The enzymology and known function of EPSPS proteins generally, and the CP4 EPSPS protein specifically, establish that this class of enzymes performs a well-described and understood biochemical role in plants. The detailed enzymology (Harrison *et al.*, 1996) and biochemical composition evaluations (Padgett *et al.*, 1996a) confirm that the CP4 EPSPS protein, as expressed in Roundup Ready canola, has the predicted metabolic effect: the production of aromatic amino acids via the shikimic acid biosynthetic pathway.

Safety evaluations were conducted including assessment of the known structural relationship of the CP4 EPSPS protein with other EPSPS proteins found in food, the comparison of the amino acid sequences with conserved identity of the active site residues, the expected conserved three-dimensional structure based on similarity of the amino acid sequence, and the fact that EPSPS proteins catalyze a non-rate-limiting step in the aromatic amino acid pathway (hence increases in the level of EPSPS proteins would not be expected to affect the flux through the aromatic amino acid pathway). With respect to amino acid sequence, there is considerable divergence among known EPSPS proteins. For instance, the amino acid sequence of CP4 EPSPS protein is 41% identical at the amino acid level to *Bacillus subtilis* EPSPS protein, whereas the soybean EPSPS protein is 30% identical to *Bacillus subtilis* EPSPS protein. Thus, the divergence of the CP4 EPSPS protein amino acid sequence from typical food EPSPS protein sequences is on the same order as the divergence among food EPSPS proteins themselves (Harrison *et al.*, 1996).

The enzyme glyphosate oxidoreductase (GOX and GOX<sub>v247</sub>) catalyzes the cleavage of the C-N bond of glyphosate, yielding aminomethylphosphonate (AMPA) and glyoxylate as reaction products (Barry *et al.*, 1992). Glyoxylate is a compound naturally present in plant cells involved in carbohydrate and amino acid metabolism. AMPA can then be non-selectively bound to natural plant constituents, further degraded to one-carbon fragments that are incorporated into natural products, or conjugated with naturally-occurring organic acids to give trace level metabolites. AMPA is the most important metabolite in biological systems and a recent review of the toxicology studies with AMPA can be found in Williams *et al.*, 2000. . Regulatory authorities and the FAO/WHO consider AMPA of no more toxicological concern than glyphosate (European Commission, 2001; US EPA, 1997; FAO/WHO,

1997). The substrate specificity of GOX was also examined and these studies establish a narrow specificity of the GOX enzyme for compounds with secondary amines and specific steric and electronic properties similar to glyphosate. Based on the known biochemical pathways in plants, there are no known chemicals that have the properties that would make them suitable GOX substrates.

Furthermore, the homology of the GOX protein to known proteins was assessed using bioinformatics evaluations of protein sequence databases. These assessments show some alignments to various D-amino acid oxidases and oxidoreductases, largely from bacterial sources but lack homology to known protein toxins. The D-amino acid oxidases and oxidoreductases are widespread, and include human functional homologues. In humans, the D-amino acid oxidases are thought to provide a protective function, as they are responsible for the metabolism of D-amino acids.

The potential consequences for human and animal health and the environment from the presence of the GOX proteins and any GOX metabolites have been assessed on several levels. Compositional analyses on GT73 seed and meal established that for all parameters measured, there were no biological significant differences between GT73 and its parental line, Westar (Tables 2-10). In addition, animal feeding studies (in broiler, rat, trout, quail) with raw and processed canola meal from GT73 also demonstrate that there are no adverse effects from consumption of the feed from GT73, the GOX protein or potential GOX metabolites.

#### *In vitro Digestion of CP4 EPSPS and GOX Proteins*

*In vitro* simulated mammalian gastric and intestinal digestive mixtures were used to assess the susceptibility of the CP4 EPSPS and GOX proteins to proteolytic digestion. Rapid degradation of the protein correlates with limited exposure to the gastrointestinal tract and little likelihood that the protein can produce pharmacological, toxic or allergenic effects. The method of preparation of the simulated digestion solutions used is described in the United States Pharmacopeia (1990).

The CP4 EPSPS protein was shown to be rapidly degraded in these *in vitro* studies (Harrison *et al.*, 1996), greatly minimizing any potential for this protein to be absorbed by the intestinal mucosa. Western blot analysis demonstrated a half-life for CP4 EPSPS of less than 15 seconds in the simulated gastric system and less than 10 minutes in the intestinal system. To put the rapid degradation of the CP4 EPSPS protein in the simulated gastric system into perspective, solid food has been estimated to empty from the human stomach by about 50% in two hours, while 50% of liquid intake has left the stomach within approximately 25 minutes (Sleisenger and Fordtran, 1989). If the CP4 EPSPS protein were not degraded in the gastric system, it would be rapidly degraded in the intestine. Proteins that are rapidly degraded in the gastrointestinal tract are unlikely to confer toxicity or allergy (Astwood *et al.*, 1996; Astwood and Fuchs, 2000).

Similarly, the GOX protein and its variant, the GOX<sub>v247</sub> protein, were shown by western blot analysis to rapidly degrade *in vitro* using simulated gastric and intestinal fluids. In simulated gastric fluid, the GOX protein degraded extremely rapidly, with more than 90% of the GOX protein degraded within 15 seconds. In simulated intestinal fluids, more than 90% of the GOX protein degraded within 30 seconds. As an additional indicator of degradation, GOX enzymatic activity was found to dissipate readily in both systems.

#### *Assessment of Acute Oral Toxicity of CP4 EPSPS and GOX Proteins*

Few proteins are toxic when ingested and those that are toxic typically act in an acute manner (Sjoblad *et al.*, 1992). To confirm the lack of acute toxicity, oral toxicity studies with CP4 EPSPS and GOX proteins as the test material was performed on mice to directly assess any potential toxicity associated with the protein. Acute administration was considered sufficient to assess the safety of the CP4 EPSPS and GOX proteins, since proteins that are toxic act via acute mechanisms. There were no treatment-related adverse effects in mice administered CP4 EPSPS protein by oral gavage at dosages up to 572 mg/kg of body weight (Harrison *et al.*, 1996). This dose represents a significant – greater than 1000-fold – safety margin relative to the highest potential human consumption of CP4 EPSPS protein and assumes that the protein is expressed in multiple crops (Harrison *et al.*, 1996).

Similarly, there were no adverse effects noted in mice administered GOX<sub>v247</sub> protein by oral gavage at doses up to 104 mg/kg body weight. Since the only human-consumed fraction derived from canola, namely canola oil, does not contain detectable levels of protein, a safety margin cannot be calculated. However, the dose used was calculated to be approximately 5000-fold over a calculated exposure based on the hypothetical presence of the GOX<sub>v247</sub> proteins in corn.

Results from these studies demonstrated that the CP4 EPSPS and GOX<sub>v247</sub> proteins are, not acutely toxic to mammals. Most importantly, human exposure to CP4 EPSPS and GOX<sub>v247</sub> proteins are practically infinitesimal since, as already indicated, there is no human consumption of canola meal and there are no detectable proteins in oil. Studies demonstrate that protein levels in canola oil are less than the limit of detection of the method, approximately 1.28 ppm.

#### *Assessment of Structural Homology of the CP4 EPSPS and GOX Proteins to Known Protein Toxins*

Another aspect used for the assessment of potential toxic effects of proteins introduced into plants is to compare the amino acid sequence of the protein to known toxic proteins. Homologous proteins derived from a common ancestor are likely to share function. Therefore, it is undesirable to introduce DNA which encodes for proteins that are homologous to toxins. Homology is determined by comparing the degree of amino acid similarity between proteins using published criteria (Doolittle *et al.*, 1990). The CP4 EPSPS and GOX proteins do not show meaningful amino acid sequence similarity when compared to known protein toxins.

#### *Assessment of Allergenic potential of the CP4 EPSPS and GOX Proteins*

Introduction of Roundup Ready canola varieties does not present increased allergenic concerns. Although there are no single predictive bioassays available to assess the allergenic potential of proteins in humans (FDA, 1992), the physicochemical and human exposure profile of the protein provides a basis for assessing potential allergenicity by comparing it to known protein allergens. Thus, important considerations contributing to the allergenicity of proteins ingested orally includes exposure, and an assessment of the factors that contribute to exposure, such as stability to digestion, prevalence in the food, and consumption pattern (amount) of the specific food (Metcalf, *et al.*, 1996; Kimber *et al.*, 1999).

A key parameter contributing to the systemic allergenicity of certain food proteins appears to be stability to the peptic and acidic conditions of the digestive system (Astwood *et al.*, 1996; Astwood and Fuchs, 1996; Fuchs and Astwood, 1996; FAO, 1996; Kimber *et al.*, 1999). Important protein allergens tend to be stable to peptic digestion and the acidic conditions of the stomach if they are to reach the

intestinal mucosa where an adverse immune response can be initiated. As noted above, the *in vitro* assessment of the CP4 EPSPS (Harrison *et al.*, 1996) and GOX protein digestibility indicates that these proteins, like other food-derived proteins, are very labile to digestion when compared to many clinically important food allergens.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentrations in foods (Taylor, 1992; Taylor *et al.*, 1987; Fuchs and Astwood, 1996). Most food allergens are present as major protein components in the specific food, in amounts ranging from 1% up to 80% of total protein (Fuchs and Astwood, 1996). In contrast, the CP4 EPSPS and GOX<sub>v247</sub> proteins are present at extremely low levels in Roundup Ready canola seed – less than 0.02% and 0.07% of the total protein, respectively – and are not detectable in canola oil, the sole product from canola that is consumed by humans.

It is also important to establish that the introduced proteins do not represent previously described human allergens and do not share potentially immunologically-relevant amino acid sequence segments or structure with a known allergen. An efficient way to determine whether the added protein is an allergen, or is likely to contain cross-reactive structures to allergens, is to compare the amino acid sequence of the introduced protein with those of all known allergens. A database of protein sequences associated with allergy and coeliac disease has been assembled and is maintained from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt). The amino acid sequences of the CP4 EPSPS and GOX proteins were compared to these sequences and shown to have no meaningful amino acid sequence similarity with any of the known allergens (Fuchs and Astwood, 1996).

In summary, these data and analyses support the conclusion that the CP4 EPSPS and GOX<sub>v247</sub> proteins are not detectable in canola products used for human food, do not pose a significant allergenic risk, are not derived from allergenic sources, do not possess immunologically-relevant sequence similarity with known allergens, and do not possess the characteristics of known protein allergens (Table 11).

In addition, the U.S. EPA granted tolerance exemptions for the CP4 EPSPS protein on August 2, 1996 (FR 61(150):40338) and for the GOX and GOX<sub>v247</sub> proteins on October 9, 1997 (FR 62(195):52505). These determinations included an assessment of potential allergenic effects of the GOX protein, and the US EPA concluded there were no significant concerns.

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**Table 11. Characteristics of known allergenic proteins**

<i>Characteristic</i>	<i>Allergens</i>	<i>CP4 EPSPS</i>	<i>GOX</i>
Stable to digestion	yes	no	no
Stable to processing	yes	no	no
Similarity to known allergens	yes	no	no
Prevalent protein in food	yes	no	no

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As described in Taylor (1992) and Taylor *et al.* (1987)

### **Compositional Analysis and Nutritional Assessment of Roundup Ready Canola**

The design of a food and feed safety assessment program for a genetically improved crop requires detailed understanding of the uses of the crop and crop products in animal and human nutrition. Roundup Ready canola is processed for use in animal feed and human food ingredients. Essentially, edible oil is the sole product from canola that is consumed by humans while heat-processed canola meal is used solely as a protein-rich livestock feed and is not consumed by humans. The major uses of compound animal feed containing canola meal are in poultry, pig and cattle production. Oil, the main edible food ingredient derived from canola, contains negligible, non-detectable quantities of protein. It is used in cooking and salad oils, salad dressings, shortening, and margarine, and is currently an important edible oil used in Canada, Japan, and the U.S.A.

As a part of the program to assess the food and feed safety of Roundup Ready canola event GT73, extensive compositional analyses were performed on materials from both GT73 and the Westar control obtained from field trials. Seed from all lines underwent proximate analysis (% protein, fat, ash, moisture, fiber, carbohydrate, and calculated calories) and were analyzed for fatty acid and amino acid composition, including the aromatic amino acids tryptophan, phenylalanine and tyrosine. In addition to analyses of nutrients, canola GT73 was monitored for anti-nutrient factors: glucosinates, sinapine, and erucic acid. Additional compositional analyses were also performed on refined, deodorized, bleached oil and toasted meal fractions.

GT73 canola and the parental variety, Westar, were generated in field trials conducted in Canada over two years. The first test was conducted in 1992 at seven locations distributed across the primary canola production areas (Manitoba, Saskatchewan, and Ontario in Canada). The 1993 field trial was also conducted in Canada at four locations in the provinces of Manitoba and Saskatchewan. Data on the parental variety, Westar was also generated as part of official trials required for registration of all new canola varieties (Co-op Tests) and these results were included in the assessment. These results are summarized in Tables 2 through 10 and demonstrate that there is no material difference between GT73 and Westar in composition.

The results of all analyses (> 1800 individual assays) show that the composition of Roundup Ready canola event GT73 seed and the processed fractions (toasted meal and refined, deodorized and bleached oil) are not materially different from the control, non-transgenic varieties, canola seed or

fractions. Furthermore, the levels of anti-nutrients (glucosinolates (Tables 5-8), sinapine, phytic acid (Table 10) and erucic acid (content in GT73 was 0.24% in 1992 and 0.04% on 1993) prepared from Roundup Ready canola seed are at or below levels currently found in commercial canola. Based on these results, Roundup Ready canola event GT73 is substantially equivalent in proximate values to Westar canola. In addition, compositional analyses of Roundup Ready canola line GT73 grown commercially in Canadian Co-op trials during 1998 and 2000 support the conclusion of substantial equivalence (Table 12).

The nutritional equivalence of Roundup Ready canola was confirmed in feeding studies with broiler, rat, trout, and quail. As an example of the results from these types of studies, detailed results from the broiler study are presented in Table 13. In each of these studies there were no adverse effects observed in test animals that were attributed to the insertion of the GT73 glyphosate tolerance trait into canola.

In conclusion, the results of numerous analyses of the compositional and nutritional content confirm that Roundup Ready canola is as safe and nutritious as conventional canola. Products derived from Roundup Ready canola meal and oil are compositionally equivalent to commercially available canola varieties except for the expression of the CP4 EPSPS and GOX proteins. Importantly, the only human food derived from canola is canola oil, in which proteins including CP4 EPSPS and GOX are not detected. The heat processing inactivates both the CP4 EPSPS and GOX proteins in canola meal that is used for consumption by farm animals only.

## **Environmental Assessment**

### *Canola (Brassica napus)*

*Brassica* is a genus of the Brassicaceae (Cruciferae), commonly known as the mustard family. *Brassica napus* Linnaeus; known as rapeseed, rape, oilseed rape and canola in some cultivars, is a mustard crop grown primarily for its seed which yields about forty percent oil and a high-protein animal feed. It produces an inflorescence of yellow, nectar-bearing flowers that are capable of both self-fertilization and intraspecific cross-fertilization. *Brassica napus* is an ancient crop plant, referred to in Indian Sanskrit writings 2000-1500 BC as well as in Greek, Roman and Chinese writings 500-200 BC (Downey and Röbbelen, 1989). Currently, the top producers of canola are Canada, China, India, and the European Union (Niewiadomski, 1990).

The origin of the species *Brassica napus* can be traced to natural crossing between two diploid species, *B. oleracea* and *B. rapa*, growing in close proximity, followed by spontaneous chromosome doubling of the hybrid (Downey and Röbbelen, 1989, Kimber and McGregor, 1995). Amphidiploids, a special case of polyploidy, are formed by mating two species with different genomes and doubling the chromosome number of the hybrid. Such a doubled chromosome configuration would be stable at meiosis and thus allow the new polyploid species to reproduce. Crossing without chromosome doubling results in sterile progeny. Cytological studies of *B. napus* have shown that it contains both the aa and the cc genomes, and is an amphidiploid derived from the monogenomic species, *B. oleracea* (cc genome) and *B. rapa* (aa genome) (Mizushima, 1980; U, 1935).

*B. napus* is principally a self-pollinating crop which is also able to cross with other plants of the same species. Honeybees are the primary pollinators. Partial sexual compatibility exists with some related

Brassica spp. and other closely related species outside the genus. Pollen movement is by means of wind and insects, mainly bees. Wind is not a particularly effective means of cross-pollination, as *B. napus* pollen is fairly heavy and sticky and cannot travel more than a few yards without insect pollinators (Downey and Röbbelen, 1989).

Some crossing between individual plants of *B. napus* does occur under field conditions (Scheffler and Dale, 1994; Rakow and Woods, 1987). The only cultivated species sexually compatible with *B. napus* under field conditions are *B. napus*, *B. rapa*, and *B. juncea*. Hybrids between *B. napus* and *Diplotaxis muralis*, *Raphanus raphanistrum*, *Erucastrum gallicum* and *Hirschfeldia incana* have been reported under field conditions. Several other wild mustard species, *B. carinata*, *B. oleracea*, *Sinapis alba* syn *B. hirta* and *B. tournefortii*, require intervention such as emasculation and manual pollination to produce progeny when crossed with *B. napus* under field conditions (OECD, 1997). Thus, oilseed rape (*B. napus*) is principally a self-pollinating crop that readily crosses (forms hybrids) with other plants of the same species, and to a lesser extent with several other members of the mustard family. Literature information concerning the frequency of intraspecific outcrossing (Becker *et al.*, 1992; Bing *et al.*, 1991; Downey, 1992; Kapteijns, 1993; Kerlan *et al.*, 1992; Metz *et al.*, 1997) is variable in its conclusions, which reflects the fact that pollinator activity, planting density, genotype, weather and distance have an impact on outcrossing (Scheffler and Dale, 1994). Values have been reported as high as 30% (Rakow and Woods, 1987; Downey, 1992; Bing *et al.*, 1991).

Numerous pollen dispersal studies using *B. napus* have been conducted within the framework of the European Commission's Biotechnology Action Program (BAP)<sup>1</sup>, and BRIDGE<sup>2</sup> program, and the United Kingdom's PROSAMO<sup>3</sup> project. Experiments were small to medium scale, and employed a transgenic pollen source (marked with a marker gene) and measured the frequency of transfer of the marker gene to surrounding (non-transgenic) canola plants. Results from the PROSAMO project, employing a 9-meter diameter circle of transgenic rape in a one-hectare field, demonstrated that the frequency of outcrossing decreased from 5% at zero distance down to 0.0003% at 47 meters distance (Scheffler *et al.*, 1993). Earlier studies, using a 3m diameter circle of transgenic canola within a 100m diameter circle of canola also demonstrated that outcrossing frequency decreased from 1.5% at 3 meters distance, to non-detectable at 24 and 48 meter distances (Scheffler *et al.*, 1993; Dale *et al.*, 1991; De Greef, 1991). The results from the BRIDGE project, using male sterile canola as the pollen trap, confirmed that outcrossing frequencies to other OSR plants is likely to be minimal.

It can be concluded that *B. napus* pollen dispersal is mainly short distance dispersal, although dispersal may occur over greater distances at a very low frequency (Staniland, *et al.*, 2000; Scheffler *et al.*, 1993; Timmons *et al.*, 1996). Perhaps most importantly, transgenic *B. napus* lines do not exhibit any greater rates of outcrossing than non-transgenic lines (Downey, 1992). Volunteer canola plants that are herbicide tolerant can be easily managed by cost effective herbicide and tillage programs and normal crop rotations.

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<sup>1</sup> Biotechnology Action Program (1985-1990) - supported by the Directorate for Science, Research and Development (DGXII) of the Commission of the European Communities.

<sup>2</sup> Biotechnology Research for Innovation, Development and Growth in Europe (1990-1993) - supported by the Directorate for Science, Research and Development (DGXII) of the Commission of the European Communities.

<sup>3</sup> Planned Release of Selected and Modified Organisms (1991-1993) - supported by a consortium of the U.K Dept. of Trade and Industry, the U.K Agriculture and Food Research Council, and industrial members.

Most rapeseed-producing countries utilize the crop domestically. Those regions that export rapeseed, primarily Canada and the EU, share about 90% of world trade almost equally between them. The main importers are China, the EU, India and the US (Kimber and McGregor, 1995). The widespread use of canola as a source of food and animal feed is recent, with the development of varieties having low erucic and glucosinolate levels in the 1970s. In 1985, canola oil received Generally Regarded As Safe (GRAS) status by US FDA.

### *Assessment of weediness*

Weediness can be broadly defined as any capacity for invasion of natural habitat. Many species of *Brassica* and related mustards are weeds or have weedy tendencies. *B. napus* is mentioned as an occasional weed, escape or volunteer in cultivated fields (Munz, 1968, Bing *et al.*, 1996). *B. juncea*, *B. nigra*, *B. rapa*, and *S. arvensis* (= *B. kaber*) to some degree are agricultural weeds, sometimes serious, in much of the United States (Gleason 1952; Slife *et al.* 1960; Reed 1970).

*B. napus* is the only *Brassica* species naturalized in the United States, and is not considered to be a weed in the United States (Holm *et al.* 1979). Generally most crop plants are bred and carefully selected to express agriculturally useful traits, and therefore, they are not usually competitive in unmanaged or untended natural environments. In other words, they are not ecologically fit to survive. Canola and other rapeseed are very well adapted for cultivation (fertilization, herbicide, and pesticide application), but not so for growth outside agricultural environments. Without favorable conditions, and intensive cultivation, domesticated types of *B. napus* cannot compete successfully with naturalized forms of *B. napus* in the United States. Naturalized types of *B. napus* are sporadically distributed in Canadian environments, and in the United Kingdom, they are widespread in the wild (Mitchell-Olds, 1992). Non-transgenic canola plants are not weeds, and the only question that arises is whether glyphosate-tolerant canola is a weed or has the potential to become a weed.

Numerous experiments have been conducted to evaluate the weediness potential of Roundup Ready canola event GT73, including US field trials in 1996 and 1997, Canadian variety trials in 1992 and 1993, and European field trials in 1995. The results of field observations have shown that Roundup Ready canola line GT73 has no increased potential of becoming a weed compared to unmodified *B. napus*. Data for days to maturity, germination, seed yield, pod shattering, and volunteer counts all demonstrate that canola line GT73 is agronomically equivalent to Westar, the nontransgenic parental control (Tables 14-19), with the exception being its tolerance to glyphosate.

Dissemination of canola genes occurs primarily by means of seeds and pollen. Canola is mainly self-pollinating and if glyphosate tolerant individuals were to arise through interspecific or intergeneric hybridization, the tolerance would not confer any competitive advantage to these plants unless challenged by Roundup agricultural herbicides. This would only occur in managed ecosystems where Roundup agricultural herbicides are applied for broad-spectrum weed control, or in plant varieties developed to exhibit glyphosate tolerance and in which Roundup agricultural herbicides are used to control weeds. As with glyphosate tolerant *B. napus* volunteers, these individuals, should they arise, would be controlled using other available chemical means during normal crop and herbicide rotation. Thus, gene flow from Roundup Ready canola to relatives is indeed possible, but would not result in increased weediness or invasiveness of these relatives.

### *Assessment of Agronomic Performance*

Roundup Ready canola GT73 was evaluated for agronomic performance in Canadian variety trials<sup>4</sup> in 1992 and 1993 as well as in US field trials in 1996, 1997 and 1998 under permits or notifications acknowledged by the USDA-APHIS. Furthermore, Roundup Ready canola has been produced commercially for six years and assessments of the products performance have been published (Harker et al., 2000).

Standard data collected in the Canadian trials included relative emergence, vegetative growth, flowering time, days to maturity, susceptibility to black leg disease, compositional analyses for crude fat, crude protein, glucosinolates, yield and shattering. Roundup Ready canola GT73 was determined to be agronomically comparable or superior to a set of conventional varieties that included Westar and other non-transgenic commercial varieties. Germination tests on seed of canola GT73 and Westar from 1992 variety trials were conducted at the Agriculture Canada seed quality-testing laboratory in Saskatoon, Saskatchewan. Germination percentages were 98% for GT73 treated with Roundup and 99% for GT73 untreated and Westar, demonstrating high germination and essentially no difference between transgenic canola and non-transgenic controls.

In February 1995, the Western Canadian Canola and Rapeseed Recommending Committee (WCCRRC) recommended several canola varieties containing event GT73 for conditional registration on the basis of its suitable agronomic performance under Canadian conditions. Following this decision, canola GT73 was grown commercially in Canada in 1996 on 50,000 acres. In 2000, 4.6 million acres were planted in Canada and 800,000 acres in the US with excellent agronomic performance comparable to other commercial varieties. Currently, over 38 canola varieties containing event GT73 have been registered in Canada and the USA.

### *Lack of Effect to Non-target Organisms*

The conventional canola varieties grown currently are not considered to be harmful to other organisms. There are no indications that Roundup Ready canola is different than other canola in this respect. The CP4 EPSPS and GOX proteins are present in Roundup Ready canola at very low levels (Table 1), have been well characterized and have been demonstrated not to have adverse impacts on trout and quail in feeding studies with these organisms. As mentioned earlier, EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Harrison *et al.*, 1996), and is thus ordinarily present in food derived from plant sources. EPSPSs from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al.*, 1985). CP4 EPSPS thus represents one of many different EPSPSs found in nature. EPSPS is considered to be ubiquitous in nature since it is present in all plants and microorganisms. Therefore, all organisms that presently feed on plants and/or microbes have historically been exposed to EPSPS proteins.

On the basis of the characterization of the introduced proteins and the compositional analyses described above, no specific interactions of Roundup Ready canola with non-target organisms are to be expected, beyond those which occur with other canola varieties. The glyphosate tolerance trait is intended to provide protection to the crop when Roundup agricultural herbicides are applied to control competing

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<sup>4</sup> The Western Canadian Canola, Rapeseed Recommending Committee, must approve any new variety of Canola developed for commercial use through the independent variety approval process involving data review and approval. These trials examine the major agronomic and quality factors associated with Canola.

weeds. Extensive observations in the field have also confirmed that there are no differences between Westar and Roundup Ready canola in phenotype, susceptibility to diseases and predators, or yield, indicating that there is no alteration in the interactions with predatory or beneficial non-target organism.

### *Impact on Biodiversity*

From the extensive testing in Canada, Australia, USA and EU, and commercial experience in Canada and the USA, there is no indication that Roundup Ready Canola containing event GT73, compared to other canola, has negative impact on biodiversity. The potential for harm may be considered in view of the intended effects of the genetic modification, as well as the potential for harm resulting from any unintended effects. The intended modification to canola was the expression of CP4 EPSPS and GOX proteins conferring tolerance to glyphosate. It has been determined that both the CP4 EPSPS and GOX proteins are safe for consumption by animals and humans.

The potential for harm resulting from any unintended effects of the modification have been assessed by:

- Observations of the interaction of Roundup Ready canola and other organisms in various environments in the agronomic situation;
- Compositional analyses for indications of unintended modifications in canola seed quality;
- Confirmatory animal feeding studies with raw and processed canola;
- Pollination processes as determined by yield.

### *Appearance of Glyphosate-Tolerant Weeds*

More than 100 herbicide-resistant weed biotypes have been identified to date; over half of them are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Shaner, 1995). Resistance has usually developed because of the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action, long residual activity of the herbicide with the capacity to control weeds year long, and frequent applications of the same herbicide without rotation to the other herbicides or cultural control practices. Using these criteria, and based on current use data, glyphosate is considered to be an herbicide with a low risk for weed resistance (Benbrook, 1991). Nonetheless, questions have been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide. This concern is based on the assumptions that the use of the herbicide will increase significantly, and possibly that it will be used repeatedly in the same location. However, increases in other uses of glyphosate over the previous years have been more significant than the projected increase associated with the introduction of Roundup Ready crops. Although it cannot be stated that evolution of resistance to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a rare event because:

1. Weeds and crops are inherently not tolerant to glyphosate, and the long history of extensive use of glyphosate has resulted in few instances of resistant weeds (Bradshaw *et al.*, 1997);
2. Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants, and lack of residual activity in soil, which make the development of resistance unlikely;

3. Selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and would, therefore, be expected to occur rarely in nature under normal field conditions.

In 1996 in Australia, it was reported that a biotype of annual rye-grass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley *et al.*, 1996). To date, after examination of thousands of samples, six locations have been confirmed as having the resistance population, indicating that the phenomenon is not widespread. A large body of biochemical and molecular biology experiments to determine the cause of observed weed control differences between Australian rye-grass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. Conclusions drawn to date are that the resistant biotype is easily controlled by conventional practices (tillage, other herbicides), and is caused by a complex inheritance pattern, unlikely to occur across a wide range of other species. Results of these studies have been presented (Pratley, 1999).

Two additional reports of resistant ryegrass in northern California are being investigated. Similar to the Australian locations, these fields are small and isolated. Again, the use of mowing and other herbicides have been very effective in controlling the ryegrass. Research continues in an effort to better understand the resistance mechanism. Most recently, a population of *Elusine indica* (goosegrass) was reported to survive labeled rates of glyphosate in Malaysia; analyses found that the resistant goosegrass has a modified EPSPS protein that is two- to four-fold less sensitive to glyphosate than in more sensitive biotypes. Research is underway to investigate the resistance mechanism.

In summary, environmental assessments indicate that the risks present with Roundup Ready canola are equivalent to or not greater than those already present with traditional canola varieties. In addition, data generated to support the registration of Roundup agricultural herbicides and almost 30 years of use experience with glyphosate demonstrate that these herbicides will not cause unreasonable adverse effects to humans, mammals or other non-target organisms under normal use conditions. In addition, the data demonstrate that the use of these herbicides in canola is not expected to cause unreasonable adverse effects to the environment.

## Summary

The introduction of Roundup Ready canola provides an improvement in weed control effectiveness and ease in herbicide management in rotations. Growers have found their rotations to be more flexible and were able to seed earlier in the spring or fall, thus benefiting from soil moisture conservation. Additional benefits reported include harvest management and use of chemical herbicides that are less toxic or which leave less soil residue. In addition, approximately 2.6 million acres in canola rotations in western Canada have been positively impacted by increased conservation tillage practices. An increased yield advantage over conventional canola has also contributed to an overall increase in the adaptation of the technology by growers (Canola Council of Canada, 2001).

The inserted genes in Roundup Ready canola are stable and the line is phenotypically and genetically stable over generations and across varied environments. Agronomic studies have shown no phenotypic differences between the Roundup Ready and conventional canola varieties, except for the expected tolerance to glyphosate.

Extensive food, feed and environmental safety assessments confirm the safety of this product. The analyses included: 1) detailed molecular characterization of the introduced DNA; 2) safety assessments of the expressed CP4 EPSPS and GOX<sub>v247</sub> proteins; 3) compositional analysis of canola seed, oil and meal; 4) animal feeding studies with broiler, rat, trout and quail and 4) environmental impact assessment of canola and Roundup Ready canola event GT73. These studies demonstrate that GT73 is as safe as conventional canola from a human and animal safety standpoint and that the introduced CP4 EPSPS and GOX<sub>v247</sub> proteins are safe to non-target organisms, including humans, and animals. Finally, there will be no significant consumption of the newly expressed proteins since only the oil is consumed by humans and there is no detectable protein in canola oil. Given the safety of the proteins and the lack of exposure, Roundup Ready canola has been shown to be as safe and nutritious as conventional canola varieties.

Information and data contained within this document have been provided to regulatory authorities for review. Regulatory review continues as we update regulatory files and make submissions to additional countries globally.

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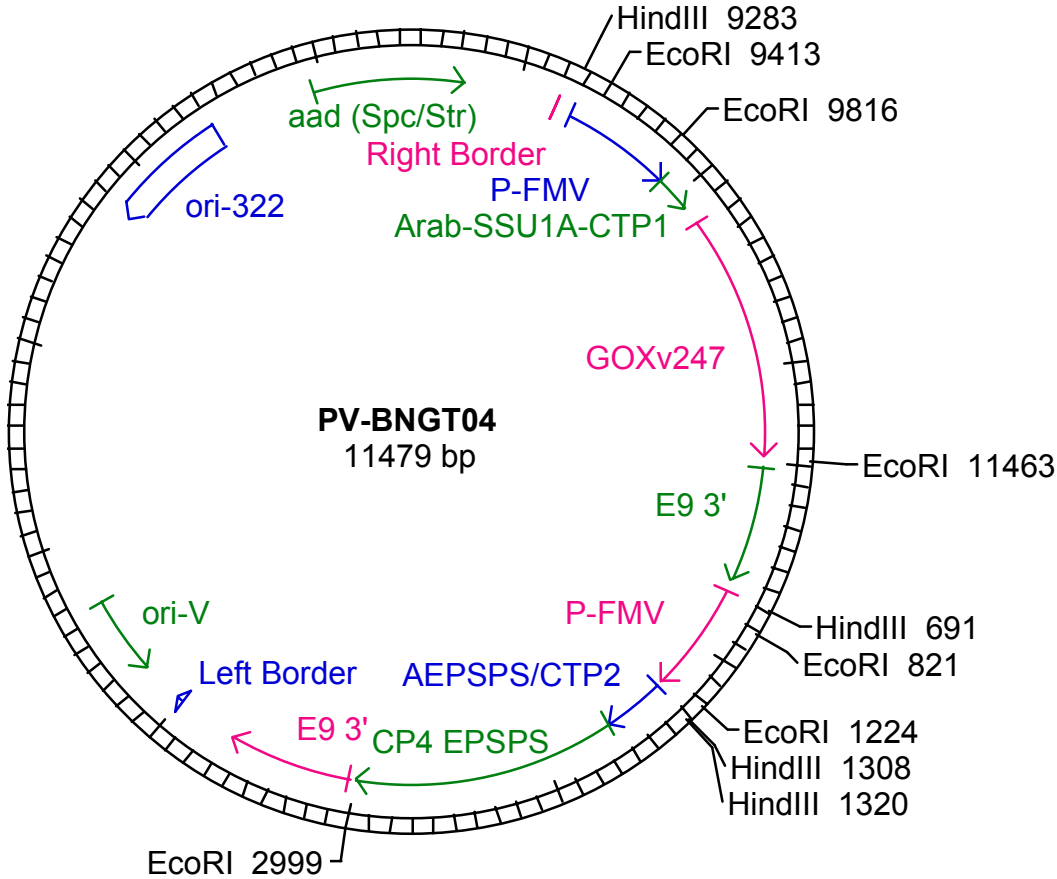
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**Figure 1:** Plasmid Map of PV-BNGT04



**Table 1. CP4 EPSPS and GOX Protein Expression in leaf and seed of Roundup Ready Canola Event GT73**

Tissue Type	CP4 EPSPS Protein (µg/mg tissue fwt)			GOX Protein (µg/mg tissue fwt)		
	1992 <sup>1</sup>	1993 <sup>2</sup>	1995 <sup>3</sup>	1992 <sup>1</sup>	1993 <sup>2</sup>	1995 <sup>3</sup>
<b>Leaf</b>						
mean:	0.034	n.a. <sup>5</sup>	0.027	0.108	n.a. <sup>5</sup>	0.133
range:	0.028-0.037		0.016-0.070	0.071-0.161		0.082-0.247
<b>Seed</b>						
mean:	0.049	0.028	0.028	0.154	0.193	0.211
range:	0.044-0.051	0.018-0.047	0.017-0.037	0.109-0.203	0.108-0.334	0.122-0.313
<b>Westar<sup>4</sup></b>						
Leaf	ND	n.a. <sup>5</sup>	ND	ND	n.a. <sup>5</sup>	ND
Seed	ND	ND	ND	ND	ND	ND

<sup>1</sup> Values for leaf and seed samples in 1992 from three field locations in Canada. CP4 EPSPS analyses were performed on single sample extracts; n=3 for leaf, n=3 for seed. GOX analyses were performed on single sample extracts; n=6 for leaf, n=6 for seed.

<sup>2</sup> Values for seed samples in 1993 from four field locations in Canada. CP4 EPSPS analyses were performed on single sample extracts at two loadings, n=8. GOX analyses were performed on single sample extracts, duplicate runs at two loadings, n=16.

<sup>3</sup> Values for leaf and seed samples in 1995 from six field locations in Europe. CP4 EPSPS analyses were performed on single sample extracts, run at two loadings, n=12 for leaf, and single loadings for seed, n=6 for seed. GOX analyses were performed on single sample extracts, run at three loadings, n=18 for leaf, and two loadings for seed, n=12 for seed.

<sup>4</sup> In each analysis, Westar canola samples were used as a negative control. Values for Westar canola samples were beneath a calculated limit of detection (LOD). The LOD is determined by computing the mean and the standard deviation for Westar control wells in ELISA. The LOD is then the mean plus three standard deviations. ND - not detected.

<sup>5</sup> n.a. - not available.

**Table 2. Protein Content of Roundup Ready Canola Event GT73 and Westar Canola from 1992 and 1993 Field Trials. (Values are % of defatted meal,  $\leq 3\%$  moisture basis)**

Sample (year)	Mean <sup>1</sup>	Range <sup>1</sup>	Number of Samples <sup>2</sup>
GT73 (92)	42.0	38.5 - 44.9	7
Westar (92)	41.1	38.4 - 42.9	7
Co-Op Westar (92)	43.3	34.8 - 48.0	52
GT73 (93)	41.8	39.6 - 44.8	4
Westar (93)	41.2	38.3 - 45.0	5
Co-Op Westar (93)	42.3	34.0- 50.8	87

<sup>1</sup> Analyses were run in triplicate using the same sample. Triplicates were averaged. The mean values are an average of the means of triplicate analyses of the same sample.

<sup>2</sup> Samples are the number of values used to calculate the means listed in this table.

**Table 3. Oil Content of Roundup Ready Canola Event GT73 and Westar Canola Seed from 1992 and 1993 Field Trials. (Values are % of whole seed,  $\leq 3\%$  moisture basis)**

Sample (year)	Mean <sup>1</sup>	Range <sup>1</sup>	Number of Samples <sup>2</sup>
GT73 (92)	45.2	43.2 - 48.8	7
Westar (92)	44.8	41.9 - 47.7	7
Co-Op Westar (92)	42.8	37.7 - 47.6	52
GT73 (93)	45.8	43.7 - 47.1	4
Westar (93)	45.1	42.4 - 47.3	5
Co-Op Westar (93)	44.8	37.9 - 51.1	87

<sup>1</sup> Analyses were run in triplicate using the same sample. Triplicates were averaged. The mean values are an average of the means of triplicate analyses of the same sample.

<sup>2</sup> Samples are the number of values used to calculate the means and ranges listed.

**Table 4. Fatty Acid Profile of Roundup Ready Canola Event GT73 and Westar Canola from Seed Samples from 1992 and 1993 Field Trials, Means and Ranges. (Values are % of total fat content)**

Fatty Acid	GT73 <sup>1</sup> (1992)	Westar <sup>1</sup> (1992)	Westar Co-op <sup>2</sup> (1992)	GT73 <sup>3</sup> (1993)	Westar <sup>3</sup> (1993)	Westar <sup>2</sup> Co-op (1993)
16:0	4.0 3.8-4.0	4.1 3.9-4.2	4.1 3.7-4.8	4.1 4.1-4.2	4.1 3.8-4.3	4.1 4.0-4.3
16:1	0.4 0.3-0.4	0.3 0.3-0.4	0.4 0.0-0.6	0.2 0.2-0.2	0.2 0.2-0.2	0.2 0.2-0.3
18:0	1.7 1.4-1.9	1.7 1.4-2.0	1.7 1.2-2.1	1.7 1.5-1.9	1.7 1.4-1.9	1.8 1.7-1.9
18:1	61.3 59.5-63.4	61.0 58.8-62.5	60.8 57.4-63.4	62.9 60.5-64.8	61.7 60.1-62.8	62.6 61.9-63.1
18:2	19.2 18.5-19.7	19.8 18.9-20.2	19.9 18.3-22.1	18.7 16.4-20.0	19.7 18.8-20.6	19.1 18.4-19.8
18:3	10.6 9.2-12.9	9.8 8.1-12.1	9.9 8.2-13.0	9.7 8.6-10.8	9.3 8.6-10.1	9.0 8.5-9.8
20:0	0.7 0.6-0.8	0.7 0.6-0.8	0.7 0.4-0.9	0.7 0.6-0.7	0.7 0.6-0.7	0.7 0.6-0.7
20:1	1.6 1.4-1.8	1.8 1.7-2.0	1.7 1.3-2.3	1.5 1.3-1.6	1.7 1.6-2.0	1.5 1.4-1.9
20:2	0.1 0.1-0.2	0.1 0.1-0.1	0.1 0.1-0.2	0.1 0.1-0.1	0.1 0.1-0.1	0.1 0.1-0.1
22:0	0.4 0.3-0.4	0.4 0.3-0.4	0.3 0.3-0.4	0.4 0.4-0.4	0.4 0.4-0.5	0.4 0.4-0.4
22:1 <sup>4</sup>	0.2 0.1-0.5	0.4 0.3-0.6	0.5 0.1-1.4	0.04 0.0-0.26	0.4 0.2-0.6	0.2 0.1-0.5

<sup>1</sup> n=7

<sup>2</sup> Values for Westar from the 1992 Co-op test, n=13, and from the 1993 Co-op test, n=8.

<sup>3</sup> n=5

<sup>4</sup> 22:1 is erucic acid

**Table 5. Alkyl Glucosinolate Content of Roundup Ready Canola Event GT73 and Westar Canola from 1992 and 1993 Field Trials. (Values are  $\mu\text{mol/g}$  defatted meal.)**

Sample (year)	Mean <sup>1</sup>	Range <sup>1</sup>	Number of Samples <sup>2</sup>
GT73 (1992)	11.2	9.0 - 14.2	7
Westar (1992)	8.8	6.2 - 11.4	7
Co-Op Westar (1992)	9.7	7.0 - 12.5	13
GT73 (1993)	10.6	8.0 - 12.9	5
Westar (1993)	8.9	6.7 - 11.1	5
Co-Op Westar (1993)	7.6	5.3 - 9.4	9

<sup>1</sup> Single samples were prepared and analyzed in quadruplicate in 1992 and triplicate in 1993. Replicates were averaged. The mean values are an average of the means of replicate analyses of the same sample.

<sup>2</sup> Samples are the number of values used to calculate the means and ranges listed.

**Table 6. Indolyl Glucosinolate Content of Roundup Ready Canola Event GT73 and Westar Canola from 1992 and 1993 Field Trials. (Values are  $\mu\text{mol/g}$  defatted meal.)**

Sample (year)	Mean <sup>1</sup>	Range <sup>1</sup>	Number of Samples <sup>2</sup>
GT73 (92)	11.6	10.8 - 12.4	7
Westar (92)	11.4	9.8 - 13.4	7
Co-Op Westar (92)	11.0	7.0 - 13.7	13
GT73 (93)	11.4	10.9 - 12.0	5
Westar (93)	11.5	11.0 - 12.5	5
Co-Op Westar (93)	11.5	10.7 - 12.5	9

<sup>1</sup> Single samples were prepared and analyzed in quadruplicate in 1992 and triplicate in 1993. Replicates were averaged. The mean values are an average of the means of replicate analyses of the same sample.

<sup>2</sup> Samples are the number of values used to calculate the means and ranges listed.

**Table 7. Thioalkyl Glucosinolate Content of Roundup Ready Canola Event GT73 and Westar Canola from 1992 and 1993 Field Trials. (Values are  $\mu\text{mol/g}$  defatted meal.)**

Sample (year)	Mean <sup>1</sup>	Range <sup>1</sup>	Number of Samples <sup>2</sup>
GT73 (92)	0.3	0.2 - 0.4	7
Westar (92)	0.3	0.2 - 0.4	7
Co-Op Westar (92)	0.4	0.2 - 0.8	13
GT73 (93)	0.3	0.2 - 0.3	4
Westar (93)	0.3	0.2 - 0.4	5
Co-Op Westar (93)	0.3	0.2 - 0.4	9

<sup>1</sup> Single samples were prepared and analyzed in quadruplicate in 1992 and triplicate in 1993. Replicates were averaged. The mean values are an average of the means of replicate analyses of the same sample.

<sup>2</sup> Samples are the number of values used to calculate the means and ranges listed.

**Table 8. Glucosinolate Profile of Roundup Ready Canola Event GT73 and Westar Canola from 1992 and 1993 Field Trials, Percent of Total Composition.**

Glucosinolate <sup>1</sup>	GT73 (1992)	Westar (1992)	GT73 (1993)	Westar (1993)
<b>ALKYL</b>				
allyl	3.00	1.56	1.46	1.47
3-Butenyl	13.84	12.56	13.83	12.5
4-pentenyl	1.79	1.75	1.37	1.43
2-OH-3-but.	30.57	26.64	31.36	28.33
2-OH-4-Pent.	0.42	0.38	0.09	0.14
<b>THIOALKYL</b>				
4-MeSbut.	0.88	0.94	0.80	0.90
4-MeSpent.	0.29	0.28	0.40	0.43
<b>INDOLYL</b>				
3-MeInd.	2.92	4.02	3.45	5.37
4-OHInd.	45.45	50.07	47.26	49.42
<b>ARYL</b>				
4-OHBenzyl	0.83	1.79	0.00	0.00

The glucosinolates are listed in structural classes.

Codes for the individual glucosinolates:

2-OH-3-but., 2-hydroxy-3-butenyl; 2-OH-4-pent., 2-hydroxy-4-pentenyl; 4-MeSbut., 4-methylthio-3-butenyl; 4-MeSpent., 4-methylthio-4-pentenyl; 3-MeInd., 3-methylindolyl; 4-OHInd.; 4-hydroxyindolyl; 4-OHBenzyl, p-hydroxybenzyl.

**Table 9. Proximate Values for Roundup Ready Canola Event GT73 and Westar Canola Seed from 1992 and 1993 Field Trials, Means and Ranges.**

Analysis <sup>1</sup>	GT73 (1992) <sup>3</sup>	Westar (1992) <sup>3</sup>	GT73 (1993) <sup>4</sup>	Westar (1993) <sup>4</sup>
% Fiber <sup>5</sup>	7.83 7.08-8.79	8.21 7.16-9.90	8.36 7.98-8.77	8.62 8.07-9.59
% Ash	3.78 3.50-4.16	3.68 3.44-3.91	4.00 3.72-4.47	4.07 3.58-4.26
% Moisture <sup>6</sup>	4.39 4.00-4.77	4.39 3.69-4.86	9.22 8.49-9.99	10.4 8.4-11.6
% Carbohydrate (by calculation)	24.6 23.0-26.9	26.4 23.6-28.0	26.1 24.4-27.1	26.4 25.8-27.9

<sup>1</sup> All results are reported on a dry weight basis except moisture.

<sup>2</sup> n=2

<sup>3</sup> n=7

<sup>4</sup> n=4

<sup>5</sup> Fiber analysis was crude fiber.

<sup>6</sup> Seed were pre-dried in 1992. In 1993, moisture analysis was performed on seed as received from the field.

**Table 10. Comparisons of Sinapine and Phytic acid content**

Analysis	Westar	GT73
Phytic Acid %DW <sup>1</sup>	3.09	3.33
Sinapine (mean and range <sup>2</sup>	12.7 (11.6-14.3)	13.1 (11.0-15.3)
Sinapine (mean and range <sup>3</sup>	15.5 (13.8-17.4)	15.1 (14.2-16.4)

<sup>1</sup> Analysis on composite sample from 4 sites, 1993 trials. Samples were dried prior to analysis.

<sup>2</sup> 1992 field trials. Mean values are an average of the means of triplicate analyses of the same sample. Values cited are ppm in defatted meal.

<sup>3</sup> 1993 field trials. Mean value is a mean of triplicate analysis from 5 sites. The values are mg/g defatted meal.

**Table 12. Compositional Analyses of Roundup Ready Canola From 1998 And 2000 Canadian Co-op Trials**

Canola Component	Average 1998 (15 varieties) (Range)		Average 2000 (36 varieties) (Range)	
	RR	Conventional <sup>1</sup>	RR	Conventional <sup>1</sup>
<b>Oil</b> (dry basis)	45.3 (40.0-51.8)	45.4 (40.1-51.8)	46.7 (42.3-55.6)	46.3 (42.1-53.9)
<b>Protein</b> (dry basis)	48.4 (41.7-53.7)	48.5 (41.1-52.7)	47.3 (38.8-54.0)	46.9 (40.4-51.1)
<b>Saturated fatty acid</b> <sup>2</sup> % total fatty acids	6.9 (6.4-7.5)	6.8 (6.5-7.1)	6.7 (6.0-8.0)	6.6 (6.1-7.2)
<b>Erucic acid</b> <sup>3</sup> % total fatty acids	0.798 (0.00-5.93)	0.397 (0.04-0.90)	0.08 (0.00-0.27)	0.05 (0.04-0.06)
<b>Glucosinolates</b> <sup>4</sup> Micromoles/gram of meal	12.9 (7.4-33.3)	14.2 (8.6-19.5)	9.2 (6.7-13.1)	10.4 (8.1-12.4)

<sup>1</sup> Average of 3 non-GM varieties: AC Excel, Defender and Legacy.

<sup>2</sup> Saturated fatty acids: (C16:0, C18:0, C20:0, C22:0).

<sup>3</sup> Erucic Acid (C22:1) of planted seed as determined from 2 sub samples of the same sample.

<sup>4</sup> Micromoles per gram of meal at 8.5 % moisture.

**Table 13. Performance and Composition of Broilers fed Roundup Ready® (Event GT73), Parental or Reference Canola Meal**

Treatment	8	7	1	2	3	4	5	6	Treatments SSD <sup>1</sup>	LSD <sup>2</sup> 5.0%	Historical Range <sup>3</sup>	Literature Range <sup>4</sup>
<b>Performance</b>												
Live weight (g/bird) day 0	40.033	40.783	40.250	40.467	40.800	40.533	40.917	40.650	NS	0.845	35.3-42.5	NA
Live weight (kg/bird) day 42	2.274	2.229	2.208	2.206	2.232	2.230	2.570	2.580	NS	0.063	1.891-2.346	1.79-2.43 <sup>a-f</sup>
Feed intake (kg/bird)	3.975	4.134	3.773	3.742	3.819	3.929	3.877	3.871	NS	0.275	3.500-3.981	NA
Feed efficiency (kg/kg)	1.773	1.842	1.725	1.696	1.697	1.753	1.713	1.696	NS	0.103	1.543-1.844	1.60-2.07 <sup>a,b,c,d</sup>
Adjusted Feed Efficiency (kg/kg)	1.608 <sup>c</sup>	1.608 <sup>c</sup>	1.623 <sup>bc</sup>	1.665 <sup>a</sup>	1.665 <sup>a</sup>	1.644 <sup>ab</sup>	1.619 <sup>bc</sup>	1.618 <sup>bc</sup>	**	0.032	1.528-1.724	NA
<b>Carcass Yield</b>												
Live weight (kg)	2.243	2.237	2.186	2.211	2.255	2.238	2.26	2.279	NS	0.072	2.102-2.313	NA
Chill weight (kg)	1.597	1.546	1.543	1.535	1.573	1.570	1.590	1.595	NS	0.055	1.440-1.645	NA
Chill weight (% of live wt.)	70.1 <sup>abc</sup>	69.2 <sup>c</sup>	69.7 <sup>abc</sup>	69.5 <sup>bc</sup>	70.4 <sup>ab</sup>	70.4 <sup>ab</sup>	70.4 <sup>ab</sup>	70.6 <sup>a</sup>	*	0.010	68.4-71.6	67.1 - 76.0 <sup>a,c,d,e</sup>
Fat pad weight (kg)	0.029	0.027	0.025	0.027	0.025	0.027	0.028	0.028	NS	0.004	0.030-0.044	0.0242 - 0.0632 <sup>a-f</sup>
Fat pad weight (% of live wt.)	0.013	0.012	0.011	0.012	0.011	0.012	0.012	0.012	NS	0.002	1.42-2.18	1.14 - 3.60 <sup>a-f</sup>
Breast meat weight (kg)	0.399	0.390	0.397	0.385	0.397	0.400	0.402	0.405	NS	0.018	0.338-0.432	0.225-0.551 <sup>a,b,d,e</sup>
Breast meat weight (% of chill wt.)	0.249	0.252	0.256	0.250	0.252	0.255	0.253	0.254	NS	0.006	23.4-26.2	11.19-32.62 <sup>a,d,e</sup>
Thighs weight (kg)	0.267	0.257	0.257	0.257	0.259	0.262	0.269	0.269	NS	0.012	0.252-0.282	0.258 - 0.318 <sup>e,f</sup>
Thighs weight (% of chill wt.)	0.167	0.165	0.166	0.167	0.164	0.166	0.169	0.169	NS	0.003	16.8-17.9	12.80 - 20.65 <sup>e,f</sup>
Drums weight (kg)	0.231	0.221	0.221	0.224	0.228	0.225	0.230	0.231	NS	0.009	0.201-0.231	0.213 <sup>f</sup>
Drums weight (% of chill wt.)	0.144	0.143	0.144	0.146	0.145	0.143	0.144	0.145	NS	0.002	13.9-14.5	10.50 <sup>f</sup>
Wings weight (kg)	0.192	0.186	0.186	0.187	0.191	0.189	0.191	0.193	NS	0.006	0.171-0.191	0.170 <sup>f</sup>
Wing weight (% of chill wt.)	0.120	0.120	0.121	0.122	0.121	0.120	0.120	0.122	NS	0.002	11.5-12.0	8.40 <sup>f</sup>
<b>Breast Meat Analysis</b>												
Moisture (%)	75.246	75.099	75.261	75.133	75.119	75.220	75.373	74.959	NS	0.467	74.4-75.3	72.7-74.3 <sup>g</sup>
Protein (% as is basis)	23.736	23.708	23.648	23.761	23.908	23.672	23.693	23.969	NS	0.518	22.4-24.3	22.9-24.3 <sup>g</sup>
Fat (% as is basis)	0.818	0.864	0.822	0.815	0.759	0.814	0.922	0.871	NS	0.251	0.78-1.20	0.770-1.80 <sup>g</sup>
<b>Thigh Meat Analysis</b>												
Moisture (%)	76.638	76.608	76.640	76.686	76.390	76.450	76.355	76.735	NS	0.751	75.5-76.9	70.0-72.4 <sup>g</sup>
Protein (% as is basis)	21.137	20.514	21.119	20.607	20.895	20.935	20.816	20.645	NS	1.045	19.9-21.4	17.7-19.2 <sup>g</sup>
Fat (% as is basis)	2.158	2.285	2.501	2.201	2.364	2.724	2.409	2.161	NS	0.646	1.79-3.09	7.50-11.6 <sup>g</sup>

<sup>1</sup> SSD, statistical significance of differences: NS, not significant; \*, P<0.05; \*\*, P<.01; Individual treatment means with the same superscript letter in the same row are not statistically different (P>0.05).

<sup>2</sup> LSD, least significant difference between means (P<0.05).

<sup>3</sup> Historical data are based upon corn feeding studies, data herein are the first to report feeding of transgenic canola. Monsanto 38 to 42 day studies numbered a) 2001-01-50-02 (Ross x Ross), b) 2000-01-39-38 (Ross x Ross), c) 2000-01-39-02 (Ross x Ross), d) 2000-01-39-01 (Cobb x Cobb), e) XX-98-081 (Ross x Ross), and f) XX-97-252 (Ross x Arbor Acres).

<sup>4</sup> a) Smith et al., 1998 (Ross x Ross); b) Lei and Van Beek, 1997 (Ross); c) Farran et al., 2000 (Ross); d) Esteve-Garcia and Llaurodo, 1997 (Ross); e) Kidd and Kerr, 1997 (Ross x Ross); f) Peak et al., 2000 (Ross x Ross, Cobb x Cobb, and Ross x Cobb); and g) Grey et al., 1983 (Ross).

**Table 14. Days to Maturity from the 1993 Canadian Co-Op Test.**

<b>Location</b>	<b>Strain or Cultivar (days)</b>	
	<b>GT73</b>	<b>Westar</b>
Lacombe	120	119
Scott	112	110
Durban	107	108
Melfort	114	113
Winnipeg	98	97
Fort Saskatchewan	116	119
Olds	129	126
Yorkton	107	105
Rosebank	99	98
High Level	119	119
Portage la Prairie	94	94
Brandon	102	102
Fort Vermilion	113	111
Saskatoon	112	110
<b>Average</b>	<b>110.1</b>	<b>109.4</b>

**Table 15. Seed Yield for Test Lines in 1993 Canadian Co-Op Trial.**  
(Values are 100X kg/ha)

Location	Strain or Cultivar	
	GT73 <sup>1</sup>	Westar
Lacombe	29.5	30.5
Loon Lake	12.8	16.9
Westock	31.8	31.6
Olds	16.8	17.0
High Level	13.9	12.1
Fort Vermilion	15.2	17.5
<b>Average Short Season Zone</b>	<b>20.0</b>	<b>20.9</b>
Scott	18.7	20.8
Lashburn	21.4	22.0
Durban	20.7	20.0
Melfort	20.7	16.6
Fort Salk.	29.2	33.8
Kelsey	20.5	22.5
Alexandra	37.8	41.5
Yorkton	13.7	13.8
Saskaton	23.3	24.0
<b>Average Mid Season Zone</b>	<b>22.9</b>	<b>23.9</b>
Thornhill	8.1	7.5
Winnipeg	7.2	9.0
Rosebank	18.8	15.1
Portage	11.9	14.8
Brandon	11.8	18.3
<b>Average Long Season Zone</b>	<b>11.6</b>	<b>12.9</b>

<sup>1</sup> - Seed of GT73 was bulked in Chile over the winter of 1992 and 1993. To meet deadlines for Co-Op introduction, this seed was harvested earlier than considered optimal. Consequently, the slight yield reduction in the 1993 Co-Op was attributed to the slightly immature seed.

**Table 16. Volunteer Canola Counts Taken in 1993 on 1992 Trial Sites.**  
(Values are plants/m<sup>2</sup>)

Location	Westar	GT73 (untreated) <sup>1</sup>	GT73 (treated) <sup>2</sup>
Minto <sup>3</sup>	10 <sup>a</sup>	12 <sup>a</sup>	13 <sup>a</sup>
Melfort <sup>3</sup>	67 <sup>a</sup>	2 <sup>b</sup>	6 <sup>b</sup>
Saskatoon <sup>3,4</sup>	no data	292 <sup>a</sup>	388 <sup>a</sup>
Saskatoon <sup>3,5</sup>	168 <sup>a</sup>	209 <sup>a</sup>	no data

<sup>1</sup> Untreated indicates that no glyphosate herbicide was applied to these plants.

<sup>2</sup> Treated indicates that the original Roundup herbicide (MON 2139) was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.

<sup>3</sup> Statistical significance for a listed location were determined using Duncan's Multiple Range Test P=0.05.

<sup>4</sup> Data from plots of a tolerance trial conducted in 1992. No data were available for Westar.

<sup>5</sup> Data from plots of a variety trial conducted in 1992. No data were available for treated GT73.

<sup>a,b</sup> Statistical significance: Averages followed by the same letter are not significantly different.

**Table 17. Average Volunteer Counts Taken on 1993 Canola Plots in 1994.**  
(Values are plants/m<sup>2</sup>)

Location	Westar	GT73 (untreated) <sup>1</sup>	GT73 (treated) <sup>2</sup>
Minto <sup>3,4</sup>	571.2 <sup>a,b</sup>	1063.2 <sup>b</sup>	188.4 <sup>a</sup>
Lethbridge <sup>3,5</sup>	119.2 <sup>a</sup>	125.0 <sup>a</sup>	129.0 <sup>a</sup>
Melfort <sup>3,4</sup>	67.6 <sup>a,b</sup>	85.6 <sup>a</sup>	34.8 <sup>b</sup>
Scott <sup>3,6</sup>	166.8 <sup>a</sup>	155.3 <sup>a,b</sup>	152.5 <sup>b</sup>
Saskatoon <sup>3,7</sup>	863.6 <sup>a</sup>	804.2 <sup>a</sup>	832.2 <sup>a</sup>
Average <sup>3</sup>	357.7 <sup>a</sup>	446.6 <sup>a</sup>	267.4 <sup>a</sup>

<sup>1</sup> Untreated indicates that no glyphosate herbicide was applied to these plants.

<sup>2</sup> Treated indicates that the original Roundup herbicide (MON 2139) was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg active ingredient/ha.

<sup>3</sup> Statistical significance for a listed location were determined using Duncan's Multiple Range Test P=0.05.

<sup>4</sup> Counts were taken from 0.25 m<sup>2</sup> plot.

<sup>5</sup> Counts were taken from 0.1 m<sup>2</sup> plots.

<sup>6</sup> Counts were taken from 0.5 m<sup>2</sup> plots.

<sup>7</sup> Counts were taken from 0.35 m<sup>2</sup> plots.

<sup>a,b</sup> Statistical significance: Averages followed by the same letter are not significantly different.

**Table 18. Results of Shattering Studies Conducted in 1993.**  
(Values are expressed as % Westar control)

Location	Westar	GT73 (untreated) <sup>1</sup>	GT73 (treated) <sup>2</sup>
Minto <sup>3</sup>	100 <sup>a</sup>	110 <sup>a</sup>	85 <sup>a</sup>
Melfort <sup>3</sup>	100 <sup>a</sup>	53 <sup>a</sup>	66 <sup>a</sup>

<sup>1</sup> Untreated indicates that no glyphosate herbicide was applied to these plants.

<sup>2</sup> Treated indicates that the original Roundup herbicide (MON 2139) was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.

<sup>3</sup> ANOVA statistical analysis was performed indicating no significant difference at each location as indicated by the letter following the value.

<sup>a,b</sup> Statistical significance: Averages followed by the same letter are not significantly different.

**Table 19. Germination Test of Seed from 1992 Trials.**

Canola Cultivar or Line	Percent Germination
Westar	99 %
GT73 (untreated) <sup>1</sup>	99 %
GT73 (treated) <sup>2</sup>	98 %

<sup>1</sup> Untreated indicates that no glyphosate herbicide was applied to these plants.

<sup>2</sup> Treated indicates that the original Roundup herbicide (MON 2139) was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.