# Monsanto Petition (11-188-01p) for Determination of Non-regulated Status of MON 88302 Canola

## OECD Unique Identifier:
## MON 883Ø2-9

# Plant Pest Risk Assessment

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**Agency Contact**
**Cindy Eck**
**Biotechnology Regulatory Services**
**4700 River Road**
**USDA, APHIS**
**Riverdale, MD 20737**
**Fax: (301) 734-8669**

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# A. Introduction

Monsanto Company (referred hereafter as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically engineered (GE) glyphosate herbicide-resistant[1] canola event MON 88302 (referred hereafter as MON 88302) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 11-188-01p, and is hereafter referenced as Monsanto 2011. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq.*)[2]. This plant pest risk assessment was conducted to determine if MON 88302 is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and is also considered a plant pest. A GE organism is also regulated under 7 CFR part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk. MON 88302 was produced by transformation of canola tissue using *Agrobacterium tumefaciens*, a plant pest, and some of the sequences (i.e., enhancer sequence from 35S promoter from figwort mosaic virus, codon-optimized *aro*A gene sequence from *Agrobacterium* sp. strain CP4., and right and left T-DNA border sequences from *A. tumefaciens*) used in the transformation process (Monsanto 2011) are also from plant pests organisms listed in 7 CFR 340.2. Monsanto has conducted introductions of MON 88302 as a regulated article under APHIS-authorized notifications since 2005 (Appendix Table A-1, p. 218, Monsanto 2011), in part, to gather information to support that it is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk characteristics associated with MON 88302 and its progeny and their use in the

[1] Monsanto has described the phenotype of MON 88302 as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America's definition of "herbicide resistance" since MON 88302 has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type" (WSSA 1998). By the WSSA definition, "resistance [to an herbicide] may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis. "Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 88302 is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, expression of the gene product, new enzymes, or changes to plant metabolism, potential impacts of genetic modifications on disease and pest susceptibilities, effects of the regulated article on nontarget organisms, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, changes to agricultural or cultivation practices that may impact diseases and pests of plants, indirect plant pest effects on other agricultural products, and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. The EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA). The post-emergence (in-crop) use of glyphosate in previously deregulated Roundup Ready canola RT73 was first approved by the EPA in March 1999 (Monsanto 2011). Monsanto has submitted a request for amended labeling to the EPA in February 2011 for EPA Registration Numbers 524-537 (Roundup WeatherMAX® Herbicide) and 524-549 (Roundup PowerMAX® Herbicide) that proposes to modify the current use pattern of glyphosate in canola based on MON 88302. The amended labeling request pertains to use of glyphosate during an expanded window of application and at rates higher than those currently recommended and authorized (p. 13, Monsanto 2011). EPA is currently reviewing the label changes to add the increased rate and later application of glyphosate to commercial herbicide-resistant canola production. EPA's assessment will analyze risks to non-target organisms to determine if the label is sufficient to meet EPA's standards for registration; "reasonable certainty of no harm to humans" and "no unreasonable adverse effects on the environment." If these standards are not met, EPA will apply appropriate risk mitigation strategies and propose label modifications to address the specific concerns. After EPA has completed it assessments and provided these to APHIS, APHIS will update this PPRA as appropriate.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its voluntary consultation process.

In compliance with this policy, Monsanto initiated a consultation with the FDA (FDA BNF No. 127) on the food and feed safety and compositional assessment of MON 88302 (p. 28, Monsanto 2011). Monsanto submitted a safety and nutritional assessment summary document to the FDA in March 2011 and received a completed consultation letter from the FDA in April 2012. A copy of the text of this letter responding to BNF 127, as well as a copy of the text of FDA's memorandum summarizing the information in BNF 127, is available for public review via the FDA Completed Consultations on Bioengineered Foods page at www.fda.gov/bioconinventory.

## B. Development of MON 88302 Canola

Canola (*Brassica* spp) is an oil seed crop primarily cultivated in China, India, Europe, and Canada and is becoming popular in the United States and Australia (OECD 2012). There are two main types of *B. napus*: 1) oil-yielding oleiferous rape, of which one subset with specific quality characteristics is often referred to as "canola" (vernacular name), and 2) vegetable and/or forage type: the tuber-bearing swede or rutabaga *(B. napus* var. *napobrassica*, rutabaga or Swede) and Siberian or rape kale (*B. napus* var. *pabularia*)(OECD 2012). At present, three species of *Brassica* (*B. napus, B. rapa, and B. juncea*) have commercialized varieties with "double low" characteristics, i.e. low erucic acid content (<2%) in the fatty acid profile and very low glucosinolate content (<30 micromoles/g) in the air-dried oil-free meal, characteristics desirable for high-quality vegetable oil and high-quality animal feed, respectively (CCC 2003). In North America these species are considered to be of "canola" quality. There are spring (annual) and winter (biennial) biotypes of canola varieties. Spring canola is planted in early spring and harvested in late summer. It is a cool season crop that is grown in western Canada, southern Australia, northern China, and in the northern Great Plains region of the U.S. Winter canola is planted in the fall, requires exposure to winter cold (vernalization) to flower, and is grown in parts of northern Europe, central China, the northwestern U.S. and in the central portions of the U.S. Great Plains (OECD 1997, 2012). The spring biotypes are typically lower yielding than the winter types, but require considerably less time to complete their life cycle (OECD 1997). Spring canola varieties are available in all three *Brassica* species while winter canola varieties are created only in *B. napus* and *B. rapa* species (CCC 2003).

In the U.S. canola is grown in three geographical regions – the Northern and Southern Great Plains and the Pacific Northwest (Figure VIII-1, p. 131 and Table VIII-2, p. 132, Monsanto 2011). For the growing season 2010, the largest U.S. canola producing state was North Dakota (1,280,000 acres or ~ 88%) followed by Oklahoma with 60,000 acres (~ 4%) (Table VIII-2, p. 132, Monsanto 2011). Nearly all the canola grown in North Dakota is the *B. napus* spring canola type (NDSU 2005) while Oklahoma primarily grows the *B. napus* winter canola type (Monsanto 2011, p.129).

MON 88302, a spring canola biotype (*B. napus*), has been genetically modified to express 5-enolpyruvylshikimate-3-phosphate synthase (termed CP4 EPSPS) enzyme. The CP4 EPSPS enzyme, encoded by the *cp4 epsps* gene, confers resistance to glyphosate-containing herbicides by the production of CP4 EPSPS protein that is less sensitive to inhibition by glyphosate compared to the endogenous plant EPSPS (Padgette et al. 1996; Funke et al. 2006). As detailed later in this document, MON 88302 utilizes an improved promoter sequence to enhance CP4 EPSPS expression in male reproductive tissues to facilitate glyphosate herbicide application at later stages of plant development without harming male reproductive tissues. According to Monsanto, such late application of glyphosate herbicide is not possible with currently available glyphosate herbicide-resistant canola varieties, and later application has the advantage of providing greater flexibility to growers to manage weeds in canola fields (p. 4, Monsanto 2011).

***Description of the genetic modifications***

As described in the petition (p. 33, Monsanto 2011) MON 88302 was developed through *Agrobacterium*-mediated transformation of hypocotyls (Radke et al. 1992) of a donor spring canola variety, Ebony, utilizing a binary plasmid vector PV-BNHT2672. The PV-BNHT2672 plasmid vector contained two parts as summarized below: 1) a Transfer DNA (T-DNA) sequence containing the *cp4 epsps* plant expression cassette, and 2) backbone sequences necessary for maintenance or selection of the plasmid vector in bacteria, but which are not expected to be transferred to the plant (Figure III-1, p. 35 & Table III-1, pp. 38-39, Monsanto 2011).

T-DNA *cp4 epsps* cassette

- Right Border (RB) Sequence: A specific DNA region from *A. tumefaciens* used for T-DNA transfer (Depicker et al. 1982; Zambryski et al. 1982).
- *FMV/Tsf1* Promoter: Chimeric promoter consisting of the promoter of the *Tsf1* (Twin Sister of Flowering Locus 1) gene from the *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989) and enhancer sequences from the 35S promoter from the FMV (Figwort Mosaic Virus) (Richins et al. 1987).
- *Tsf1* Leader Sequence: 5' untranslated leader (exon 1) from the *A. thaliana Tsf1* gene encoding elongation factor EF-1α (Axelos et al. 1989).
- *Tsf1* intron: Intron from the *A. thaliana Tsf1* gene encoding elongation factor EF-1α (Axelos et al. 1989).
- *CTP2* targeting sequence: Targeting sequence from the *shkG* gene encoding the chloroplast transit peptide region of *A. thaliana* EPSPS (Herrmann 1995; Klee et al. 1987) that directs transport of the CP4 EPSPS protein to the chloroplast.
- *cp4 epsps* coding sequence: Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al. 2001; Padgette et al. 1996).
- *rbcS2-E9* gene terminator: 3' untranslated sequence from the *rbcS2* gene of *Pisum sativum* (pea) encoding the Rubisco small subunit (Coruzzi et al. 1984).
- Left Border (LB) sequence: A specific DNA region from *A. tumefaciens* used for T-DNA transfer (Barker et al. 1983; Zambryski et al. 1982).

Backbone sequence

- Origin of replication (*oriV*): Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al. 1981).
- Repressor of primer (*rop*) protein coding sequence: Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang 1989).
- Origin of replication (*ori-pBR322*): Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe 1979).
- Promoter-coding sequence-Terminator sequence *for aadA* gene: Bacterial promoter, coding sequence, and 3′ untranslated region for an aminoglycoside-modifying enzyme,
- 3"(9)-O-nucleotidyl-transferase from the transposon Tn7 (Fling et al. 1985) that confers
- Spectinomycin and streptomycin antibiotic resistance.
- In addition to the above sequences, both the *cp4 epsps* cassette and the backbone contain four or five intervening sequences of various lengths to facilitate DNA cloning.

The binary plasmid vector PV-BNHT2672 is disarmed. Disarmed binary plasmid vectors lack T-DNA sequences from Ti (Tumor-inducing) – plasmids normally responsible for the formation of crown gall tumors upon *A. tumefaciens* infection (Hoekema et al. 1983; Hellens et al. 2000). Furthermore, following transformation the plant tissue was treated with antibiotics carbenicillin, ticarcillin disodium and clavulanate potassium to inhibit the growth of excess *Agrobacterium* (Hellens et al. 2000; p. 33, Monsanto 2011). Plant pathogen sequences inserted into MON 88302, i.e. the T-DNA border sequences from *Agrobacterium* and the enhancer sequences from FMV do not cause plant disease.

Monsanto provided evidence demonstrating that,

- the DNA inserted into the MON 88302 canola genome is present at a single locus, and contains one functional copy of *cp4 epsps* gene expression cassette with truncated portions of the T-DNA right border (42 bp) and left border (273 bp) (Table IV-2, p. 46 and section IV.A., pp. 47-50, Monsanto 2011);
- the final product does not contain any of the backbone sequences from the plasmid PV- BNHT2672 outside of the T-DNA region or *cp4 epsps* expression cassette borders as determined through Southern blot analysis (section IV-B., pp. 51-55, Monsanto 2011);
- the T-DNA sequence in MON 88302 (Table IV-2, p.46, Monsanto 2011) is identical to the corresponding T-DNA sequence of the original donor plasmid PV-BNHT2672 (as confirmed by DNA sequence analysis) (section IV.C., pp. 56-57, Monsanto 2011);
- the inserted *cp4 epsps* expression cassette DNA was stably inherited across four breeding generations (section IV.E., pp. 60-62, Monsanto 2011); and,

- the *cp4 epsps* expression cassette in MON 88302 resides at a single locus within the canola genome and is inherited according to Mendelian principles of inheritance (as determined by Chi-square analysis for three segregating populations) (section IV-F., pp. 63-66, Monsanto 2011).

Insertion of foreign genetic materials tends to induce mutations at sites of insertion (generally referred to as insertional mutations) in recipient genomes (Nacry et al. 1998; Laufs et al. 1999). Monsanto examined the T-DNA insertion site in MON 88302 and corresponding conventional control line using Polymerase Chain Reaction (PCR) and sequence analyses (Appendix B, pp. 223-224, Monsanto 2011) and discovered that MON 88302 contained transformation induced mutations at the flanking site adjacent to the 3' end of the T-DNA insert (section IV.D., pp. 58-59, Monsanto 2011). There was a 9 base pair insertion, a 29 base pair deletion, and a single nucleotide difference between the conventional control sequence and the genomic DNA sequence flanking the 3' end of the T-DNA insert in MON 88302. According to Monsanto, the observed insertion-deletion mutation (indel mutation) presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta 1998) while a single nucleotide difference was most likely caused by a single nucleotide polymorphism (SNP) segregating in the canola population (Trick et al. 2009). As discussed later in this document, none of these mutations altered the function of *cp4 epsps* gene or exhibited deleterious phenotypes in MON 88302.

## C. Expression of the Gene Product, Enzymes or Changes to Plant Metabolism

USDA-APHIS assessed whether changes in plant metabolism or composition in MON 88302 canola are likely to alter its plant pest risk. The assessment encompasses a consideration of the expressed protein or enzyme and its effect on plant metabolism and an evaluation of whether the nutrients and anti-nutrient levels in harvested seed derived from MON 88302 are comparable to those in the conventional canola control variety Ebony or to other reference canola cultivars considered for the composition analysis. Host plant quality (including such components as carbon, nitrogen, amino acid sources, trace elements, and defensive metabolites) is known to affect herbivore performance and fecundity; and higher-trophic level interactions, such as the performance of predators and parasitoids, may also be affected (reviewed by Awmack and Leather 2002). Similarly a vast array of secondary metabolites in plants is known to provide defense against microbes (Dixon, 2001). Thus APHIS assessed whether changes in host plant quality could have the potential to affect MON 88302 canola's performance against pest and disease incidences.

The enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), catalyzes one of the enzymatic steps of the shikimic acid pathway, and is the target for the broad spectrum herbicide glyphosate, the active ingredient in Roundup agricultural herbicides (Haslam 1993; Herrmann and Weaver 1999; Steinrücken and Amrhein 1980). Glyphosate - herbicide resistance in MON 88302 comes from the expression of a bacterial (*Agrobacterium* sp. strain CP4) gene *cp4 epsps*. The *cp4 epsps* gene encodes CP4 EPSPS protein, which like plant EPSPS protein, catalyzes the transfer of the enolpyruvyl group

from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings 2001). Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules necessary for plant growth. In conventional plants, glyphosate blocks the biosynthesis of EPSP thereby depriving plants of essential amino acids (Steinrücken and Amrhein 1980; Haslam 1993). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (Padgette et al. 1996). Therefore, in glyphosate herbicide-resistant plants, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al. 1996).

In MON 88302 the *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Figure III-3, p. 40, Monsanto 2011; Padgette et al. 1996). Because CP4 EPSPS protein has a long history of use in various genetically engineered (GE) crops to provide herbicide-resistance, its physiochemical and functional properties along with food and/or feed safety data were previously examined on an identical version of CP4 EPSPS protein produced in *E. coli* (Harrison et al. 1996). Thus *E. coli* produced CP4 EPSPS protein has become an industry standard for comparative studies. A comparison of the MON 88302-produced CP4 EPSPS protein to the *E. coli*-produced CP4 EPSPS protein confirmed the structural and functional identity of the MON 88302-produced CP4 EPSPS protein with that of *E. coli* produced CP4 EPSPS protein (Appendix C, pp. 226-244, Monsanto 2011). Furthermore, since CP4 EPSPS proteins isolated from other Roundup Ready crops have been previously demonstrated to be equivalent to the *E. coli*-produced CP4 EPSPS protein, then according to Monsanto by inference the MON 88302-produced CP4 EPSPS protein must be equivalent to the CP4 EPSPS proteins expressed in other Roundup Ready crops that have been deregulated by USDA-APHIS (Section V, pp. 68-70, Monsanto 2011).

Monsanto estimated CP4 EPSPS protein levels in eight tissue types covering forage, seed, leaf and root (Appendix D, pp. 245-249, Monsanto 2011) collected from three experimental fields in the U. S. (Idaho, Minnesota and North Dakota) and three in Canada (two sites in Manitoba and one in Saskatchewan) during the 2009 growing season. According to Monsanto, the six selected field sites were representative of canola producing regions suitable for commercial production in the U.S. and Canada. Monsanto observed MON 88302 CP4 EPSPS protein expression in all the various tissue types: its level was highest in leaf (180 - 230 μg/g dry weight (dw)) followed by forage, root and seed (27 μg/g dw) (Table V-1, p. 71, Monsanto 2011). The CP4 EPSPS protein expression levels in leaf (forage) and seed tissue in MON 88302 reported on a fresh weight basis (Table V-1, p. 71, Monsanto 2011) are within the range reported for the previously deregulated glyphosate resistant canola RT73 (Table 6, p. 36, Monsanto 1998). Although data on CP4 EPSPS protein in pollen were not reported in the petition, other studies suggest that it is more highly expressed in MON 88302 compared to RT73. Feng et al. (2010) describes the difference in sensitivity to glyphosate between RT73 and MON 88302, the 'second generation RR2' GR canola. Pollen viability drops to zero percent when RT73 is sprayed at 4-10-leaf stages with 1.33 times the maximum single

application rate of 0.6 kg acid equivalents (a.e.)/hectare ha, whereas no reduction in pollen viability is seen in MON 88302 pollen even with 6 times this application rate. Also at this 6 x rate, MON 88302 is able to produce approximately 1.5 times more average seed weight per plant than RT73 (Figure 3.2 in Feng et al. 2010). MON 88302 also suffers no leaf chlorosis when sprayed with an 8 x rate of glyphosate.

Monsanto carried out a compositional assessment of seed samples obtained from MON 88302, control, and seven commercial reference varieties (Appendix Table E-1, p. 250, Monsanto 2011) using the principles and analytes outlined in the OECD consensus document for canola composition (OECD 2001) to assess whether levels of key nutrients, toxicants and anti-nutrients in MON 88302 were equivalent to levels in the conventional control and to the composition of commercial reference varieties. The OECD consensus document does not recommend analysis of canola forage, as canola forage is rarely consumed by animals and is not a source of nutrition for humans. The seven different commercial reference varieties were included across all sites of the field production to provide data on natural variability of each compositional component analyzed. The samples for compositional assessment were collected in the 2009 growing season from two U.S. sites (Minnesota and North Dakota) and three Canadian sites (two sites in Manitoba and one site in Saskatchewan). The five field sites were chosen to represent the typical canola growing regions of the United States and Canada. In addition to the conventional weed control programs, MON 88302 plots were treated at the 5-6 leaf stage with a glyphosate application at a target rate of 1.6 lb a.e. per acre (1800 g a.e./ha) (p. 77, Monsanto 2011).

Nutrients assessed included proximates (ash, carbohydrates, moisture, protein, and total fat), fiber, amino acids, fatty acids, vitamin E, and minerals. The toxicants assessed in seed included erucic acid and glucosinolates. The anti-nutrients assessed in seed included phytic acid and sinapine (see Appendix E for analytical methods. pp. 250-259, Monsanto 2011). In all, according to Monsanto, 70 different components were measured. Of those 70 components, 18 nutrients and one toxicant (18 fatty acids, including erucic acid, and one mineral) had more than 50% of the observations below the assay limit of quantitation (LOQ), and therefore were excluded from the statistical analyses.

Monsanto employed six statistical comparisons on the MON 88302 compositional data. One comparison was based on compositional data combined across all five field sites (combined-site analysis) and five separate comparisons were conducted on data from each of the individual field sites. Data from the commercial reference varieties were combined across all sites and used to calculate a 99% tolerance interval for each compositional component to define the natural variability of each component in canola varieties that have a history of safe consumption, and that were grown concurrently with MON 88302 and the conventional control in the same trial (see p. 78, Monsanto 2011 for further details about data interpretations).

No statistically significant differences were observed between MON 88302 canola and the control canola mean values for a majority of the analyzed nutrients except for total dietary fiber (TDF) and seven fatty acids (16:1 palmitoleic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 20:0 arachidic acid, and 22:0 behenic

acid) (Tables VI-1 and VI-2, pp. 83-100, Monsanto 2011). However, mean values for all of the nutrient components found to be significantly different ($\alpha = 0.05$) from the combined-site analysis of MON 88302 were within the 99% tolerance interval established from the commercial reference varieties grown concurrently (Tables VI-1 and VI-2, pp. 83-100, Monsanto 2011); and with the exception of TDF, for which no commercial reference values have been published, all of the compositional components identified as significantly different from the conventional control were also within the natural variability of these components in commercial canola composition as published in the scientific literature (Table VI-4, p. 102-103, Monsanto 2011).

Similar to nutrient analysis, MON 88302 and control were similar in anti-nutrient composition except for alkyl glucosinolate, which was slightly reduced in MON 88302 (Tables VI-1 to VI-3, pp. 83-101, Monsanto 2011). Although in the combined-site analysis a significant difference in alkyl glucosinolates was observed for MON 88302; the mean value was lower than the conventional mean value, and the difference was not consistently observed at the individual sites. The combined-site mean alkyl glucosinolates value for MON 88302 was within the natural variability of commercial canola defined by the 99% tolerance interval established from the concurrently grown commercial reference varieties that have a history of safe consumption, and the value was within the safety threshold for canola (pp. 81-82, Monsanto 2011). Higher glucosinolinates in some Brassica species have also been reported to reduce the feeding rates of larvae of the pest species White cabbage butterflies (*Pieris rapae*) and the fecundity of the Cabbage aphid (*Brevicoryne brassicae*) (Awmack and Leather 2002). However, as noted in the next section of this PPRA, pest damage ratings for cabbage worms (Pieridae) and aphids were not different between the 88302 canola and control canola (Table G7, p. 331, Monsanto 2011, nor was there any significant difference in aphid abundance (Table G-9, p. 333, Monsanto 2011).

Results of the comparison indicate that the composition of the seed of MON 88302 is similar to that of the conventional control and within the natural variability of commercial reference varieties. These findings support the conclusion that nutrients in seed from MON 88302 are compositionally equivalent to those in conventional canola varieties with a history of safe usage.

Previously CP4 EPSPS protein was part of 13 deregulated crop events, including canola (Petitions for Nonregulated Status Granted or Pending by APHIS, http://www.aphis.usda.gov/biotechnology/not_reg.html; Information Systems for Biotechnology, http://www.isb.vt.edu/search-petition-data.aspx). So far there are no reports of plant pest characteristics exhibited by them. Based on all the above noted considerations, APHIS concludes that MON 88302 poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional canola varieties.

## D. Potential Impacts on Disease and Pest Susceptibilities

USDA-APHIS assessed whether MON 88302 is likely to have significantly increased disease and pest susceptibility because of the introduced *cp4 epsps* gene compared to the

control canola variety. This assessment encompasses a thorough consideration of introduced traits, their impact on agronomic traits (discussed later in the document) and plant composition (discussed earlier), and quantitative and/or qualitative data on pest and disease responses. Important changes are those which would (1) affect not only the new GE crop, but that would also result in significant introduction or spread of a damaging pest or disease to other plants; and/or (2) result in the introduction, spread, and/or creation of a new disease or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility should be evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode, or weed programs exist (see http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml ); however none specifically target pests of canola.

The complex of insects that feed upon the Brassicas is one of the important factors limiting the production of commercial Brassica crops (Lamb 1989: Weiss et al. 2009). Brassicaceous plants produce a family of sulphur compounds called glucosinolates whose breakdown products are attractants and stimuli for feeding and oviposition but, on the other hand, act as deterrents or toxins for herbivores not adapted to plants of the Brassicaceae. Some of the more important insect pests of canola are listed in Table VIII-4 (p. 148, Monsanto 2011). Likewise, Brassica crops are subject to a broad range of pathogens (Kharbanda et al. 2001). Out of all the diseases affecting Brassica crops, the three most troublesome diseases are blackleg or stem canker (*Leptosphaeria maculans*); Sclerotinia stem rot (*Sclerotinia sclerotiorum*); and clubroot (*Plasmodiophora brassicae*).

As detailed in the petition (p. 116, Monsanto 2011), in the 2009 growing season, 17 trial locations (8 in the U.S. and 9 in Canada) (Table VII-3, p. 118, Monsanto 2011) were selected in order to provide agronomic and pest and disease observations representative of the major spring annual canola growing regions in the U.S. and Canada, where commercial canola production and sales of MON 88302 canola are expected. Agronomic practices used to prepare and maintain each field site were characteristic of each respective region. Mon 88302 and the conventional control were planted at all field sites, while 24 commercial reference varieties included in the field trials (see Appendix Table G-1, p. 314, Monsanto 2011) were site-specific, locally-adapted genotypes, including a few glyphosate or glufosinate herbicide-resistant varieties. In addition, insect pests and diseases at the Canada locations are typical of those found in the U.S. All plots of MON 88302, the conventional control, and the commercial reference varieties at each site were uniformly managed in order to assess whether the introduction of the glyphosate-tolerance trait altered the phenotypic and agronomic characteristics or the environmental

interactions (biotic and abiotic stress) of MON 88302 compared to the conventional control. Glyphosate herbicide was not applied to any of the plots in the trial. The same observations and data collection were not made for every site. Although some data were missing or excluded from statistical analyses or observational interpretations for a variety of technical and logistic reasons (Appendix G, Table G3, pp. 321-322, Monsanto 2011), the sample size included for statistical analyses on each variable was large enough to perform the necessary statistical analyses.

Monsanto conducted three types of pest and disease analyses—(1) qualitative assessment at all 17 sites for disease damage (Appendix Table G-6, p. 330, Monsanto 2011) and arthropod related damage (Appendix Table G-7, p. 331. Monsanto 2011); (ii) quantitative assessment at four sites for flea beetle (Chrysomelidae) and seedpod weevil (*Ceutorhynchus obstrictus*) damage (Appendix Table G-8, p. 332, Monsanto 2011); and, (iii) abundance of pest arthropods at four sites (Appendix Table G-9, p. 333-334, Monsanto 2011). A detailed description about the data collection sites, techniques, and statistical analyses has been provided in Appendix G (pp. 312-338, Monsanto 2011).

In a qualitative assessment of plant response to disease damage and arthropod damage, no differences were observed between MON 88302 and the conventional control for any of the 141 comparisons involving 16 assessed diseases or for any of the 165 comparisons for any of the 13 assessed arthropods among all observations at the sites (Table VII-5, p. 123; Appendix Tables G-6 and G-7, pp. 330-331, Monsanto 2011). Although a minor yet statistically significant difference was detected in flea beetle damage at one site for one observation (Table G-8, p. 332, Monsanto 2011), in the combined-site analysis for a quantitative assessment of flea beetle (Chrysomelidae) damage and seedpod weevil damage, no statistically significant differences were detected between MON 88302 and the conventional control from the four sites evaluated (Table VII-6, p. 124, Monsanto 2011). Furthermore, pest and beneficial arthropods (the discussion on 'beneficial arthropods' is presented in the following section "Potential Effects on Nontarget Organisms…") were collected at the four experimental locations four times during the 2009 growing season (Section G.8, p. 337, Monsanto 2011 for sampling strategy and statistical analyses). According to Monsanto, the arthropods assessed often varied between collections from a site and between sites due to differences in temporal activity and geographical distribution of the taxa (Section G.8, p. 317, Monsanto 2011). Pest arthropod abundance data did not reveal any consistent trends that would suggest an increase in pest abundance for MON 88302 compared to the control variety for aphids (Aphididae), Bertha armyworms (*Mamestra configurata*), diamondback moth larvae (*Plutella xylostella*), flea beetles (Chrysomelidae), lygus bugs (Miridae), or thrips (Thripidae) (Table G-9, pp.333-334, Monsanto 2011) and no significant difference was detected for any of the 36 observations carried out across the four experimental sites (Table VII-5, p. 123, Monsanto 2011).

As discussed earlier there were no significant changes in MON 88302 compositions that would render MON 88302 more susceptible to pests and diseases over its control or reference canola varieties. As presented later in this document, the observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 88302 is or could be relatively more susceptible to pests and diseases over control or

reference canola varieties. Thus MON 88302 is expected to be susceptible to the same plant pathogens and insect pests as conventional canola. The introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage on MON 88302 over the control line. For this reason, there is also unlikely to be any indirect plant pest effects on other agricultural products.

## E. Potential Impacts on Nontarget Organisms, Including Those Beneficial to Agriculture

MON 88302 is not engineered for pest resistance, thus there are no 'target' species, and thus no 'nontarget' species either. However, APHIS assessed whether exposure or consumption of herbicide-resistant MON 88302 containing the CP4 EPSPS protein would have an adverse effect on beneficial species or wildlife associated with canola. Monsanto provided the following information justifying the safety of MON 88302 canola (Section V.D., pp. 72-75, Monsanto 2011):

(i) The donor organism, *Agrobacterium* sp., strain CP4 is not known for human or animalpathogenicity, *Agrobacterium* sp. strain CP4 has been previously reviewed as a part of the safety assessment of the donor organism during Monsanto consultations with the FDA regarding Roundup Ready soybean (1994), Roundup Ready canola (1995), Roundup Ready cotton (1995), Roundup Ready 2 corn (1996), Roundup Ready sugar beet (1998), and Roundup Ready Flex cotton (2005). Further, the Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (EPA 1996).

(ii) The CP4 EPSPS protein present in MON 88302 is similar to EPSPS proteins consumed in a variety of food and feed sources. CP4 EPSPS protein is homologous to EPSPS proteins naturally present in plants, including food crops (e.g., soybean and maize) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe human consumption (Harrison et al. 1996; Padgette et al. 1996).

(iii) The CP4 EPSPS protein originates from *Agrobacterium* sp. strain CP4 an organism that has not been reported to be a source of known allergens. Bioinformatics analyses demonstrated that the CP4 EPSPS protein does not share amino acid sequence similarities with known allergens and, in vitro digestive fate experiments conducted with the CP4 EPSPS protein demonstrate that the protein is rapidly digested in simulated gastric fluid and in simulated intestinal fluid.

(iv) An acute oral toxicology study with mice indicated that the CP4 EPSPS protein did not cause any adverse effects in mice at the highest dose level (572 mg/kg) tested (Harrison et al., 1996).

(v) The potential route of human exposure to MON 88302 is through canola oil. The primary human food currently produced from canola is refined, bleached, and deodorized (RBD) oil. Because RBD oil contains negligible amounts of protein (Martín-Hernández

et al., 2008), oil produced from MON 88302 will contain negligible levels of CP4 EPSPS protein that would minimize the dietary exposure to this protein from consumption of foods derived from MON 88302. Furthermore, the safety of CP4 EPSPS has been extensively assessed (Harrison et al., 1996) and several Roundup Ready crops that produce CP4 EPSPS have been reviewed by FDA and other regulatory agencies. They concluded the Roundup Ready crops were safe for consumption. Likewise Monsanto provided data showing that there is minimal exposure to MON 88302 CP4 EPSPS in relation to the total protein consumed by animals such as dairy cows, pigs, and chickens (FDA BNF No. 127).

Therefore, based on the food and feed safety analyses, including toxicity and allergenicity data for CP4 EPSPS, it can be inferred that MON 88302 is unlikely to cause any significant adverse effects on nontarget organisms (including beneficial species or wildlife associated with canola) compared to other commercial canola varieties.

Additionally, as indicated earlier in this PPRA, compositional analysis of the seed showed that the mean values for nutritional components, anti-nutrients and toxicants from the combined site analysis were within the 99% tolerance interval for the commercial reference varieties, so there is unlikely to be nontarget effects resulting from changes in composition.

Direct observations detected no differences for beneficial arthropods between MON 88302 and the conventional control over 15 observations across 4 sites (as summarized in Table VII-5, p. 123, Monsanto 2011. Beneficial arthropod observation data were collected for Chironomid midge, Lacewings (Chrysopidae), Ladybird beetles (Coccinellidae), micro- and macro-parasitic hymenoptera, *Orius* spp., Spiders (Aranaea), and Sphecid wasps (Sphecidae); however with the exception of *Orius* spp., they were rarely observed in either MON 88302, the conventional control, or in the commercial reference varieties (Table G10, pp. 335-337). Although honeybees were not assessed in the beneficial arthropod study, honeybees tend to show a preference for oilseed rape pollen (Cook et al. 2003; Keller et al. 2005). Monsanto did provide observations on pollen morphology, and data on pollen diameter and viability from growth chamber grown plants showed no significant difference between MON 88302 and the control (Appendix H, pp. 339-342, Table H-1, p. 340, Monsanto 2011). Therefore, no direct or indirect effects from the transformation process are expected to have an adverse impact on beneficial species or wildlife associated with canola compared to other commercial canola varieties.

## F. Potential for Enhanced Weediness of MON 88302 Canola

APHIS assessed whether MON 88302 canola has attained characteristics as a result of genetic engineering that would enhance its weediness compared to the nontransgenic progenitor and whether the engineered trait affects methods of control for canola in situations where it is managed as a volunteer in subsequent crops or in feral populations.

Canola is a domesticated *Brassica* species. Canola is not identified as a noxious weed in the Federal Noxious Weed List nor does it appear in any state weed lists (USDA-NRCS 2012). However, canola does possess a few attributes commonly associated with weeds, such as a large seed crop and harvest yield loss (Thomas et al. 1991; Brown et al. 1995), prolonged seed dormancy of 2-5 years, and an ability to persist as feral populations in disturbed habitats (Gulden et al., 2004). Monsanto collected major agronomic data relevant to weedy traits such as seed germination, seed dormancy, seedling emergence, plant height, lodging, pollen viability and morphology, seed quality and seed moisture, seed yield, pod shattering, and abiotic stress tolerance (Table VII-1, p. 109, Monsanto 2011). Except for seed dormancy, germination, and pollen characteristics investigation, all other phenotypic and agronomic data were collected from the experimental set up described earlier under the section "Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities."

For assessment of the seed germination and dormancy characteristics, the seed lots of MON 88302, the conventional control and four commercial reference varieties were produced in the field in Grand Forks County, ND in 2009, a geographic area which represents an environment with conditions suitable for canola production (Appendix Table F-1, p. 309, Monsanto 2011). In addition to the Association of Official Seed Analysts recommended temperature regime of 15/25 °C (AOSA 2009a, 2009b), seed was tested at five additional temperature regimes of constant 5, 15, 25 or 30 °C, and alternating 5/25 °C to assess seed germination properties. The details of the experimental materials and methods are presented in Table VII-1 (pp. 108-109, Monsanto 2011) and Appendix F (p. 307-311, Monsanto 2011). According to Monsanto, no statistically significant differences were detected between MON 88302 and the control at any of the temperature regimes for normal and abnormal germination and dead seed and between MON 88302 and the control at the 5 ºC temperature regime for viable nondormant seed (Table VII-2, p. 115, Monsanto 2011). According to Monsanto, statistical comparisons were not made due to a low number of dormant seed in the AOSA-recommended temperature regime (15/25 ºC), MON 88302 numerically had fewer dormant seeds than the conventional control (0.0% vs. 0.3%). A reduction in the number of dormant seed would not increase plant weediness since non-dormant seed would be more likely to germinate reducing the potential for persistence in the soil seed bank (Gulden et al., 2003). Although, no statistical comparisons were made for viable non-dormant seed in the 15 ºC, 25 ºC, 30 ºC and 5/25 ºC temperature regimes, the magnitude of the difference for seed of MON 88302 and the conventional control was small and there were no observable trends in the mean difference in this category across the different temperature regimes (p. 114, Monsanto 2011).

The experimental set up and data analysis for agronomic and phenotypic data was described earlier in this document (see section "Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities"; the methods and detailed results of the individual site data comparisons are presented and discussed in Appendix G, pp. 312-339, Monsanto 2011). A total of 12 phenotypic and agronomic characteristics were evaluated (early stand count, seedling vigor, days after planting to both first flowering and seed maturity, lodging, plant height, both visual and quantitative measures for pod shattering, seed moisture and quality, yield, and final stand count) (Table VII-4, p. 119,

Monsanto 2011). Only two statistically significant differences were detected between MON 88302 and the conventional control in the combined-site analysis: days to flowering and seed moisture content (Table VII-4, p. 119, Monsanto 2011). MON 88302 reached first flowering later than the conventional control (61.1 vs. 56.2 days). However, the mean value of MON 88302 for days to first flowering was within the natural variability of the commercial reference varieties (45.9 – 67.5 days). Therefore, the difference in days to first flowering is unlikely to be biologically meaningful in terms of increased weediness potential. Although early flowering is one of the potential indicators of enhanced weediness (Campbell et al. 2009; Ridley and Ellstrand 2009), MON 88302 canola is a late flowering type and the observed difference in flowering is unlikely to facilitate any weediness potential to MON 88302 canola. In a separate study on growth-chamber grown plants, another reproductive feature, pollen morphology, did not exhibit any difference between MON 88302 and control, as no statistically significant differences were detected between MON 88302 and the conventional control for percent viable pollen or pollen grain diameter (Table VII-7. p. 126, Monsanto 2011). MON 88302 had higher harvested seed moisture than the conventional control (13.2% vs. 11.7%). However, the mean value of MON 88302 for harvested seed moisture was within the natural variability of the commercial reference varieties (7.5% – 14.8%). Therefore, according to Monsanto the difference in seed moisture is unlikely to be biologically meaningful in terms of increased weediness potential. Likewise, Monsanto did not observe significant qualitative differences between MON 88302 and the control canola for the nine abiotic stress tolerance traits examined, with the exception of one observation for frost damage at one site, which was more severe for MON 88302 but within the range observed for the reference varieties (Table VII-5, p. 123, and Table G5, p. 329, Monsanto 2011). Stress responses examined included cold, heat, frost, hail, drought flood, wind, nitrogen deficiency and compaction.

Seed shattering or seed yield loss during harvest combined with extended seed dormancy have the potential to create volunteer and weed problems for subsequent crops. Indeed canola is known to shatter seeds with about 2-7% of the seed yield lost during seed harvest (Table 2 in Gan et al. 2008). Despite significant seed loss during harvest, a majority of fallen seeds in the soil tend to germinate (> 90%) in the first season after harvest, and the remaining seeds generally exhibit 1-2 years of dormancy (Gulden et al. 2003). A few research reports also noted canola seed dormancy periods extending beyond 3 years (Légère et al. 2001; Simard et al. 2002; D'Hertefeldt et al. 2008), yet it was also observed that very few canola volunteers emerge during and after the third year of the post-harvest (Simard et al. 2002). Nonetheless, volunteer canola plants have still been documented at low densities four and five years after production (Simard et al. 2002). A significant body of research exists on the ability of canola to form feral populations (Simard et al. 2002 and references therein; Schafer et al. 2011) and there is a widespread concern that herbicide-resistant feral populations may become an unmanageable weed problem around field edges and minimally managed agroecosystems. Unlike highly domesticated crops such as corn and soybean, canola is a relatively newly domesticated crop plant and possesses a few traits (e.g. prolonged seed dormancy, large seed yield, seed shattering) that facilitate canola to persist as feral populations (Crawley and Brown 1995; Pessel et al. 2001). On the contrary, a mere

possession of the potential weedy traits (Baker 1965) does not appear to predispose a plant taxon to become a weed (Perrins et al. 1992; Sutherland 2004).

Despite possessing some of the weed traits, canola is unlikely to become an unmanageable weed with the introduced trait. Like other crop plants, canola has several domesticated traits such as high seed output under optimum agronomic practices, self-pollination, etc., that make canola less competitive in unmanaged or minimally managed ecosystems (Crawley et al. 1993; Crawley et al. 2001; Salisbury 2002). The agronomic characteristics and germination data discussed earlier in this section provide evidence that the genetic modification resulting in MON 88302 canola did not alter any major characteristics of the plant that would allow for development of weedy characteristics different from other canola varieties. Furthermore, the herbicide-resistance trait conferred by the *cp4 epsps* gene is unlikely to provide a selective advantage in unmanaged ecosystems, but rather only in settings where glyphosate is being applied for weed control.

As described in the petition (section VII.D., p. 126, Monsanto 2011), herbicide-resistant canola is no more likely to form feral populations than unmodified canola, nor is it more likely to be more invasive or competitive or persistent in habitats where the target herbicide is not applied (Warwick et al. 2009; Andersson and de Vicente 2010). Even in those areas where herbicide-resistant canola has been grown extensively in the last several years, there is no indication of altered weediness or invasiveness potential imparted to feral canola or volunteer canola populations (Hall et al. 2005).

Volunteer herbicide-resistant canola (MON 88302 canola, as well as other commercially available glyphosate, glufosinate, and imidazolinone resistant varieties) should be controlled before planting canola cultivars with different herbicide resistance traits to reduce the potential for gene flow to result in stacked herbicide resistance, as such stacked herbicide resistance in canola has already been documented (Hall et al. 2000; Shaefer et al. 2011) (discussed in more detail in the following section). Although herbicide-resistant and nonresistant canola varieties are documented to persist as volunteers or establish feral populations, there are alternative herbicides that can be used to control them. Paraquat and diuron (alone or in various combinations, some including glyphosate) were shown to be effective herbicides for control prior to planting in conservation tillage systems in the Pacific Northwest (Rainbolt et al. 2004). Producers need to consider which crop will follow the canola when making herbicide selections. Canola best follows cereal grains or fallow in rotation, and is rarely planted within one or two years following canola and other crops highly susceptible to sclerotinia (a stem rot) e.g. sunflower, dry edible beans or crambe, and sugar beet (Berglund et al. 2007). Glyphosate resistant canola is typically rotated with other crops, typically wheat in a two year rotation or with wheat and soybean in a three year rotation, and other rotation crops include oats, barley, and flax (Berglund et al. 2007). Several (26) herbicide formulation options provide at least good to excellent control of volunteer glyphosate resistant canola, particularly at the 3-6 leaf stage, as described in the 2012 North Dakota Weed Control Guide, and several can be used in the most common rotation crops of canola (NDSU 2012, p. 115; Table VIII-12, p. 164, Monsanto 2011). Tillage can also be used. CP4 EPSPS does not confer cross resistance

to other herbicide modes of action. Therefore, MON 88302 is expected to be sensitive to the same herbicides as other glyphosate resistant canola already commercialized.

Therefore, based on this characterization, MON 88302 is no more likely to establish feral populations than either existing transgenic or nontransgenic herbicide-resistant or nontransgenic herbicide sensitive canola varieties, and such feral populations can be controlled using current weed control practices.

## G. Potential of MON 88302 to Impact the Weediness of Other Plants with which It Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Soltis and Soltis 1993; Rieseberg 1997; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars. However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (see Table 1 in Ellstrand et al. 1999).

APHIS considers two primary issues when assessing weediness of sexually compatible plants because of transgene flow: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

Canola is predominantly self-pollinating, but outcrossing does occur via wind and insect pollination (William 1984; William et al. 1987). Depending on the size of the crop and distance between plants or fields, a variety of outcrossing rates (12-55%) were observed for canola (See Table 1 in Beckie et al. 2003). Most outcrossing between fields generally occurs within the first 10-20 m of the recipient field, and rates decline with distance (Table 1 in Beckie et al. 2003; Table IX-2, p. 181, Monsanto 2011).

Canola is grown in very few places in the U.S. and a majority (~88%) of canola production occurs in North Dakota, while the remaining cultivation comes from Minnesota, Idaho, Washington, Montana, Oklahoma, and Oregon (USDA-NASS 2011; Table VIII-2, p. 132, Monsanto 2011). Spring and winter canola varieties are generally grown in different regions of the U.S. based on climate zones most suitable for the varieties (pp. 129-130 and 138 in Monsanto 2011). Brassica crops involve a number of diploid and polyploidy species and the family Brassicaceae involves a number of major weed species (OECD 2012).

In a majority of crop species, gene flow is idiosyncratic depending upon biology and ecology of both crop and sexually compatible relatives (Gliddon et al. 1999; Ingram 2000; Warwick et al. 2009). Accordingly, there are several important considerations for a successful gene flow and introgression between MON 88302 and sexually compatible crop and weedy relatives such as spatial proximity, overlapping phenology, F1 hybrid

fertility, self-sustaining reproductively fertile hybrid-derived (backcrossed) populations, and neutral or beneficial introgressed genes (Devos et al. 2009). In addition, the stage at which the crop is harvested, e.g. before or after flowering or seed set, will also influence the likelihood of successful gene flow.

Monsanto provided a summary of published literature on unassisted hybridization under field conditions with *B. napus* as a the male parent to various crops and wild relatives of canola that occur in the U.S. (Table IX-3, p. 189-193, Monsanto 2011) and the likelihood of hybridizations with canola based on information on the success rate of hand pollinations or spontaneous and natural hybridization, weediness, and presence of the species in winter or spring canola growing areas (section IX.D., pp. 180-193, Monsanto 2011). From this analysis Monsanto concludes: "There are reports of hybridization under field conditions with *B. napus* as the pollen donor with six species including *Brassica rapa*, *B. juncea*, *B. oleracea*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapsis arvensis* (Table IX-3). The species *B. rapa*, *B. juncea* and *B. oleracea* are cultivated for crop production. The other species listed, *H. incana*, *R. raphanistrum* and *S. arvensis* are not cultivated for crop production, but are found in the environment. In all cases the resulting hybrids had decreased environmental fitness evidenced by a variety of characteristics including decreased pollen viability, seed production, seedling survival, *etc*. when compared to parental varieties." (p. 182, Monsanto 2011). APHIS agrees with this conclusion, which is well supported by published literature.

Genus *Brassica* and related genus *Raphanus* contain oil seed, vegetable, and forage crop species (Ellstrand 2003; FitzJohn et al. 2007) such as *B. napus* (oil seed, swede) and *B. rapa* (oilseed, turnip and Chinese cabbage), *B. oleraceae* (cauliflower, cabbage, broccoli), *B. juncea* (Indian mustard), and *R. sativus* (radish). Three *Brassica* species and one species in the related genus *Sinapis* are 'mustards': *B. carinata* (Ethiopian mustard), *B. juncea* (Indian mustard), *B. nigra* (black mustard) and *Sinapis alba* (white mustard). Cultivation of *B. carinata* as an oilseed and vegetable crop is largely restricted to Ethiopia and India (Hemingway 1995; Stewart 2002). Some forms of *B. napus*, *B. oleracea*, *B. rapa* and *R. sativus* are also grown as fodder crops (FitzJohn et al. 2007).

The three *Brassica* species forming the foundation of the Triangle of U showing genome relationships among cultivated Brassicaceae (Nagaharu 1935) are *B. rapa, B. nigra,* and *B. oleracea*. *B. napus* and *B. rapa* outcross readily with each other, while *Brassica napus* and *B. juncea* share a common set of chromosomes, enhancing the likelihood of interspecific hybridization and gene flow (Myers 2006). The A genome is common to the three major oilseed *Brassica* species, which explains the success of interspecific crossing, and the ability to transfer genes among these species.

The Brassicaceae family contains a number of major weeds, including those in the genera *Sinapis, Capsella, Thlaspi, Erucastrium, Raphanus*, and others (OECD 1997). Concerns have been raised about the potential for the transfer of transgenes from the cultivated oilseed *Brassica* species to their weedy relatives in Europe and North America where *Brassica* crop species are widely grown. These *Brassica* crop species can also outcross, albeit rarely, with a wide range of wild and weedy species (summarized in OGTR 2002). Some *Brassica* crops and their wild relatives will hybridize only under artificial

conditions in laboratories or highly contrived field conditions; whereas others will hybridize at very low rates under natural conditions (Raybould 1999; Barton and Dracup 2000). Through an extensive literature survey, Warwick and Black compiled an exhaustive list of interspecific and intergeneric hybridization among the members of the tribe Brassiceae, including large-scale artificial intergeneric hybridizations between various members of the tribe (Table 1 in Warwick and Black 1993) and reported very few natural hybrids. As noted earlier, several reproductive and ecological barriers between canola and its wild species prevent formation of successful introgressed, self-sustaining hybrid derived populations (see detail descriptions in section IX.D., pp. 180-193 in Monsanto 2011).

Feral canola is a quite common occurrence along canola field edges and transportation routes (Bagavathiannan and Van Acker 2008; see Table 1 in Devos et al. 2012). The major concern raised about herbicide-resistant GE canola is about the ability of feral canola populations to act as a sink for the accumulation (stacking) of multiple herbicide resistance genes from the four different herbicide-resistant (GE-glufosinate resistance, GE-bromoxynil resistance, GE-glyphosate resistance, non-GE imidazolinone-resistant) canola currently under cultivation in Canada (Hall et al. 2000). There are reports indicating that canola cultivars with different HR traits resulted in volunteers with multiple resistance at a field site in western Canada (Hall et al. 2000). Therefore, the stacking of HR traits has the potential to facilitate the evolution of invasive feral canola populations (Ellstrand 2003; Knispel et al. 2008; Warwick et al. 2009).

In 1997 in northern Alberta, a field of glyphosate resistant canola was grown adjacent to a field of glufosinate resistant and imidazolinone resistant canola. Volunteers were selected with glyphosate in 1998 (Hall et al. 2000). These volunteers flowered and produced seeds that contained individuals resistant to glyphosate and glufosinate; glyphosate and imazethapyr; and glyphosate, imazethapyr, and glufosinate. Two triple herbicide-resistant individuals were detected, with one plant located 550 m from the glyphosate resistant pollen source. More recently, a study at 11 sites in Saskatchewan, Canada, where glyphosate HR *B. napus* canola was grown adjacent to glufosinate HR *B. napus* canola, documented gene flow to the limits of the study areas—a maximum distance of 800 m—on the basis of occurrence of double-HR volunteers (Beckie et al. 2003). The results of both studies suggest that herbicide-resistant gene stacking is common in *B. napus* canola volunteers in western Canada. In a similar study Knispel et al (2008) surveyed for the presence of single and multiple herbicide resistance traits and assessed the extent of gene flow within escaped canola populations. Seed was collected from 16 escaped canola populations along the verges of fields and roadways in four agricultural regions in southern Manitoba from 2004 to 2006. Glyphosate resistance was found in 14 (88%) of these populations, glufosinate resistance in 13 (81%) populations, and imidazolinone resistance in five (31%) populations. Multiple herbicide resistance was observed at levels consistent with previously published canola outcrossing rates in 10 (62%) of the tested populations. These reports indicate that intraspecific gene flow results in stacking of herbicide resistance traits in individuals within escaped canola populations (Knipsel et al. 2008). Similar multiple herbicide-resistant canola feral populations were also reported from Japan around transportation routes, although Japan

never cultivates GE canola varieties and only imports them for food and feed purposes (Aono et al. 2006; Kawata et al. 2009).

Gene flow from MON 88302 was evaluated thoroughly with respect to plant pest risk. The introduced *cp4 epsps* gene in MON 88302 is not expected to change the ability of the plant to interbreed with other plant species. Furthermore, the APHIS-BRS evaluation of data provided by Monsanto (2011) of agronomic and phenotypic properties of MON 88302, including those characteristics associated with reproductive biology, indicated no unintended changes likely to affect the potential for gene flow from MON 88302 to sexually compatible species. In addition, gene flow has been occurring between non-GE canola (both herbicide-resistant and other canola varieties) and sexually compatible species even before the advent of GE canola varieties. Therefore, the consequences of gene flow and introgression of the glyphosate-resistant trait from MON 88302 to the same or sexually compatible species are anticipated to be the same as for existing commercial glyphosate-resistant canola varieties.

Successful hybridization of canola and a wild/weedy relative is highly unlikely and even if those successful rare events occur, the herbicide-resistance trait would only provide selective advantage in situations in which the weedy hybrid was in contact with the herbicide (*i.e.*, in an agricultural field or treated rights of way). One such situation is when feral canola populations or wild relatives of canola are exposed to herbicide due to herbicide drift, whereby some herbicides are carried by winds and airflow beyond the intended area of application. Londo et al. (2010) demonstrated the potential for changes in the movement and persistence of glyphosate- resistance transgenes in weedy plant communities due to gene flow and fitness traits under the presence of glyphosate drift. As authors noted in their research paper, the above-mentioned research results were obtained from outdoor sunlit mesocosms (simulated and controlled natural system) and were not performed as a field experiment. Furthermore, Londo et al. (2010) advocated for future field-based studies to validate their observations and to test for other environmental factors that influence the gene flow dynamics of weedy populations exposed to glyphosate drift. Therefore, so far there is no strong evidence that genetically engineered glyphosate herbicide-resistant canola has substantially altered the gene flow dynamics between GE canola and its wild and weedy relatives.

Any herbicide-resistant feral and hybrid-derived populations are likely to be controlled using other available chemical or mechanical means. Many herbicides that are effective for control of glyphosate resistant canola are also effective for control of wild mustards (NDSU 2012, pp. 115, 117, 119, and 120). As described by Beckie and colleagues (see Beckie et al. 2004 and references therein) the following cultural or mechanical practices are recommended to growers to manage multiple-HR canola volunteers: (1) leaving seeds on or near the soil surface as long as possible after harvest because a high percentage will germinate in the fall and be killed by frost, whereas seeds incorporated into the soil may develop secondary dormancy that will increase persistence; (2) using tillage immediately before seeding; (3) silaging and green manuring to prevent seed set in volunteers; (4) isolating fields of canola with different herbicide resistance traits to reduce outcrossing; (5) following canola with a cereal crop and rotating canola in a 4-yr diverse cropping sequence to deplete volunteers from the seedbank over time (which also facilitates use of

alternative herbicides with different modes of action) and growing competitive crops to minimize volunteer canola interference (by choice of species and manipulation of agronomic practices such as higher seeding rates and precision fertilizer placement); (6) scouting fields for volunteers not controlled by weed management treatments and preventing seed set; (7) using pedigreed seed to reduce the probability of the presence of off-types with different herbicide resistance traits; and (8) reducing seed loss during harvest by swathing at the correct crop development stage and properly adjusting combine settings. Herbicide treatments such as metribuzin, 2,4-D, or MCPA, alone or in a mixture, can control single or multiple herbicide-resistant canola volunteers when densities warrant, either pre-seeding or in-crop where registered. Previous studies have shown no difference in fitness among non-herbicide, single herbicide-resistant, or multiple herbicide-resistant canola. These findings indicate that multiple herbicide-resistant canola can be controlled equally well as non-herbicide-resistant or single herbicide-resistant plants by alternative herbicides within an integrated weed management program (Beckie et al. 2004).

Large-scale cultivation of herbicide-resistant canola has occurred for nearly 15 years in Canada and the United States. To date, there are no reports of problems with interspecific crosses and introgression of herbicide-resistance genes into cultivated or wild relatives of canola (Andersson and de Vicente 2010). The International Survey of Herbicide Resistant Weeds has no confirmed cases of glyphosate resistant weeds that are wild or weedy relatives of canola (Heap 2013). The MON 88302 is not expected to expand the amount of acreage planted to canola or to glyphosate resistant canola, but rather to provide growers an opportunity to control troublesome weeds of canola crops with the glyphosate herbicide application during later stages of plant development than currently possible with other varieties of glyphosate-resistant canola already commercially available.

Therefore, it is highly unlikely that canola plants in the United States will be found outside of an agricultural setting, except along roadsides along seed transportation routes. It is also highly unlikely that gene flow and introgression will occur between MON 88302 plants and wild or weedy species in a natural environment. Herbicides are available to control volunteer glyphosate-resistant canola and weedy relatives. USDA has therefore determined that any adverse consequences of gene flow from MON 88302 to wild or weedy species in the United States are highly unlikely.

## H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered whether there are likely to be significant changes to agricultural practices associated with cultivation of MON 88302, and if so are they likely to significantly exacerbate plant diseases or pests, especially those for which APHIS has a control program.

Glyphosate-resistant MON 88302 is not a new type of GE crop, as three different types of herbicide-resistant canola varieties (conventionally derived imidazolinone-resistant (Clearfield); GE glyphosate-resistant (Roundup Ready RT73); and GE glufosinate-resistant (InVigor)) are already available for cultivation in the U.S. (Brown et al. 2008).

In addition, a significant acreage of genetically modified herbicide-resistant canola (predominantly glyphosate-resistant) has been planted commercially in the U.S. since 1999. For instance, in 2008, genetically engineered herbicide-resistant canola was estimated to be 95% of the U.S. canola crop (Brookes and Barfoot 2010). One major difference between MON 88302 and earlier glyphosate resistant canola varieties is that MON 88302 is modified to withstand a later application of glyphosate compared to the earlier herbicide-resistant canola varieties. According to Monsanto, MON 88302 is a second-generation glyphosate-resistant spring canola variety designed to provide growers with improved weed control through greater flexibility of glyphosate herbicide application. By virtue of enhanced CP4 EPSPS expression in male reproductive tissues, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at later stages of development and at higher rates than is possible with the current product (Feng et al. 2010; p. 196 in Monsanto 2011).

Current label directions indicate glyphosate agricultural herbicides can be applied post-emergence on glyphosate-tolerant canola from emergence to the 6-leaf stage of development up to 0.39 to 0.56 lb a.e./acre in spring canola varieties and 0.77 lb a.e./acre for winter canola varieties to prevent weed competition. The first application of glyphosate to glyphosate-tolerant canola is made at the two- to three-leaf stage to prevent early weed competition and an additional application is made up to the six-leaf stage to control any late-emerging weeds. Two applications of glyphosate were made on approximately 42% of the canola acreage in the largest canola producing state, North Dakota, in 2008 (Table VIII-8, p. 153, Monsanto 2011), however, after the 4-leaf stage a single application of glyphosate greater than 0.39 lb a.e./acre in spring canola and greater than 0.56 lbs a.e./acre prior to the 6-leaf stage in the fall for winter canola can result in crop injury. In winter canola, a sequential application up to 0.77 lbs a.e./acre can be made the following spring prior to the bolting stage, as long as the two in-crop applications do not exceed a total of 1.55 lbs. a.e./acre (Section VIII.G., pp. 156-157, Monsanto 2011).

MON 88302 provides greater crop tolerance which will permit higher rates and a wider period for application of glyphosate in spring and winter canola compared to the first-generation Roundup Ready canola system. The higher glyphosate rates and extended timing for applications up to the first flower stage will provide improved control of difficult to control weed species such as Canada thistle, dandelion, sow thistle, common lambsquarters, kochia, smartweed and wild buckwheat (CCC 2003), however glyphosate resistant kochia has now been reported in crop land in six states (including significant canola-producing states, North Dakota and Montana, as well as in Alberta, Canada (Heap 2013). If the glyphosate label is amended by the EPA, MON 88302 will permit two in-crop sequential glyphosate applications up to 0.77 lbs. a.e./ acre each in spring canola or one application up to 1.55 lb a.e./acre prior to the 6-leaf growth stage. Total in-crop applications will be increased from 0.78 to 1.55 lbs of glyphosate a.e./acre for spring canola. Recommended application rates for glyphosate in winter canola will likely remain unchanged with the introduction of MON 88302 (Section VIII.G., p. 157, Monsanto 2011). Although MON 88302 commercial cultivation would likely increase the total amount of the glyphosate herbicide use in canola crops for an extended period of time during crop cultivation, similar cultivation practice has already been in use with

several GE herbicide-resistant crops that are planted every year across millions of acres of agricultural lands. Moreover, the increased glyphosate use on MON 88302 would be negligible (1%) compared to the amount of glyphosate usage on other glyphosate herbicide-resistant crops such as corn, cotton, and soybean which are grown over much larger acreages (NDSU 2007; Benbrook 2009). Issues related to weed control and herbicide resistance as a result of changes in herbicide use are covered in the NEPA document prepared for this petition.

Recent studies (Anderson and Kolmer 2005; Feng et al. 2005, 2008) have reported that glyphosate is active against certain fungal diseases (e.g. in wheat and soybean) and when applied to GR crops provided disease suppression that benefit from the persistence of glyphosate. As this is primarily a glyphosate effect, this issue will be more fully evaluated in the NEPA document prepared for this petition. A more recent review by Duke et al. (2012) indicates that although it is clear that glyphosate does increase severity of disease on glyphosate susceptible plants, overall the baseline disease resistance or susceptibility of the host plant, not the presence of the glyphosate resistance gene or treatment with glyphosate, is the major contributor to susceptibility. None of the referenced studies on disease response following glyphosate treatment in glyphosate resistant crops included glyphosate resistant canola.

As discussed throughout this document, MON 88302 is similar to conventional canola in its agronomic, phenotypic, environmental intercalations, and compositional characteristics and has levels of tolerance to insects and diseases comparable to conventional canola. Therefore, no significant impacts on current cultivation and management practices for canola are expected following the introduction of MON 88302.

## I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 88302 Canola Cannot Interbreed

The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998) and sequencing of large numbers of genomic sequences (Choi and Kim 2007). HGT contributed to major transitions in evolution of prokaryotic organisms (Woese 2002) and has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in viruses, bacteria, and eukaryotes. Although, gene exchange has been documented for nearly all types of genes and between unrelated organisms at an evolutionary scale (Gogarten et al. 2002; Yoshida et al. 2010), the frequency of HGT among higher organisms are shown to be extremely rare and, consequently, such transfers did not play any major role in their evolution (Kurland et al. 2003).

APHIS examined the potential for the new genetic material inserted into MON 88302 to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. Although MON 88302 contains genetic sequences from the plant pathogenic organisms *Agrobacterium* and figwort mosaic virus, the expressed gene product does not

have plant pathogenic properties or cause disease in plants. Furthermore, many organisms, including pathogens and symbionts, already contain variations of genes encoding EPSPS with varying degrees of sensitivity to glyphosate (see Duke et al. 2012). Furthermore, horizontal gene transfer and expression of DNA from a plant species to other fungal, bacterial, or parasitic species is unlikely to occur based on the following observations.

Although there are many opportunities for plants to directly interact with fungi, bacteria, and parasitic plants (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), so far there are no reports of significant horizontal gene transfer between evolutionarily distant organisms (as reviewed in Kurland et al. 2003; Keese 2008). Accumulated evidence show that there are universal gene-transfer barriers, regardless of whether transfer occurs among closely or distantly related organisms (Koonin et al. 2001; Wood et al. 2001; Kaneko et al. 2002; Brown 2003; Sorek et al. 2007). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al. 2002; Wood et al. 2001). There is no evidence that these organisms contain genes derived from plants. In cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003), so also the case with the recent report about HGT between sorghum and purple witchweed. According to authors (Yoshida et al. 2010), the incorporation of a specific genetic sequence occurred between sorghum and purple witchweed before speciation of purple witchweed (*Striga hermonthica*) and related cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots, from their common ancestor. In other words, HGT is an extremely rare event, and a majority of those rare events occur over millions of years.

Transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA 1998: http://www.fda.gov/Food/GuidanceComplianceRegulatory Information/GuidanceDocuments/Biotechnology/ucm096135.htm). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur from MON 88302 to microorganisms, and thus no significant plant pest risk is expected from horizontal gene transfer.

## J. Conclusion

APHIS has reviewed the information submitted by the petitioner and conducted a plant pest risk assessment on MON 88302 (including its progeny). APHIS concludes that MON 88302 canola is highly unlikely to pose a plant pest risk for the following reasons:

a. Neither the introduced sequences nor the method of transformation has resulted in disease symptoms, pathogen infection, or expression of a pathogen in MON 88302.
b. Composition of Mon 88302 seed is similar to that of the conventional control and/or within the natural variability of commercial reference varieties. Therefore, changes in gene expression, enzymes or metabolism from introduced genes in MON 88302 are unlikely to pose a plant pest risk.
c. MON 88302 is expected to be susceptible to the same plant pathogens and insect pests as conventional canola and it is unlikely to bring about any indirect plant pest effects on other agricultural products.
d. MON 88302 is not expected to adversely impact wildlife or other organisms beneficial to agriculture any more than conventional canola varieties.
e. Introduced genes in MON 88302 did not significantly alter any major characteristics of the plant (i) that would facilitate the development of weedy characteristics in the crop or (ii) that would enhance its gene flow potential to wild/weedy relatives and consequently produce or alter weedy characteristics in wild/weedy relatives.
f. The glyphosate resistance trait in MON 88302 and the anticipated changes in agricultural practices related to glyphosate resistance in MON 88302 are not expected to increase pests or diseases or impact their control in canola or other crops based on prior experience with previously deregulated glyphosate resistant canola.
g. Genes encoding variants of the EPSPS protein already exist among pathogens and symbionts in the environment, and horizontal transfer of the inserted glyphosate resistance gene from MON 88302 to other organisms with which it cannot interbreed is highly unlikely, and thus should not pose a plant pest risk.

## K. References

Alibhai, M.F. and Stallings, W. C. 2001. Closing down on glyphosate inhibition - With a new structure for drug discovery. Proceedings of the National Academy of Sciences (U.S.A.) 98:2944-2946.

Anderson, J. A., and Kolmer, H. A. 2005. Rust control in glyphosate tolerant wheat following application of the herbicide glyphosate. Plant Disease 89:1136-1142.

Andersson, M. S., and de Vicente, M.C. 2010. Canola, oilseed rape. Pp. 73-123 in M. Andersson and M. de Vicente, M. (eds.) Gene Flow Between Crops and Their Wild Relatives. The John Hopkins University Press, Baltimore, Maryland.

Aono, M., Wakiyama, S, Nagatsu, M., Nakajima, N., Tamaoki, M., Kubo, A., and Saji, H. 2006. Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. Environmental Biosafety Research 5:77-87.

AOSA (Association of Official Seed Analysts). 2009a. AOSA Rules for testing seeds. Volume 1. Principles and Procedures. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA (Association of Official Seed Analysts). 2009b. AOSA Rules for testing seeds. Volume 4. Seedling Evaluation. Association of Official Seed Analysts, Lincoln, Nebraska.

Awmack, C. S., and Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47:817-844.

Axelos, M., Bardet, C., Liboz, T., Le Van Thai, A., Curie, C., and Lescure, B. 1989. The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1α: Molecular cloning, characterization and expression. Molecular and General Genetics 219:106-112.

Bagavathiannan, M. V., and Van Acker, R. C. 2008. Crop ferality: Implications for novel trait confinement. Agriculture, Ecosystems and Environment 127:1-6.

Baker, H. B. 1965. Characteristics and modes of origin of weeds. Pp. 147-169 *in* H. G.

Baker and G. L. Stebbins (eds.) The Genetics of Colonizing Species. Academic Press, London.

Barker, R.F., Idler, K. B., Thompson, D. V., and Kemp, J.D. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology 2:335-350.

Barry, G.F., Kishore, G. M., Padgette, S. R. and Stallings, W. C. 2001. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 6,248,876, U.S. Patent Office, Washington, D.C.

Barton, J. E., and Dracup, M. 2000. Genetically Modified Crops and the Environment. Agronomy Journal 92:797-803.

Benbrook, C. 2009. Impacts of genetically engineered crops on pesticide use: The first thirteen years. The Organic Center, Boulder, Colorado.

Berglund, D.R., McKay, K., and Knodel, J. 2007. Canola Production. Publication A-686, Revised August 2007. North Dakota State University, Fargo, North Dakota. http://www.ag.ndsu.edu/pubs/plantsci/crops/a686w.htm (Accessed 3/18/2012)

Beckie, H. J., Séguin-Swartz, G., Nair, H., Warwick, S. I., and Johnson, E. 2004. Multiple herbicide–resistant canola can be controlled by alternative herbicides. Weed Science 52:152-157.

Beckie, H. J., Warwick, S. I., Nair, H., and Se´guin-Swartz, G. 2003. Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecological Applications 13:1276-1294.

Brookes, G., and Barfoot, P. 2010. GM crops: global socio-economic and environmental impacts 1996-2008. PG Economics Ltd, Dorchester, UK.

Brown, J. R. 2003. Ancient horizontal gene transfer. Nature Review Genetics 4:121-132.

Brown, J., David, J. B., Lauver, M., and Wysocki, D. 2008. U.S. Canola Association Canola Growers' Manual. U.S. Canola Association. University of Idaho & Oregon State University. http://www.uscanola.com/site/files/956/102387/363729/502632/Canola_Grower_Manual_FINAL_reduce.pdf (Accessed 2/2/2011)

Brown, J., Erickson, D. A., Davis, J. B., and Brown, A. P. 1995. Effects of swathing on yield and quality of spring canola (Brassica napus L.) in the pacific North West. Pp. 339–341 in Proceedings of the 9th International Rapeseed Congress; Cambridge, U.K. Volume 1. Cambridge, U.K.

Campbell, L. G., Snow, A. A., and Sweeney, P. M. 2009. When divergent life histories hybridize: insights into adaptive life-history traits in an annual weed. New Phytologist 184:806-818.

CCC (Canola Council of Canada). 2003. Canola Growers Manual. Canola Council of Canada. Winnipeg, Manitobahttp://www.canolacouncil.org/canola_growers_manual.aspx (Accessed 4/24/2012)

Choi, I-G., and Kim, S-H. 2007. Global extent of horizontal gene transfer. Proceedings of the National Academy of Sciences (U.S.A.) 104:4489-4494.

Cook, S. M., Awmack, C.S., Murray, D. A., and Williams, I. H. 2003. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecological Entomology 28:622-627.

Coruzzi, G., Broglie, R., Edwards, C., and Chua, N-H. 1984. Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1, 5-bisphosphate carboxylase. EMBO Journal 3:1671-1679.

Crawley, M. J., and Brown, S. L. 1995. Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proceedings of the Royal Society of London. Series B: Biological Sciences 259:49-54.

Crawley, M. J., Brown, S. L., Hails, R. S., Kohn, D. D., and Rees, M. 2001. Biotechnology: Transgenic crops in natural habitats. Nature 409:682-683.

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D., and Buxton, J. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363:620-623.

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., and Goodman, H. M. 1982. Nopaline synthase: Transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1:561-573.

Devos, Y., De Schrijver, A., and Reheul, D. 2009. Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. Environmental Monitoring and Assessment 149:303-322.

Devos, Y., Hails, R. S., Messe´an, A., Perry, J. N., and Squire, G. R. 2012. Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic Research 21:1-21.

D'Hertefeldt, T., Jørgensen, R. B., and Pettersson, L. B. 2008. Long-term persistence of GM oilseed rape in the seedbank. Biology Letters 4:314-317.

Dixon, R.A. 2001. Natural products and plant disease resistance. *Nature* **411:**843-847.

Dröge, M., Puhler, A., and Selbitschka, W. 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. Journal of Biotechnology 64:75-90.

Duke, S.O., Lydon, J., Koskinen, W.C., Moorman, T.B., Chaney, R.L., and Hammerschmidt, R. 2012. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. Journal of Agricultural and Food Chemistry 60: 10375-10397.

Ellstrand, N. C. 2003. Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives, The John Hopkins University Press, Baltimore.

Ellstrand, N. C., Prentice, H. C., and Hancock, J. F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. Annual Review of Ecology and Systematics 30:539-563.

EPA (U.S. Environmental Protection Agency). 1996. Pesticide tolerances for glyphosate. Federal Register 61: 15192-15196.

FDA (U.S. Food and Drug Administration). 1998. Draft Guidance: Use of antibiotic resistance marker genes in plants. http://www.fda.gov/Food/GuidanceCompliance RegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm (Accessed on 1/6/2012).

Feng, P. C. C., Baley G. J., Clinton, W. P., Bunkers, G. J., Alibhai, M. F., Paulitz, T. C., Kidwell, K. K. 2005. Glyphosate inhibits rust diseases in glyphosate-resistant wheat and soybean. Proceedings of National Academy of Sciences (U.S.A.) 02:17290-17295.

Feng, P.C.C., Clark, C., Andrade, G.C., Balbi, M.C., Caldwell, P. 2008. The control of Asian rust by glyphosate-resistant soybeans. Pest Management Science 64:353-359.

Feng, P. C. C., Jacob, C. A., Martino-Catt, S. J., Cerny, R. E., Elmore, G. A., Heck, G. R., Huang, J., Kruger, W. M., Malven, M., Miklos, J. A., and Padgette, S. R. 2010. Glyphosate resistant crops: Developing the next generation products. Pages 45-65 in Glyphosate Resistance in Crops and Weeds : History, Development, and Management. V.K. Nadula (ed.). John Wiley & Sons, Inc., Hoboken, New Jersey.

FitzJohn, R., Armstrong, T., Newstrom-Lloyd, L., Wilton, A., and Cochrane, M. 2007. Hybridisation within *Brassica* and allied genera: evaluation of potential for transgene escape. Euphytica 158:209-230.

Fling, M.E., Kopf, J., and Richards, C. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. Nucleic Acids Research 13:7095-7106.

Funke, T., Han, H., Healy-Fried, M. L., Fischer, M., and Schonbrunn, E. 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. Proceedings of the National Academy of Sciences (U.S.A.) 103:13010-13015.

Gan, Y., Malhi, S. S., Brandt, S. A., and McDonald, C. L. 2008. Assessment of seed shattering resistance and yield loss in five oilseed crops. Canadian Journal of Plant Science 88:267-270.

Giza, P.E. and Huang, R. C. C. 1989. A self-inducing runaway-replication plasmidexpression system utilizing the Rop protein. Gene 78:73-84.

Gliddon, C., Boudry, P., and Walker, S. 1999. Gene flow - a review of experimental evidence. Pp. 65-79 *in* C. Gliddon, A. J. Gray, and F. Amijee (eds.) Environmental Impact of Genetically Modified Crops, DETR, London.

Gogarten, J. P., Doolittle, W. F., and Lawrence, J. G. 2002. Prokaryotic evolution in light of gene transfer. Molecular Biology and Evolution 19:2226-2238.

Grant, V. 1981. Plant Speciation. Columbia University Press, New York.

Gulden, R. H., Shirtliffe, S. J., and Thomas, A. G. 2003. Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. Weed Science 51:904-913.

Gulden, R.H., Thomas, A. G., and Shirtliffe, S. J. 2004. Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. Weed Science 52:382-388.

Hall, L. M., Rahman, M. H., Gulden, R. H., and Thomas, A. G. 2005. Volunteer oilseed rape: will herbicide-resistance traits assist ferality? Pp. 5-79 *in* J. Gressel (ed.) Crop Ferality and Volunteerism. Taylor and Francis Books, Boca Raton, Florida.

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

Harrison, L.A., Bailey, M. R., Naylor, M. W., Ream, J. E., Hammond, B. G., Nida,D. L., Burnette, B. L., Nickson, T. E., Mitsky, T.A., Taylor, M. L., Fuchs, R. L., and Padgette, S. R. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is

not toxic to acutely gavaged mice. Journal of Nutrition 126:728-740.

Haslam, E. 1993. Introduction, commentary and overview. Pp. 1-50 *in* Shikimic Acid:Metabolism and Metabolites. John Wiley & Sons, Inc., Chichester, England.

Heap, I. 2013. The International Survey of Herbicide Resistant Weeds. Online. Internet. Tuesday, March 05, 2013. Available www.weedscience.org.

Hegde, S.G., Nason, J. D., Clegg, J. M., and Ellstrand, N. C. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. Evolution 60:1187-1197.

Hellens, R., Mullineaux, P., Klee, H. 2000. Technical Focus: A guide to *Agrobacterium* binary Ti vectors. Trends in Plant Science 5:446-451.

Hemingway, J. S. 1995. Mustards - *Brassica* spp. and *Sinapis alba* (Cruciferae). Pp. 82-86 *in* I. J. Smartt, N. W. Simmonds (eds.) Evolution of Crop Plants, Longman Scientific and Technical, Harlow, U.K.

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. Plant Cell 7:907-919.

Herrmann, K.M., and Weaver. L.M. 1999. The shikimate pathway. Annual Reviews of Plant Physiology and Plant Molecular Biology 50:473-503.

Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J., Schilperoort, R.A. 1983. A binary plant vector strategy based on separation of vir and T region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303:179-180.

Ingram, J. 2000. Report on the separation distances required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. MAFF Project No. RG0123.

Kaneko, T., Nakamura, Y., Sato, S., Minamisawa, K. et al. 2002. Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium *Bradyrhizobium japonicum* USDA110. DNA Research 9: 189–197.

Kawata, M., Murakami, K., and Ishikawa, T. 2009. Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Environmental Science and Pollution Research 16:120-126.

Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. Environmental Biosafety Research 7:123-149.

Keller, I., Fluri, P, and Imdorf, A. 2005. Pollen nutrition and colony development in honey bees: part1. Bee World 86:3-10.

Kharbanda, P. D., Fitt, B. D. L., Lange, R. M., West, J. S., lamey, A. H., and Phillips, D. V.. 2001. Diseases of Rapeseed = Canola (*B. napus* L. and *Brassica rapa* L. (= *B. campestris* L.). APS (American Phytopathological Society), St. Paul, MN. www.apsnet.org/publications/commonnames/Pages/Rapeseed.aspx (Accessed 3/1/2012)

Klee, H.J., Muskopf, Y. M., and Gasser, C. S. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210:437-442.

Knispel, A. L., McLachlan, S. M., Van Acker, R. C., and Friesen. L. F. 2008. Gene flow and multiple herbicide resistance in escaped canola populations. Weed Science 56:72-80.

Koonin, E. V., Makarova, K. S., and Aravind, L. 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Annual Review of Microbiology 55:709-742.

Kurland, C. G., Canback, B., and Berg, O. G. 2003. Horizontal gene transfer: A critical review. Proceedings of the National Academy of Sciences (U.S.A.) 100:9658-9662.

Lamb, R.J. 1989. Entomology of oilseed *Brassica* Crops. Annual Review of Entomology 34:211-229.

Laufs, P., Autran, D., and Traas, J. 1999. A chromosomal paracentric inversion associated with T-DNA integration in Arabidopsis. Plant Journal 18:131-139.

Légère, A., Simard, M. J., Thomas, A. G., Pageau, D., Lajeunesse, J., Warwick, S. I., Derksen, D. A. 2001. Presence and persistence of volunteer canola in Canadian cropping systems. Pp. 143-148 *in* The BCPC Conference – Weeds 2001: Proceedings of an International Conference, November 12-15, 2001, British Crop Protection Council, Brighton, UK.

Martín-Hernández, C., Bénet, S., and Obert, L. 2008. Determination of proteins in refined and nonrefined oils. Journal of Agricultural and Food Chemistry 56:4348-4351.

Monsanto. 1998. Petition for Determination of Nonregulated Status for Roundup Ready® Canola (*Brassica napus*) Line RT73. Submitted by R. C. Dobert, Monsanto Company, St. Louis, MO. (See Table at http://www.aphis.usda.gov/biotechnology/not_reg.html)

Monsanto. 2011. Petition for the Determination of Nonregulated Status for Glyphosate-Tolerant Canola MON 88302. Submitted by M. Wideman, Monsanto Company, St. Louis, MO. (See Table at http://www.aphis.usda.gov/biotechnology/not_reg.html)

Myers, J. 2006. Outcrossing Potential for Brassica Species and Implications for Vegetable Crucifer Seed Crops of Growing Oilseed Brassicas in the Willamette Valley. Oregon State University Extension Service, Special Report 1064.

Nacry, P., Camilleri, C., Courtial, B., Caboche, M., and Bouchez, D. 1998. Major chromosomal rearrangements induced by T-DNA transformation in Arabidopsis. Genetics 149:641-650.

Nagaharu, U. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Japanese Journal of Botany 7:389-452.

NDSU (North Dakota State University). 2007. Tame mustard production. A-935, North Dakota State University Extension Service, Fargo, North Dakota.

NDSU (North Dakota State University). 2012. North Dakota Weed Control Guide. Publication W-253. Compiled by R. Zollinger, with contributions by M. Christoffers, G. Endres, G Gramig, K. Howatt, B. Jenks, R. Lym, J. Stachler, A. Thostenson, and H. Valenti. NDSU Extension Service and Agricultural Experiment Station. January 2012. http://www.ag.ndsu.edu/weeds/weed-control-guides/nd-weed-control-guide-1 (Accessed on 3/18/2012)

OECD (Organization for Economic Co-operation and Development). 1997. Consensus Document on the Biology of Brassica napus L. (Oilseed Rape). Organisation for Economic Cooperation and Development, Paris. http://www.oecd.org/dataoecd/28/22/27531440.pdf (Accessed on 11/18/2011)

OECD (Organization for Economic Co-operation and Development). 2001. Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola). ENV/JM/MONO(2001)13. Organisation for Economic Cooperation and Development, Paris. http://www.oecd.org/dataoecd/15/59/46815125.pdf (Accessed on 10/15/2011)

OECD (Organization for Economic Co-operation and Development). 2012. Consensus Document on the Biology of the *Brassica* Crops (*Brassica* spp.). Organisation for Economic Cooperation and Development, Paris. http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)41&doclanguage=en (Accessed on 1/10/2013)

OGTR (Office of the Gene Technology Regulator). 2011. The Biology *Brassica napus* L. (canola), Version 2.1. Office of the Gene Technology Regulator, Department of Health and Ageing, Canberra, Australia. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/canola-3/$FILE/biologycanola08_2.pdf (Accessed on 12/10/2011)

Padgette, S.R., Re, D. B., Barry, G. F., Eichholtz, D. E., Delannay, X., Fuchs, R. L., Kishore, G. M., and R.T. Fraley, R. T. 1996. New weed control opportunities: Development of soybeans with a Roundup Ready™ gene. Pp 53-84 *in* S.O. Duke (ed.) Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. CRC Press, Inc., Boca Raton, Florida.

Perrins, J., Williamson, M., and Fitter, A. 1992. A survey of differing views of weed classification: implications for regulation of introductions. Biological Conservation 60:47-56.

Pessel, F. D., Lecomte, J., Emeriau, V., Krouti, M., Messean, A., and Gouyon, P. H. 2001. Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. Theoretical and Applied Genetics 102:841-846.

Peterson, C.D., Pearman, D. A., and Dines, T. D. 2002. New Atlas of the British flora. Oxford University Press, London, U.K.

Radke, S.E., Turner, J. C., and Facciotti. D. 1992. Transformation and regeneration of *Brassica rapa* using *Agrobacterium tumefaciens*. Plant Cell Reports 11:499-505.

Rainbolt, C.R., Thill, D.C., and Young, F.L. 2004. Control of volunteer herbicide-resistant wheat and canola. Weed Technology 18:711-718.

Raybould, A. F. 1999. Transgenes and agriculture - going with the flow? Trends in Plant Science 4**:**247-248.

Richins, R.D., Scholthof, H. B., and Shepherd, R. J. 1987. Sequence of figwort mosaic virus DNA (caulimovirus group). Nucleic Acids Research 15:8451-8466.

Ridley, C. E., and Ellstrand, N. C. 2009. Evolution of enhanced reproduction in the hybrid-derived invasive, California wild radish. Biological Invasions 11:2251-2264.

Rieseberg, L. H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg, L.H., and Wendel, J. F. 1993. Introgression and its consequences in plants. Pp. 70–109 i*n* R.G. Harrison (ed.) Hybrid Zones and the Evolutionary Process. Oxford University Press, Oxford, U.K.

Salisbury, P. 2002. Genetically modified canola in Australia: agronomic and environmental considerations. Australian Oilseeds Federation, Wilberforce, N.S.W.

Salomon, S., and Puchta, H. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal 17:6086-6095.

Schafer, M. G., Ross, A. A., Londo, J. P., Burdick, C. A., and Lee E.H., et al. 2011. The establishment of genetically engineered canola populations in the U.S. PLoS ONE 610: e25736.

Simard, M-J., Legere, A., Pageau, D., Lajeunesse, J., and Warwick, S. 2002. The frequency and persistence of volunteer canola (*Brassica napus*) in Québec cropping systems. Weed Technology 16:433-439.

Soltis, D.E., and Soltis, P. S. 1993. Molecular data and the dynamic nature of polyploidy. Critical Reviews in Plant Sciences 12:243-273.

Sorek, R., Zhu,Y., C. J. Creevey, Francino, M. P., Bork, P., and Rubin, E. M. 2007. Genome-wide experimental determination of barriers to horizontal gene transfer. Science 318:1449-1452.

Stace, C.A. 1987. Hybridization and the plant species. Pp. 115–127 *in* K.M. Urbanska (ed.) Differentiation Patterns in Higher Plants. Academic Press, New York.

Stalker, D.M., Thomas, C. M., and Helinski, D. R. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Molecular and General Genetics 181:8-12.

Steinrücken, H.C., and Amrhein, N. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic-acid-3-phosphate synthase. Biochemical and Biophysical Research Communications 94:1207-1212.

Stewart, A. V. 2002. A review of *Brassica* species, cross-pollination and implications for pure seed production in New Zealand. Agronomy New Zealand 32:63-82.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the Escherichia coli plasmid pBR322. Pp. 77-90, Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, New York.

Sutherland, S. 2004. What makes a weed a weed: life history traits of native and exotic plants in the U.S.A. Oecologia 141:24-39.

Thomas, D. L., Breve, M. A., and Raymer, P. L. 1991. Influence of timing and method of harvest on rapeseed yield. Journal of Production Agriculture 4:266-272.

Trick, M., Long, Y., Meng, J., and Bancroft, I. 2009. Single nucleotide polymorphism (SNP) discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing. Plant Biotechnology Journal 7:334-346.

USDA-NASS (USDA National Agricultural Statistics Service). 2011. Crop production: 2010 summary January 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda01.library.cornell.edu/usda/nass/CropProdSu//2010s/2010/CropProdSu-01-12-2010.pdf (Accessed on 12/15/2011)

USDA-NRCS (USDA Natural Resources Conservation Service). 2012. Invasive and Noxious Weeds. http://plants.usda.gov/java/noxiousDriver (Accessed on 12/10/2011)

Warwick, S. I., Beckie, H. J., and Hall, L. M. 2009. Gene Flow, invasiveness, and ecological impact of genetically modified crops. Annals of the New York Academy of Sciences 1168:72-99.

Warwick, S.I., and Black, L. D. 1993. Guide to the Wild Germplasm of Brassica and Allied Crops. Part III. Inrerspecific and Intergeneric Hybridization in the Tribe Brassiceae (Cruciferae). Technical Bulletin 1993-16E. Centre for Land and Biological Resources Research, Agriculture and Agri-Food Canada, Ottawa, Ontario.

Weiss, M. J., Knodel, J. J., and Olson, D. 2009. *in:* E. B. Radcliffe,W. D. Hutchison & R. E. Cancelado (eds.) Insect pests of canola, , Radcliffe's IPM World Textbook, University of Minnesota, St. Paul, Minnesota. http://ipmworld.umn.edu/chapters/Weiss_et_al_canola.htm (Accessed on 2/10/12)

Williams, I. H. 1984. The concentrations of air-borne rape pollen over a crop of oil-seed rape (*Brassica napus* L.). The Journal of Agricultural Science 103:353-357.

Williams, I., Martin, A., and White, R. 1987. The effect of insect pollination on plant development and seed production in winter oil-seed rape (*Brassica napus* L.). Journal of Agricultural Science 109:135–139.

Woese, C. R. 2002. On the evolution of cells. Proceedings of the National Academy of Sciences (U.S.A.) 99:8742-8749.

Wood, D. W., Setubal, J. C., Kaul, K., Monks, D. E. et al. 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science 294:2317-2323.

WSSA (Weed Science Society of America). 1998. Technology Notes. Weed Technology 12: 789-790.

Yoshida, S., Maruyama, S., Nozaki, H., and Shirasu, K. 2010. Horizontal gene transfer by the parasitic plant *Striga hermonthica.* Science 328:1128.

Zambryski, P., Depicker, A., Kruger, K., and Goodman, H. M. 1982. Tumor induction by *Agrobacterium tumefaciens*: Analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1:361-370.