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Biology Document **BIO1996-10**

The Biology of *Glycine max* (L.) Merr. (Soybean)

A companion document to Directive 94-08 (Dir94-08), "*Assessment Criteria for Determining Environmental Safety of Plant with Novel Traits*"

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Part A - General Information

A1. Background

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

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

Before PNT's may be authorized for unconfined release, they must be assessed for environmental safety. Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*", has been developed to define criteria and information requirements that must be considered in the environmental assessment of PNT's to ensure environmental safety, in the absence of confinement conditions.

A2. Scope

The present document is a companion document to Dir94-08. It is intended to provide background information on the biology of *Glycine max* (L.) Merrill, its centres of origin, its related species and the potential for gene introgression from *G. max* into relatives, and details of the life forms with which it may interact.

Such species-specific information will serve as a guide for addressing some information requirements of Part D of Dir94-08. Specifically, it will be used to determine whether there are significantly different/altered interactions with other life forms, resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

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

Part B - The Biology of *G. max*

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

Cultivated soybean, *G. max* (L.) Merr., is a diploidized tetraploid (2n=40), in the family *Leguminosae*, the subfamily *Papilionoideae*, the tribe *Phaseoleae*, the genus *Glycine* Willd. and the subgenus *Soja* (Moench). It is an erect, bushy herbaceous annual that can reach a height of 1.5 metres. Three types of growth habit can be found amongst soybean cultivars: determinate, semi-determinate and indeterminate (Bernard and Weiss, 1973). Determinate growth is characterized by the cessation of vegetative activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes. Determinate genotypes are primarily grown in the southern USA (Maturity Groups V to X, see B2.0). Indeterminate genotypes continue vegetative activity throughout the flowering period and are grown primarily in central and northern regions of North America (Maturity Groups 000 to IV). Semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period. None of the soybean varieties are frost tolerant, and they do not survive Canadian winter conditions.

The primary leaves are unifoliate, opposite and ovate, the secondary leaves are trifoliolate and alternate, and compound leaves with four or more leaflets are occasionally present. The nodulated root system consists of a taproot from which emerges a lateral root system. The plants of most cultivars are covered with fine trichomes, but glabrous types also exist. The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals (one banner, two wings and two keels), one pistil and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma. The pod is straight or slightly curved, varies in length from two to seven centimetres, and consists of two halves of a single carpel which are joined by a dorsal and ventral suture. The shape of the seed, usually oval, can vary amongst cultivars from almost spherical to elongate and flattened.

Soybean is grown primarily for the production of seed, has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use. In Canada, the main food use is as purified oil, utilized in margarines, shortenings and cooking and salad oils. It is also used in various food products, including tofu, soya sauce, simulated milk and meat products. Soybean meal is used as a supplement in feed rations for livestock. Industrial use of soybeans ranges from the production of yeasts and antibodies to the manufacture of soaps and disinfectants.

The major world producers of soybeans are the USA, China, North and South Korea, Argentina and Brazil. Ontario is the major producer of soybean in Canada, accounting for 90% of the total production in 1995. From 1945 to 1995, production increased from 18,000 to approximately 820,000 hectares in Canada (Canadian Grains Council, 1995).

Soybean is commonly considered one of the oldest cultivated crops, native to North and Central China (Hymowitz, 1970). The first recording of soybeans was in a series of books known as Pen Ts'ao Kong Mu written by the emperor Sheng Nung in the year 2838 B.C., in which the various plants of China are described. Historical and geographical evidence suggests that soybeans were first domesticated in the eastern half of China between the 17th and 11th century B.C. (Hymowitz,1970). Soybeans were first introduced into the United States in 1765 (Hymowitz and Harlan,1983), and into Canada in 1893, where production began in Ontario as a hay crop.

B2. Brief Outlook of Agronomic Practices for Soybean

Soybean is a quantitative short day plant and hence flowers more quickly under short days (Garner and Allard, 1920). As a result, photoperiodism and temperature response are important in determining areas of cultivar adaptation. Soybean cultivars are identified based on bands of adaptation that run east-west, determined by latitude and day length. There are thirteen maturity groups (MG), from MG 000 in the north (45˚ latitude) to MG X near the equator. Within each maturity group, cultivars are described as early, medium or late maturing.

The seed will germinate when the soil temperature reaches 10˚C and will emerge in a 5-7 day period under favourable conditions. In new areas of soybean production an inoculation with Bradyrhizobium japonicum is necessary, for optimum efficiency of the nodulated root system. Soybeans do not yield well on acid soils and the addition of limestone may be required. Soybeans are often rotated with such crops as corn, winter wheat, spring cereals, and dry beans.

B3. The Reproductive Biology of *G. max*

The stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self-fertilization and cross pollination is usually less than one percent (Caviness, 1966).

A soybean plant can produce as many as 400 pods, with two to twenty pods at a single node. Each pod contains one to five seeds. Neither the seed pod, nor the seed, have morphological characteristic that would encourage animal transportation.

There are no reports of vegetative propagation under field conditions in Canada.

B4. The Centres of Origin of the Species

G. max belongs to the subgenus *Soja*, which also contains *G. soja* and *G. gracilis*. *G. soja*, a wild species of soybean, grows in fields, hedgerows, roadsides and riverbanks in many Asian countries. Cytological, morphological and molecular evidence suggest that *G. soja* is the ancestor of *G. max*. *G. gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *G. gracilis* may be an intermediate in the speciation of *G. max* from *G. soja* (Fekuda, 1933) or a hybrid between *G. soja* and *G. max* (Hymowitz, 1970).

B5. Cultivated *G. max* as a Volunteer Weed

Soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation. If this should occur, volunteers do not compete well with the succeeding crop, and can easily be controlled mechanically or chemically. The soybean plant has no weedy tendencies and is non-invasive in natural habitats in Canada. It does not grow in unmanaged habitats.

B6. Summary of Ecology of *G. max*

G. max is not found outside of cultivation in Canada. In managed ecosystems, soybean does not effectively compete with cultivated plants or primary colonizers.

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

Part C - The Close Relatives of *G. max*

C1. Inter-Species/Genus Hybridization

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

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

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

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

The subgenus *Soja*, to which *G. max* belongs, also includes *G. soja* Sieb. and Zucc. (2n=40) and *G. gracilis* Skvortz. (2n=40), wild and semi-wild annual soybean relatives from Asia. *G. soja* (2n=40) is a wild viny annual with small and narrow trifoliate leaves, purple flowers and small round brown-black seeds. It grows wild in Korea, Taiwan, Japan, Yangtze Valley, N.E. China and areas around the border of the former USSR. *G. gracilis*, an intermediate in form between *G. soja* and *G. max*, has been observed in Northeast China (Skvortzow, 1927). Interspecific, fertile hybrids between *G. max* and *G. soja* (Sieb and Zucc.) (Ahmad et al.,1977; Hadley and Hymowitz, 1973; Broich, 1978), and between *G. max* and *G. gracilis* (Karasawa, 1952) have been easily obtained.

In addition to the subgenus *Soja*, the genus *Glycine* contains the subgenus *Glycine*. The subgenus *Glycine* consists of twelve wild perennial species, including *G. clandestina* Wendl., *G. falcata* Benth, *G. latifolia* Benth., *G. latrobeana* Meissn. Benth., *G. canescens* F.J. Herm., *G. tabacina* Labill. Benth., and *G. tomentella* Hayata. These species are indigenous to Australia, South Pacific Islands, China, Papua New Guinea, Philippines, and Taiwan (Hymowitz and Newell, 1981; Hermann, 1962; Newell and Hymowitz, 1978; Grant, 1984; Tindale, 1984,1986). Hybrids between diploid perennial *Glycine* species show normal meiosis and are fertile.

Early attempts to hybridize annual (subgenus *Soja*) and perennial (subgenus *Glycine*) species were unsuccessful. Although pod development was initiated, these eventually aborted and abscised (Palmer, 1965; Hood and Allen, 1980; Ladizinsky et al., 1979). Intersubgeneric hybrids were later obtained in vitro through embryo rescue, between *G. max* and *G. clandestina* Wendl; *G. max* and *G. tomentella* Hayata (Singh and Hymowitz, 1985; Singh et al., 1987); and *G. max* and *G. canescens*, using transplanted endosperm as a nurse layer (Broué *et al.*, 1982). In all cases, the progeny of such intersubgeneric hybrids was sterile and obtained with great difficulty.

C2. Potential for Introgression of Genes from *G. max* into Relatives

Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR, and do not exist naturally in Canada. These species are not naturalized in North America, and although they could occasionally be grown in research plots, there are no reports of their escape from such plots to unmanaged habitats. There is therefore no potential for gene flow from cultivated soybean plants to wild soybean relatives in Canada.

Part D - Potential Interactions of *G. max* with Other Life Forms During its Life Cycle

Table 1 is intended to guide applicants in their considerations of potential impacts the release of the PNT in question may have on non-target organisms, but should **not be considered as exhaustive**. Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may also need to be considered.

Table 1. Examples of potential interactions of *G. max* with other life forms during its life cycle.

Other life forms **(Common Name)**	**Interaction with *G. max*** **(Pathogen; Symbiont or Beneficial Organism; Consumer; Gene Transfer)**
Septoria glycines (Brown spot)	Pathogen
Peronospora trifoliorum var. *manshurica* (Downy mildew)	Pathogen
Phialophora gregata or *Acremonium strictum* (Brown stem rot)	Pathogen
Phytophthora megasperma (Phytophthora root and stalk rot)	Pathogen
Diaporthe phaseolorum var. *caulivora* (Stem canker)	Pathogen
Rhizoctonia solani (Rhizoctonia stem and root rot)	Pathogen
Pythium spp. (Pythium root rot)	Pathogen
Fusarium spp. (Fusarium wilt, blight, and root rot)	Pathogen
Sclerotinia sclerotiorum (Sclerotinia stem rot)	Pathogen
Diaporthe phaseolorum var. *sojae* (Pod and stem blight)	Pathogen
Pseudomonas syringae (Bacterial Blight)	Pathogen
Soybean mosaic virus (SMV)	Pathogen
Colletotrichum truncatum (Anthracnose)	Pathogen
Cercospora kikuchii (Purple seed stain)	Pathogen

Microsphaera diffusa (Powdery mildew)	Pathogen
Meloidogyne spp. (Root knot)	Pathogen
Acari: Terranychidae (Spider mite)	Consumer
Heterodera glycines (Soybean cyst nematode)	Consumer
Lepidopterans (Soybean looper, white fly)	Consumer
Soil insects	Consumer
Birds	Consumer
Animal browsers	Consumer
Pollinators	Symbiont or Beneficial Organism; Consumer
Mychorrhizal fungi	Symbiont or Beneficial Organism
Soil microbes	Symbiont or Beneficial Organism
Earthworms	Symbiont or Beneficial Organism
Other *G. max*	Gene Transfer

Part E - Acknowledgments

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