The Biology of *Helianthus annuus* L.

Biology Document BIO2005-01: A companion document to the 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

The Canadian Food Inspection Agency's (CFIA) Plant Biosafety Office (PBO) is responsible for regulating the intentional introduction of plants with novel traits (PNTs) into the Canadian environment.

PNTs are plants containing traits not present in plants of the same species already existing as stable, cultivated populations in Canada, or are expressed outside the normal statistical range of similar existing traits in the plant species.

PNTs that are subject to an environmental safety assessment are those plants that are potentially not substantially equivalent, in terms of their specific use and safety for the environment and for human and animal health, to plants currently cultivated in Canada, with regard to weediness/invasiveness, gene flow, plant pest properties, impacts on other organisms and impact on biodiversity.

Consistent with the Canadian approach, the CFIA recognizes that it is the presence of a novel trait in a plant that potentially poses environmental risk, and hence is subject to regulatory oversight, as opposed to how the traits are specifically introduced, e.g., introduction of novel traits by traditional breeding, mutagenesis, recombinant DNA techniques, etc.

Before PNTs may be authorized for unconfined environmental release, they must be assessed for their environmental safety. The CFIA's Directive 94-08 (Dir94-08), entitled "Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits", describes the criteria and information requirements that must be considered in the environmental assessment of PNTs to ensure environmental safety in the absence of confined conditions.

The assessment criteria are designed to be used in conjunction with species-specific biology documents that describe the biology of the species to which the modified plant belongs, including details of other life forms with which it interacts. The assessment is part of the continuum of research, development, evaluation and potential commercialization of PNTs.

A2. Scope

The present document is a companion document to Dir94-08. It is intended to provide background information on the biology of *Helianthus annuus* L., its centre of origin, its related species, the potential for gene introgression from *H. annuus* 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 of the information requirements of Appendix 4 of Dir94-08. Specifically, it will be used to determine if there are significantly different/altered interactions with other life forms resulting from the PNTs 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 *H. annuus* only relate to plants of this species with no novel traits.

Part B - The Biology of *H. annuus* L.

B1. General Description, Cultivation and Use as a Crop Plant

Helianthus is a genus in the tribe Heliantheae of the Compositae family. The genus consists of annual and perennial species. The cultivated species *H. annuus* known also as sunflower has close wild species relatives.

The cultivated *H. annuus* as described by Heiser, 1978 and Seiler, 1997 for the most part are tall, but varieties have been developed that range from 50 to 500 cm. The stems are typically unbranched and along with most other parts of the plant vary from glabrous to very densely pubescent. Stem length is determined by the number of internodes. The first leaves are always opposite but in some varieties become alternate. The leaves are usually petiolate and three nerved, vary in shape from linear to ovate and are usually entire or serrated. The color intensity could vary from light to dark green. The heads are radiate and the ray flowers are neutral or pistillate. They are usually large and yellow but the color can range from lemon-yellow, orange to reddish.

The inflorescence is a capitulum or head, characteristic of the Compositae family. It consists of 300 to 1000 flowers but could be higher in non-oil cultivars. The outer whorl of disk flowers open first, at about the time that ray flowers spread out from their folded position against the buds of disk flowers. Successive whorls of one to four rows of disk flowers open daily for 5 or more days.

The attitude of the head is variable. The head shape varies, being concave, convex or flat.

The achene, or fruit of the sunflower consists of a seed, often called the kernel, and adhering pericarp, usually called the hull. In the absence of fertilization, the achenes will be empty, with no kernel. Achenes vary from 7 to 25 mm in length and 4 to 13 mm in width. They may be linear, oval or almost round.

Early cultivation of sunflower in North America was mainly for silage and to some extent as scratch feed for poultry (Dedio *et al.*, 1980). It was not until the crop was re-introduced from Russia that it received attention as a possible oilseed crop (Putt, 1978). Because of its long season, its centre of production in Canada is in southern Manitoba, extending to northern States, with small amounts grown in Saskatchewan and Alberta. Its fair drought resistance and susceptibility to disease - particularly sclerotinia - makes it suitable for production in drier areas of the country.

The sunflower is cultivated primarily for its seeds, which yield one of the worlds most important sources of edible oil. Sunflower oil is considered a premium oil because of its light colour, high level of unsaturated fatty acids, lack of linolenic acid, lack of trans fat, bland flavour, high oxidative stability and high smoke points. The oil is used for cooking, margarine, salad dressings, baby formula, lubrication, bio-fuel, hydraulic fluids, soaps, illumination and certain types of paints, varnishes and plastics. The meal left after the oil has been extracted is a valuable animal feed with 50-60% protein.

Lately, traditional sunflower acreage in Canada has shifted somewhat to non-oil sunflower varieties. Their large achenes are lower in oil and higher in protein than those of the smaller oilseed type. These seeds are used for human consumption either raw, roasted, salted, made into flour or as dehulled kernels in bread baking. They are also used as birdfeed and as a high protein meal for livestock. The flowers are used as a yellow dye, and the plant itself can be used for fodder, silage and as a green-manure crop.

Some sunflowers are grown as ornamentals, and varieties have been developed with exotic colors.

B2. The Centres of Origin of the Species

H. annuus L. is a native of North America. Its wild relatives and other *Helianthus* species are distributed widely across the Central Plains of Canada from north to south. Heiser, 1954 reports 67 species, but there is much doubt whether all are distinct species as hybridization has been found to occur. Archeological evidence seems to indicate the crop was domesticated in the central part of USA. There are both annual and perennial species, with polyploidy occurring in the latter group.

B3. The Reproductive Biology of *H. annuus*

The sunflower is an annual crop that is propagated by seed only and can hybridize spontaneously with several wild/weedy relatives (Burke *et al.*, 2002). Until the 1960's the cultivars grown were open-pollinated and cross-pollinated mostly by insects. They, along with the wild species, were highly self-incompatible. Current commercial sunflower varieties are self-compatible, however environmental conditions can influence the level of self-fertility expressed (Snow *et al.*, 1998).

Pollen transfer is via insect pollinators, principally bees. The pollen is spiney and adapted to be transported by insects. Little is pollinated by wind, as the pollen is rather heavy (Fick, 1978). It may be viable for several days. Although the anthers containing the pollen and the stigma are on the same floret, the two lobes of the stigma are initially not exposed to their own pollen. However, they are susceptible to pollination from other florets of the same head by insects, wind or gravity.

B4. Breeding, Seed Production and Agronomic Practices for Sunflower

Early breeding was by mass selection, which involved selecting heads for some specific trait. Because sunflower is a highly cross-pollinating crop, there was no control of pollination. In this way, varieties for characters such as disease resistance, oil content and seed characteristics were developed. Later on, Pustovoit (1964) in USSR in the 1920's developed a much more successful technique called the method of reserves. It involves testing of seed from individual heads for various characters in an evaluation nursery for 2 years, followed by controlled pollination of selected heads. In this way, a dramatic increase in oil content was achieved along with improvement in yield.

Although inbreeding as a method for improving sunflower was used as early as 1922 (Cardon), the first hybrids from this method such as Advance and Advent were commercially grown in Canada in the 1940's and 1950's. The hybrids were produced by using a highly self-incompatible female and crossing it with a highly self-compatible male line. The resulting hybrid seed provided a considerable yield advantage, but in commercial practice, the proportion of actual hybrids was rather low. When the high oil varieties from the USSR were made available in Canada these hybrids were abandoned.

It was the discovery of cytoplasmic male sterility by Leclercq (1969) in France, followed by identification of fertility restorer genes that heterosis could be fully exploited. Hybrid seed that are 100% hybrid was now possible and by the late 1960's, the switch was rapid to these hybrids when breeders incorporated the system in their breeding programs.

The field standards for the production of certified hybrid and open-pollinated seed requires that there be 0.8 km isolation from other varieties, non-certified crops of the same variety, volunteer sunflowers or wild sunflowers. Many states require that for hybrid seed production, a minimum of 1.6 km isolation. Seed producers in the Sacramento Valley of California have set even greater isolation distances.

Sunflower is normally seeded in May, as it requires a long season to mature (Sunflower Production Guide). It has good frost tolerance up to the four-leaf stage. Row crop seeder is usually used to seed at populations of about 40,000 plants per hectare, but could be considerably higher for oil-type dwarf cultivars, at rows 76-96 cm apart. It is essential that the seed is placed deep enough, up to 10 cm if needed to reach sufficient moisture levels, although a shallower seeding where moisture is sufficient results in more rapid germination (Dedio *et al.*, 1980).

The sunflower is considered to be somewhat of a drought tolerant plant and will grow in a variety of soil types from sands to clays, and a wide range of soil pH's from 5.7 to over 8. Sunflowers do however possess a low salt tolerance and require well drained soil. Fertility nutrients required by sunflower are nitrogen, phosphorus and occasionally potassium. Because of its deep roots, the crop can utilize the nutrients that have been leached into the deeper zones from previous applications. As sunflower is sensitive to fertilizer, the latter should be side banded 2.5 cm to the side and 2.5 cm below the seed.

Weed control is essential in the early stages of growth, as the crop does not compete well. This is usually done with herbicides or inter-row cultivation. Later on when the canopy becomes heavy, competition from weeds is considerably reduced.

A four-year rotation is recommended for sunflower mostly because of its high susceptibility to sclerotinia. In the intervening years, other broad leaf crops such as beans, peas, and canola should be avoided as they act hosts for the disease. The crop is also susceptible to other disease (e.g. verticillium, rust and downey mildew) but most varieties now grown have at least partial resistance. In case of downey mildew, it can be controlled chemically by seed coating with a fungicide.

Sunflower is considered mature when backs of heads are yellow and the bracts are turning brown. It usually takes 2 or more weeks before the moisture is low enough for the crop to be harvested. Frost or chemical desiccation speeds up the drying. Sunflower threshes easily and harvesting is done in October when the moisture content is below 12%.

Various insects attack the crop. At emergence, cutworms and wireworms could reduce the stand substantially in spots. The sunflower beetle can cause extensive defoliation shortly after emergence and later on at bud stage. Several seed-eating insects have been a big problem for growers. The banded sunflower moth is almost a perennial problem as it winters in the Canadian prairies, but so far no chemical control has been recommended. More recently the sunflower midge and the seed weevil has been a problem for non-oil sunflower producers in the Red River valley.

B5. Cultivated *H. annuus* as a Volunteer Weed

As with other cultivated crops that are harvested at the field scale, some seed may escape harvest, mostly from shattering, particularly when infected with sclerotinia. The seed remains in the soil until the following season when it germinates either before or following the seeding of succeeding crop. In some instances, the volunteers may give considerable competition to the seeded crop and warrants chemical or mechanical control. Because of its short dormancy period most of volunteers are destroyed the following year but some that are buried will germinate two years later. Besides reducing yield of the infested crop, volunteers make crop sequence not as effective in controlling diseases and insects.

Part C - Related species of *H. annuus* L.

C1. Inter-species/Genus Hybridization

Important in considering the potential environmental impact following the unconfined release of genetically modified *H. annuus* is an understanding of the possible development of hybrids through interspecific and intergeneric crosses between the crop and related species and resulting in:

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

Because the cultivated *H. annuus* was originally a cross pollinating crop and rather self-incompatible, it crosses readily with other *Helianthus* species. The genus has about 67 species (Heiser, 1978), which includes the annuals and the polyploid perennials, but it is the annual species that the cultivated sunflower crosses with. These include *H. argophyllus*, *H. bolanderi*, *H. dibilis*, *H. neglectus*, *H. paradox* and *H. praecox*.

The cultivated and wild *H. annuus* have many opportunities for hybridization as they often grow in close proximity in many locations. These species overlap in flowering time and are visited by the same pollinators, namely honeybees, bumblebees and solitary bees (Arias and Rieseberg, 1994). The wild *H. annuus* is a very common roadside weed in the southern parts of the prairies particularly in Manitoba extending into central United States. A subspecies, *H. annuus* subspecies annuus, which grows in waste places, is most closely related to the cultivated sunflower. *H. petiolaris*, another annual species that occurs in pockets in Canada, has been known to hybridize with wild *H. annuus*.

Several perennial species occur in Canada. The most conspicuous is the *H. maximiliani* which flowers on the roadside in late summer and fall. Some *H. giganteus* occurs in pockets and the *H. tuberosus* (Jerusalem artichoke) is found primarily on riverbanks. This species has been cultivated to a small extent for its tubers. Artificial methods are required to cross *H. annuus* with these species.

Hybridization with perennial species that are found in Canada occurs naturally very rarely. Some crosses with the cultivated sunflower have been achieved using special techniques. The following species have been reported by Whelan, 1978 to be successfully crossed with the cultivated sunflower when used as the female parent:

- *H. niveus ssp. canescens*
- *H. petiolaris*
- *H. neglectus*
- *H. debilis*
- *H. praecox*
- *H. argophyllus*
- *H. bolanderi*
- *H. paradox*
- *H. decapetalus*
- *H. hirsutus*
- *H. rigidus*
- *H. giganteus*
- *H. maximiliani*
- *H. grosseserratus*

C2. Potential for Introgression of Genetic Information from Cultivated *H. annuus* into Relatives

By far, the most likely introgression of genes from cultivated *H. annuus* would be into wild *H. annuus*. It is a very weedy plant, occurring in large numbers on roadsides and disturbed soils all across the Central Plains including the southern Canadian prairies. Once hybrids between crop and wild *H. annuus* have been formed, F1 hybrids have been found to germinate in their first year, given suitable conditions (Snow *et al.*, 1998). Even after 3 years of burial at depths of 10-20 cm, approximately 20% of the hybrids were viable. These dormancy characteristics could most likely allow seed banks to persist and become established following disturbance and favourable conditions. In addition, cultivated *H. annuus* genes are very likely to persist in wild populations of *H. annuus* (Whitton *et al.*, 1997).

Natural hybridization of wild *H. annuus* has been found to occur with other Helianthus species but mostly with annual species. Most of these species are found only in the southern US except for *H. petiolaris* which occurs in pockets in the northern plains. Thus the most likely transfer of genes into other *Helianthus* species would be through wild *H. annuus*.

In the case of a sunflower expressing a novel trait, the impact of gene movement from cultivated into wild populations must be considered. Depending on the trait, there may be fitness or non-target effects from the wild sunflower expressing the novel trait (Snow *et al*, 2003) or no significant impact on the wild *H. annus* population (Burke and Reiseberg, 2003).

C3. Summary of Ecology of Relatives of *H. annuus*

The wild *H. annuus* thrives in areas that have been disturbed by humans, such as roadsides, waste places, empty city lots and edges of fields and can become a serious weed problem. In addition, it poses a serious problem in seed production. The hybrids from outcrosses with the cultivated sunflower stand out in the field as tall, late maturing, branching plants.

The other annual species, *H. petiolaris*, occurs in patches and only occasionally becomes a weed.

Several perennial species of the Divaricati section, which are characterized by rhizomes or tubers can be found in Canada. Hybridization among the species within the section resulting in offspring with various degree of polyploidy is common and species boundary is not well defined. *H. maximiliani* is conspicuous in the fall with masses of yellow flowers along the roadside. Along with this species some *H. giganteus* L. and *H. nuttallii* T& G can be found.

H. tuberosus, which is a triploid and has large tubers, prefer the wetter areas such as banks of creeks and low lying undisturbed spots.

Part D - Potential Interactions of *H. annuus* L. with other Life Form During its Life Cycle

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

The intention is not to require comparison data between the PNT and its *H. annuus* counterparts for all interactions. Sound scientific rationale will be required to justify the decision that data would be useless or irrelevant for the remaining interactions. For example, the applicant might choose not to provide data on the potential for gene transfer from the PNT to related species if it can be clearly shown that the novel trait will not affect reproductive characteristics of *H. annuus*, either directly or indirectly.

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

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

Table 1 - Examples of Potential Interactions of *H. annuus* L. with Other Life Forms During its Life Cycle.

Other life forms (Common Name)	Interaction with *H. annuus* (Pathogen; Symbiont or Beneficial Organism; Consumer; Gene Transfer)
Plasmopara halstedii (Farl) Berl. & de Toni (Downey mildew)	Pathogen
Puccinia helianthi Schw. (Rust)	Pathogen
Sclerotinia sclerotiorum (lib.) de Bary (Sclerotinia wilt, head rot)	Pathogen
Verticillium Dahliae Klebahn *Verticillium albo-atrum* Reinke & Berth (Verticillium wilt)	Pathogen
Sclerotinica Bataticola Taub (Charcoal)	Pathogen
Alternaria zinniae Pape *Alternaria helianthi* (Hansf.) Alternaria	Pathogen
Phialophora asteris	Pathogen
Phoma oleracea *Phoma macdonaldii Boerma*	
Septoria helianthi Ell. & Keth (Septoria Blotch)	Pathogen
Botrytis cinerea (Gray-mold rot)	Pathogen
Rhizopus spp.	Pathogen
Phomopsis helianthi Munt. & Cyet (Sunflower Phomopsis)	Pathogen
Erysiphe Cichoracearum (D.L.) (Powdery mildew)	Pathogen
Pythium, *Phytophtora*, *Fusarium* spp. (Damping-off fungi)	Pathogen
Pseudomonas spp. *Erwinia carotovora* (Bacterial blight / rot)	Pathogen
Phyotplasma (Aster yellows)	Pathogen
Albugo tragopogonis (D.C.) (White rust)	Pathogen

Pollinators / Birds	Symbiont or Beneficial Organism
Homoesoma electellum (Sunflower moth)	Consumer
Phalonia hospes (Banded Sunflower moth)	Consumer
Suleima helianthana (Budworm)	Consumer
Smicronyx fulvus (Seed weevil)	Consumer
Smicronyx sordidus (Seed weevil)	Consumer
Haplorhynchites aeneus (Seed weevil)	Consumer
Contarina schulzi (Sunflower midge)	Consumer
Gymnocarena diffusa (Tephritid complex)	Consumer
Neotephritis finalis (Tephritid complex)	Consumer
Zygogramma exclamationis (Sunflower beetle)	Consumer
Cynthia cardui (Painted lady)	Consumer
Strauzia longipennis (Sunflower maggot)	Consumer
Cylindrocopturus adspersus (Stem weevil)	Consumer
Bothynus gibbosus (Carrot beetle)	Consumer
Euxoa spp. (Cutworms)	Consumer
Grasshoppers	Consumer
Plodia interpunctella (Indian meal moth)	Consumer
Birds	Consumer
Aphids	Consumer
Animal browsers	Consumer
Soil insects	Symbiont or Beneficial Organism
Soil microbes	Symbiont or Beneficial Organism
Earthworms	Symbiont or Beneficial Organism

Other *H. annuus*	Gene Transfer

Part E - Acknowledgements

W. Dedio, Former Research scientist as Sunflower breeder, Agriculture Canada, Morden, developed this document.

Part F - Bibliography

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