The Biology of *Medicago sativa* L. (Alfalfa)

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Biology Document Bio2005-02: 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

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 environmental safety. Regulatory Directive Dir94-08: *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 plants with novel traits.

A2. Scope

The present document is a companion document to Dir94-08: *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits.* It is intended to provide background information on the biology of *Medicago sativa* L., its centre of origin, its related species and the potential for gene introgression from *M. sativa* L. 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 Part D of Dir94-08. Specifically, it will be used to determine if significantly different/altered interactions occur 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 *M. sativa* L. only relate to plants of this species with no novel traits.

Part B - The Biology of *Medicago sativa* L.

B1.0 General Description

Alfalfa (*Medicago sativa* L.) is a widely grown perennial herbaceous legume (Lesins and Lesins, 1979) that is a tetraploid (2n = 4x = 32).

Valued as a forage crop due to its high feeding value and wide adaptability, alfalfa is an important rotational crop, providing soil structure, nitrogen contribution, and pest management benefits.

The general alfalfa plant morphology was considered by Teuber and Brick (1988) and Barnes and

Sheaffer (1995). The mature alfalfa plant is characterized by a strong taproot. This taproot may eventually surpass 6 or more metres in length with several to many lateral roots connected at the crown when alfalfa is grown in deep, well drained, moist soils. The crown, a complex structure near the soil surface, has perennial meristem activity, producing buds that develop into stems. Tri- or multi-foliolate leaves form alternately on the stem, and secondary and tertiary stems can develop from leaf axils. A plant in a typical forage production field has between 5 and 15 stems and can reach nearly one metre in height. Flowers vary in colour yet purple, variegated, yellow, cream and white are the most common. After pollination, these flowers will most commonly produce spiral-shaped seed pods.

Following winter dormancy or harvest, vegetative regrowth occurs either directly from crown-produced buds, or from auxiliary buds developed in the remaining stubble, respectively. Flowers, borne in clusters in a raceme and attached to the central rachis, develop in leaf axils at stem apices. Stems are indeterminate so that vegetative and reproductive growth occurs simultaneously. Flowering requires that a minimum day-length (ca. 14 h) be maintained, during which flowering will continue for several weeks, until either the plant is harvested or the stem becomes senescent.

Alfalfa is adapted to a range of climatic and soil conditions, but prefers deep loam soils that are not acidic (pH 6.5). Soils with good drainage are essential for alfalfa growth and crop persistence. When inoculated, alfalfa benefits from a symbiotic relationship with nitrogen-fixing bacteria (*Sinorhizobium meliloti*) inhabiting the plant roots. Alfalfa can also form symbiotic relationships with the common fungal arbuscular mycorhizae to facilitate the uptake of nutrients such as phosphate.

An extensive review of the alfalfa family is found in *Alfalfa and Alfalfa Improvement* (Hanson *et al.*, 1988).

B2.0. Use as a Crop

Alfalfa is recognized as the oldest plant grown solely for forage. Alfalfa is the most important forage crop species in Canada and is recognized as the most widely adapted agronomic crop. Alfalfa (pure stand plus mixtures) is the third largest crop by area in Canada with over 4.5 million hectares under production (Statistics Canada, 2002).

Alfalfa is grown in all provinces, but is of greatest significance in the three Prairie Provinces accounting for 75 percent of the total national production area of pure alfalfa or alfalfa mixtures, while Ontario and Quebec account for approximately 20 percent of the total alfalfa area under production.

Canada is the world's leading exporter of alfalfa pellets (350,000 tonnes annually) and second largest exporter of alfalfa cubes (225,000 tonnes annually) (Agriculture and Agri-Food Canada, 2003).

Canada exported 5.2 and 8.8 million kilograms of certified and other than certified alfalfa seed, respectively in 2001/02 (Canadian Seed Trade Association, 2003). Greater than 95 percent of the alfalfa seed for export produced in Canada originates in the three Prairie Provinces.

Alfalfa is highly valued for animal feed because of its high protein content, high intake potential, and digestibility. Alfalfa can provide the sole plant component in many livestock feeding programs when supplemented with the proper minerals. The majority of alfalfa produced in Canada is grown in mixture with perennial forage grasses to extend the usefulness of the crop and reduce alfalfa induced bloating in animals. Alfalfa will tolerate rotational grazing, but stands may be weakened under heavier grazing pressure. Alfalfa may be grown in pure stands for quality livestock feed high in crude protein, for on-farm storage as dried hay or haylage or for dehydration processing into meal or pellets.

Alfalfa is an important rotational crop. Alfalfa improves soil structure due to the effects of a large deeply penetrating taproot that contributes to soil aeration and organic matter content. Established alfalfa when plowed down, contributes significantly to the nitrogen requirement of following crops in the rotation. A thick forage stand containing at least 50% legume such as alfalfa, will contribute 100 kg/ha nitrogen to the nitrogen requirement of the following crop (Ontario Ministry of Agriculture, Food and Rural Development, 2003a). Alfalfa also serves as an important break crop for pests specific to other crops, especially cereal and corn crops.

Alfalfa, along with other cultivated crops such as clover and canola, is a source of pollen for foraging honey bees. Canada is the sixth largest producer of honey in the world and in 2002, 8,884 beekeepers maintained 585,683 beehives and produced 33,297 tonnes of honey of which half was exported (Agriculture and Agri-Food Canada, 2003).

B3.0 The Centres of Origin

Alfalfa, including both cultivated alfalfa and closely related subspecies, originated in Asia Minor, Transcaucasia, Turkmenistan, and Iran and is endemic throughout the Mediterranean region, North Africa, the Middle East, most of Europe, Siberia, northern India, and China (Ivanov, 1988; Michaud *et al.*, 1988; Quiros and Bauchan, 1988).

B4.0 Taxonomy and Genetics

Medicago sativa L. belongs in the order Fabales, family *Fabaceae*, tribe *Trifolieae*, genus *Medicago*. The genus *Medicago* is very extensive, consisting of more than 60 different species; two thirds of the species are annuals and one third are perennials (Quiros and Bauchan, 1988).

Commercially cultivated alfalfa properly belongs to the *M. sativa* complex, a group of closely related subspecies that are interfertile and share the same karyotype. The most commonly cultivated alfalfa in the world is *M. sativa* subsp. *sativa*, but subspecies *falcata* is also cultivated on a limited basis, primarily under rangeland conditions and in colder regions. Other subspecies in the complex include subsp. *glutinosa*, subsp. *coerulea*, subsp. x *tunetana*, subsp. x *varia*, subsp. x *polychroa*, and subsp. x *hemicycla* (Quiros and Bauchan, 1988). Two other closely related species, *M. prostrata* and *M. glomerata*, can be considered capable of limited natural hybridization with alfalfa (Quiros and Bauchan, 1988). *M. prostrata* and *M. glomerata* do not occur naturally in North America (Table I). *M. glomerata* is generally listed as one parent of subsp. x *tunetana*, which occurs in North Africa (Lesins and Lesins, 1979).

Cultivated alfalfa, *M. sativa* subsp. *sativa*, is a tetraploid ($2n = 4x = 32$), characterized by purple flowers and coiled pods (Quiros and Bauchan, 1988). Subspecies *falcata* occurs both as tetraploid and diploid ($2n = 2x = 16$) accessions and has yellow flowers and straight to sickle-shaped pods. Purple-flowered *M. sativa* ssp. *coerulea* is a diploid form of *M. sativa* ssp. *sativa*. Interploidy gene flow is possible through the production of unreduced (2n) gametes (McCoy and Bingham, 1988). All other members of the *M. sativa* complex readily cross-pollinate with cultivated alfalfa; subsp. x *varia* is actually the hybrid of subsp. *sativa* and *falcata*.

B5.0 Reproductive Biology

Alfalfa is exclusively an insect-pollinated crop that, unlike other insect-pollinated crops, is pollinated by a small number of insect species, primarily bees. Alfalfa flowers have a tripping mechanism, which is triggered by bees visiting the flower to collect nectar or pollen. Once tripped, the stigma of the flower becomes lodged into the groove of the standard petal of the flower. Tripped flowers generally cannot be fertilized again. Because of the nonreversible "tripping" mechanism within the alfalfa flower, each alfalfa bloom may be pollinated only a single time, by a single pollinating insect. Moisture, genotype and pollinator, however, can all affect the level of tripping. Flowers do not shed pollen to the wind. After pollination, alfalfa seed requires four to six weeks of adequate growing conditions to ripen. Rainfall during the ripening period will cause poor seed quality and decrease seed yield. Commercial production of the alfalfa seed crop, therefore, is largely confined to the Prairie Provinces where late season rain is unlikely.

A relatively small number of bee species can effectively pollinate alfalfa flowers. Predominant species that are important for alfalfa seed production include leafcutter bees (*Megachile rotunda*), honeybees (*Apis mellifera*) and alkali bees (*Nomia melanderi*). In alfalfa, the leafcutter bee is the preferred pollinator due to high proficiency, in situations where alfalfa seed is being produced for commercial purposes. Leafcutter bees are purposely stocked into the seed producing alfalfa fields.

Many alfalfa plants exhibit various forms of genetic self-incompatibility or self-sterility and will not successfully self-pollinate (Viands *et al.*, 1988). Alfalfa is adversely affected by inbreeding, i.e., self-fertilized plants commonly demonstrate a dramatic reduction in forage and seed yield potential (Rumbaugh *et al.*, 1988). Inbreeding depression may be due to the loss of heterosis and/or accumulation and unmasking of deleterious recessive alleles that occur as a result of self-pollination and/or pollination among close relatives.

B6.0 Breeding and Seed Production

Commercial alfalfa breeding programs are structured to avoid significant inbreeding and the resulting negative effects of inbreeding depression (Rumbaugh *et al.*, 1988). A typical alfalfa variety may have 10 to 200 parent plants that were initially crossed in isolation to form the breeder seed generation. The breeder seed of commercial alfalfa varieties is produced by the random intercrossing (open pollination) of all parent plants. An alfalfa variety is maintained through two to three seed generations beyond breeder seed *via* the open pollination of their progeny in isolation from other alfalfa varieties or pollen sources. Plant varieties bred in this way are called synthetic varieties (Rumbaugh *et al.*, 1988).

Plants within a synthetic variety are genotypically and phenotypically heterogeneous, i.e., no two individuals within the variety are exactly alike. Synthetic alfalfa varieties are closed populations that segregate, within a defined range, for most morphological traits and naturally occurring genetic markers. Because alfalfa varieties are segregating heterogeneous populations, alfalfa varieties are routinely described in terms appropriate to populations (mean or % trait expression).

Most commercial alfalfa cultivars are synthetics produced from advanced generation progenies of superior clones. A common practice used to develop an improved synthetic is to vegetatively propagate promising plants to produce clonal lines, test their polycross progenies to determine the relative combining ability of each clone, and test the clones with high general combining ability in specific combinations (Hanson and Barnes, 1978). Combining ability is a measure of the performance of progeny resulting from the cross of a clone to other potential clones in the population. Cultivars are identified from the evaluation of advanced generations of synthetics with superior performance (yield, traits).

To reduce the amount of bee-mediated gene movement between varieties during seed production, alfalfa varietal purity is achieved by maintaining adequate isolation distances between alfalfa seed production fields. In Canada, an alfalfa crop grown for the production of Certified seed, must be isolated by a minimum distance of 50 metres from a different variety or non-pedigreed crop of the same kind (Canadian Seed Growers' Association, 2003). For the production of Foundation seed grown from Breeder seed, this isolation distance is increased to 200 metres for fields exceeding 5 acres or to 300 metres for fields that are 5 acres or less.

Alfalfa forage feeding and nutritive value declines with onset of flowering, and is an important consideration in gene flow in alfalfa. Harvesting of forage alfalfa periodically removes the entire plant canopy where blooms or seed might form. Growth of the canopy must be reinitiated from vegetative crown buds (as occurs in the Spring) or from the elongation of lower stem axillary buds. Alfalfa managed for forage production is generally cut with multiple harvests within a growing year (two to three cuttings per year depending upon geographic region). The harvest interval is dependent upon weather conditions and stage of alfalfa development. For high quality dairy feed, first cutting should occur at the mid- to late-bud stage, while cutting at the early flower to 10% bloom growth stages is more appropriate for beef cow hay (Ontario Ministry of Agriculture, Food and Rural Development, 2003b). Therefore, properly managed forage alfalfa will have minimal contribution to pollen-mediated gene flow due to the limited presence of mature flowers that would be a source of pollen. Some gene flow could occur, however, during the early flowering stage or from poorly managed forage production fields.

For seed production, an alfalfa stand seeded from Breeder seed can produce Foundation seed for five years, followed by three years of Certified seed production. Alternatively, Certified seed may be harvested for eight years if Foundation seed was used to establish the alfalfa stand (Canadian Seed Growers' Association, 2003).

B7.0 Gene Flow During Commercial Seed Production

In situations where alfalfa seed is being produced for commercial purposes, bees are purposely stocked into the field.

To reduce the amount of bee-mediated gene movement between alfalfa varieties during seed production, alfalfa varietal purity is achieved by maintaining adequate isolation distances between alfalfa seed production fields. In the U.S. current official seed production isolation standards are 274 meters and 50 meters for foundation and certified seed classes, respectively.

Forage Genetics International and Monsanto have conducted studies to measure the effectiveness of current isolation standards. A brief summary of the results and conclusions drawn from these studies follows. Studies were conducted using the Roundup Ready trait as a marker to measure gene flow in moderate or small sized plots. This research examined the movement of the *cp4 epsps* gene from Roundup Ready alfalfa to conventional alfalfa pollen trap plots under seed production conditions and provides data that can be used to assess the potential for gene flow between commercial-scale seed production fields (Fitzpatrick, *et al.*, 2003). Leafcutter bees were introduced as pollinators in all of the studies to ensure pollination. Combined information from three years of research showed that the upper bound (99.9% confidence) of gene flow at 274 metres was approximately 0.3 percent and at 152 meters was approximately 1.7 percent and gene flow was not detected 610 metres or at distances 825 metres, in 2000 and 2002, respectively.

Information obtained from these studies confirms that spatial isolation remains an effective means to maintain the purity of both conventional and Roundup Ready alfalfa varieties.

B8.0 Gene Flow During Forage Production

Pollen dispersal, also called pollen flow, is one of the ways genes can move between plants. However, not all pollen dispersal results in gene flow. Gene flow is defined as the *successful* transfer of genetic material. Because pollen-mediated gene flow only can occur between individual plants of the same or sexually compatible species, gene flow from pollen occurs only when pollen (the male gamete) is deposited on the stigma of a plant, fertilizes the ovule (female gamete) of that plant, and viable seed is produced.

Alfalfa is cultivated for its animal feeding value, and the nutritive value of alfalfa decreases after flowering. Alfalfa managed for forage production is generally cut on a calendar schedule with multiple harvests within a growing year (two to eleven cuttings per year depending upon North American geographic region). The harvest interval is dependent upon weather conditions and optimally coincides with the early flower to 10% bloom growth stages. For many regions, this interval is 28-35 days in length during the growing season, an interval inadequate to initiate full bloom or ripen seed. Alfalfa requires four or more weeks of adequate temperature and photoperiod to grow and form floral buds and an additional four to six weeks to form mature viable seed on pollinated blooms. Forage harvest periodically removes the entire plant canopy where blooms or seed might form. Growth of the canopy must be reinitiated from vegetative crown buds (as occurs in the spring) or from the elongation of lower stem (stubble) axillary buds. Therefore, alfalfa managed as forage will have little contribution to pollen-mediated gene flow under production conditions because there will be few if any open flowers in the standing canopy or mature seeds in the harvested forage.

As noted above, gene flow from Roundup Ready alfalfa grown for forage to conventional alfalfa hay production fields will be predictably far less than that which would be expected to occur when alfalfa is grown for seed. However, some gene flow could occur during the early flowering stage or from mismanaged forage production fields.

B9.0 Cultivated *Medicago sativa* L. as a Volunteer Weed

As a hardy perennial plant, alfalfa can survive the winter and potentially persist in following crops as a volunteer weed. In Manitoba, where 652,000 hectares of alfalfa/alfalfa mixtures are grown (Statistics Canada, 2002), volunteer alfalfa was reported to be the 29th most abundant weed in agricultural fields in that province (Thomas *et al.* 1997) indicating this plant is present as a weed in agricultural systems but that it is of limited significance.

Mature alfalfa seed often has an impermeable seed coat that prevents the uptake of water; these seeds are referred to as "hard seeds" (Bass *et al.*, 1988). To reduce the percentage of hard seed to acceptable levels (typically 10%), commercial alfalfa seed may be mechanically scarified to slightly scratch the seed coat, allowing the seed to imbibe water; unscarified seed can lie dormant in the soil for many years (Bass *et al.*, 1988). The size of the alfalfa seed bank or the length of time seeds survive in soil is unclear.

Chemical methods are available for the control of volunteer alfalfa in fallow land, prior to seeding, and in-crop treatment in several crops. In situations where tillage is utilized, mechanical methods will further contribute to the control of volunteer alfalfa.

B10.0 Summary of Ecology

Alfalfa is a plant species that is well adapted to most environments as evidenced by its worldwide distribution. Characteristics of the plant that aid adaptation are, the perennial growth habit, ability to survive winter temperatures as low as -20°C (McKenzie *et al.*, 1988), production of hard seed and nitrogen-fixing (legume) symbiosis. These characteristics also facilitate survival outside of cultivation. A U.S. six state survey (Forage Genetics and Monsanto unpublished data) confirmed that alfalfa survives to some extent outside of cultivation. Feral populations were found in close proximity to cultivated alfalfa. Anecdotal evidence suggests that a similar situation exists in Canada. Gene flow between feral and cultivated alfalfa has been confirmed by Jenczewski *et al.* (1999). While alfalfa is a highly adaptable plant species, *M. sativa* is not listed as a noxious weed in the Weed Seeds Order (1986). In managed ecosystems, alfalfa does not effectively compete with cultivated plants, nor is it recorded as being invasive of natural ecosystems. There is no evidence in Canada that *M. sativa* has weed or pest characteristics.

Part C - Related Species of *Medicago sativa* L.

C1.0 Inter-Species/Genus Hybridization

Medicago is in the tribe Trifolieae, which also includes *Trifolium* (true clovers), *Melilotus* (sweetclover), and *Trigonella* (fenugreek). *Medicago* does not hybridize with any of these (or other) genera. Within *Medicago*, at least 56 species are recognized. Small and Jomphe (1989) described 83 species in their review, the most recent complete taxonomic study. Of these species, roughly two-thirds have an annual life cycle; the others are perennial and include cultivated alfalfa, *M. sativa*. Because annual x perennial hybrids cannot be produced artificially, and no evidence exists for their occurrence in nature, naturally hybridization to the annual species is not discussed further in this document. Although one report of a hybrid was published (Sangduen *et al.*, 1982), it did not produce seed, no further research on the hybrid was reported, and the experiment has never been repeated. *M. sativa*. is the only cultivated perennial species in the genus.

The *M. sativa* complex has been successfully hybridized via pollination with 12 other perennial species (McCoy and Bingham, 1988), summarized in Table I and by Quiros and Bauchan [1988 (see Table 3-6 in reference)]. However, many of these interspecific hybrids have only been successful by using embryo rescue culture of the hybrid or other extraordinary procedures (McCoy and Smith, 1986) making them highly unlikely to occur in nature. Putative hybrids with *M. lupulina* are not included in the list of 12 successful hybridizations nor by Quiros and Bauchan (1988), nor by McCoy and Bingham (1988) as considerable doubt exists as to whether these plants were actually of hybrid origin (Lesins and Lesins, 1979; Turkington and Cavers, 1979; Fridriksson and Bolton, 1963). In addition to these, a hybrid between *M. sativa* and *M. arborea*, an annual species, was developed through protoplast fusion (Nenz *et al.*, 1996). Thus, the realistic extent of natural gene flow from cultivated alfalfa is only to other members of the *M. sativa* species complex, and possibly to the closely related *M. prostrata* (with both 2n=2x=16 and 2n=4x=32 types) and *M. glomerata* (2n=2x=16) species. It is with only these two species that *M. sativa* appears to have the natural potential to form viable seed when gametes of the same ploidy unite, but, the interspecific hybrids have demonstrated impaired fertility and/or meiotic irregularities (summarized by McCoy and Bingham, 1988).

Table I. *Medicago* Species Hybridized to Alfalfa and their Distribution[1]

Species	Distribution	Hybridization Method	Result
M. glomerata	Southern Europe to North Africa (Quiros and Bauchan, 1988)	Hand pollination Natural	Successful Putative ancestor to subsp. x *tunetana*
M. prostrata	Eastern Austria and Italy, eastern Adriatic coast to Greece	Hand pollination	Successful, esp. when *prostrata* is female
M. cancellata	Southeastern European Russia, north of Caucasus	Hand pollination	Successful, but ploidy may interfere in crosses of certain genotypes because *cancellata* is a hexaploid.
M. rhodopea	Mountain ranges of Bulgaria	Hand pollination Ovule/embryo culture	Successful, but aberrant ploidies in progeny. Successful with normal chromosome complements.
M. rupestris	Crimean mountains	Hand pollination Ovule/embryo culture	Not successful. Successful, but F_1 plants had very low fertility and backcross progeny were only produced using ovule/embryo culture.
M. saxatilis	Crimean mountains	Hand pollination	Successful, particularly when alfalfa was maternal parent.
M. daghestanica	Mid-mountain zone of Daghestan, Russia	Hand pollination Ovule/embryo culture Hand pollination using	No seed produced. Successful. Alfalfa was hand crossed to a *daghestanica x pironae* hybrid that had been colchicine doubled to a tetraploid; resulted in hybrid seed.

		trispecies bridge	
M. pironae	Eastern Alps in northeast Italy	Ovule/embryo culture; Trispecies bridges	As for *daghestanica*, *viz*. o/e culture worked directly, but for hand pollination, a trispecies bridge was required.
M. papillosa	Pontus mountains of north-eastern Anatolia to adjacent Caucasus mountains	Hand pollination	Successful when using uneven ploidy levels.
M. dzhawakhetica	Mountains of Transcauscasia	Hand pollination	Successful when using uneven ploidy levels. F_1 were triploid and produced nonviable pollen. Backcrosses to alfalfa possible.
M. marina	Mediterranean and Black Sea shores, Atlantic coast of Iberia and France	Hand pollination Ovule/embryo culture	Unsuccessful. Weak hybrids that did not produce flowers.
M. hybrida	Corbier Mtns. and east Pyrenees	Ovule/embryo culture	Successful, no other data.
M. lupulina[23]	Europe, most of Asia, North Africa, North America	Hand pollination	Unsuccessful (Fridriksson and Bolton, 1963) or doubtful [one report of hybrid formation may have been due to misidentification of selfs (see Turkington and Cavers, 1979, Lessins and Lessins, 1979)].
M. arborea	Southern Europe from Canary Islands to Greece	Protoplast fusion only	Viable hybrids formed between these sexually incompatible species (Nenz *et al.*, 1996).
M. scutellata[2]	Mediterranean region	Hand pollination, with growth	Single plant only, no progeny produced; never replicated (Sangduen *et al.*, 1982).

		regulator applied	

1 All data is taken from Lesins and Lesins (1979) or McCoy and Bingham (1988) unless otherwise noted. Table excludes all references to natural cross-pollination among subspecies in the *M. sativa* complex (see main text for further information).

2 *M. lupulina* is an annual, biennial, or short-lived perennial species, *M. scutellata* is an annual species; all others are perennial species.

3 The report of *M. lupulina* and *M. sativa* hybridization was never able to be confirmed and is in conflict with a previous report (Fridriksson and Bolton, 1963, also see Turkington and Cavers, 1979, Lessins and Lessins, 1979). Two separate comprehensive literature reviews of McCoy and Bingham (1988) and Quiros and Bauchan (1988) do not recognize *M. lupulina* as having formed viable hybrids with *M. sativa*.

C2.0 Potential for Introgression of Genetic Information from *Medicago sativa* L. into Relatives

Alfalfa can potentially cross with other *Medicago* species as discussed in Section C1.0, but the potential for such gene flow is limited by geographic isolation and/or unequal ploidy among the species for which hybridization has been reported. *M. lupulina* (black medick) is the only *Medicago* relative of alfalfa listed as occurring in Canada (Darbyshire *et al.*, 2000) or in North America. *M. lupulina* is a widely naturalized plant in Canada existing in annual, biennial or short-lived perennial forms (Turkington and Cavers, 1979). In North American *M. lupulina* populations, a chromosome number of 2n=16 was reported for this species (Mulligan, 1957). The ability of *M. lupulina* to produce fertile hybrids with *M. sativa* L. has been summarized by Turkington and Cavers (1979). Early reports of hybridization may have utilized faulty methods and self-fertilization may have occurred.With expert consensus that *M. sativa* L. x *M. lupulina* hybrids are impossible or exceedingly unlikely to occur in nature, negligible risk exists for interspecific hybridization of *M. sativa* with any other species in North America.

In other areas of the world, particularly Europe, Asia, the Middle East, and North Africa, native populations of various members in the *Medicago sativa* complex, as well as other perennial *Medicago* species, are present (Sinskaya, 1961; Lesins and Lesins, 1979; Ivanov, 1988). Introgression of genes from *Medicago sativa* L. to wild relatives is possible in these areas of the world as expected.

C3.0 Summary of the Ecology of Relatives of *Medicago sativa* L. that Occur in Canada

As stated in Section C1.0, there are no *Medicago* species capable of interspecific hybridization with *M. sativa*. that occur in Canada. Experts in the taxonomy, genetics, breeding, cytology and cytogenetics of *M. sativa*. do not support the putative finding of interspecific hybridization between *M. sativa* and *M. lupulina*. However, for completeness, the distribution and importance of *M. lupulina* in Canada is presented.

Turkington and Cavers (1979) summarized the distribution of *M. lupulina* in Canada. This weed is prevalent throughout much of Canada's agricultural production area from British Columbia to Nova Scotia, although it is not common in Manitoba, Saskatchewan or Alberta. *M. lupulina* inhabits a variety of disturbed habitats such as wasteland, roadsides, newly seeded landscapes, abandoned pastures, gravel pits, forest margins. In managed turfgrass, *M. lupulina* is a common weed in Central and Eastern Canada, well adapted to this environment due to a prostrate growth habit.

M. lupulina is not a competitive plant and is rarely a weed of concern in managed agricultural systems. In both agricultural and turfgrass systems, *M. lupulina* is readily controlled by cutting or applications of

2,4-D herbicide.

M. lupulina is not listed as a noxious weed in Canada (Weed Seeds Order, 1986).

Part D. Potential Interaction of *Medicago sativa* L. with Other Life Forms

Table II 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 *M. sativa* L. counterpart(s) for all interactions. Depending on the novel traits, applicants might decide to submit data for only some of the interactions. Sound scientific rationale will be required to justify the decision that data would be appropriate or irrelevant for the remaining interactions. For example, the applicant might chose not to provide data on weediness potential of the PNT if it can be clearly shown that the novel trait will not affect reproductive or survival characteristics of *M. sativa* , either directly or indirectly.

Some of the life forms are listed as categories (i.e. pollinators, mycorrhizal fungi, animal browsers, birds, soil microbes, and soil insects). Should concerns be raised about interactions between a PNT and a non-target organism, applicants may be required to provide detailed information on interactions with indicator species in each category.

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

Table II. Examples of Potential Interactions of *Medicago sativa* L. with Other Life Forms During it's Life Cycle

"X" indicates the type of interaction between the listed organisms and *Medicago sativa*.
(information requirements may be waived if valid scientific rationale is provided)

Other Life Form Common Name	Other Life Form Scientific Name	Pathogen	Symbiont or Beneficial Organism	Consumer	Gene Transfer
alfalfa blotch leafminer	*Agromyza frontella*			X	
alfalfa caterpillar	*Colias eurytheme* Bdv.			X	
alfalfa leafcutting bee	*Megachile rotundata* (F.)		X	X	
alfalfa looper	*Autographa californica*			X	
alfalfa plant bug	*Adelphocoris lineolatus*			X	
alfalfa seed	*Bruchophagus roddi*			X	

chalcid					
alfalfa snout beetle	*Otiorhynchus ligustici* (L.)			X	
alfalfa webworm	*Loxostege cereralis*			X	
alfalfa weevil	*Hypera postica*			X	
alkali bee	*Nomia melanderi*		X		
animal browsers	Various			X	
anthracnose	*Collectotrichum trifolii*	X			
Aphanomyces root rot	*Aphanomyces euteiches*	X			
army cutworm	*Euxoa auxiliaris*			X	
armyworm	*Pseudaletia unipuncta*			X	
bacterial wilt	*Clavibacter michiganense*	X			
Beet webworm	*Loxostege sticticalis* (L.)			X	
birds	Various			X	
crown rot	*Fusarium spp., Rhizoctonia solani, Phoma medicaginis*	X			
deer	*Odocoileus virginianus*			X	
downy mildew	*Peronospora trifoliorum*	X			

earthworms			X		
Fusarium wilt	*Fusarium oxysporum f.sp. medicaginis*	X			
grasshopper	Various			X	
groundhog	*Marmota monax*			X	
honeybee	*Apis mellifera L.*		X	X	
leaf spot	*Cercospora medicaginis*	X			
Leptosphaerulina leaf spot	*Leptosphaerulina briosiana*	X			
Lygus bug	*Lygus hesperus*			X	
mycorrhizal fungi	Various		X		
nitrogen-fixing bacteria	Rhizobium meliloti		X		
pea aphid	*Acyrthosiphon pisum*			X	
Phytophthora root rot	*Phytophthora medicaginis*	X			
potato leafhopper	*Empoasca fabae*			X	
rabbit	*Silvilagus floridanus*			X	
snow mold (winter crown rot)	*Coprinus psychromorbidus, Microdochium nivale,*	X			

	Typhula ssp.				
soil insects	Various			X	
soil microbes	Various	X	X		
sweet clover weevil	Sitona cylindricollis			X	
Verticillium wilt	Verticillium albo-atrum	X			
Bacterial wilt	Clavibacter michiganense	X			

Acknowledgements

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