Appendix O. Colony Collapse Disorder and Glyphosate-Tolerant Alfalfa

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Colony Collapse Disorder and Glyphosate-Tolerant Alfalfa

1.0 Introduction

1.1 Overview of the Biology and Culture of Alfalfa

The biological and cultural characteristics of alfalfa are summarized by a Council for Agricultural Science and Technology Task Force (CAST) Special Publication (CAST, 2008). Briefly, alfalfa (*Medicago sativa* L.) is an herbaceous short-lived perennial forage legume (CFIA, 2005; Lesins and Lesins, 1979). Introduced to the Americas, it is the most important forage crop species in Canada and the United States and is widely adapted and grown in all 50 U.S. states. The three states with the largest amount of alfalfa hay acreage are South Dakota, Montana, and Wisconsin, according to the 2007 Census of Agriculture. Hybridization is restricted to certain species in the Genus *Medicago* and the crop relies on a variety of bee species for pollination (Van Deynze et al., 2008). Alfalfa is self-incompatible and predominately outcrossing and is not capable of natural hybridization with any other species found in North America (CFIA, 2005). Alfalfa grows optimally in fertile, well-drained soils, but it can also survive as feral plants growing outside of cultivation (Kendrick et al., 2005). In the United States, the vast majority of alfalfa is harvested as forage for use as animal feed. Forage is harvested as dry hay, haylage, and greenchop and grazed in pastures, which will be collectively referred to herein as "hay" or "forage." In 2007, approximately 23.5 million acres of alfalfa were grown in the United States (USDA-NASS, 2007). Only about 122,000 acres (0.5 percent) of the total U.S. alfalfa production acreage is harvested for seed production (USDA-NASS, 2007). Approximately 70 percent of all alfalfa seed grown in the United States is used for the establishment of domestic hay fields, with the balance sold in export markets (Van Deynze et al., 2008; Putnam, 2006). Because weeds and pests consistently reduce the yield of pure alfalfa and negatively affect forage nutritional quality, virtually all alfalfa planting seed produced in the United States is grown using insecticides and or herbicides (Peters and Linscott, 1988), which precludes its use for organic or sprouting (i.e., food) purposes.

1.2 Alfalfa Forage Production

In North America, alfalfa can be sown when there is available moisture and a sufficient frost-free growth period. Most alfalfa is predominately sown in the spring, except in the western and southern United States where fall planting is more common (Hower et al., 1999). Once alfalfa seedlings are established, the hay fields are harvested two to ten times per year depending upon location and seasonal climate. Following forage cutting, re-growth is from buds associated with the perennial root structure (the crown) or the lower stem nodes. Flower bud initiation requires long day length. Alfalfa forage production fields remain economically viable for approximately three to five years after initial planting.

Most alfalfa fields in the United States are harvested in the pre-flower (vegetative) or early bloom stages of maturity to optimize forage yield and feeding quality. Mature alfalfa stems with open

flowers or seed result in poor feed quality and significantly reduced market value (Sheaffer et al., 1988). Accordingly, most alfalfa forage producers have a strong economic disincentive to allow extensive flowering or mature seed set in hay production. Since forage production practices periodically remove the entire plant canopy where flowers, pollen, or seed might form, greater than 98 percent of alfalfa in the United States is without flowers (vegetative) or in an early stage of flower development (Van Deynze et al., 2008; Sheaffer et al, 1988; Putnam, 2006; Putnam, 2007) at any given time. Alfalfa fields in flower are thus infrequent and sporadic. Exceptions are most notable in alfalfa seed production (seed increase) fields of the Western United States and in non-irrigated, low-input forage production fields in the Plains States wherein a combination of drought and the short growing season often limit forage yield and quality potential (McCaslin, 2007).

1.3 Alfalfa Seed Production -- The Role of Bees

Alfalfa seed production is a distinct commercial practice from forage production (Hanson et al., 1988). Alfalfa seed production requires a long growing season with very warm temperatures and very low humidity during seed ripening. Alfalfa seed yield is highly influenced by grower inputs, weeds, insect pests, and seasonal weather fluctuations. Due to the limited number of skilled growers and suitable acres, U.S. alfalfa seed production occurs exclusively in niche areas of the western United States on approximately 100,000 to 120,000 acres that are of high value, intensively managed, and irrigated (USDA-NASS, 2007).

Alfalfa cultivars are developed by plant breeders using a combination of phenotypic and or genotypic recurrent selection to identify parent plants. Seeds of the cultivar (or variety) are produced commercially in spatial isolation from other cultivars (AOSCA, 2003). Cultivars are, with few exceptions, open-pollinated varieties. Alfalfa is not wind pollinated and it is very rarely self-pollinated (Teuber, 2007b; Viands et al., 1988). Cultivated (seed producing) alfalfa is exclusively bee cross-pollinated (CFIA, 2005; Olmstead and Wooten, 1987). Most professional seed producers use cultured bees and specialized equipment associated with bee culturing. The cost and availability of cultured pollinator bees is highly variable (many cultured bees are imported from western Canada). As seed is not desired on managed forage fields, pollination and bees are of no direct consequence or value to the hay crop.

Alfalfa seed production fields are usually planted in the fall and mowed in late spring so that subsequent bloom within the field is uniform, synchronous, and optimally timed for the warm dry season and optimal bee pollinator activity. Weed and in-crop volunteer plants are controlled using herbicides and cultivation prior to the start of pollination or after seed harvest. Insecticides, primarily for *Lygus spp* control, and other pesticides are applied prior to bee release to avoid insecticide damage to bees (Hower et al., 1999). Flowering begins approximately in mid June. At approximately 50 percent flower (ca. early to mid July), cultured bees (discussed below) are gradually moved with their domicile or hive for local shelter into the seed field for pollination. The bees are allowed to pollinate the plants for approximately one month, the seeds are allowed to ripen for approximately four to six more weeks, and then, chemically desiccated or swathed several days prior to combining the seed. At the end of the pollination period and several weeks prior to field desiccation, the pollinating generation of bees is either at the end of its lifecycle (leafcutter or alkali bees), or, in the case of honey bees, transported by the bee

keeper to a different location to forage on fall-flowering plant species. Post-pollination, it takes approximately four to six weeks for the alfalfa seed to ripen and become physiologically mature under optimal seed production conditions (Bass et al., 1988). High winds and or drought stress may induce flowers to mechanically auto-pollinate; however, the resulting self-pollination (inbreeding) most commonly results in no seed or few seeds with inferior vigor (Viands et al., 1988). Seed is harvested in mid August to late September, depending on geography.

As mentioned earlier, alfalfa seed production is entirely dependent upon several different bee species to "trip" flowers (discussed in more detail below) and release pollen for ovule fertilization and seed production. Teuber et al. (2005) developed an extensive literature review on honey bee pollination in alfalfa. A general recent review of principles of bee pollination may be found in *Crop Pollination by Bees* (Delaplane and Mayer, 2000). A low frequency of feral solitary bee species may visit blooming alfalfa fields to collect pollen or nectar (Hammon et al., 2006); however in most alfalfa seed-growing areas, naturally occurring populations of alfalfa-pollinating bees are either nonexistent or at levels insufficient for adequate pollination (Arrnett, 2002). Most professional seed producers use prudent, cultured bee management to control the bee species, bee hive field placement, bee stocking date(s), and bee stocking rate(s). In addition, most professional seed producers are careful to apply bee compatible insecticides in their seed fields.

Honey bees (*Apis mellifera* L.) are used primarily in the irrigated valleys of the Desert Southwest (California, Arizona, etc.) and cultured alfalfa leafcutting bees, *Megachile rotundata* (F.), are used primarily in the cooler Pacific Northwest. In certain niche geographies where suitable climate and soil beds exist (e.g., southern Washington), natural and managed colonies of the ground-nesting alkali bee, *Nomia melanderi* Cockerell, contribute significantly to commercial alfalfa pollination, and are estimated to contribute 20 percent pollination for alfalfa seed fields proximal to an alkali bee bed. Alkali bees have a demonstrated preference for alfalfa flowers (Mader et al., 2010). However, alkali bee pollination is often augmented by adding cultured leafcutter bees. Occasionally, some seed producers use a blend of two managed cultured bee species for pollination to increase the rate of seed set or shorten the pollination period. Feral honey bees and native bees including *Bombus* spp., *Osmia* spp., *Agapostomen* spp., and *Megachile* spp. can also be found visiting alfalfa flowers in varying numbers. These species may sometimes pollinate alfalfa flowers but their importance in alfalfa pollination is minor (Hammon et al., 2006; Arnett, 2002). Hammon et al. (2006) identified nine native bee species visiting alfalfa flowers in Colorado. In the northern, central and southern plains where a small portion of the U.S. alfalfa seed tonnage is produced, seed yield and grower inputs per acre are very low. In these marginal seed growing geographies, seed producers typically do not augment bee populations and typically rely on feral and native bee populations. Other non-bee insect pollinators are not effective pollinators of alfalfa; thus, some authors have estimated that about 60 percent of all pollinators in alfalfa hay and seed fields are honey bees (Morse and Calderone, 2000; Johnson, 2007).

Bee pollinator activity is required to simultaneously deposit non-self pollen onto the alfalfa flower's stigma surface, rupture the protective stigmatic cuticle, and effect pollination (Teuber, 2007b; Viands et al., 1988). This process is called "tripping" and is triggered by bees visiting the flower to collect nectar or pollen. After it is tripped, the stigma of the flower becomes lodged

into the groove of the standard petal of the flower. Because of the nonreversible tripping mechanism within the alfalfa flower, each alfalfa flower may be pollinated only a single time by a single pollinating bee. Honey bees are hit in the head by this violent tripping process, which they learn to avoid by sipping nectar slantwise from the flower (Milius, 2007). Accordingly, honey bees demonstrate a strong behavioral aversion to foraging for nectar in flowering alfalfa (Arnett, 2002; Pitts-Singer, 2007; Teuber et al., 2005). Because honey bees avoid the tripping mechanism they also are less efficient commercial alfalfa pollinators (Arnett, 2002; Cane, 2002). Indeed, Cane (2002) determined that honey bees only pollinate ("trip") about 22 percent of the alfalfa flowers they visit. In non-seed production areas, flowering weeds or other crop species are more important sources of nectar and pollen for honey bees (Arnett, 2002; Pitts-Singer, 2007; Teuber, et al. 2005). Researchers are working to improve honey bee pollination through selection of useful honey bee traits such as increased hive pollen storage, where in a typical honey bee hive only a small portion of the workers actually collect pollen instead of nectar (for honey). The hope is that bees selected for high pollen storage will also be good alfalfa pollen collectors, thus making them better pollinators (Mueller, 2008). Because of these shortcomings of the honey bee and competition from other crops for pollinating hives (e.g., almonds), alfalfa seed producers have increasingly used the alfalfa leafcutter bee for alfalfa pollination.

The leafcutter bee (*M. rotundata*) was accidentally introduced into North America from Eurasia in the 1930s and by the 1950s, entomologists recognized the bee as a superior pollinator of alfalfa (Greer, 1999; Bosch and Kemp, 2005). Currently, alfalfa leafcutting bees are the preferred bee pollinator for more than two-thirds of all alfalfa seed production acres in North America (Bosch and Kemp, 2005). In the 1970's, leafcutter bee keepers started experiencing difficulty in maintaining leafcutter bee populations. Population losses (the difference between parental adults released and live progeny recovered) of 50 percent are now common in the Pacific Northwest. Leafcutter bees are increasingly being introduced from Canada to maintain adequate pollination for seed production. Chalkbrood, a disease caused by pathogenic fungus, *Ascosphaera* spp., is thought to be a major contributing factor negatively affecting leafcutter bee populations.

All bees have a limited range over which they will search to collect pollen. The maximum foraging radius for each of the three commercially available bee species *A. mellifera, M. rotundata,* and *N. melanderi* depends heavily on the abundance (or dearth) of nectar and pollen resources. Leafcutter bees have the shortest routine foraging radius (<1/4 mile to 1 mile), followed by the honey bee, (ca. 1 to 3 miles), and the alkali bee (ca. up to 5 miles) (Hammon et al., 2006; Mader et al., 2010; Teuber et al., 2005; Pitts-Singer, 2008). Honey bees may infrequently transport alfalfa pollen and effect pollination up to 3 miles from the source (St. Amand et al., 2000; Teuber et al., 2004; Hammon et al., 2006). Accordingly, managed bee colonies are placed either in or in very close proximity to the crops they are intended to pollinate. Therefore, it is a common practice for certified and many non-certified seed producers to mitigate cross-pollination by unknown alfalfa pollen by using spatial field isolation and stocking their fields with recommended rates of cultured bees to synchronously and quickly pollinate the field and make it less attractive to feral bees. Likewise, conventional seed producers that have concerns regarding biotechnology-derived crops may use isolation and prudent bee management strategies to help mitigate cross-pollination to external alfalfa.

1.4 Hazard Assessment of Biotechnology-derived Crops and Associated Technologies on Honey Bees

Before commercialization, biotechnology-derived crops, including glyphosate-tolerant (GT) alfalfa and other GT crops, undergo extensive testing and evaluation as part of an environmental assessment to determine if there are any potential environmental effects. In particular, potential effects on non-target organisms (NTO) are considered. These potential effects are addressed via a science-based tiered assessment approach (Mendelsohn et al., 2003; Romeis et al., 2006, 2008; USDA/APHIS, 1997; U.S. EPA, 2007). A key component of that process is a problem formulation step that defines protection endpoints and management goals from which risk hypotheses are developed to evaluate risk. For biotechnology-derived crops expressing the herbicide-tolerance trait, a petition must be submitted to the United States Department of Agriculture / Animal and Plant Health Inspection Servive (USDA/APHIS) that addresses effects on NTO among other relevant factors. In most cases, the Environmental Protection Agency (EPA) must be provided with information to support an amendment to the relevant herbicide label demonstrating that application of the herbicide to the herbicide-tolerant crop will not cause unreasonable adverse effects on the environment. For biotechnology-derived crops expressing insecticidally active proteins (termed PIPs or Plant Incorporated Protectants by EPA), extensive evaluation including testing for potential effects on NTOs such as the honey bee have been conducted. The results of those analyses show that there are no negative effects of PIP-containing crops or their expressed proteins on honey bees (Mendelsohn et al., 2003; O'Callaghan et al., 2005; OECD, 2007; Rose et al., 2007; U.S. EPA Biopesticides Registration Action Documents) or bumble bees (*Bombus* spp.) (Babendreier et al., 2008). A recent meta-analysis by Duan and coworkers concluded that there was no evidence for any negative effects from use of registered PIPs on honey bees (Duan et al., 2008). It should be noted that GT alfalfa does not contain a PIP.

1.5 EPSPS Protein Specificity and Mode of Action

The EPSPS (5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC2.5.1.19)) family of enzymes is ubiquitous in plants and microorganisms and their properties have been extensively studied (Dill, 2005). EPSPS proteins catalyze the transfer of the enolpyruvol group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and shikimic acid-3-phosphate (EPSPS), the penultimate product of the shikimic acid pathway (Alibhai and Stallings, 2001; Dill, 2005). EPSPS enzymes have extreme substrate specificity and bind only PEP and glyphosate, an EPSPS inhibitor. Shikimic acid is a substrate for the biosynthesis of aromatic amino acids (Phe, Trp and Tyr) as well as many secondary metabolites, such as tetrahydrofolate, ubiquinone and vitamin K. The shikimate pathway and, hence, EPSPS proteins, are absent in mammals, fish, birds, reptiles and insects including bees (Alibhai and Stallings, 2001).

The *cp4 epsps* gene is an *epsps* homolog isolated from *Agrobacterium sp*. strain CP4, a common soil-borne bacterium, (Padgette et al.. 1996a, b). The *cp4 epsps* gene encodes the CP4 EPSPS protein. The CP4 EPSPS protein expressed in glyphosate-tolerant plants is functionally identical to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS naturally displays reduced affinity for glyphosate, the active ingredient in Roundup® agricultural herbicides

(Padgette et al., 1996a, b). In conventionally bred plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of 5-hydroxyl of shikimate-3-phosphate, thereby starving plants of essential amino acids and secondary metabolites (Steinrücken and Amrhein, 1980; Haslam, 1993). In GT plants, the production of aromatic amino acids and other metabolites that are necessary for normal growth and development are met by the continued action of the glyphosate-tolerant CP4 EPSPS enzyme (Padgette et al., 1996a, b; Cerdeira and Duke, 2006, 2007). This difference in the glyphosate binding affinity is the basis for glyphosate tolerance in crops that produce CP4 EPSPS (Franz et al., 1997).

1.6 Safety Assessment of CP4 EPSPS on Arthropods

Safety assessments of GT crops, such as GT alfalfa, have shown that insertion of the *cp4 epsps* gene and the glyphosate-tolerant CP4 EPSPS protein does not alter the levels of nutrients and anti-nutrients in glyphosate treated and untreated plants (Heck et al., 2005; Nida et al., 1996; Padgette et al., 1996a; Ridley et al., 2002; Nickson and Hammond, 2002; Cerdeira and Duke, 2006; U.S. FDA, 2008). For GT alfalfa, compositional analyses were performed for 35 nutritional components including proximates (protein, fat, ash and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, minerals, and carbohydrates (McCann et al., 2006; U.S. FDA, 2004). The values for all of the biochemical components assessed were similar to those of the conventional alfalfa grown in the trials or were within the range observed for conventional alfalfa. In addition, the levels of naturally occurring toxicants and anti-nutrients were addressed. Specifically, the levels of lignin (an anti-nutritional factor affecting digestibility), coumestrol (an estrogenically active phytoestrogen), as well as saponins and soluble forage proteins (both are associated with frothy bloat in ruminants), were all shown to fall within the ranges that occur in conventional alfalfa varieties (OECD, 2005). These data support substantial equivalence components of the risk assessment and establish that there should be no unintended effects of GT alfalfa, due to its equivalence with conventional alfalfa, on non-target organisms (Romeis et al., 2008).

Given the specificity of the EPSPS family of enzymes and their absence of demonstrated impacts in mammals, fish, birds, reptiles and insects (including bees), there is no known scientific basis to justify direct testing of this protein on specific non-target organisms such as bees (Cerdeira and Duke, 2006, 2007; Romeis et al., 2008). CP4 EPSPS containing crops, including alfalfa, canola, cotton, maize, and soybean have now been grown on many hundreds of thousands of hectares in excess of 10 years without any suggestion of negative effects on non-target organisms (Cerdeira and Duke, 2006; Dill et al., 2008). It may be noted that most of these crops serve as occasional nectar and or pollen sources for foraging honey bees. Even though the likelihood of hazard is low for the CP4 EPSPS protein, a number of researchers conducted laboratory and field investigations with different types of arthropods and other non-mammalian organisms exposed to GT crops containing the CP4 EPSPS protein, as discussed below.

Representatives of pollinators, soil organisms, beneficial arthropods and pest species were exposed to tissues from GT crops that contain the CP4 EPSPS protein[1]. These studies vary in design; some for relatively short duration and others for multiple generations (*Collembola*). Precise estimates of exposure of the test animals to the CP4 EPSPS protein in most of these test

[1] See document entitled "Food safety and environmental impact of the CP4 EPSPS protein.

systems were not assessed. Importantly, no toxicity was observed in arthropods exposed to these GT crops, reinforcing the conclusion of minimal hazard.

The risk assessment for CP4 EPSPS protein in GT crops reveals no evidence of unreasonable risk to NTOs because minimal hazard is predicted based on widespread occurrence of the EPSPS proteins and no scientifically plausible mechanism of toxicity. Although minimal ecological risk is predicted in the formal risk assessment process, several field-monitoring studies were conducted for GT crops to reinforce the conclusion and address any potential uncertainties in the risk assessment process. Plots were monitored for arthropod abundance in Roundup Ready® 2 Corn, Roundup Ready® Soybeans, Roundup Ready® Sugar Beet and Roundup Ready® Canola. Results of these studies are consistent with the predictions of the ecological risk assessment in that there is no evidence of unreasonable risk demonstrated in these studies. These results support the argument that the CP4 EPSPS expressed in GT crops such as GT alfalfa do not negatively affect non-target organisms such as honey bees.

1.7 Safety Assessment of Glyphosate on Arthropods

Glyphosate (N-(phosphonomethyl) glycine) is a non-selective systemic herbicide, first commercialized in 1974. Glyphosate herbicide is now one of the most widely used herbicides in the world (Franz et al., 1997; Giesy et al., 2000). Formulations of glyphosate have been extensively tested for a wide range of potential environmental effects and have proven safe for a wide range of organisms, including honey bees (extensively reviewed by Giesy et al., 2000 and see especially Agriculture Canada, 1991; U.S.EPA, 1993a; WHO, 1994). Regarding honey bees Giesey et al. 2000, states:

> "Honey bees are not affected by glyphosate formulations, either by ingestion or direct overspray, at maximum use rates. The majority of other beneficial arthropods are unaffected by Roundup®. Although screening tests under extreme exposure conditions indicate toxicity of glyphosate formulations to some beneficial arthropods, at the maximum use rates these effects were reduced or eliminated when more realistic exposure conditions were used. These data demonstrate minimal risk to beneficial arthropods in areas adjacent to treated fields. Within treated fields, vegetation changes resulting from herbicide use can lead to significant changes in beneficial arthropod populations."

1.8 Exposure Assessment of Glyphosate-Tolerant Alfalfa and Honey Bees

1.8.1 Exposure in Glyphosate-Tolerant Alfalfa Production

As of 2002, there were an estimated 2.4 million bee colonies in the United States, of which the majority belong to commercial migratory (managed) beekeepers (Johnson, 2007). About one-third of these colonies are in California and Florida. Another 7 percent are found in the Dakotas and 5 percent are each located in Texas and Montana. Minnesota, Idaho, Michigan, Washington, Wisconsin, Oregon, and New York, collectively account for an additional 20 percent. Commercial migratory honey bee providers may move their hives two to five times per growing season. Estimates show that the majority of rented hives are used in apple and almond production, followed by clover seed, cherry, and pear producers. Collectively, across all alfalfa

cropping practices (seed and hay) it has been estimated that about 60 percent of all alfalfa pollinators are honey bees (Johnson, 2007; Morse and Calderone, 2000) but the number of acres with pollinating bees varies widely by crop purpose (seed vs. hay) and by geography. Therefore, the 60 percent estimate is not informative for the estimation of exposure since pollinators are not typical in hay crop production (99.5 percent of the U.S. alfalfa acres) and the choice of bee pollinator species in alfalfa seed production is highly dependent on geography and seed grower management.[2] A worst-case exposure estimate can be generated based on alfalfa seed production acreage (USDA-NASS, 2007) and typical pollinator species for the geography (Van Deynze et al., 2008).

Forage Genetics International (FGI) is the sole seed-producing licensee of GT (Roundup Ready®) alfalfa (FGI, 2007, 2008). As such, FGI contractually specifies which seed growers, field sites and under what protocols the GT alfalfa seed crop can be produced (i.e., licensed for variety increase). Of the growers in the eleven (11) western U.S. states that are eligible to grow GT alfalfa for seed[3], only those in the Desert Southwest States (CA, AZ, etc.) rely predominately on commercially managed honey bees as pollinators. In other geographies where wild or commercial honey bees may be present (e.g. the Dakotas) GT alfalfa seed increase will either not be licensed[2] and/or seed producers stock non-*Apis* bee species. There are approximately 44,000 acres of alfalfa seed in the southwestern United States, of which approximately 30,000 acres (Van Deynze et al., 2008; McCaslin, 2007) is produced for export—this is a market sector that is sensitive to biotechnology-derived traits and unlikely to adopt GE varieties at this time (McCaslin, 2007; Van Deynze et al., 2008). Therefore, only the domestic market acres (ca. 14,000 acres) under honey bee pollination are projected as likely candidates for licensed seed increase for GT alfalfa varieties. For exposure calculation purposes, 14,000 acres and 44,000 acres will be used as the likely and high acreage exposure estimates, respectively, and per Mueller (1989), the maximum hive stocking rate will be three colonies per acre. Using these data, the following worst-case exposure estimates were made (table O-1). These estimates show that the exposure of managed honey bee colonies to GT alfalfa seed production fields in the United States would range from 1.75 percent (projected) to potentially 5.50 percent (worst case) of the total managed honey bee population in a given year.

[2] Data cited by Johnson, 2007, reference Morse and Calderone, 2000. Morse and Calderone, 2000, provide a table entitled: "The Value of Honey Bees as Pollinators of U.S. Crops in 2000," that shows that for both seed and alfalfa hay production about 60 percent of all alfalfa pollinators are honey bees (Johnson, 2007; Morse and Calderone, 2000). While honey bees are used in the production of alfalfa seed, they are not used for alfalfa hay production. The attr bution by the authors of 60 percent of the value of alfalfa hay to honey bees was meant to capture the indirect value honey bees contr bute to the production of alfalfa hay and not to imply that honey bees are used or required for the production of hay once the grower has purchased alfalfa seed (Calderone, pers. comm.).

[3] FGI will limit the contracting of Roundup Ready alfalfa seed production acres to 11 western states--AZ, CA, CO, ID, MT, NV, OR, TX, UT, WA and WY, under terms of an industry coexistence consensus plan sponsored by The National Alfalfa & Forage Alliance (NAFA 2008). Seed production requires that Roundup agricultural herbicide be applied to the seed field. Such supplemental labeling is only available in these 11 states plus OK and NM where contracts have/will not be issued.

Table O-1. Projected Likely and Highest ("Worst Case") Exposure Estimates for Managed Honey Bee Colonies in Glyphosate-Tolerant Alfalfa Seed Production

Honey Bee Colonies in U.S.	Alfalfa Seed Production Acres, Total U.S.	Acres Pollinated by Honey Bees in Southwest U.S.	Acres Licensed for GT Alfalfa Seed Increase & Pollinated by Honey Bees	Colonies per Acre	Colonies Pollinating GT Alfalfa Seed Acres	Percent of Colonies Pollinating GT Alfalfa Seed Acres
2,400,000	120,000	44,000	Highest potential; all acres (44,000)	3	132,000	5.50 %
2,400,000	120,000	44,000	Non-exported acres; likely potential (14,000)	3	42,000	1.75 %

1.9 Exposure in Honey Production

As stated above, although bees serve no direct agronomic (pollination) purpose in hay production fields, some alfalfa hay growers opt to allow their late-summer hay crop to flower extensively, precluding the harvest of maximum hay quality or yield. Working with honey bee keepers, these non-irrigated hay growers may allow their hay crop to flower and be used as an intentional source of honey bee forage instead of cutting for other livestock feeding purposes. In this situation, the bees are not "pollinators" used to produce the alfalfa crop *per se*; rather they are placed there as "foragers" to produce a honey crop and bee progeny (not seed).

While it is theoretically possible for exposure of honey-producing bees to GT alfalfa flowers found in fields where harvesting was delayed due to weather or on unmanaged feral plants to occur intermittently in some geographies, because of factors of scale, hay harvest routines and the seed purchase license restrictions, the extent and duration of any unintentional exposure is expected to be minor in comparison to intentional seed crop pollination use (see Van Deynze et al. 2008; Kendrick et al.; 2005). Hay growers that elect to plant GT alfalfa must sign a Technology/Stewardship Agreement that contractually obligates them to read and follow applicable sections of the Monsanto's Technology Use Guide (TUG). For GT alfalfa, the TUG includes information on hay and forage management practices to promote high forage quality (i.e., before 10 percent bloom) and to prevent seed development.

Honey producers interested in producing organic honey are likely to avoid placing hives near GT alfalfa. Unintended exposure to GT alfalfa flowers can be minimized by beekeepers if they opt to selectively place their hives near only conventional alfalfa variety fields rather than growers using GT alfalfa. The hay growers who purchase GT alfalfa will do so as a means to more efficiently produce high yielding, high quality forage alfalfa; these higher management alfalfa producers are unlikely to currently host bee hives because delayed harvest is incompatible with optimal forage harvest cycles. Such intensively managed pure-stand alfalfa is not needed by beekeepers and it is not optimal for fields used in honey production. Instead, honey producers

are more likely to work with a conventional alfalfa grower using marginal lands or lower inputs in geographies such as the upper plains states where extensive bloom is common (and geographically, growers are not eligible for GT seed production contracts). When alfalfa flowers are used as a source of nectar, the alfalfa may be grown mixed with clover. GT alfalfa varieties will not be grown in clover mixtures because clover is not glyphosate tolerant. Other flowers may also occur in the vicinity, decreasing the amount of foraging on alfalfa flowers. The majority of honey bees in a colony selectively collect nectar (not pollen) for honey production and only about 25 percent of honey bees actually forage for pollen (Arnett, 2002; Mueller, 1989). Because of the numerous biological and cultural mitigation factors, and the non-preference of alfalfa as a nectar source, exposure of honey bees outside of GT alfalfa seed producing areas is expected to be minimal.

Accurate estimates for the acreage of alfalfa devoted to honey production are not available, nor are there data for the number of pounds of alfalfa honey produced per year or the amount of honey produced per acre of alfalfa forage. Extension publications contain general references of "one-third of honey produced is from alfalfa" but verifiable numbers are not collected (Hannaway and Larson, 2004; Mulder, 2008). Honey production per acre of alfalfa is difficult to estimate since production is dependent on a number of abiotic and biotic factors including plant variety, geography, management practices and weather. However, a conservative worst-case estimate can be made by assuming that, if approximately one-third of the U.S. honey production is predominately alfalfa-based, then approximately one-third of honey bees are foraging predominately on alfalfa (table O-2). Therefore, of the 2.4 million colonies in the United States (Johnson 2007), approximately 800,000 potentially forage on alfalfa. Using a stocking rate estimate of two to three colonies per acre (Mueller, 1989), then, it may be calculated that approximately 266,667 to 400,000 full-bloom alfalfa acres are used to support 800,000 colonies. The total acreage of alfalfa grown in the United States for hay and or seed production was nearly 24 million in 2007 (USDA, 2007). Therefore, approximately 1.2 to 1.8 percent of the total U.S. alfalfa (hay and seed) acreage is potentially used to support honey bee colonies during the longer days of summer when alfalfa has sufficient photoperiod to bloom and it remains unclipped.

As discussed above, due to the prohibitions in the Monsanto TUG and the negative economic impacts of extensive flowering on forage quality and yield, optimally managed alfalfa hay fields (e.g., GT varieties) are very unlikely host fields for honey bee keepers. It is therefore likely that honey bees will only be intentionally placed on GT alfalfa when it is grown for seed increase (not forage), and then, only when the seed acres are planted in the irrigated desert valleys of California and Arizona where honey bees are the pollinator of first choice. In total, there are approximately 44,000 acres of alfalfa seed production in the Desert Southwest States (McCaslin, 2007; USDA, 2007) of which approximately 30,000 seed acres would be unlikely to plant GT alfalfa because they produce conventional seed for the export market (Van Deynze et al., 2008; McCaslin, 2007). One possible conservative exposure scenario for honey production would be to assume that all of the remaining seed production acres in these southwestern geographies (i.e., ca. 14,000 acres) are planted to GT alfalfa seed production, honey bees are used as the sole pollinator species and honey is harvested. Under this scenario, the following conservative exposure estimates can be developed (table O-2):

- 0.06 percent of the total alfalfa acres in the United States would be GT *and* intentionally used to host honey bee colonies producing honey (i.e., 14,000 of 24 million acres).
- 3.5 to 5.3 percent of the current acres used to support alfalfa honey production acres (i.e., 14,000 of the 266,667 to 400,000 acres, overall) would encounter a change from conventional alfalfa flowers as bee forage to GT alfalfa flowers and all of these acres would be under seed producer variety increase contracts and license.
- Likewise, 3.5 to 5.3 percent of all U.S. alfalfa honey production would encounter a change from conventional alfalfa flowers as bee forage to GT alfalfa flowers and, essentially all of these bee keepers (and bees) would be working in close association as a pollinating service for a contracted, licensed GT alfalfa seed producer. Relative to this figure, only minimal additional exposure would be likely from delayed harvest forage fields or dispersed feral plants.
- 1.2 to 1.8 percent of all U.S. commercial honey bee colonies would be intentionally foraged on GT alfalfa flowers for the stocking rates of three and two colonies per acre on 14,000 acres, respectively (i.e., 28,000 to 42,000 of the 2.4 million bee colonies would be intentionally foraged on the GT alfalfa, inclusive of all hay and seed uses). Over 98 percent of cultured honey bee colonies would have little or no intentional foraging exposure to GT alfalfa.
- Assuming that one-third of this honey is sourced from alfalfa and exposure is estimated to be 1.2 to 1.8 percent of all honey bee colonies annually, a worst case estimate for honey sourced from GT alfalfa is 2.2 percent of the U.S. honey production. Honey sourced from GT alfalfa can be a concern for organic honey producers, but organic beekeepers maintain specific production practices to ensure the organic nature of their products regardless of conventional or genetically modified crops.

Table O-2. Post-Commercialization Exposure Estimates for the 2.4 Million U.S. Bee Colonies

	3 colonies/acre		**2 colonies/acre**	
Alfalfa acreage supporting honey bees (seed and hay)	266,667		400,000	
Acres of conventional alfalfa	252,667	94.70%	386,000	97.50%
Acres of GT alfalfa	14,000	5.30%	14,000	3.50%
Colonies supported by alfalfa flowers (seed and hay)	800,000			
# colonies on GT alfalfa	42,000	5.30%	28,000	3.50%
# colonies kept on conventional alfalfa	758,000	94.70%	772,000	97.50%
Estimated colonies kept on GT alfalfa flowers	42,000	1.80%	28,000	1.20%
Estimated colonies kept on all other sources of flowers	2,358,000	98.20%	2,372,000	98.80%

1.10 Honey Bees and Colony Collapse Disorder

Honey bees are the major bee species used for crop pollination worldwide and in 2000 their contribution to U.S. agriculture, including alfalfa production, was valued at $14.6 billion (Greenleaf & Kremen, 2006). Currently, approximately 2.4 million managed honey bee colonies are used to pollinate crops in the United States (Johnson, 2007). This number represents a significant decrease from the estimated 4 million managed honey bee colonies present in 1981. This downward trend continues in both the United States and Europe. The National Research Council's (NRC) "Status of Pollinator's Committee" report (USDA-CCD, 2007) detailed the following problems facing the beekeeping industry including:

- Catastrophic problems with invasive parasitic mites (and resistance to pesticides meant to control them);
- Africanized honey bees which are aggressive and poor honey producers;
- The bacterial American foulbrood disease (and resistance to the antibiotic used to control it);
- The small hive beetle;
- A variety of viruses, bacteria, and fungi (including microsporidia);
- Insecticides with known toxicity to honey bees used in production agriculture; and
- Increased production costs and competition from cheap imported honey.

As listed above, honey bees face a number of abiotic and biotic stressors that can seriously disrupt or destroy a hive (Flottum, 2008a; MAAREC, 2007; USDA-CCD, 2007; Williams, 2008). Cultural practices by managed bee keepers can also potentially affect honey bee health. These include immune-suppressing stress from poor nutritional forage sources, apiary overcrowding, a lack of pollen or nectar, air pollution, drought, and migratory stress from long-distance transport of hives for pollination services (Endres, 2008; McFrederick et al., 2008; Williams, 2008). Crowding can also increase pathogen transmission. More recently Colony Collapse Disorder (CCD) has emerged (or possibly re-emerged) as a potential and poorly understood threat. Because of the importance of honey bees to U.S. agriculture, the USDA formed an ad hoc CCD Working Team comprised of academic, private, and Federal scientists to determine causes contributing to, and potential mitigation plans for, CCD (USDA-CCD, 2007).

CCD describes a phenomenon first reported in the United States in 2006 in which honey bee colonies (*A. mellifera*) apparently disappear without a trace (USDA-ARS, 2010). A similar phenomenon has been observed in Europe (Belgium, France, the Netherlands, Germany, Greece, Italy, Portugal, Spain, Switzerland and the United Kingdom), Canada, West Africa, and Taiwan (Dupont, 2007; Flottum, 2008c; Kievits, 2007; Molga, 2007; Steinberger, 2007). Large scale disappearances of honey bees are not new and are recorded as far back as 950 and 992 in Ireland, and more recently in the United States in the 1880s, the 1920s and the 1960s (Oldroyd, 2007). Isolated disappearances occurred in the Cache Valley of Utah in 1903 and in Pennsylvania in 1995-1996 (USDA-CCD, 2007). These disappearances have been variously named autumn collapse, disappearing disease, fall dwindle disease, May disease, and spring dwindle (Oldroyd, 2007). It is unknown whether these losses were related to the causal agent(s) of CCD or to some other stressor(s). Some experts argue, for example, that the case definition for CCD is ambiguous and that CCD may not be a new disorder because the symptoms for CCD are indistinguishable from those of other the normal diseases, such as winter colony collapse (Anderson and East, 2008; Oldroyd, 2007). At this time, however, most experts are classifying CCD as a unique disease (USDA-CCD, 2007) the symptoms of which include:

- Sudden loss of the colony's adult bee population with very few bees found near the dead colonies;
- Several frames with healthy, capped brood and low levels of parasitic mites which indicates that colonies were relatively strong shortly before the loss of adult bees and that the losses cannot be attributed to a recent infestation of mites;
- Food reserves that have not been robbed, despite active colonies in the same area, suggesting avoidance of the dead colony by other bees;

- Minimal evidence of wax moth or small hive beetle damage; and
- A laying queen often present with a small cluster of newly emerged attendants.

Some scientists believe that these symptoms are consistent with a disease or disease/pathogen interaction, perhaps aggravated by other sub-optimal cultural practices (CCD-USDA, 2007; Flottum, 2008a, 2008b, 2008d; Cox-Foster, 2008; Williams, 2008). Leading candidates are Nosema infections, mites and viruses.

Nosema apis and *Nosema ceranae* are microsporidian diseases of the western (*A. mellifera*) and eastern honey bee (*A. cerana*), respectively (Klee et al., 2007). In 2007, Klee and co-workers confirmed the presence of virulent *N. ceranae* in the western honey bee, first in Spain and Taiwan, and now world wide. Colonies can be simultaneously infected with both *Nosema* species. Symptoms of Nosema infection can be somewhat non-specific and thus confused with other diseases. They generally involve diarrhea and loss of female workers, which appear to be the most susceptible. Nosema infection shortens worker lifespan and can seriously weaken the colony leading to loss of hives.

Mite infestations of honey bees generally involve either the tracheal mite (*Acarapis woodi*) or the Varroa mite (*Varroa destructor*). A resistant strain of honey bee, as well as cultural controls, has been developed to control the tracheal mite. The Varroa mite is an obligate external parasite of both eastern and western honey bees (*A. cerana* and *A. mellifera*). Besides the visual presence of mites, other symptoms of mite infestation include the presence of dead bees in the hive and invasion of the hive by other pests as the hive weakens. Because mites feed on the hemolymph of bees and move between brood and adults, they are also capable of vectoring viruses such as the Deformed Wing Virus, a major pathogen affecting honey bee colonies (Lanzi et al., 2006).

A number of different viruses can infect honey bees (Chen et al., 2006). Many of the symptoms of CCD are consistent with a pathogen infection; therefore, viruses are likely candidates for the disease (Stokstad, 2007). For example, some investigators found that sanitation via irradiation of CCD affected combs allowed successful repopulation of the combs by honey bees (Williams et al., 2008). To try to identify a potential pathogen(s) as a causal agent of CCD, Cox-Foster and colleagues (2007) used an unbiased metagenomic approach to survey microflora in CCD hives, normal hives, and imported royal jelly. Their results showed a strong correlation of the incidence of CCD with the Israeli Acute Paralysis Virus (IAPV), which is either a new viral species or an unclassified virus possibly related to Kashmir bee virus (KBV) (Cox-Foster et al., 2007; Chen et al., 2007). IAPV is a single-stranded RNA virus in the family Dicistroviridae (genus *Iflavirus*) that can be vectored by the Varroa mite. IAPV is one of the top candidates as the causal agent of CCD. Although the CCD Working Group is developing new recommendations regarding data collection to identify the cause of CCD, one conclusion reached very quickly for the CCD Working Group (USDA-CCD, 2007; MAAREC, 2007) was that:

> *"Several other factors have been suggested as causal mechanisms of CCD, for example, the use of genetically modified (GMO) crops. However, large bee die-offs have also occurred in Europe, where GMO crops are not widely grown. Also, in the United States, the patterns of CCD-affected colonies do not appear to correlate with the distribution of GMO-crops such as Bt-corn. Furthermore,*

extensive laboratory and field testing has indicated a lack of acute and sub-lethal effects on bees exposed to GMO-pollen."

The CCD working group further stated that it is not currently investigating GMO crops because, *"Some GMO crops, specifically Bt Corn, have been suggested as a potential cause of CCD. While this possibility has not been ruled out, CCD symptoms do not fit what would be expected in Bt affected organisms. For this reason GMO crops are not a "top" priority at the moment."* (MAAREC, 2007)

2.0 Conclusion

CCD is a serious threat to honey bee populations worldwide. Although the exact cause(s) of CCD is unknown, diseases and colony stress have been proposed as possible candidates. The temporal and geographic distribution of CCD, as well as the pathology associated with the disorder, are factors cited by the CCD working group. These factors limit the likelihood that any glyphosate-tolerant crop, including GT alfalfa, might be involved in CCD. A causal link between CCD and GT crops such as GT alfalfa is unlikely due to the limited exposure of honey bees to GT alfalfa and the lack of hazard associated with the CP4 EPSPS protein. In addition, the currently approved Bt crops referenced by the CCD working group occupy much of the same general agricultural acreage as GT crops, including the acreage that GT alfalfa is expected to occupy. Given the dynamics of GT alfalfa seed and forage production, the current worst case exposure estimates of commercial honey bee colonies to GT alfalfa flowers are 1.2 to 1.8 percent. Safety assessments and field observations, based on the mode of action of the CP4 EPSPS protein, suggest no potential negative effects on any animal including honey bees. Given the pathology and distribution of CCD and the lack of hazard or exposure of GT alfalfa to commercial honey bees, we conclude that there will be no negative impact on honey bees from the deregulation and commercial production of GT alfalfa.

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