Appendix V: Gene Flow within Alfalfa and Mitigation Strategies

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Gene Flow within Alfalfa and Mitigation Strategies

1.0 Biology and Culture of Alfalfa

Alfalfa (*Medicago sativa* L.) is a short-lived perennial herbaceous legume (Lesins and Lesins 1979) and is exclusively an insect-pollinated crop that is pollinated by a small number of insect species, all of which are bees. *Medicago* is in the tribe Trifolieae, which also includes *Trifolium* (true clovers), *Melilotus* (sweetclover), and *Trigonella* (fenugreek). *Medicago* species members do not hybridize with any of these (or other) genera. The M. sativa complex has been successfully hybridized with 12 other perennial *Medicago* species (McCoy and Bingham 1988). However, many of these interspecific hybrids have been successful only by using embryo culture of the hybrid (McCoy and Smith 1986), making them highly unlikely to occur in nature. No perennial *Medicago* species are present naturally in the Americas, Australia, New Zealand, or South Africa. Therefore, no risk for interspecific hybridization exists in the United States. However, natural cross-pollination to the scattered naturalized (feral) populations of M. sativa is possible. A detailed discussion of the potential for interspecific gene flow is presented in appendix I. Alfalfa is an introduced species to the Americas and has become the most important forage crop species in the United States and Canada. It is recognized as a widely adapted agronomic crop and grows in all continental States, Alaska and Hawaii. Alfalfa grows optimally in fertile, well-drained soils; however, because of its adaptability, it also survives outside of cultivation. It is not a weedy species and it is not listed as a Federal noxious weed or on other weed lists such as: Federal Noxious Weed List (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html), Washington State Weed Lists (http://www.nwcb.wa.gov/weed_list/weed_listhome.html), California Weed Species Lists (http://www.extendinc.com/weedfreefeed/list-b.htm), Montana County Noxious Weed List (http://www.weedawareness.org/weed%20list.html), North Dakota Noxious Weeds (http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1103w.htm.

1.1 Biological Characteristics

A concise, recent review of several salient features of the biology of alfalfa within the context of gene flow was recently developed by an expert panel assembled by the National Alfalfa & Forage Alliance (Van Deynze et al. 2008). This white paper is an in-depth discussion of the potential risks of pollen and seed mediated gene flow in alfalfa growing under cultivated or feral conditions. The key relevant biological characteristics were summarized by these authors and others are as follows:

"Growth and Flowering

An alfalfa plant starts its initial growth from a seed during establishment, but after each harvest or winter, it re-grows from buds arising from the perennial root structure (the crown). The vegetative growth interval (i.e. harvest schedule) during most times of the year is 22 to 40 days, and harvest for forage is typically done three to eight times per year depending upon location and seasonal climate. Most of alfalfa in the US is managed to limit growth to the juvenile (vegetative) state in order to optimize forage phytomass production (yield) and nutritional quality of the hay. Hay with late maturity (presence of open flowers or seed) is of poor feed quality and market value. It is therefore atypical, that farmed hay fields produce any viable seed. In seed fields, of course, flowering and seed production are promoted. In most fields, flower buds begin to form on stems approximately 4 to 6 weeks after field mowing during long-day photoperiods and warm weather. Flowering is not triggered under short days or cool weather (i.e., late summer through mid spring). Once flowering ensues, alfalfa flowers indeterminately, and its duration is dependent upon moisture, temperature and other factors (Van Deynze et al. 2008).

Pollination

Alfalfa is a perennial crop that requires cross-pollination for fertilization, which is performed by insect pollinators. Wind cross-pollination in alfalfa does not occur (Viands et al. 1988). The primary insect pollinators are alfalfa leafcutter bees (*Megachile rotundata*), honey bees (*Apis mellifera*), and alkali bees (*Nomia melanderi*). Alfalfa must bloom, be cross-pollinated by insects, set fruit, and then dehisce seed in order to have offspring. Alfalfa requires at least four weeks of appropriate environmental conditions (temperature, sunlight, nutrients, and water) before forming reproductively mature floral buds, and then four to six weeks after that to form mature seeds after pollination (Rogan and Fitzpatrick 2004).

Alfalfa requires bees to "trip" flowers to release pollen for ovule fertilization and seed production. In the United States, alfalfa seed production fields are pollinated primarily with leafcutter bees (*Megachile rotundata* F.) in the Pacific Northwest and honey bees *(Apis mellifera)* in California. Some growers in niche areas of southern Washington use alkali bees (*Nomia melanderi* C.) and certain seed producers use a blend of cultured species for pollination. Native bees, including *Bombus* spp, *Osmia* spp, and *Agapostomen* spp. and native *Megachile* spp. can be found visiting alfalfa in varying numbers. While bee species are the dominant pollinators employed by alfalfa farmers, other insect pollinators can also serve as pollinators of alfalfa (Van Deynze et al. 2008).

Seed Formation

After pollination of the flowers, alfalfa seed embryos require 4 to 6 *additional* weeks of adequate growing conditions to ripen. Rainfall, low temperature, or snow during the ripening time will cause decreased seed yield and poor seed quality (e.g., reductions in seedling vigor and reduced percent germination because of fungal pathogen infection of the seed, or seed will sprout prematurely and die while it is still in the pod). Alfalfa seed is borne in a coiled leguminous pod, and is non-shattering. Natural, non-mechanized,

seed dispersal is very local. Alfalfa seed is too dense and smooth for effective wind dispersal (Van Deynze et al. 2008).

Hard Seed

It is typical for a proportion of the alfalfa seeds in any seed lot to exhibit post-harvest latency ("dormancy") related to "hard seed" (Mallory-Smith and Zapiola 2008). A hard, water-impervious seedcoat temporarily prevents water uptake and delays germination until the hard seedcoat is weathered, aged, or abraded. In contrast, seeds maintained dry in storage may remain viable for decades. Alfalfa does not exhibit true, physiological seed dormancy and in most situations the majority of seeds readily germinate. In alfalfa, hard seed is defined as the percentage of seed that do not imbibe water during a standard 72 hr germination test. However, these hard seed have been commonly observed to germinate under field conditions. Undersander et al. (1993) examined rate and extent of germination of alfalfa seed lots varying in hard seed content and found no correlation between the laboratory hard seed rating (percent hard seed) and germination in the field (Van Deynze et al. 2008).

Autotoxicity

Alfalfa plants and alfalfa debris produce compounds that elicit an autotoxic reaction to germinating alfalfa seeds. The autotoxic reaction and inter-plant competition severely limit germination and seedling vigor of alfalfa sown or dropped into existing or newly terminated alfalfa stands. Cultivated fields do not successfully self-seed. Attempts to thicken existing alfalfa stands by deliberately inter-planting new seed into them typically fail, which is why most agronomists do not recommend the practice (Canevari et al. 2000, Orloff et al. 1997). Therefore, secondary seedlings are a very unlikely avenue for effective gene flow into existing solid-seeded alfalfa plantings. A portion of seed growers plant their fields in rows instead of solid plantings. In these situations, in-crop volunteers from dropped seeds occur and the resulting seedlings could be a means of gene flow to subsequent crops. However, in order to maintain required varietal and species purity, these seed growers routinely control germinating alfalfa seedlings and weeds using cultivation, irrigation, and or soil-active herbicides that do not impact the pre-established, growing crop. The high likelihood of autotoxicity is one reason why growers must rotate to a different crop for at least one full year following stand take-out (Van Deynze et al. 2008).

Longevity

Alfalfa is a short-lived perennial. Fields grown for hay production are typically maintained for 3 to 6 years or longer in some areas. Commercial production of the alfalfa seed crop is exclusively confined to the western regions of the United States where late season (post-pollination) rain is unlikely, irrigation is carefully managed and specialized alfalfa seed growers, equipment, and infrastructure are available. To assure varietal purity, commercial seed production contracts typically require that stands be terminated after three years. Alfalfa is effectively terminated using a variety of mechanical, cultural, and or chemical methods. Glyphosate, although somewhat effective in the control of unwanted conventional alfalfa, is not typically used or it is used in combination with other stand take-out practices. Glyphosate will not control Roundup Ready alfalfa (GT

alfalfa); however, other herbicides and cultural practices remain effective. After cultivated stands are terminated, both seed and hay farmers rotate the field to a different crop species for one or more years wherein alfalfa volunteers can be controlled, if necessary. (Van Deynze et al. 2008)

1.2 Alfalfa in the Landscape (feral alfalfa).

Feral plants are crop plants that grow and reproduce outside of cultivation. Feral alfalfa plants can sometimes be found on road edges, in fence lines and in abandoned fields. In the US, feral alfalfa populations have occurred through escapes from intentional plantings of cultivated varieties ("escapes" from cultivation) or, in some cases, they originated from intentional planting of the abandoned fields, roadsides, or marginal lands. Feral alfalfa occurs at very low density and scale relative to cultivated alfalfa grown for seed or hay. Biogeographic survey data from six States indicates that for most agricultural areas feral alfalfa plants do not occur or they are sparse (Kendrick et al. 2005). In a 2001/2002 multi-State survey, feral plants were found as dispersed plants or patches within 1.25 miles (2 km) of cultivated alfalfa at only 22 percent of the survey sites (Kendrick et al. 2005). Feral alfalfa plants are sometimes managed on roadsides by clipping, either with hay being harvested or simply left on the ground along with the other roadside vegetation. Feral plants are sometimes completely unmanaged and given adequate moisture and timely presence of pollinators, can flower and set seed. Feral plants are susceptible to the environmental (e.g. drought in the irrigated West) and insect (e.g. Lygus bugs in the West and potato leafhopper in the East) stresses common to the local area. Although alfalfa was introduced to North America more than 200 years ago, it is not considered weedy, noxious or invasive in cultivated or feral settings."

Where alfalfa exists outside of cultivation, it is typically not targeted for control. In some instances on lands where unmanaged or feral alfalfa now occurs, the planting was intentional (e.g., feral plants exist in relegated sown pastures, abandoned alfalfa fields, or on roadsides once sown with alfalfa seed [see Petition page 375]). Alfalfa can be planted for further alternative usages of the crop products (e.g., composting, organic alfalfa meal fertilizer, green manure cover crop, intentional sowing that is not managed after planting, as part of a long term soil building program, cover crop left to flower to attract beneficial insects). These populations may serve as avenues for unintended escape of the Roundup Ready alfalfa. All feral alfalfa in the U.S, like alfalfa under cultivation, originated from introduced varieties. Section 5, part 5.5 summarizes the extent of feral populations within six major alfalfa production States of the United States, confirming that feral populations exist to a minor extent in areas where alfalfa seed or forage is produced. In situations where control of feral alfalfa is desired, just like cultivated alfalfa, it can be controlled or discouraged using cultural or chemical methods (see Petition section VII. Part F and appendix V-4). Because it is unmanaged and genetic resistance to pests is largely absent, feral alfalfa likely is more negatively impacted by endemic pests than alfalfa under cultivation. As a result, the competitiveness and number of viable, vigorous seeds produced by feral alfalfa plants likely are significantly constrained as compared to cultivated alfalfa of improved genetics under management (Van Deynze et al. 2008). Additional discussion of feral alfalfa is presented in section 5 of this document. The issue of the persistence of hard seed and hard seeds contributing to the weed potential of alfalfa is extensively discussed in letters from weed control

experts during the USDA public comment period (Docket 04-085-1; e.g., Doll comment #505). Alfalfa does not naturally propagate from vegetative plant parts (e.g., Docket 04-085-1 comment Beuselinck #501).

2.0 Alfalfa Hay Production

Alfalfa grown for forage is the third-ranked crop in the United States by value and fourth ranked by total acreage. The number and location of alfalfa forage acres are closely associated with livestock operations, especially dairy. Alfalfa is highly valued for animal feed because of its high protein content, high intake potential, and digestibility. It can provide the sole plant component in many livestock feeding programs when supplemented with the proper minerals.

Alfalfa is a deep-rooted perennial that has many economic, soil, and environmental benefits when used alone or in rotation (Hanson et al. 1988). In the United States more than 22 million acres currently are grown for forage production (figure V-1). The alfalfa acreage in the United States has declined gradually over the past 40 years from a high of approximately 30 million acres (Petition table VII-1, page 255).

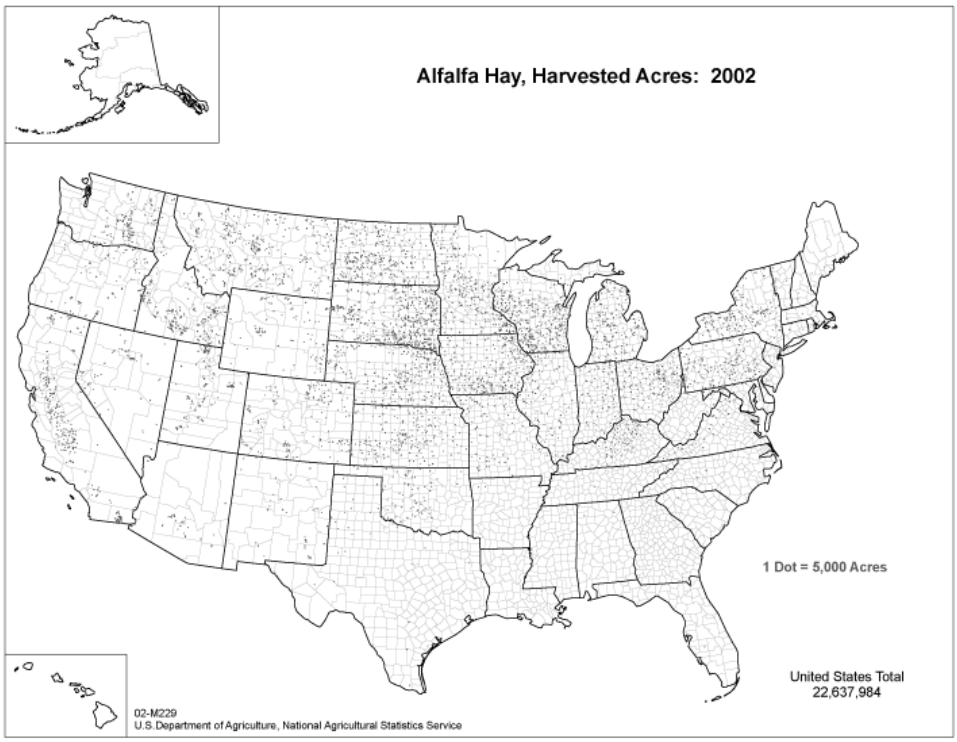

Figure V-1. Geographic distribution of alfalfa forage acres, 2002 (USDA FS 2002).

Alfalfa grown for hay does not require the presence of pollinators as the end use product is the vegetative plant material for forage. Therefore, alfalfa grown for hay will be mowed before setting seed in most circumstances. There are numerous citations that claim that alfalfa hay destined for the dairy industry is typically mowed before 10 percent bloom (table V-1). Because alfalfa hay comprises the majority of the geographic footprint of alfalfa, this assumption (harvest at <10 percent bloom) and possible exceptions to this assumption are explored in this appendix. Key information for estimating potential pollen flow from alfalfa hay is how much the field blooms before it is harvested. Monsanto requires that farmers sign a Monsanto

Technology/Stewardship Agreement (MTA)(Monsanto 2008) which stipulates that farmers must harvest GT alfalfa hay prior to 10 percent bloom and farmers must follow the Monsanto Technology Use Guide (TUG) (Monsanto 2010). Presumably farmers that prefer to harvest later would not adopt GT alfalfa or would change their harvesting time. No information on farmer compliance with the MTA was found. The discussion in this section investigates the premise that alfalfa hay is only rarely harvested later than 10 percent bloom. The discussion does not apply to GT alfalfa if farmers comply with the MTA. There are two basic groups of hay users, dairy producers and all other groups. Dairy producers demand alfalfa with high digestibility and protein, and low fiber, and will pay extra for this class of hay. For other uses, including feed for beef cattle, nonlactating dairy cows, and horses, lower quality hay is considered acceptable (Orloff et al. 2002).

2.1.1 Recommended Harvest Timing

Some of the factors that influence farmers' timing of hay field harvests include:

- Predicted quality – Younger plants have higher forage digestibility and protein content, therefore hay from young plants receives the highest grades (and highest price per ton).
- Predicted yield – Yield is based on the biomass of the harvested portion of the plant. Alfalfa reaches its greatest biomass and therefore greatest yield potential at full bloom. During seed set yield decreases. A full bloom field may produce the highest tonnage, but the hay receives a lower grade based on digestibility and protein content.
- End hay user needs (self or buyer) – The highest grade hay is best for dairy cattle because it maximizes milk production and merits its higher price. Heifers (immature dairy cattle), beef cattle, horses, and other livestock do not benefit from the highest grade hay in a way that provides economic gain to farmers; therefore lower grades are acceptable and even desirable for these animals.
- Stand health – Mowing patterns can influence stand health and longevity.
- Root reserves – Stand life can be reduced if alfalfa is repeatedly cut before root reserves are restored. In regions that experience harsh winters adequate root reserves are essential for plant survival. Root reserves are at their lowest during the bud stage and are at their highest from full bloom to seed set (Shroyer et al. 1984; Hesterman and Durling 1991; Howley and Wright 1991).
- Weed control – In fields with severe weed problems, early mowing can rescue a stand (Orloff et al. 1997).
- Pest control – Mowing can reduce insect pests (Lee et al. Undated).
- Weather – Wet weather can delay mowing and impact drying (for baled hay).

The factors presented above are illustrated by examples in table V-1. Most of the sources of information in table V-1 are State university Agricultural Extension publications. There is general agreement that the best balance between high quality and yield is to harvest at or before 10 percent bloom (table V-1; Undersander and Pinkerton 1989). Table V-1 also shows that delayed final harvest is sometimes recommended for the roots to store enough resources to overwinter well. Companion cropping (also known as catch cropping) is often done with alfalfa

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
South Dakota	North Central	10.70	6.82	Alfalfa for hay is harvested in generally one to four cuttings per year, depending on location within the State. Western areas of the State often receive only one cutting per year due to low rainfall, whereas southeast South Dakota usually can support four and occasionally five cuttings per year. Highest quality hay is taken when alfalfa plants are in pre-bud to early bud.	Wilson Undated
Montana	Winter Hardy Inter-mountain	7.80	2.60	Alfalfa plants are injured or weakened if cut when the root reserves are low-usually three weeks after growth begins or after cutting. Harvest timing is most critical in the fall. Alfalfa plants must be able to make sufficient fall growth to store large quantities of carbohydrates in the roots. These reserves help to prevent winterkill and provide rapid spring growth. To assure adequate food reserves in the roots, the alfalfa plants should have at least 30 days of regrowth before being killed by frost. Determine when the first killing frost for your area occurs, and make your last cutting 30 days before that date. Alfalfa regrowth should not be harvested or pastured in the fall until several killing frosts cause the plants to become dormant. Average dates of killing frosts for most areas of Montana are available and should be considered when scheduling the last hay harvest. Harvesting at the early-bloom stage will reduce leaf and stem shatter and cut the losses. Harvesting the forage as silage can save considerable feed value. Weather forecasts should be watched closely, since it is better to delay cutting than to suffer excessive rain damage.	Baldridge et al. 1985

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
North Dakota	North Central	7.35	10.09	NEW PLANTINGS--Alfalfa harvested during the establishment year should grow to the 10 to 25 percent bloom growth stage before harvesting the first cutting to enable the young plants to become well established, although earlier harvest has not been detrimental to stands in high-moisture areas. Alfalfa seeded with a companion crop usually does not grow tall enough after removing the companion crop for an economical forage harvest. If sufficient growth is obtained, it usually occurs in September, and harvest should be delayed until air temperatures have dropped low enough to restrict regrowth, or until just prior to or immediately after the first killing frost. ESTABLISHED STANDS--should be harvested using a combination of growth stage and calendar date to determine the best harvest date. The first cutting must usually be taken before mid-June to allow time for three cuttings prior to August 20-25 in an average year. The first crop should be harvested by the 10 percent bloom stage (late bud to early bloom), especially in the Red River Valley area or under irrigation where three annual cuttings usually are obtained. Delayed harvest lowers the quality of the first harvest the most. Advantages of an early harvest are that a near maximum yield of quality forage is obtained, root reserves for regrowth have been adequately replenished, and soil water usually remains to initiate new growth. Forage quality of second and third-cut alfalfa is less affected by delayed harvest. Harvesting third cutting at 10 to 50 percent bloom will allow buildup of root reserves to aid in overwintering, and forage will be of high quality.	Meyer and Helm 1994
Wisconsin	North Central	6.91	14.38	There is no simple alfalfa cutting schedule recipe or rule of thumb that will apply every year or for every farm. However, for an alfalfa enterprise to be profitable, producers must optimize forage yield, quality, and stand persistence. Estimating first cut forage quality before the crop is actually harvested is now possible with the availability of scissor cut results on an area basis and making PEAQ estimates on an on-farm basis. Use these tools to make first cut harvest decisions. Summer cuts will typically be made at 35 to 40 day intervals unless environmental factors deviate significantly from normal. Plan to be out of alfalfa fields by about August 25th and make late fall cutting decisions based upon forage need under the realization that stand persistence and subsequent plant vigor will be put at risk	Rankin Undated

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
Minnesota	North Central	6.02	10.44	In stands which are in the first season of production (the seeding year), one fourth of growers take no cuttings, one quarter take one, one quarter take two, and one quarter take three cuttings. In established stands, a majority of Minnesota growers (about 70 percent) take three cuttings per year. About one-fourth take two cuttings per year, and a fourth cutting is taken by about 10 percent of growers. Most growers harvest alfalfa when the stand is at 10 percent bloom; if four cuttings are made, a greater percentage of growers harvest when the stand is at pre-bloom. Mid-August through mid-September is when almost all Minnesota growers take the last cutting of the season.	Nelson 2000
Iowa	North Central	5.57	4.50	For maximum yields, it is important to have 20-30 plants per square foot during the seedling year for protection against weed competition. Do not harvest alfalfa seeded in late summer until the following spring. Allow new seedlings to start to bloom before the first harvest. Cut alfalfa three-four times a year when stand is 25 percent flowered.	Welter Seed Undated
California	Moderate Winter Hardy Inter-mountain/ Southwest	4.63	6.48	In the Intermountain Region, it takes 3 to 4 weeks to restore root reserves and another 7 to 10 days to add surplus carbohydrates to the roots so the plant is ready for another cutting. Hay intended for this [dairy] market must be cut early (late-bud stage at the latest) for the necessary quality to be achieved. Conversely, hay intended for beef cattle or horses can be cut later, at 10- to 30-percent bloom, to maximize yields with acceptable quality for these classes of livestock.	Orloff and Putnam 2007; Orloff et al. 1997
Michigan	East Central	4.01	0.35	(1) Schedule your cuttings based upon alfalfa stage of growth and your goals for forage production. Harvest at the early bloom stage of growth to maximize nutrient yield per acre and ensure that root reserves have been restored to a reasonably high level. Harvesting earlier maximizes forage quality but does not ensure adequate levels of stored root reserves. Harvesting later maximizes yield and stored root reserve levels but forage quality is lowered. (2) Offset the risk of winter injury by selecting multiple disease resistant, winter hardy varieties, maintaining young stands, keeping soil fertility levels high, growing alfalfa on well drained soils, and retaining snow in the winter if possible. (3) Delay the first cutting of winter-injured stands until full bloom. If alfalfa plants were frost heaved, cut above the normal height to avoid crown injury.	Hesterman and Durling 1991

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
Kansas	Great Plains	3.79	0.32	On established stands the first cutting should be made when regrowth at the crown is observed; subsequent cuttings can be made at one-tenth bloom or 28-30 day intervals if weather conditions allow. Severe leaf loss may initiate cutting to salvage the hay crop and maintain stands. If this occurs the next cutting should be delayed to allow the root reserves to be replenished. Cutting at the pre-bud and bud stage produces a higher quality forage than at later stages, but repeatedly cutting at early stages reduces root reserves which results in poor stands and lower yields. Cutting when regrowth at the crown appears and at one-tenth bloom maximizes forage yield, quality and benefits stand longevity. The last cutting before fall dormancy should allow four to five weeks of growth so that root reserves are replenished.	Shroyer et al. 1984
Colorado	Winter Hardy Inter-mountain	3.57	4.38	If highest quality is the primary consideration, harvest at the bud stage during each regrowth cycle. Many growers, however, produce alfalfa hay that is sold or fed on the basis of less than premium quality standards. For these producers, the optimum growth stages for harvest are mid- to late bloom, because higher yields can be obtained and quality is still acceptable. Alfalfa grown in Colorado below 7,000 feet can be harvested in late summer or early fall with little concern for timing relative to the first killing frost (28 degrees F). Regardless of the amount of regrowth at the time of frost, fall conditions almost always are mild enough to allow for adequate recharge of reserves. Above 7,000 feet, it generally is best not to cut within four weeks of the average date of the first killing frost.	Smith et al. 1998
Wyoming	Winter Hardy Inter-mountain	2.67	0.84	Alfalfa should be harvested at the late bud to early bloom (10 percent bloom) stage for premium hay. Delaying harvest will reduce the hay quality. Taking the first cutting early will control weevil problems. In fall-planted alfalfa, first harvest occurs in late May to June, depending on the region and time of planting. Subsequent harvests are approximately every 28 days, as long as the late bud to early bloom stage of development has been reached (second harvest usually in July, third harvest September 1 - 15). The last harvest of the season should be at least two weeks before the first killing frost. At high elevations, 8 - 14 drying days are needed between cutting and baling the hay.	McDonald et al. 2004

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
Ohio	East Central	2.27	0.50	When harvests are made at late bud-early bloom stage of development, the fall cutting schedule is important for stand maintenance. Make the last regular harvest by the calendar: northern Ohio, September 1 to 7; central Ohio, September 3 to 12; southern Ohio September 5-15. This means that on those fields with an intensive cutting schedule, no harvest should be taken from early-mid September to mid-October. At least 30 days between the last regular harvest and killing frost are needed to adequately restore root and crown reserves.	Myers and Van Keuren Undated
Pennsylvania	East Central	2.27	0.60	During the year of establishment, seedlings need a high level of energy reserves in order to persist through the winter. For spring seedings made without a companion crop, two harvests can generally be made the first year, provided the crop has adequate rainfall and optimum levels of soil nutrients. The first harvest can be made before flowers begin to appear, but waiting for the alfalfa to flower will ensure greater energy reserves in the roots. Alfalfa will generally reach this stage of development between 60 and 70 days after emergence. The second harvest should either be made before September 1, to ensure an adequate buildup of energy reserves for winter, or be delayed until after the first killing frost (24°F) in the fall or after mid-October. Occasionally, when the second harvest is made before September and there are good fall growing conditions, a third harvest may be made after a definite killing frost. When mid-October or later harvests are made, a high stubble (6 inches) should be left for ground cover to protect the crowns and to catch snow for added insulation.	Bosworth et al. Undated
Missouri	East Central	2.01	0.58	For spring-seeded established stands in the seeding year, take the first harvest at the mid- to full-bloom stage. Make following harvests as flowers begin to appear. For established stands, take the first (May 10 to 20) and second (June 15 to 25) cuttings when the plants are just beginning to bloom. For persistence of the stand, make two more harvests at about 35-day intervals before Sept. 15. Harvesting alfalfa in the bud stage produces five cuttings of high-quality hay before Sept. 15. This practice, however, reduces stand life to three or four years.	Henning and Nelson 1993

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
New York	East Central	2.01	0.16	As a general guideline, all perennial forages should be harvested at early stages of maturity when both palatability and nutrient content are high. For alfalfa, the neutral detergent fiber (NDF) value should be approximately 40 percent. This occurs at a late bud stage for the spring harvest of alfalfa. Early harvest also allows time for regrowth and subsequent harvests during the growing season. Alfalfa can be harvested two to four times a year in New York, depending on the growing season and soil type. Alfalfa stands will persist longer if there is a forty- to forty-five-day interval between harvests. All studies comparing various harvest schedules indicate that four harvests per year decrease the long-term persistence of stands compared to three harvests per year. Frequent harvests, with a shorter interval between, may be more advantageous in short rotations, where stands of alfalfa are only kept down for two to three years.	Cherney et al. 2001
Washington	PNW-Inter-mountain	2.01	0.56	High-quality dairy hay, which brings the highest price, is harvested during the late bud stage of growth before any sign of bloom. Growers can discern bud stage by simply walking through the field and feeling the terminals of the tallest stems. Buds about to open have a hard lump inside the terminal growth point. Dairy-quality hay is harvested on an average of every 30 days, starting in late May or early June and continuing through September. Alfalfa hay for the horse market requires lower protein and higher fiber. More mature hay with up to 50 percent bloom can be desirable for this market. This growth stage is attained by delaying harvest to 35- to 37-day intervals. In the southern growing areas, 4 to 5 harvests are taken, while in the northern areas 3 to 4 harvests are taken each year.	Kugler and Woodward 2006
Oklahoma	Great Plains	1.43	0.04	The best time to harvest will vary, depending on projected use of hay. If hay will be sold as high-quality forage (for dairy cattle), alfalfa should be cut at bud stage or earlier (28 day cycle or less). If alfalfa is being used as feed for a cow-calf operation where high quality is not as critical, it should be cut at 25-50 percent bloom stage (35-42 day cycle) to maximize yield.	Caddel et al. 2001
Kentucky	East Central	1.16	0.01	Once established, the crop is harvested at 30 to 38 day intervals between May 1 and September 15.	Townsend et al., 2002

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
Arizona	Moderate Winter Hardy Inter-mountain/ Southwest	1.16	0.24	In general, cutting alfalfa when half of the regrowth buds are three-quarters of an inch or longer is a good compromise between yield and quality and will help maintain an adequate stand.	Tickes and Ottman 2008
New Mexico	Moderate Winter Hardy Inter-mountain	1.07	0.33	Late bud to first flower for first cutting, first flower to one-tenth bloom for second and later cuttings.	McWilliams et al. 2005
Virginia	East Central	0.49	0.14	Harvesting usually occurs at late bud to 1/4 bloom, except the first cutting. The first cutting should be made in (hay or silage) bud stage or when orchard grass begins to head. Alfalfa may be cut 3-5 times/year at 30- to 40-day intervals, depending upon location in the State and average rainfall. Make the last cutting three to four weeks before average date of first killing frost in fall or in time to allow 6-8 inches of regrowth. Allow at least one harvest to reach 1/10 bloom to help persistence.	Schooley et al. 2004
Tennessee	East Central	0.16	0.00	New spring seedlings should be harvested when they reach full bloom. All other harvests should be made when 10 percent of the plants are blooming. Fall seeded and established stand may be harvested at the bud stage for the first cutting, and then at the 10 per cent bloom stage for all later cuttings. Last harvest should be made before mid-September.	Bates 1998
New Jersey	East Central	0.11	0.00	Usually, when following cutting intervals closely, the alfalfa will be in the pre-bud to bud stage, which is ideal for harvesting, providing the highest nutrient levels and greatest digestible content for livestock. Farmers are encouraged to harvest the crop early rather than use pesticides if pest populations are over thresholds.	Lee et al. Undated

Table V-1. Alfalfa Dry Hay Harvest Statistics and Recommendations

State	Growing Region	Percent of Harvest Acres (2005)	Percent of Organic Harvest Acres (2005)	Harvest Advice	Source
Massachusetts	East Central	0.06	0.00	Careful management of new seedings is especially important in the seeding year to ensure a long-lived productive stand. New seedings should not be harvested until plants have come to at least the one-half bloom stage to ensure adequate storage of total nonstructural carbohydrates (TNC) in the roots. This management should be followed for all harvests in the seeding year. Established stands of alfalfa can survive earlier harvesting in the spring than that recommended for new seedings. Removal of first cuttings at the full bud stage in the spring does not reduce the annual forage yields appreciably. It may even be necessary to spread the harvest period of each cut or may be desirable to improve forage quality and to prevent over-mature hay crops. Because the effects of early cutting are accumulative, the practice of cutting at early growth stages should be rotated among alfalfa fields. Alfalfa stand will not be reduced from a single cut at the bud stage, provided successive cuttings are permitted to reach at least the one-tenth bloom stage of maturity.	Herbert 1996

2.1.2 Quality of Hay is Dependent on Timing of Harvest

Quality of hay can be measured by several different variables. Although quality can be partially predicted by time of harvest based on percentage of bloom, many buyers require nutrient testing (Robinson et al. 2007). USDA Agricultural Marketing Service (AMS) Market News uses the following visual physical descriptions for quality (http://www.ams.usda.gov/mnreports/lsamlgr311.pdf):

- Supreme: Very early maturity, pre bloom, soft fine stemmed, extra leafy. Factors indicative of very high nutritive content. Hay is excellent color and free of damage.
- Premium: Early maturity, i.e., pre-bloom in legumes and pre head in grass hays, extra leafy and fine stemmed-factors indicative of a high nutritive content. Hay is green and free of damage.
- Good: Early to average maturity, i.e., early to mid-bloom in legumes and early head in grass hays, leafy, fine to medium stemmed, free of damage other than slight discoloration.
- Fair: Late maturity, i.e., mid to late-bloom in legumes, head-in grass hays, moderate or below leaf content, and generally coarse stemmed. Hay may show light damage.
- Utility: Hay in very late maturity, such as mature seed pods in legumes or mature head in grass hays, coarse stemmed. This category could include hay discounted due to excessive damage and heavy weed content or mold. Defects will be identified in market reports when using this category.

The stages of maturity mentioned in the list above are defined as follows (Bagg 2003):

Late vegetative – No visible buds, stem at least 12 inches tall.
Early bud – Visible flower buds on at least 1 stem.
Mid bud – 50 percent of stems have at least 1 bud.
Late bud – 75 percent of stems have at least 1 bud, no visible flowers
First bloom – Flowers on at least 1 stem.
1/10 bloom (Early bloom) – 10 percent of stems have at least 1 flower.
Mid bloom – 50 percent of stems have at least 1 flower.
Full bloom – 75 percent of stems have at least 1 flower.

Hay is tested by certified laboratories for acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and dry matter (DM). Total digestible nutrients (TDN) are calculated from ADF. Table V-2 presents the numeric criteria for these hay quality components. Note that only 10 percent grass (either from livestock edible weeds or companion crops) is allowed.

Table V-2. USDA AMS Alfalfa Guidelines (for domestic livestock use and not more than 10 percent grass)

Quality	ADF	NDF	*RFV	**TDN-100 percent	**TDN-90 percent	CP
Supreme	<27	<34	>185	>62	>55.9	>22
Premium	27-29	34-36	170-185	60.5-62	54.5-55.9	20-22
Good	29-32	36-40	150-170	58-60	52.5-54.5	18-20
Fair	32-35	40-44	130-150	56-58	50.5-52.5	16-18
Utility	>35	>44	<150	<56	<50.5	<16

*RFV calculated using the Wis/Minn formula. **TDN calculated using the western formula. Quantitative factors are approximate, and many factors can affect feeding value. Values based on 100 percent dry matter (TDN showing both 100 percent & 90 percent). Guidelines are to be used with visual appearance and intent of sale (use).

USDA's Natural Resources Conservation Service (NRCS) provides the following key to nutrient values and maturity levels for legumes, grass, and grass/legume mixtures (table V-3).

Table V-3. Quality standards for legume, grass, and grass and legume mixture (USDA NRCS 2002)

Maturity	ADF	NDF	RFV
Pre-bloom	<31	<40	>151
Early bloom	31-35	40-46	151-125
Mid bloom	36-35	47-53	124-103
Full bloom	41-42	54-60	102-87
Rain damaged	43-45	61-65	86-75
Severe damage	>45	>65	<75

The AMS maturity-to-protein-content categories do not seem to agree with the NRCS table V-3. For example, the Fair category is described as mid to late bloom in the AMS narrative standards and has an ADF of 32-35, NDF of 40-44, and RFV of 130-150. In the NRCS table similar nutrient levels are categorized as early bloom. The one difference between the two sources is that AMS alfalfa guidelines are for legumes with less than 10 percent grass and the NRCS table is for legume, grass, and grass and legume mixtures of unspecified percentages. Because grass has lower nutrient value than legumes, legume/grass mixtures would have to be harvested earlier to achieve the same nutrient ratings as pure legumes. Therefore, for alfalfa stands with less than 10 percent grass, the AMS table may be more accurate.

Iowa State University Extension has created a tool for alfalfa farmers in Iowa that can help them predict the RFV of the first harvest of the year (Lang 2001). The Predictive Equations for Alfalfa Quality (PEAQ) allow growers to use height of tallest stem (in inches) and the maturity of the most mature stems to predict RFV, then subtract 10 to 20 RFV units for losses during harvest. This tool is helpful because climatic differences from year to year make calendar date harvesting unreliable. The tool has been validated in the Midwest and other environments in California and New York. Lang (2001) recommends 150 RFV for milking dairy herds, and 120 to 130 for heifers, stocker cattle and lactating beef cattle. So for hay to achieve RFV of 150, late vegetative (no visible buds) corresponds with 28 inches in height, bud stage (no flowers) corresponds with 26 inches in height, and flower stage (1 or more nodes with open flowers) corresponds with 24 inches in height. So clearly maturity based on bloom is not fully predictive of nutrient content.

Orloff and colleagues (Orloff et al. 2002) have developed a tool for balancing quality and yield for intermountain alfalfa growers. With this tool the price differential between the classes of hay can determine the best recommended harvest timing. For example, for the extremes in the market, 1996 and 1999, different harvest decisions would be recommended by using the decision tool. In 1996 the price differential between Premium and Fair was only 24.4 percent, so the tool recommended cutting for yield (Fair quality) on both the first and second cutting. On the other hand, in 1999, the price differential was wide which resulted in a decision to cut earlier for both cuttings. Other considerations also influence harvest, such as long-term stand life, machinery costs, and overall system viability, but the basic economic decision starts with the yield-quantity trade-off.

If the AMS narrative criteria could be taken at face value, then a crude estimate of the tonnage of hay alfalfa that goes to mid and late bloom could be estimated by examining the quantity of hay sold in the good and fair categories. USDA Market News tracks alfalfa hay prices on a weekly basis for some regions. The alfalfa hay market for June, 2008 is summarized in table V-4. Only data for California, Nevada, Idaho, Washington, and Oregon included information on tonnage. For other States and regions average prices were reported, but not tonnage. Factors that contribute to the inaccuracy of this crude estimate include:

Nursery crops – Annual companion crops that are interseeded with alfalfa to reduce weed growth during stand establishment (first year) typically have lower protein content than alfalfa. Therefore the presence of companion crop in a harvest can lower the quality of the hay regardless of the maturity of the alfalfa. This factor applies mainly to the first year of stand life because established alfalfa is a good competitor and companion plants are reduced.
Weeds – The prevalence and protein content of weeds in any given batch of alfalfa is unknown and highly variable. In general weeds lower alfalfa protein quality and overall quality regardless of alfalfa maturity level at harvest.
Tonnage ≠ Acreage – Yield (tons/acre) is not consistent between the quality categories. Because the biomass of the alfalfa is lower when the plants are younger, harvesting supreme and premium hay results in lower yields. If the alfalfa is permitted to gain more biomass yield is higher, but the quality of the hay drops to good or fair. Therefore tonnage does not indicate acres. In addition yield also fluctuates due to many other factors, both environmental and farm management.

Based on June, 2008 data for the five Western States listed in table V-4, 57 percent of the alfalfa hay sold was in the supreme and premium categories, which are harvested before flowers mature. Forty three percent of the tonnage was in the good to fair categories. These market numbers only include dry hay. Other hay harvests missing from table V-4 include haylage and any hay that is contracted or directly purchased. These missing categories are usually high quality. Therefore the percentages in table V-4 are not representative of the alfalfa hay harvest as a whole. It only serves to illustrate that Fair hay does make it the market. Factors that can result in lower quality hay include mixtures of alfalfa/grass, weeds, rain damage during harvest, dustiness, heat or mold damage during storage, improper adjustment of bailing equipment, harvest at late bloom, and abiotic or biotic stress prior to harvest. Plant height also affects quality. For example, first cut

forages in California can have zero to a few percent flowers, but can have quality ranging from very low to premium.

Commercial hay testing laboratories provide information that can be used to insure that livestock diets are being properly formulated to meet daily nutrient requirements. The commercial testing laboratory Dairy One publishes their testing data online (www.dairyone.com). For legume hay, 132,027 samples taken between 2000 and 2010 had an average of 30 ± 3.9 percent acid detergent fiber. Table V-4 indicates that range is achieved by harvesting at or before early bloom.

Table V-4. Alfalfa Hay Market – Tons and Quality Rating (Month of June 2008)[1]

State	Market	Supreme	Premium and good/ premium	Good	Fair	Utility	Total
California	Antelope Valley		2990	100	1500		4590
	Blythe		4800	9750	9700		24250
	Chino Los Angeles San Diego	800	6170	8082	4650		19702
	Escalon Modesto Turlock	23500	8325	5925	5875		43625
	Hanford Corcoran Tulare Visalia	3215	3937	3334	1015		11501
	Imperial Valley	1100	18353	15550	3908		38911
	Kern County	1435	12300	9326	8975		32036
	Los Banos Dos Palos Merced	19995	12587	4515	3552		40649
	Northern Intermountain	5800	1200	1050			8050
	Petaluma	3050	400	2400	3900		9750
	Sacramento Valley	2895	13993	7618	12210		36716
	South Central-Coastal		1925				1925
	Tracy Patterson Stockton	13894	3300	6450	2000		25644
	Tulare Visalia Hanford Bakersfield	17390	16690	14180	7915		56175
	Fresno	4839	4779	1450	1150		12218

[1]Current:http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateN&navID=MarketNewsAndTransportationData&leftNav=MarketNewsAndTransportationData&page=LSMarketNewsPageHay
Idaho, June, 2008: http://search.ams.usda.gov/mndms/2008/06/LS20080601Mmlgr312.pdf
Nevada, June, 2008: http://search.ams.usda.gov/mndms/2008/06/LS20080601Mmlgr314.pdf
Oregon, June, 2008: http://search.ams.usda.gov/mndms/2008/06/LS20080601Mmlgr313.pdf
Washington, June, 2008: http://search.ams.usda.gov/mndms/2008/06/LS20080601Mmlgr310.pdf
California, June, 2008: http://search.ams.usda.gov/mndms/2008/06/LS20080601Mmlgr311.pdf

Table V-4. Alfalfa Hay Market – Tons and Quality Rating (Month of June 2008)[1]

State	Market	Supreme	Premium and good/ premium	Good	Fair	Utility	Total
	Madera Firebaugh						
Subtotal CA		97913	111749	89730	66350		365742
Subtotal CA percent		26.77	30.55	24.53	18.14		100.00
Nevada	Northern	2380					2380
	Western	2200	5450				7650
	Central/East Central	700	650				1350
Subtotal NV		5280	6100				11380
Subtotal NV percent		46.40	53.60				100.00
Idaho		18275	19999	9235	7975		55484
Subtotal ID percent		32.94	36.04	16.64	14.37		100.00
Washington	Columbia Basin	4005	54282	63575	9600	200	131662
Subtotal WA percent		3.04	41.23	48.29	7.29	0.15	100.00
Oregon	Crook Deschutes Jefferson Wasco		1200				1200
	Klamath Basin	400	2840				3240
	Lake County	500	120	130			750
	Harney County	50					50
	Eastern Oregon		650	200	1110		1960
Subtotal OR		950	4810	330	1110		7200
Subtotal OR percent		13.19	66.81	4.58	15.42		100.00
Total Tons		126423	196940	162870	85035	200	571468
Total percent		**22.12**	**34.46**	**28.50**	**14.88**	**0.03**	**100.00**

3.0 Alfalfa Seed Production

Cross-pollination and thus gene flow is required for seed production in alfalfa. Alfalfa seed farmers would like to keep pollen flowing within a field, but not between fields. This presents problems when the most common alfalfa pollinators, bees, do not respect farmer field boundaries. Therefore, controlling how pollen is distributed within and among alfalfa fields is dependent on knowledge of bee behaviors and ability to design seed production systems that regulate pollen flow within certain areas, and at the same time restrict pollen flow to other unintended areas. It should be noted that pollen flow and gene flow are not synonymous terms even though they are sometimes used interchangeably. For example, pollen may land on a flower and pollinate it, but if the plant is mowed before seed sets, then gene flow has not occurred.

Alfalfa seed growers wish to maintain the genetic integrity of their crops, and in order to maintain variety purity, seed crops are grown under conditions that are designed to keep gene flow restricted to individuals grown in the same field. Two factors that influence the severity and likelihood of breakdowns in seed crop integrity are cross-contamination due to equipment carry-over and gene flow due to cross-pollination.[2] The Association of Official Seed Certifying Agencies (AOSCA) and the international Organization for Economic Co-operation and Development (OECD) have developed alfalfa seed production practices that enable growers to meet the Federal Seed Act (7 CFR 201) requirements of greater than 99 percent variety purity for certified seeds and greater than 99.9 percent variety purity for foundation seeds.

With the introduction of glyphosate-tolerant (GT) alfalfa, farmers who want to grow seed or hay crops that have no GT traits[3] questioned whether the AOSCA standards are adequate to meet their needs. Different thresholds for percentage of unintended presence are discussed below:

- Zero (GE-free)[4] – Zero is not statistically provable. All testing methods destroy the sample. Therefore, to be useful, the test can only be run on a portion of the lot.
- No transgene DNA detected by PCR – PCR is the most sensitive test available and a negative result is considered to represent zero, even though true zero is not verifiable on a large sample. PCR is expensive ($250-$325 each test) and currently needs to be performed in a laboratory (Mueller 2005).
- No transgene product (protein) detected by antibody strip test – Tests for transgene protein are not as sensitive as DNA tests, however, they can be field portable and much cheaper ($5-$10 each) than PCR tests (Mueller 2005). They are also commercially available and are a standard in the field of testing for GM traits. No detection using an antibody strip test is also often referred to as "zero," even though statistically it is not. Using a standard strip test protocol to test five 600 seed samples from one seed lot, if all

[2] Intentional presence due to sabotage is also possible, but is not discussed in this report. Inadvertent seed spread due to losses during production and transportation (leaky handling system) is also not addressed in this report (Mallory-Smith and Zapiola, 2008).
[3] "GT trait" refers to both the DNA and the protein it encodes which result in the glyphosate-tolerant phenotype. Transgene refers to the DNA, except when followed by "product" or "protein."
[4] Genetically modified (GM), genetically modified organism (GMO), and genetically engineered or genetically enhanced (GE) are all roughly synonymous terms.

five samples test negative for the transgene protein, then there is a 95 percent confidence that any undetected impurity is less than 0.1 percent (FGI 2007).

- Forage Genetics International (FGI) has a company goal of less than 0.5 percent cross-pollination for all their seed concerning the GT trait. Their current Best Practices achieve 0.180 percent or less unintended presence.
- If more than 0.9 percent transgene protein is detected then the lot must be labeled "genetically modified organism" (GMO) in the European Union.
- If more than one percent transgene protein is detected, then the lot must be labeled GMO in Australia and Brazil.
- If more than three percent transgene protein is detected, then the lot must be labeled GMO in Korea.
- If more than five percent transgene protein is detected, then the lot must be labeled GMO in Japan.
- In the U.S. National Organic Program (NOP), "certified organic" crops are grown without any intentional inclusion of transgenic seed. Testing for transgene DNA or protein is not required.

Alfalfa grown for seed production purposes occurs almost exclusively in niche areas of the Western United States on approximately 100 to 120 thousand acres (figure V-2). Although alfalfa forage is grown in all continental states, the crop value is highest in the West, Plains and upper Midwest. Additional information on by-county seed production may be found at: http://www.nass.usda.gov/Census/Pull_Data_Census.

3.1 Breeding Systems Cultivar Development

Alfalfa cultivars typically are developed by plant breeders using a combination of phenotypic and or genotypic recurrent selection to identify parent plants. Cultivars are, with few exceptions, open-pollinated synthetic varieties. Seed of the cultivar (or variety) is produced commercially using bee pollination in isolation from other cultivars (AOSCA 2003). More than 250 registered conventional alfalfa cultivars are commercially available for planting, and multiple varieties are available from numerous U.S. vendors (>40 seed brands). A list of registered alfalfa cultivars and marketers that sell them are listed in an alfalfa variety list publication offered by the National Alfalfa & Forage Alliance (http://www.alfalfa.org). Although U.S. plant variety protection is available to cultivar developers, for the past 20 years, relatively few of the AOSCA registered conventional varieties were submitted by the developer for U.S. plant variety protection. Since 2005, approximately 30 Roundup Ready alfalfa cultivars have been registered within the AOSCA National Alfalfa and Miscellaneous Legume Variety Review Board process. In addition to registered public and proprietary varieties, numerous conventional unnamed brands and common seeds of alfalfa are available for planting.

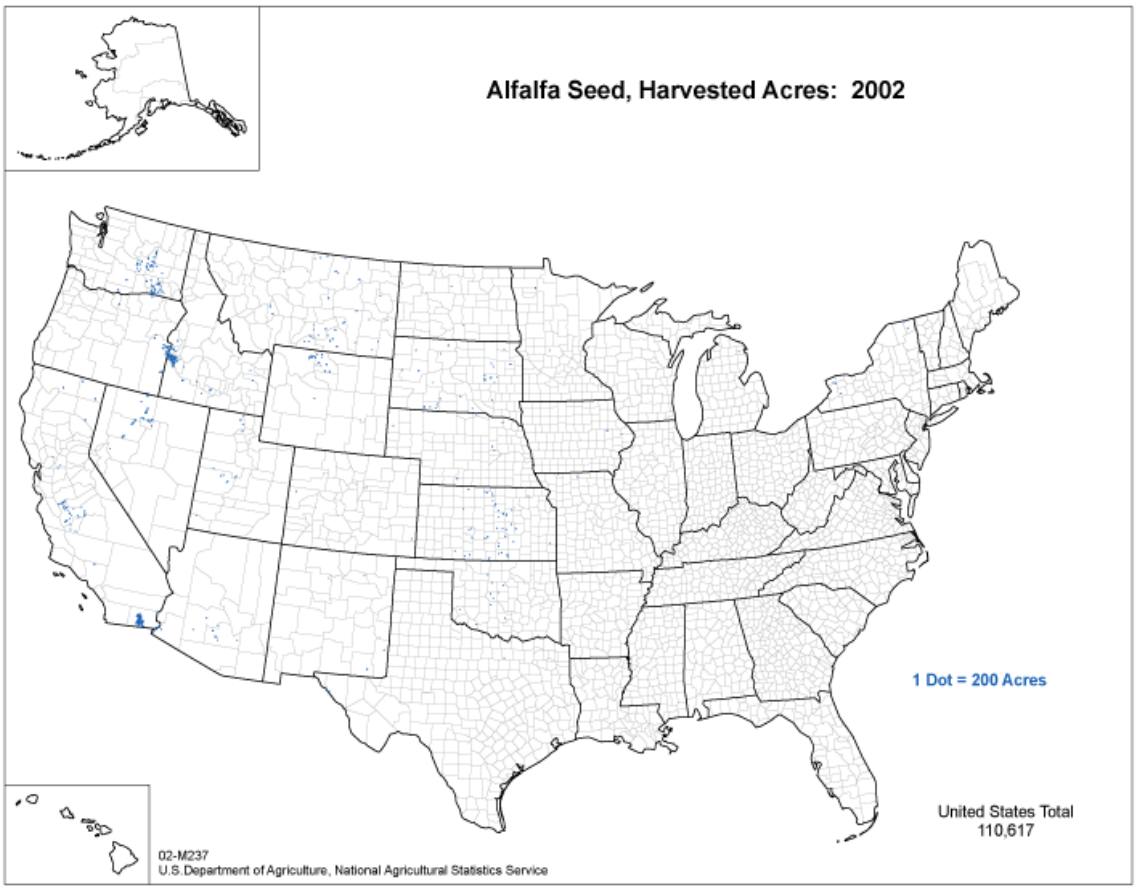

Figure V-2. Geographic distribution of alfalfa seed acres, 2002 (USDA-FS 2002).

3.2 Commercial Planting Seeds

The numerous seed alternatives differ in seed quality, adaptation, pest reaction, yield potential, forage quality, tolerance to grazing, degree of winter activity, and many other agronomic features. All alfalfa varieties have some inherent level of genetic variation and there are currently many different varieties and cultivars of alfalfa available to farmers. For example, large alfalfa seed producers have many varieties of alfalfa available for farmers, often tested for optimal growth in specific geographies. For example, Dairlyland seeds have 23 cultivars and Cal West Seeds has 75+ cultivars. Alfalfa seed may be sold raw or more typically it is sold pre-inoculated with one or more strains of *Sinorhizobium meliloti* (the alfalfa-nodulating, nitrogen-fixing bacteria) and or coated with lime or clay that may contain a fungicide (e.g., metalaxyl), seed colorants and or micronutrients. Proprietary cultivars often are sold as treated or coated seeds. The retail price for alfalfa seed is related to its agronomic performance, value-added features, quality of the seeds (germination, purity), brand name/seed supplier reputation, annual supply and demand, etc. The choice of seed is especially important because the decision is made only once in the life of each multi-year stand. The seed price is only two to three percent of the overall alfalfa stand establishment cost. Therefore, the use of high quality seed of superior varieties is a widely recommended practice by university and extension forage specialists. With

respect to seed quality requirements and seed labeling laws, State and Federal seed control agencies conduct seed lot inspections to ensure truth in labeling. Inadequately cleaned seed lots (purchased alfalfa, grower-saved or bin-run companion crop seeds) can be a potential source of weeds in newly seeded alfalfa.

In most cases, an alfalfa seed producer grows seed of a specific named variety under terms of a seed company contract. Such contracts may require third-party inspection of production conditions and they may prohibit or restrict the practice of saving proprietary seeds grown under the contract. Commercial seed industry practices are discussed in section VII.B.5 and B.7 (pages 259 and 260) of the Petition and further addressed below.

3.3 Commercial Seed Production

In the United States, alfalfa commercial seed production occurred on approximately 110,000 to 122,000 acres during 2002 and 2006, respectively. Alfalfa seed production occurs almost exclusively in the Western states where late-summer seed ripening may occur without damage from rain, heavy dew or high humidity (figure V-2). Alfalfa seed production is usually a distinct commercial practice from forage production (Hanson et al. 1988). Due primarily to the climate in the West, the United States is a major alfalfa seed-producing country. The value of the U.S. annual seed crop is estimated to be in excess of $90 million. Approximately 70 percent of the seed crop is used domestically.

The value of alfalfa seed varies widely between years, variety type (proprietary versus common; certified versus non-certified; value-added traits versus none) and quality specifications (germination, species purity, etc.). Official statistics on seed-grower settlement prices are lacking, but on average, it may be approximately one-half to two-thirds of the wholesale price of seed.

Nearly all alfalfa seed is used for the establishment of hay fields, with a minor amount used as seed field stock seed (variety increase) or for sprouting purposes. Alfalfa seed is not consumed as a grain and therefore not used directly as a food or feed product. Essentially all alfalfa planting seed produced in the United States is grown using insecticides and or herbicides, the use of which precludes the seeds' use for food/sprouting purposes. The use of biologically contaminated canal or wastewaters or livestock manures to fertilize alfalfa sprout seed fields also are prohibited by food safety regulations. Therefore seed products entering the planting and sprouting seed channels are kept as distinct. A small percentage of U.S. alfalfa seed may be used for sprouting seed. Bass et al. (1988) estimated that 7 percent of U.S. alfalfa seed is used for sprouting, but this has not been confirmed. Acreage or production of sprout-destined seed is not reported, because field locations for such production are not recorded, they are not known officially. It is believed that most of the sprout seeds are imported because economic alfalfa seed production in the United States generally requires the use of some food-prohibited practices.

3.3.1 Commercial Seed Production Culture

Alfalfa seed production requires a long growing season with very warm temperature, very low humidity during seed ripening and specialized equipment. Most professional seed producers use

cultured bees and specialized equipment associated with bee culture. Therefore, professional seed production only occurs in niche areas of the western United States primarily under intensive management and irrigated field conditions (figure V-2).

Cultural practices used to produce seed typically are distinct from those used to produce forage. Professional seed growers usually grow seed under terms of a two or three year term seed company contract, by variety name. The contracting seed company supplies the stock seed (e.g., foundation seed) to the seed producer and genetic source variety of the seed is documented. In contrast, seed companies purchasing or growing "common seed" or "catch crop" seed typically use lower management and inputs, the genetic identity of the stock seed is often unspecified /unknown and the resultant product quality is highly variable and cannot be certified as to cultivar or variety identity. Common seed may be collected from fields that were previously harvested for forage.

Typically, seed fields are planted in the fall and clipped back in late spring so that bloom within the field is uniform, synchronous and optimally timed for the warm dry season and optimal pollinator activity. Weed and in-crop volunteer controls (herbicides and cultivation) are applied mainly prior to the start of pollination or after seed harvest. Flowering begins in approximately mid June. Insecticides (primarily for *Lygus* control) and other pesticides are applied prior to bee release to avoid insecticide damage to the bees. At approximately 50 percent flower (ca. early to mid July), cultured bees are gradually moved into the seed field for pollination with their domicile or hive for local shelter. The field is actively pollinated for approximately one month, allowed to ripen seed for approximately four more weeks, and then, chemically desiccated or swathed several days prior to combining the seed. At the end of the pollination period and just prior to desiccation, the pollinating generation of bees is either at the end of their lifecycle (i.e., leafcutter or alkali bees) or are transported by the honeybee keeper to a different location to forage on fall-flowering plant species. Seed is harvested in mid August to late September depending on geography. In long-growing season regions, the cool-season alfalfa forage growth between seed crops is sometimes mechanically harvested or grazed.

Usually, stands of alfalfa grown for seed production only are maintained an average of three production seasons. Seed production contracts and AOSCA variety certification standards generally predetermine the length of the seed stand. Because most seed production is planted in widely spaced rows and are not cut monthly, relative to forage stands, weeds in seed fields have more time and open area to proliferate and compete with the alfalfa. Therefore, weeds (and insect) pests are intensively managed in seed production systems. Weed seeds and weed debris in grower seed lots directly reduce the purity and yield of alfalfa seed and drive up growers' costs to remove them. The presence of prohibited weeds, such as dodder (*Cuscuta* spp.) makes the alfalfa seed very costly to condition and or not saleable.

In 2002, alfalfa seed was grown only on 110,600 acres on 1,234 farms, whereas, the numbers for forage production were 23 million acres and 400,000 (USDA NASS 2002)(Note: 2002 is the most recent year for which NASS alfalfa seed production full census data are available). In the past 10 years, the alfalfa seed production industry has consolidated and the number of alfalfa variety developers (breeding companies) is small relative to those for other U.S. field crops. Due to the specialized expertise, high-cost of perennial, tetraploid breeding programs and in-part due

to a *conventional* alfalfa variety developer's limited ability to protect their proprietary variety from unauthorized increase and sale via common seed channels, there are a small number of alfalfa variety developers. One reason for the small number of variety developers is that, unlike most other crop breeding systems, in conventional alfalfa cultivars, there are no unique, true-breeding genetic markers to help ensure intellectual property, patent rights or plant variety protection rights are respected by unauthorized seed producers (i.e., alfalfa varieties cannot be "fingerprinted" like corn inbreds). Common, variety not stated and or "brown bag" seeds are seed market channels through which unauthorized producers sell improved germplasm of named proprietary varieties without variety developer authorization or knowledge. The unique, event-specific genetic sequence of transgenes will allow genetic fingerprinting of germplasm lines in alfalfa and thereby allow a means to certify cultivar origin, patent rights and trait stewardship. In 2006, there were only four major alfalfa seed companies that developed AOSCA registered varieties *and* produced seed: Forage Genetics International, Pioneer Hi-Bred International, Cal/West Seeds and Dairyland Seeds. In the past five years (2003-2007), in addition to these four companies, approximately three other seed companies and two public institutions have developed and registered new alfalfa varieties (http://www.NAAIC.org : NAMLVRB annual lists). In total, there are approximately 60 independent U.S. alfalfa seed companies/conditioners (primary processors) among which FGI is the largest company; approximately 29 percent of U.S. seed production acreage is produced under an FGI seed contract (Fitzpatrick et al. 2007b).

The AOSCA National Alfalfa and Miscellaneous Legumes Variety Review Board (AOSCA 2010b) lists the following companies and describes the varieties for each company, Cal/West Seeds (22 varieties), Dairyland Seed Co. (19 varieties), FGI (20 varieties), Pioneer (4 varieties), Legacy Seeds (2 varieties), and the following companies/research groups each with 1 variety, Claborn Hay Company, Cornell University, Great Plains Research Company, Inc., New Mexico Agricultural Experiment Station, S&W Seed Company. The Organic Materials Review Institute (OMRI) lists 25 varieties of organic alfalfa seeds from the following 8 companies, Welter Seed and Honey Production, High Mowing Organic Seeds, Johnnys Selected Seeds, Oasis Organics, Blue River Hybrids, Great Harvest Organics, Albert Lea Seed House, Williams Hudson bay Farm (OMRI 2010). Many of the organic seeds are for sprouts. None of the varieties listed by AOSCA or OMRI are glyphosate tolerant. Out of the 65 varieties listed by AOSCA from the 4 largest seed producers it is unclear how many of those varieties would be offered with the glyphosate-tolerant trait and then no longer offered in the non-GT form. Presumably the companies would make the decision to offer a particular variety with a set of traits based on market demand.

3.3.2 Seed Certification Value And Standards

Certified cultivar alfalfa seed commands a higher price than uncertified seed; the price of certified seed is also relatively more stable over years. During the 10-year period, 1996-2005, the average and range of retail prices paid by U.S. farmers for certified and uncertified alfalfa seeds were, respectively, $2.82 ($2.77-$2.91) versus $1.76 ($1.57-$2.05) (USDA-NASS 2006). Hay producers buying certified seeds pay a 60 percent premium over non certified seeds. Seed price is approximately 2 to 3 percent of the forage stand establishment cost.

Gene flow between alfalfa populations is a natural occurrence and bee-mediated cross-pollination among plants within a cultivar is necessary for commercial seed production. Alfalfa seed producers use spatial isolation to separate cultivars and manage bee and pollen flow between fields of different cultivars. The minimum isolation standard for foundation and certified seed fields more than 5 acres in size is 600 and 50 ft, and for fields 5 acres or less, the standard is 900 and 165 ft, respectively [Association of Official Seed Certifying Agencies (AOSCA), 2003]. State seed certifying organizations that are members of AOSCA may adopt the same or more stringent local standards for certified alfalfa seeds. For example, Idaho Crop Improvement Association (2007) requires a greater isolation distance (900 ft) than AOSCA between certified seed fields when one field is conventional and the other is of a genetically modified type (e.g., Roundup Ready). In addition to field isolation, certified seed production applies standards for field history, known genetic origin of the stockseed and in-crop volunteer control to maintain a variety true to type. Variety certification is distinct from organic certification of seeds: variety certification is a product-based certification wherein specified tolerances for off-types and impurities are recognized. USDA organic certification is a process-based certification only; organic seeds or products are not certified according to genetic purity. In a web search of organic seed suppliers, none were found that offered organically grown seeds of a certified variety (FGI: August 14, 2007).

Production of GT alfalfa seed requires a specific contract from FGI or that the grower must be an FGI authorized FGI seed contractor. FGI's Best Practices, described in more detail in section IV.B.3 and appendix V.2, are a part of the mandatory stewardship program for licensed GT alfalfa seed growers.

In 2006, FGI initiated a seed quality assurance program to determine the efficacy of the FGI Best Practices in commercially grown GT alfalfa seed. Data from this trial found that adventitious presence of the GT alfalfa trait in FGI conventional seed lots occurred infrequently and, if detected, was at a low-level from 0.004 - 0.180 percent. Specifically, low level gene flow via pollen/pollinators ranged from 0 to 0.18 percent and commixture during seed conditioning was not detected when FGI Best Practices were implemented for field isolation and at the seed conditioning facility. The amount of pollen-mediated gene flow was found to be related to the species of bee used for commercial pollination and isolation distance (Fitzpatrick 2007a Additionally only FGI conventional seed lots were tested for the trait.) More information on testing for the efficacy of BPM programs can be found in appendix V, 5.8.1.

3.3.3 Seed Purity Standards and Requirements

Alfalfa farmers follow State mandated certification standards when cultivating seed crops. These standards vary based on the State and the level of certification desired, and outline guidelines for the management of seed crops. The Association of Official Seed Certifying Agencies (AOSCA) is an overview body that assists these State-level offices in producing, identifying, distributing, and promoting various classes of seed. The AOSCA provides the alfalfa seed certification standards for each State to the public (www.aosca.org). These are based on the Federal Seed Act (7 CFR part 201), which is as follows (only standards for alfalfa and applicable footnotes are shown)(http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=b2641469d5186de8667eaef9eb 58e654&rgn=div8&view=text&node=7:3.1.1.7.27.0.327.109&idno=7):

Title 7: Agriculture
PART 201—FEDERAL SEED ACT REGULATIONS
Certified Seed 4.2.1.1.1
§ 201.76 Minimum Land, Isolation, Field, and Seed Standards.
In the following table V-5 the figures in the "Land" column indicate the number of years that must elapse between the destruction of a stand of a kind and establishment of a stand of a specified class of a variety of the same kind. A certification agency may grant a variance in the land cropping history in specific circumstances where cultural practices have been proven adequate to maintain genetic purity. The figures in "Isolation" column indicate the distance in feet from any contaminating source. The figures in the "Field" column indicate the minimum number of plants or heads in which one plant or head of another variety is permitted. The figure in the "Seed" column indicates the maximum percentage of seed of other varieties or off-types permitted in the cleaned seed.

Table V-5. Minimum Land, Isolation, Field and Seed Standards (7 CFR § 201.76)

	Foundation				Registered				Certified			
Crop	**Land**	**Isolation**	**Field**	**Seed**	**Land**	**Isolation**	**Field**	**Seed**	**Land**	**Isolation**	**Field**	**Seed**
Alfalfa												
Nonhybrid	4[1]	600[44,48] (182.88m[59])	1,000	0.1	3[1]	300[3,44,48] (91.44m[59])	400	0.25	1[1,2]	165[44,49] (50.29m[59])	100	1.0
Hybrid	4[1]	1,320[43] (402.34m[59])	1,000[42]	0.1					1[1,2]	165[3,43,44] (50.29m[59])	100[42]	1.0

[1]The land must be free of volunteer plants of the crop kind during the year immediately prior to establishment and no manure or other contaminating material shall be applied the year previous to seeding or during the establishment and productive life of the stand.
[2]At least 2 years must elapse between destruction of indistinguishable varieties or varieties of dissimilar adaptation and establishment of the stand for the production of the Certified class of seed.
[3]Isolation distance for certified seed production shall be at least 500 feet (152.07m) from varieties of dissimilar adaptation.
[42]The ratio of male sterile (A) strains and pollen (B or C) strains shall not exceed 2:1.
[43]Parent lines (A and B) in a crossing block, or seed and pollen lines in a hybrid seed production field, shall be separated by at least 6 feet (1.83m) and shall be managed and harvested in a manner to prevent mixing.
[44]Distance between fields of certified classes of the same variety may be reduced to 10 feet (3.05m) regardless of the class or size of the fields.
[48]This distance applies for fields over 5 acres (2ha). For alfalfa fields of 5 acres (2ha) or less that produce the Foundation and Registered seed classes, the minimum distance from a different variety or a field of the same variety that does not meet the varietal purity requirements for certification shall be 900 feet (274.32m) and 450 feet (137.16m), respectively.
[49]There must be at least 10 feet (3.05m) or a distance adequate to prevent mechanical mixture between a field of another variety (or non-certified area within the same field) and the area being certified. The 165 feet (50.29m) isolation requirement is waived if the area of the "isolation zone" is less than 10 percent of the field elig ble for the Certified class. The "isolation zone" is that area calculated by multiplying the length of the common border(s) with other varieties of alfalfa by the average width of the field (being certified) falling within the 165 feet (50.29m) isolation. Areas within the isolation zone nearest the contamination source shall not be certified.
[59]Indicates metric equivalent in meters.
[59 *FR* 64516, Dec. 14, 1994, as amended at 65 *FR* 1710, Jan. 11, 2000]

California, the largest producer of seed alfalfa, outlines typical guidelines for field eligibility (past use and spatial isolation) and seed purity standards (http://ccia.ucdavis.edu/seed_cert/alfalfa_seedcert_standards.htm). For cultivating Foundation seed, seed of the highest purity, alfalfa must not have grown on the land in the previous four years, and for Certified seed, one to two years, depending on the intervening crops. All volunteer plants and noxious weeds must be eradicated and definite boundaries to the field set before field use. Foundation seed fields must be isolated from alfalfa of different varieties by 900 feet, while Certified fields must be isolated

by 165 feet. However, the 10 percent rule is followed for Certified Seed fields, where if 10 percent or less of the Certified Seed field is in the 165 foot isolation zone, the entire field is considered Certified, but if more than 10 percent is in the isolation zone, then that part of the field must be separated and not harvested as Certified seed. These rules are followed by most States.

Seed purity standards vary slightly across States, but remain high, especially for Foundation seed stock. As apparent in table V-6, which shows seed purity standards for California, Idaho, Wisconsin and Montana(http://www.idahocrop.com/standards.aspx)(http://www.wisc.edu/wcia/2008StandardW.pdf)(http://ag.montana.edu/msga/Seed%20Standards/alfalfa%20standards.pdf), at least 99 percent of each seed harvest must contain the pure seed variety, and there are strict limits on the allowable amounts of other crops, weeds and inert matter as well. After seed crops have been evaluated by seed labs, they are tagged with seed labels in accordance with law. Seed labs perform multiple tests mandated by the AOSCA on a representative sample from each submitted crop. All types of seed crops are accurately labeled, while Foundation and Certified seeds are denoted with a special tag. Information on the tag includes variety, kind, origin, net weight, percent pure seed, percent other materials, amount of noxious seed and weeds, and the information of the company performing the analysis (figure V-3).

FGI has validated their Best Practices for seed production and believes they can produce seed reliably with >99.5 percent purity (FGI 2007). To put this in context, one seed in 200 could be for an off-variety, such as GT alfalfa in conventional alfalfa. Assuming that the contaminating variety has the same germination and fitness as the certified variety, then one can calculate the number of plants in an acre that would be off-type.[5] A thriving alfalfa hay field can have 15 plants per square foot (Orloff et al. 1997), which equals 653,400 plants in an acre; 0.5 percent of 653,400 is 3267. If the contaminating variety is mixed evenly in the seed batch, then there might be an off-type plant every 13.3 square feet. In older stands where plant density may be closer to 5 plants per square foot, there might be an off-type plant every 40 square feet. FGI's 2000 to 2002 field studies, which assayed 30,000 seedlings, detected 0.000 percent gene flow (99.9 percent confidence interval is 0.01 percent), 0.01 percent contamination is 1 seed in 10,000, which is 1 plant in 667 square feet at a stand density of 15 plants per square foot.

[5] Only 60 percent of the seeds germinate and emerge and only 40 percent of emerged seedlings survive the first year (Orloff et al., 1997).

Table V-6. Seed Purity Standards by State[6 7 8 9]

State	Type of Seed	Pure Seed (Min percent)	Other Crops (max percent)	Other Varieties (max percent)	Other Material (max percent)	Isolation Distance, < 5 acres (ft)
California	Foundation	99.5	0.1	0.1	0.5	900
	Certified	99.5	0.1	0.2	0.5	165
Idaho	Foundation	99	0.1	0.0	1.1	900
	Registered	99	0.1	0.0	1.2	450
	Certified	99	0.25	1.0	1.25	330*
Wisconsin	Foundation	99	0.2	0.1	0.85	900
	Certified	99	0.75	0.25	0.95	165
Montana	Foundation	99.5	0.1	0.1	0.6	1320
	Registered	99.5	0.1	0.25	0.7	660
	Certified	99.5	0.1	1.0	0.8	330

*GMO from non-GMO: 900 ft

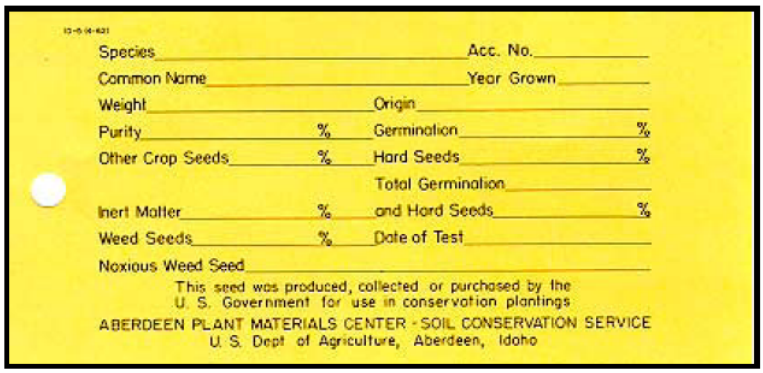
Species _____ Acc. No. _____
Common Name _____ Year Grown _____
Weight _____ Origin _____
Purity _____ % Germination _____ %
Other Crop Seeds _____ % Hard Seeds _____ %
Total Germination
Inert Matter _____ % and Hard Seeds _____ %
Weed Seeds _____ % Date of Test _____
Noxious Weed Seed _____
This seed was produced, collected or purchased by the U. S. Government for use in conservation plantings
ABERDEEN PLANT MATERIALS CENTER - SOIL CONSERVATION SERVICE
U. S. Dept of Agriculture, Aberdeen, Idaho

Figure V-3. Example of a seed tag (Hoag et al. 2002)

3.3.4 Proprietary and Public Cultivar (Variety) Conventional Alfalfa Seed Production

High quality, high value and genetically pure alfalfa seed production requires specialized equipment, pollinators, isolation, climate and unique crop management practices that are not used for forage production. The majority of alfalfa seed tonnage is produced by large professional, specialized alfalfa seed growers using irrigation under multi-year seed company contracts primarily within seven western states (California, Idaho, Nevada, Oregon, Washington, Montana and Wyoming). For example, in 2002, 54 percent of the U.S. seed crop was grown by the 98 large-scale seed growers who harvested greater than 250 acres each (NASS 2002). The smaller-scale seed growers (< 49 acres each), in total, produced only seven percent of the U.S.

[6] http://ag.montana.edu/msga/Seed%20Standards/alfalfa%20standards.pdf
[7] http://www.idahocrop.com/standards.aspx
[8] http://www.wisc.edu/wcia/2008StandardW.pdf
[9] http://ag.montana.edu/msga/Seed%20Standards/alfalfa%20standards.pdf

seed crop. For the past 20 years, most of the U.S. alfalfa seed crop was produced by registered variety name (known seed source), within official State field isolation inspection programs and under contract for a relatively small number of seed companies that develop or produce proprietary varieties. Less than ten alfalfa variety breeders (companies and public institutions) have officially registered >95 percent of the AOSCA registered proprietary alfalfa cultivars during the past 20 years (NAAIC-NAMLVRB 2007). Of the more than 250 listed alfalfa cultivars, fewer than ten public varieties have been released in the past 20 years. The majority of the U.S. alfalfa seed crop is of AOSCA registered proprietary cultivars that are intensively managed by contracted seed growers, plantings are grown to optimize alfalfa seed yield and seed quality using sufficient field isolation, in-crop volunteer control and field history to minimize genetic off-types due to unintended gene flow between conventional cultivars (e.g., using AOSCA field isolation standards (AOSCA 2003)). Seed of proprietary alfalfa varieties grown to meet official certification standards typically commands a 300 percent higher market price than common alfalfa seed (US Trade Office statistics for export 2005). Alfalfa seed companies contracting seed production of a proprietary named variety require that the seed grower plant a company-supplied stockseed lot of the variety (e.g., foundation class stockseed). Seed growers of certified seeds of public varieties must use foundation seed lots for the variety grown.

3.4 Common and Organic Seed Production

Depending on the relative current prices of hay versus seed and amount of rainfall, a highly variable amount of "common" seed is intentionally produced on converted (un-harvested) forage fields or seed fields grown without variety identification, isolation requirement or official seed certification oversight. Common seed, including catch-crop seed and grower-saved seed, is not regulated. Common seed sold inter-state or internationally is subject to phytosanitary regulations (e.g., absence of prohibited weed seeds, diseases and insect, etc.) but cultivar genetic identity or genetic purity cannot be certified.

Organic seed is certified to have been organically produced, but very little if any organic-certified seed is available that is also variety-certified (most organic seed is of common or uncertified varieties sold under a brand name, not by registered variety name). Seed sold under brand name only without a variety name or of an unregistered variety name cannot be variety-certified.

Common seed production occurs mainly in marginal, dryland, hay/seed production geographies (e.g., portions of western South Dakota, North Dakota, western Kansas, western Nebraska, Oklahoma and the Imperial Valley of California, etc.) as a "catch crop" in years of seed shortage (higher seed price), low hay price and or drought sufficient to limit hay yield or feed quality. Most catch crop hay/seed growers are relatively low-input growers and they may or may not apply cultured pollinators to the field and consequently, seed yield and seed quality can be very, very low on catch crop seed. For example, mean seed yield in Nebraska and Kansas was 72 and 100 lbs per acre (1988-1992) versus greater than 500 lbs per acre for states in prime seed growing states (Hower et al. 1999).

Roundup Ready alfalfa seed may not be legally grown as common seed (appendix V-2). In locations of common seed production, Roundup Ready forage producers are obligated to cut

their forage fields at or before 10 percent bloom as a means to mitigate gene flow to conventional common seed fields; see appendix V-2 and terms of Monsanto Technology/ Stewardship Agreement (Monsanto 2007). Additionally, farmers growing GT alfalfa for seed are required to respect any non-GE alfalfa seed production zone designated as such by a consensus of local seed growers (NAFA 2008d).

In a survey conducted to gather information on harvesting timing of GT alfalfa, surveyed farmers reportedly harvested 82 percent of their total GT alfalfa crops at or before 10 percent bloom. Farmers harvested 14 percent of their crops at 11 to 21 percent bloom, while 4 percent of the fields were harvested at 21 percent bloom or later. For the 18 percent of crops harvested after 10 percent bloom, farmers reported bad weather as being the cause for this delay 24 percent of the time (Market Probe 2010). The effect of late harvesting on gene flow to non-GT alfalfa is not known.

Although a small amount of organic alfalfa seed is purchased in the United States from U.S. seed distributors, little or none of the organic alfalfa seeds appear to have been originally grown in the United States (McCaslin 2007 declaration). A National Organic Program Standards exception allows organic forage producers to establish their fields using non-organic (conventionally grown) seeds if their chosen cultivar or cultivar type is not available to them as organic, and after 3 years of adhering to an organic production plan, old hay fields previously managed/planted as conventional may be "converted" into the organic program for organic forage and or possibly for attempted organic seed production. Also, there is an exception for very small organic producers with gross annual revenue of less than $5,000; no NOP certification is required for the small producer to garner official certification to label their hay or seed product as organic. It is possible that a low number of economically small organic producers are supplying some organic alfalfa seed to U.S. organic hay producers and because no certification of any type is required, the organic seed and acres used to produce it are unaccounted for in USDA-AMS market census statistics (the volume of seed and the location of production is not officially known). Therefore, although approximately 0.9 percent of U.S. alfalfa acreage is certified as organic (USDA 2005), only a small fraction of these acres were ever established using organic alfalfa planting seed; i.e., because it is not a requirement and there is little organic seed available. Organically certified and conventionally grown seed lots are routinely marketed to U.S. organic forage producers for the establishment of organic alfalfa forage fields (there are numerous examples on seed company Web sites).

Across the western United States, *Lygus* species bugs are a very serious insect pest on alfalfa seed production (Hower et al. 1999). In California and the Pacific Northwest, the principle U.S. alfalfa seed production regions, virtually every acre of alfalfa seed production is treated with insecticides one or more times each year to control Lygus bugs and other insects, and with herbicides to control weeds. In most years and in most fields in the West, complete alfalfa seed crop failure will result without chemical control of Lygus. For this reason, organic alfalfa seed production is not economically viable in the prime western U.S. seed growing region.

Organic alfalfa seed sold in the United States by U.S. seed companies is therefore most likely to have been wholly or largely imported from organic producers in Canada or elsewhere, where insect pests in alfalfa seed production are less catastrophic and base production costs for seed are much lower (McCaslin, 2007 declaration). After importation from the country of origin, the seeds may be packaged by a U.S. seed company prior to sale to U.S. organic forage growers; most of this seed is raw or treated only with NOP approved seed treatments and approved *Sinorhizobium* inoculant formulations.

4.0 Gene Flow: Extent, Mitigation, Industry Stewardship and Coexistence Strategies in Cultivated and Noncultivated Settings

Gene flow is a measure of exchange of genes between populations, and it may occur among and between various alfalfa populations growing in cultivated and noncultivated environments. Therefore, the discussion of alfalfa gene flow is presented between the cultivated and noncultivated environment sections of this document as it applies to both environments. In plants this can occur through outcrossing or mating between two different individuals. Some plants primarily self-pollinate, which minimizes gene flow because genetic information is less commonly exchanged between individuals. In contrast, other plant species such as alfalfa mostly outcross, and therefore face the possibility of gene flow between populations. Alfalfa very rarely self pollinates due to self-incompatibility resulting in failed germination of viable pollen or abnormal pollen tube growth (Bauchan et al. 1990). As a consequence, successful pollination and seed production in alfalfa production relies on insects for pollination.

The concern is how much gene flow is occurring between populations of GT alfalfa and non-GT alfalfa. Some farmers may want very low (<0.5 percent) or zero presence of the GT trait in their crops in order to meet the needs of their customers. Because of cropping scale and plant density, the opportunity for gene flow will be greater in cultivated settings than in noncultivated settings (Van Deynze et al. 2008).

There are several key factors that influence the probability of successful gene flow between alfalfa populations (adapted from Putnam 2006):

- Flowers: Presence or absence; synchronous flowering between populations; flowering duration; relative abundance of in-field and non-field pollen sources.
- Pollinators: Presence or absence; pollinator species; pollinator density; climatic and landscape barriers to pollinator movement; pollinator cultivation practices (hive placement).
- Proximity: Physical distance between fields (alfalfa populations) that mitigates pollinator movement, wind-blown pollinators, animal, water, wind or agricultural transfer of seeds between populations.
- Population Size: differences in the size of source and sink populations.
- Probability of seed maturation: Sufficient time for embryo development following pollination; effects of weather conditions on seed survival.
- Probability of seed germination: Dispersal of seeds beyond parental plant autotoxicity; successful germination of seeds.
- Cultivar selection and cultural practice: Stand renovation and crop removal; crop rotation; seed or hay harvest timelines; equipment cleaning and planting seed selection; product handling and segregation; gene frequency in local cultivars; etc.

An in-depth white paper review of gene flow potential and impacts in alfalfa was sponsored by The National Alfalfa & Forage Alliance (NAFA) (Van Deynze et al. 2008). The publication systematically addresses gene flow according to the population interface type, i.e., for gene flow to/from seed fields, to/from hay fields and to/from feral populations—in a total of nine possible combinations as shown in table V-11 (excerpted from Van Deynze et al. 2008). NAFA also sponsored the development of four additional topic papers that were written to assist the alfalfa industry in understanding and managing GM-trait coexistence. Within each topic paper, alfalfa gene flow is discussed with respect to impact and mitigation measures. The four topics are: (1) export hay, (2) export seed, (3) organic alfalfa, and, (4) Best Practices for Roundup Ready Seed Production (see above sections). These topic papers and the gene flow white paper are available from the NAFA Web site http://www.alfalfa.org.

Table V-7. Potential Scenarios for Pollen-mediated Fene Flow in Alfalfa

↓From To→	Hay	Seed	*Feral*
Hay	Hay-to-Hay	Hay-to-Seed	*Hay-to-Feral*
Seed	Seed-to-Hay	Seed-to-Seed	*Seed-to-Feral*
Feral	*Feral-to-Hay*	*Feral-to-Seed*	*Feral-to-Feral*

Source: Ven Deynze et al., (2008)

4.1 Cultural Practices, Trait Stewardship Tools and Biology Influencing Gene Flow in Alfalfa Systems

As presented above, alfalfa is grown exclusively for livestock forage production purposes on over 22 million acres annually. Greater than 99.5 percent of alfalfa planted in the United States is used exclusively for alfalfa hay (forage) production (figure V-1). Approximately 110,000 acres or, 0.5 percent of the total U.S. alfalfa acres are harvested for seed production (figure V-2).

Alfalfa is a herbaceous, short-lived perennial forage crop species that is predominantly cross-pollinated and entirely dependent upon bees for cross-pollination. Wind cross-pollination in alfalfa does not occur (Teuber 2007b; Viands et al.1988). Insect (bee) pollinator activity is required to simultaneously deposit non-self pollen onto the stigma surface and rupture the protective stigmatic cuticle and effect pollination (Viands et al. 1988). Alfalfa flowers have an explosive tripping mechanism that may be 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. While true embryos are found in developing alfalfa pods approximately five days after fertilization (Bass et al. 1988), it takes approximately four to six additional weeks for embryos to ripen to become physiologically mature seed under optimal seed production conditions. High winds and or drought stress may induce flowers to mechanically trip and auto-pollinate; however, it is known that the resulting self-pollination (inbreeding) most commonly results in no seed or few seed and progeny of inferior vigor (Viands et al. 1988).

Flower buds begin to form on stems approximately four to six weeks after field mowing during long-day photoperiods and warm weather. After flowering ensues, alfalfa flowers indeterminately.

After pollination, alfalfa seed embryos require four to six additional weeks of adequate growing conditions to ripen. Rainfall or snow during the ripening time will cause decreased seed yield (seed shatters from the pod to the ground) and poor seed quality (e.g., reductions in seedling vigor and reduced percent germination because of fungal pathogen infection of the seed, or seed will sprout prematurely and die while it is still in the pod). Commercial production of the alfalfa seed crop, therefore, is almost exclusively confined to the western regions of the United States where late season (post-pollination) rain is unlikely, irrigation is carefully managed and specialized alfalfa seed growers, equipment and infrastructure is available (figure V-2). Mean seed yield per acre is greatest in the Pacific Northwest and California (approximately 500-750 lb/A) and very low in the Plains states (70-200 lb/A) (Hower et al. 1999).

The potential for gene flow between alfalfa populations is dependent upon many factors and it is closely tied to agronomic management practice (whether each of the populations or fields are managed for forage production, for seed production, or, if the population is without management, e.g., feral or neglected). Alfalfa is also used for composting, as organic alfalfa meal fertilizer, as green manure cover crop, as part of a long term soil building program, or as a cover crop left to flower to attract beneficial insects. Alfalfa used for these applications has the potential to serve as sources of alfalfa seeds. Feral populations of alfalfa would also need to be managed to mitigate the potential for gene flow to and from the plants. Numerous environmental filters that mitigate the risk of gene flow between alfalfa populations growing in various alfalfa settings were described by Daniel Putnam, University of California, Alfalfa and Forage Extension Agronomist (e.g., Putnam 2006 and 2007), and, Larry Teuber, Ph.D., University of California, Professor of Plant Breeding and Agronomy, Director of the California Foundation Seed Program and Executive Director of the California Crop Improvement Association (Teuber 2007b). These experts are authorities in alfalfa forage and seed production and breeding systems. In addition, superimposed over the environmental filters are agricultural filters to further mitigate the risk of gene flow. Agricultural filters include customary agronomic best management practices, such as crop rotation, field separation (isolation), equipment sanitation, planting certified variety seeds of known origin, or in some cases, contract-specified identity preservation, or trait stewardship practices (e.g., grower licensing requirements for use of seeds containing GM traits).

4.2 Flowering Time

Flowering of seed fields and hay fields may not be synchronized, depending on mowing schedules, inputs (irrigation, fertilizer), and cultivar. For cross-pollination to occur, the flowers must be blooming at the same time. Within a field this is fairly simple to synchronize because the seeds are planted at the same time and varieties are bred to have low variability in germination time within varieties.

Alfalfa flowers for an amount of time that is dependent on factors such as region where it is grown, management practices and the species of pollinators used. In general, alfalfa will bloom longer and open flowers will accumulate when fewer pollinators are present, but there will be a decrease in standing crop of open flowers in the presence of many pollinators. After the alfalfa has been pollinated, it no longer devotes energy to flower production, but instead diverts energy to fruit development. This is evidenced by the fact that after pollination, nectar production

ceases and wilting occurs in about 4 hours at warm temperatures, or 24 hours at cool temperatures (Strickler and Vinson 2000). Different pollination management strategies will affect the blooming duration for alfalfa. For example, in regions where alfalfa leafcutter bees are used, they are released quickly after bloom and large numbers are released at once (up to 52,000 females per hectare). This would result in a short duration of open alfalfa flowers. In regions where honey bees are used for pollination, such as California, fewer bees are used and pollination occurs at a more moderate pace, leading to a longer bloom time for alfalfa (Strickler and Freitas 1999)

In a study in Idaho (Strickler and Freitas 1999), alfalfa leafcutter bees were released when the alfalfa had started blooming, and because the bees need a couple of weeks to establish their nests, stabilize themselves, and reach constant rates of foraging (Bosch and Kemp 2005), the amount of flowers reached a peak two weeks after bee release. After this point and once the pollinators began steadily foraging, the amount of flowers decreased exponentially and moved to seed production. Twenty-five percent of the peak bloom was present four weeks after pollinator release. This pattern was also observed in fields in Oregon (Bosch and Kemp 2005), where flowers accumulated for the first three weeks of the study, and by week four, one to two weeks after alfalfa leafcutter bee release, the standing crop of flowers decreased rapidly.

Hay fields are treated differently than seed fields, and are typically cut when the field is at one-tenth bloom. About four weeks are allowed to pass between cuttings, to allow fields to reach one-tenth bloom again. Figure V-4 shows one possible typical pattern of alfalfa blooming for seed fields, including pollinator introduction (alfalfa leafcutter bees) and seed formation, with hay field bloom and cutting overlaid. If these two fields were geographically close enough that pollinators could visit both fields, the potential for pollen exchange would only occur when the curves overlap. In figure V-4 the hay and seed field start synchronized but flowering becomes out of phase as cutting commences in the former but not the latter. The figure illustrates the relatively short amount of time that hay and seed field bloom would overlap in such a scenario where the hay field is harvested at 10 percent bloom.

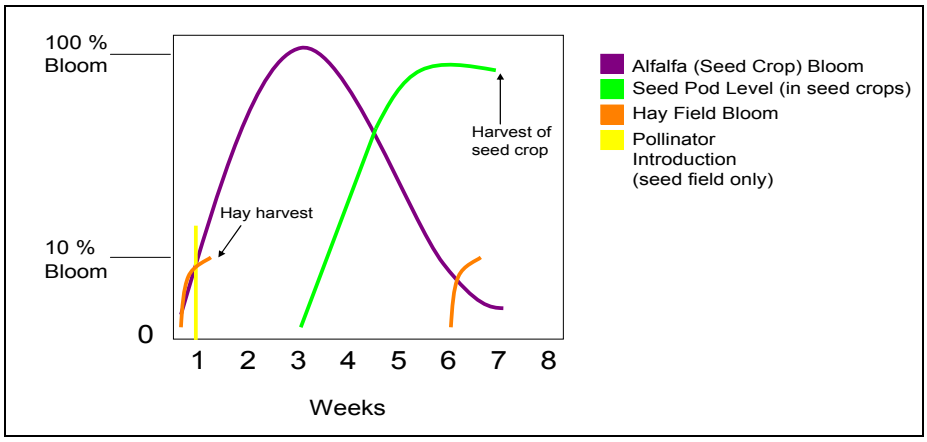

Figure V-4. Flowering overlap between hay and seed fields

4.3 Pollinators of Alfalfa

In most alfalfa seed-growing areas, naturally occurring populations of those species of bees capable of tripping and producing cross-pollination are either nonexistent or in such low number that commercial production of seed alfalfa would be impossible without the deliberate production, introduction, and management of certain bee species to provide adequate pollination (Arnett 2002). Professional alfalfa seed growers use at least one of three species of bees for pollination. They are the honey bee (*Apis mellifera*), the alfalfa leafcutting bee (*Megachile rotundata*), and in very small niche geographies with scattered alkali beds (e.g., Touchet, WA) the alkali bee (*Nomia melanderi*) is used often heavily augmented with cultured leafcutting bees. Feral and native bee species are present in low numbers and pollinate alfalfa. In addition to alkali, leafcutters, and honeybees, Hammon et al. (2007) studied alfalfa fields in Colorado, and collected data on the insects observed visiting the alfalfa. That list is in table V-8, in order of abundance, followed by estimated ranges collected from various sources. In general, the alfalfa leafcutter bee is the preferred pollinator for alfalfa, but it is not used widely in the warmer regions of the United States because it has a low heat tolerance (Rogan and Fitzpatrick 2004). Bee habits vary based on range and climate. In general, bees will forage where they need to in order to collect pollen and nectar. If there is an abundant source of both close to the nest, then their average foraging distances will be lower than if the bees must forage further to find adequate pollen and nectar. Honey bees, alfalfa leafcutter bees, and alkali bees will increase their foraging distance as the distance to high-reward resources (high nectar and pollen amounts) increases, and as closer resources become scarce. Patchiness of the environment also affects this foraging distance, as bees can prefer certain flowers over others (depending on the species and season), and might need to travel through or around obstacles in the environment, so they will adapt foraging habits accordingly (Greenleaf et al. 2007).

Table V-8. Pollinator Foraging Distance

Pollinator (*Species*)	Forage Distance from Nest
Alfalfa leafcutter bee *(Megachile rotundata)*	~1 mile (Pitts-Singer, 2008)
Honey bee *(Apis mellifera)*	Measured up to 6.21 miles away, average distance depends on availability of pollen/nectar, typically 1.86 miles (Beekman and Ratnieks, 2000)
Alkali bee *(Nomia melanderi)*	Will forage up to 4 or 5 miles away; typical pollination within 2 mile radius (USGS, 2008)
Long-horned bee *(Melissodes sp)*	Data not found
Mud/Digger bee *(Anthophora spp)* (2)	Data not found
Bumblebee *(Bombus morrisoni)*	Data not found
Bumblebee *(Bombus griseocullis)*	Data not found
Sweat bee *(Lasioglossum sisymbrii)*	Data not found
Sweat bee *(Halictus tripartitus)*	Data not found
Sweat bee *(Halictus confusus)*	Data not found
Leafcutter bee *(Megachile texana)*	Data not found
Mason bee *(Osmia latisulcata)*	Data not found

Cane (2002) performed a study on the primary pollinators of alfalfa and measured their pollination effectiveness. Alfalfa has a mechanical trip for pollination, where once the bee lands

on the flower, the mechanical pressure releases the stamen and pistils, which then fly up and either deposit pollen on the bee's underside or picks up pollen if the bee has already visited another alfalfa flower. This is an irreversible process, so one visit is enough for pollination to occur. However, because honey bees dislike getting hit by the flower, they often cheat by approaching the flower from the side to get nectar, which does not pollinate the flower. This accounts for the varying pollination rates of different bee species. Females of the alfalfa leafcutter bee and the alkali bee are the most effective pollinators, tripping 81 and 78 percent of flowers visited, respectively. Males of these species are less effective, with efficiencies of 61 and 51 percent, respectively. Honey bees trip only 22 percent of flowers visited.

Agricultural farmers manipulate bee colonies to control pollination of their crops. Alfalfa farmers purposely stock bees only in seed farming, as they do not want pollination of hay fields, and because most regions that cultivate alfalfa seed do not have naturally occurring populations of alfalfa pollinators, farmers must produce, introduce and manage these pollinators to ensure adequate pollination (Rogan and Fitzpatrick 2004). Alfalfa leafcutter bees, a typical managed species, are established next to the target crop of alfalfa. The release of these bees can be controlled by directing incubation temperatures, which signal the bee developmental processes. Farmers judge when blooming of the crop will be at a peak, and will time the incubation and subsequent release of the bees accordingly. See section 3.4 for discussion of the interaction between flowering time and pollination, for both hay and seed fields. Once the released bees have completed their season and die, the larvae laid throughout the season are collected and cleaned, then stored at a temperature that keeps them in a pupating stage until the desired release time next year. Honey bee colonies, however, are moved from field to field as they are highly portable and stay with their queen. They also tend to forage at much larger distances, and this increases the chances of pollen being spread from one location to another. Unintentional and wide dispersal of bee populations is possible when farmers deploy large amounts of bees at their fields, and competition for resources is increased (up to 40,000 to 60,000 leafcutter bees are typically used for pollinating alfalfa seed crops) (USDA 2009). Table V-10 presents factors that influence bee activity (adapted from Greenleaf et al. 2007, Strickler and Vinson 2000).

The effects of flower number per plant and degree of flowering in a population on the relative rate of gene flow from alfalfa is not known. However, given the interaction between synchronous flowering and pollinator behavior, it is unlikely that the amount of flowering and potential for gene flow is a linear relationship, complicating gene flow predictions based on percent flowering. As summarized by Bagavathiannan and Van Acker 2009, feral populations often have low flower abundance which can lead to high rates of cross-pollination due to pollinator foraging behavior (pollinators forage further). If there are more flowers per plant, pollinators remain in close proximity and a substantial reduction in seed/plant can occurs as a result of increased self-incompatibilty. Pollinator behavior could also contribute to reduced gene flow between fields and feral plants if the field is in high flower (relative to feral populations) as pollinator behavior will contribute to low forage distances (Bagavathiannan and Van Acker 2009 and references within). In summary, commercial fields with low flower percentage relative to feral populations that flower synchronously with feral plants within pollinator forage ranges would likely pose the highest risk for potential transgene flow out of alfalfa fields.

Table V-9. Factors That Influence Bee Activity

Factor	Description
Weather (rain, wind, cloud cover)	Honey bees do not fly in rain or in wind over approx. 15 mph, and have less activity on cloudy days
Temperature	There is a minimum temperature for bees to forage at, usually about 55ºF for honey bees
Season	Bees forage at specific times in the season based on species; some bees forage throughout the late spring, summer and early fall while others might only forage for a few weeks in the summer
Flower availability	Bees will forage further from the nest in order to reach high reward flowers
Time of day	Bees used for alfalfa pollination typically only forage during the day
Hive placement	Hives placed at the edges of fields will pollinate the field in a different pattern than if they are placed in the middle of a field
Number of bees	If there are too many bees at a field and competition is too high, bees may leave the field to establish new colonies or nests
Flower biology	The number of days flowers are in bloom can affect pollination efficacy—the longer flowers bloom, the more chances for pollination
Plant competition	Bees will preferentially forage on more attractive flowers if given the option
Strength of colony	Strong colonies (higher numbers) have more flying bees and will forage in lower temperatures than weak colonies
Disease and parasites	Infected colonies and bees will forage less than uninfected colonies and bees

4.3.1 Commercial Pollinator Species

The vast majority of certified alfalfa seed is grown under contract in the Pacific Northwest (PNW) and California where seed growers typically introduce and culture either leafcutter bees or honeybees, respectively. Certain seed growers use a blend of pollinator species to optimize seed yield and harvest date. Honey bees tend to tolerate higher temperatures than leafcutter bees. In unique, small geographies of the PNW, the ground-nesting alkali bee may used to supplement leafcutter bee pollination of alfalfa seed fields or *vice versa*. Bees are introduced and provided with nesting sites (hives, domiciles, or permanent beds) near to or within the seed field. Each of the three commercial pollinator species has different attributes regarding their culture and behavior. When resource-limited, however, bees will scout and range to greater distances but their maximum possible flight distance has not been determined.

The bees are a necessary and costly input for professional seed growers. Growers time the bee introduction release date(s) to coincide with advanced bloom (> 50%) and as bloom resources gradually increase, bee stocking rates are proportionately increased to rapidly and efficiently set the seed crop. These are routine pollinator management adjustments that help ensure that a grower's valuable bees pollinate and remain largely near their home domicile. Approximate stocking rate for leafcutter bees and honeybees ranges from two gallons of loose cell pupae or two hives per acre, respectively, to 4 or 6 gallons of bees per acre (approximately 10,000 bees per gallon; USDA-ARS 2009); and more or less are applied depending on local bee prices, density of bloom, and seed contract settlement price.

FGI Best Practices for seed growers (http://www.foragegenetics.com/News.asp) and Roundup Ready seed grower contracts stipulate the predominant pollinator species to be used, field location and bee management restrictions to be used. If the grower stocks a blend of pollinators, the grower's isolation minimum is set based on the pollinator stocked with the longest foraging range. Once bees are placed in a Roundup Ready alfalfa seed field, the grower is prohibited from moving the bee colony/domicile to a conventional seed field for the rest of the growing season.

4.3.2 Distance of Cultured and Native Pollinator Travel and or Foraging

Although maximum foraging radius for each of the three commercial bee species is dependent on the abundance (or dearth) of nectar and pollen resources, leafcutter bees are considered to have the shortest routine foraging distance (<1/4 to 1 mile) followed by the honey bee (ca. 1 to 3 miles), and the alkali bee (ca. 5 miles) (Hammon et al. 2006; Mader et al. 2010; Teuber et al. 2005b; Pitts-Singer 2008). There is evidence that 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). St. Amand et al. (2000) recorded outcrossing rates of 22.2 percent at 1000 m due to leafcutter bees, and recommended a minimum isolation distance of 1557 m to prevent gene flow between alfalfa fields.

Although they are frequently used as managed alfalfa pollinators, honey bees are mainly nectar collectors and are inefficient pollinators for alfalfa. Honey bees prefer not to gather pollen and nectar from alfalfa because, when visited, the alfalfa flower is 'tripped' and hits them (Pitts-Singer 2008). Cane (2002) determined that honey bees pollinate ("trip") only 22 percent of the flowers they visit; therefore, most of their foraging is for nectar, not pollen, and the nectar is obtained by "side feeding" without deposition of pollen or pollination. The most effective way to pollinate alfalfa with honey bees is to use large numbers of young bees that have not yet learned to avoid the plant's tripping mechanism by side feeding or by ignoring the plant altogether. Recent data suggests that honey bees may represent a higher risk of transgene escape relative to other bees, regardless of plant density (Brunet and Stewart 2009).

In addition to these three cultured bee species, low density populations of bumble bees (*Bombus* spp.) other bee species occasionally visit alfalfa to collect nectar and pollen and may sometimes pollinate alfalfa flowers but their importance in alfalfa pollination is minor (Hammon et al. 2006; Arnett 2002). Native bees, including *Bombus* spp, *Osmia* spp, and *Agapostomen* spp. and native *Megachile* spp. can be found visiting alfalfa in varying numbers. Non-cultured honey bees are not native bees, but may occur among the feral bees and although they prefer other plants, they sometimes pollinate alfalfa. Other insect pollinators have not shown to be effective pollinators of alfalfa. Hammon et al. (2006) found evidence to support that, "Most alkali bee foraging was within seed fields or local in nature," and that, "Alkali bees were probably of minor importance in long distance pollen movement." In the Hammon et al. pollinator collection survey (2006), in addition to commercial pollinating bee species, they collected low populations of nine native taxa of bees that may be short, mid and long-range pollinators of alfalfa. Most of the native bees are solitary in nature. A study of the native solitary bees of Germany by Gathmann and Tscharntke (2002) found that, "solitary bees have a rather small foraging range so local habitat structure appears to be more important than large-scale landscape structure." They also

determined that, "maximum foraging distance between nesting site and food patch was 150-600 m for the 16 bee species examined," and that, both foraging distance and mean foraging trip duration (6 to 28 min) were correlated positively with body length. Therefore, large bees such as *Bombus* spp. may be likely to travel further distances but because they occur in very low numbers and forage on flowers of many other plant species, bumble bees pose a very small risk of significant long-distance gene flow among alfalfa populations.

It is possible that wind storms could blow bees carrying GM pollen long distances; however, due to factors of scale and dilution, the risk of resultant subsequent transgene flow to other alfalfa would be extremely small. In all gene flow studies to date, in addition to the stocked pollinator, native and feral bees were present, so their potential contribution to gene flow under commercial conditions has been accounted for in the available data sets. A general recent review of principles of bee pollination may be found in *Crop Pollination by Bees* (Delaplane and Mayer 2000). The risk of gene flow at various distances for each pollinator species is discussed in detail in other sections of this document.

4.3.3 Passive Physical Pollen Transfer among Bees Leading to Gene Flow

It is theoretically possible that within colony or when in physical contact, bee-to-bee passive surface transfer of pollen could occur which could lead to a subsequent pollination event and gene flow (Mueller 2005). DeGrandin-Hoffman (DeGrandi-Hoffmann et al. 1986) tested the ability of honey bees to passively transfer pollen to nestmates and found that in-hive pollen transfer was nearly universal, while a study using honeybees in mango trees showed in-hive pollen transfer had occurred, based on increased hybridization in the fruit crops the bees had pollinated (Dag et al. 2001). This suggests that there is some potential for gene flow that might result from passive pollen transfer among cohabitating bees despite very little accumulation of viable pollen on the surface of bees that make repeated foraging trips to an alfalfa field or feral grouping of plants. However, there would be essentially no risk of within-nest transfer of pollen for most of the native bee species because they are solitary nesting bees.

4.3.4 Pollen Viability in the Environment

For bees whose hives are moved between fields throughout a season, there is a question of whether viable pollen could survive in the hive and be transported. Pollen environmental viability has been tested for the wind pollinated forage grass, tall fescue (Wang et al. 2004). Pollen viability is highly sensitive to environmental conditions. This particular study examined differences between transgenic pollen and non-transgenic pollen, which were non-significant. There is an optimal temperature peak (approx 25ºC or 77ºF), above and below which the pollen viability decreases. Both types of pollen dropped to 5 percent viability in 30 minutes in sunny conditions, while they dropped to the same level in 150 minutes in cloudy conditions (figure V-5a). In growth chamber conditions with controlled temperature and humidity, both types of pollen decreased to about 5 percent viability in 12 hours, with a complete loss of viability after 22 hours (figure V-5b). This rapid decrease of pollen viability in ambient atmospheric conditions is also found in wheat (none after 65-70 min), triticale, a wheat-rye hybrid (none after 110-120 min) and maize (none after 120 min). From these data it appears that if honey beehives are transported more than two hours away, residual pollen would be unviable in the new environment. The degree to which tall fescue and alfalfa have pollen with similar longevity is

unknown. In general pollen is only viable for hours or days in the environment (Mallory-Smith and Zapiola 2008). The FGI Best Practices do not permit hives to be moved from GT alfalfa to non-GT alfalfa within a growing season.

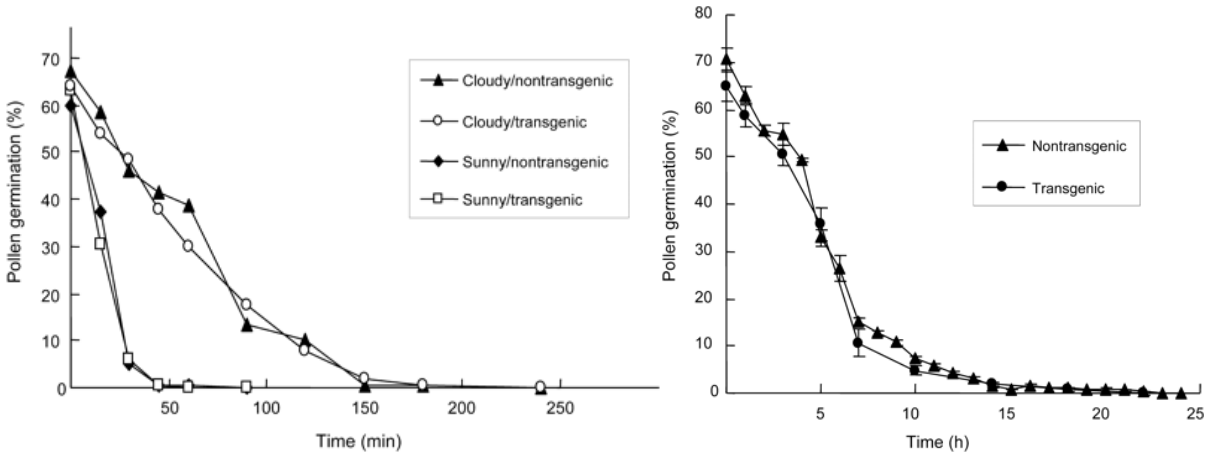

Figure V-5. Pollen longevity in tall fescue (Wang et al. 2004)

Figure V-7a: Longevity of pollen of tall fescue under ambient atmospheric conditions. Pollen was collected from seed-derived plants and transgenic progenies of tall fescue, and germination was evaluated under sunny (Sunny/nontransgenic, Sunny/transgenic) and cloudy (Cloudy/nontransgenic, Cloudy/ transgenic) conditions.
Figure V-7b: Longevity of pollen of tall fescue under controlled conditions (24°C, relative humidity 54 ± 5 percent) in a growth chamber as measured by percentage germination (means ± 1 SE). Pollen was collected from seed- derived plants (nontransgenic) and transgenic progenies (transgenic) of tall fescue. (Wang et al, 2004)

4.3.5 Honey Production

Alfalfa honey makes up one-third of the annual production of U.S. honey, and there is a market for organic honey. Because honey bees are known to forage over long distances, there is a chance that honey bees would bring pollen containing GT trait back to honey-producing hives if there was a GT alfalfa field nearby (Hubbard 2008).

4.4 Forage and Seed Field Proximity

The Agricultural Resources and Environmental Indicators Database has a mapping tool that converts agricultural data to map form.[10] It contains data on alfalfa hay and seed production for the United States from 1997, and shows the approximate distribution of this production. As the color gets darker, production increases. This gives a visual idea of the regions of the country with the most alfalfa, and shows that seed production is far less than hay production and is more geographically clustered. Figure V-6 presents alfalfa hay acreage and figure V-7 presents alfalfa seed acreage.

[10] (http://maps.ers.usda.gov/AgResources/index.asp?Describ=Alfalfa+hay+%28tons%2C+dry%29%2C+harvested+%28acres%29&TableNo=28&CatalogVal=Field+Seeds%2C+Grass+Seeds%2C+Hay%2C+Forage%2C+and+Silage&Year=D97&Type=Total&drawtype=drawmap&action=&boundaryflag=&labelflag=&drawtypesaved=drawmap&sort=&sorttype=&printflag=&yearvalue=D97&x=280&y=187).

Figure V-6. 1997 Alfalfa hay (tons, dry), harvested (acres)

Figure V-7. 1997 Alfalfa seed (pounds), harvested (acres)

Forage and seed field data for 2007 are presented in table V-10. Alfalfa hay and seed production occurs mainly in the west and northwest United States. The biggest producers of hay (pounds) are California, South Dakota, and Idaho, while the biggest producers of seed (pounds) are California, Washington, and Idaho. When looking at acreage, however, South Dakota, Montana and Wisconsin have the largest hay production, while California, Washington and Idaho have the most acreage for seed production. Acreage might be a more important concern when looking at gene flow, as the spatial distribution of alfalfa fields is a factor in determining the potential of pollination. California, Washington and Idaho would be the States that would most likely have farmers concerned about the adventitious presence of the GT trait, as they not only have the most acreage devoted to seed production, but also produce the most seed in the country.

Roundup® is only registered for alfalfa seed production in 13 States: Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oklahoma, Oregon, Texas, Utah, Washington, and Wyoming. There are several States which produce seed but would not be able to produce GT alfalfa seed because Roundup® is not approved for use on seeds: South Dakota, North Dakota, Minnesota, Kansas, and Nebraska (FGI 2007).

Table V-10. Top 20 States for Alfalfa Hay and Seed Acreage and Quantity, 2007 U.S. Census of Agriculture

Dry Hay					Seeds				
State	Number of Farms Harvested	Acres Harvested	Quantity (pounds) Harvested	Percent of Total Hay Acres	State	Number of Farms Harvested	Acres Harvested	Quantity (pounds) Harvested	Percent of Total Pounds Harvested
United States	290726	20244497	65349074	100.00%	United States	806	121467	62115239	100.00%
South Dakota	12653	1996599	4414338	9.86%	California	114	36625	19083458	30.72%
Montana	9711	1868756	3936445	9.23%	Washington	82	17127	10860608	17.48%
Wisconsin	30810	1517522	3673619	7.50%	Idaho	92	12788	9346709	15.05%
North Dakota	8985	1457604	3072682	7.20%	Wyoming	62	10548	5915816	9.52%
Nebraska	14820	1085921	3955881	5.36%	Nevada	19	6498	4237101	6.82%
Idaho	8817	1037520	4254543	5.12%	Montana	80	10338	3729635	6.00%
California	3587	986982	7057014	4.88%	Oregon	32	4959	3183375	5.12%
Minnesota	20398	944775	2671173	4.67%	Utah	54	3803	2077813	3.35%
Colorado	8648	861053	2887865	4.25%	Arizona	53	5206	1902669	3.06%
Iowa	22040	830440	3054729	4.10%	South Dakota	47	6014	428447	0.69%
Kansas	9643	793140	2986134	3.92%	Oklahoma	29	2004	281121	0.45%
Michigan	16431	698595	1707036	3.45%	Texas	24	546	79885	0.13%
Wyoming	4007	674284	1696438	3.33%	Minnesota	17	611	63461	0.10%
Utah	7780	548570	2172218	2.71%	Missouri	19	399	40540	0.07%
Pennsylvania	14402	475873	1357225	2.35%	North Dakota	6	(D)	34784	0.06%
New York	7707	450144	1119421	2.22%	New Mexico	15	310	29907	0.05%
Washington	4294	448588	2192001	2.22%	Kansas	5	342	22430	0.04%
Ohio	15354	437658	1256174	2.16%	Nebraska	29	545	21216	0.03%
Oregon	3569	428812	1777894	2.12%	Michigan	10	(D)	15610	0.03%
Oklahoma	3781	334990	1131938	1.65%	New York	3	27	6180	0.01%

D = data withheld to protect the identity of individual farms

4.5 Seed-mediated Gene Flow – Secondary Seedlings

Secondary seedlings (seedlings that are not planted directly by the farmer but rather sprout unintentionally) are a possible but unlikely avenue for effective gene flow into existing solid-seeded alfalfa plantings since alfalfa plants and alfalfa debris produce compounds that elicit an autotoxic reaction to germinating alfalfa seeds. The autotoxic reaction and inter-plant competition severely limit germination and seedling vigor of alfalfa sown or dropped into existing or newly terminated alfalfa stands. Cultivated fields do not successfully self-seed. Attempts to thicken existing alfalfa stands by deliberately inter-planting new seed into them typically fail, which is why most agronomists do not recommend the practice (Canevari et al. 2000).

A portion of seed growers plant their fields in rows instead of solid plantings. In these situations, in-crop volunteers from dropped seeds occur and the resulting seedlings could be a means of gene flow to subsequent crops. However, in order to maintain required variety and purity of the alfalfa crop, these seed growers routinely control germinating alfalfa seedlings and weeds using irrigation and/or soil-active herbicides that do not impact the pre-established, alfalfa crop.
There are several possible pathways for the unintentional distribution of alfalfa seeds that may lead to gene flow due to secondary seedlings. A list of potential sources for gene flow due to secondary seedlings includes:

- Factors associated with the harvest, processing or storage of alfalfa seeds. Secondary seedlings may occur if alfalfa harvests for seed production result in high rates of seed shattering in fields, leaving seeds in fields for subsequent volunteers. As mentioned above, seedlings that result from shatter of seeds into established alfalfa fields are unlikely to sprout soon after alfalfa cultivation due to alfalfa autotoxicity. If these seeds have dormancy and the field is rotated to another crop, they may germinate in a subsequent year. Improper cleaning of shared harvesting and processing equipment could result in secondary seed dispersal (Mallory-Smith and Zapiola 2008). Finally, spillage during transportation (failure to tarp, insecure gates, etc.) could contribute to dispersal.
- Factors associated with weather. Extreme weather phenomenon could contribute to the dispersal of seeds and result in secondary seedlings. Natural dispersal due to extreme weather events cannot be prevented (Mallory-Smith and Zapiola 2008). For example, tornados and high winds could potentially blow swaths of alfalfa prior to harvest, flooding could result in the transportation of swaths and seeds between adjacent fields, and escape into irrigations ditches due to storm water overflow could all contribute to the potential for secondary seedlings.
- Factors associated with animals. Seed predation of ripe seeds both on standing plants and shattered seeds left in harvested fields could be transported by birds or other animals. Natural dispersal of seeds due to wildlife can contribute to gene flow and cannot be prevented (Mallory-Smith and Zapiola 2008). Rodents could potentially

spread seeds if storage bins are infested. Finally, if alfalfa seeds survive passage through the digestion of hay and forage products consumed by livestock or wildlife, unintentional escape of secondary seedlings is possible.

No studies were identified to quantify the relative role of secondary seedlings in the movement of alfalfa between populations.

5.0 Pollen-mediated Gene Flow Studies

There are different ways to define "populations" of alfalfa. The three population definitions used in this appendix and in table V-11 are as follows (based partly on Bagavathiannan and Van Acker 2008):

- Hay field population – agricultural field that is intentionally planted with alfalfa and is harvested for hay (may also include some grazing).
- Seed field population – agricultural field that is intentionally planted with alfalfa and is harvested as seed stock.
- Feral and other alfalfa –
    - Feral – alfalfa growing on any non-agricultural land (including roadsides, fences, and waste lots) that reproduces without intentional human inputs, including reseeding. This is considered the "naturalized" population in the United States because alfalfa was introduced to the continent at least 200 years ago (Putnam et al. 2001).
    - Habitat/rehabilitation/erosion control – alfalfa that is intentionally sown (most likely in a seed mix), but is not managed after planting.
    - Rangeland – seed may be sporadically sown for grazing, but land is not mowed for regular hay harvest, populations are mostly self sustaining.
    - Volunteer – alfalfa growing out of rotation in an agricultural field with another crop (e.g., corn).
    - Escaped volunteer – alfalfa from seed that escaped from an agricultural field (this is the first generation of a feral population). Escaped volunteers may not persist past a generation or two, depending on conditions.

Table V-11 presents nine possible scenarios of gene flow between the above identified populations of alfalfa. Each of these scenarios are discussed in this appendix, but are summarized in table V-11.

Table V-11. Relative Potential for Gene Flow Between Populations of Alfalfa (requires that viable seed is produced) (adapted from Van Deynze et al. 2008)

Pollen Donor	Pollen Acceptor		
	Seed Field	**Hay Field**	**Feral and Other Alfalfa**
Seed field	Without distance between fields, cross-pollination between fields would occur at an undesirable rate (if flowering time overlapped). FGI Best Practices that include distance between fields can manage cross-pollination to below 0.5 percent.	Lowest risk of gene flow because hay is cut before seed is produced.	Feral populations should be controlled near seed fields to preserve seed purity. However, if feral plants are present, they will likely be cross-pollinated by seed field pollen.
Hay field	The percent bloom at harvest will influence how much pollen could potentially be transported to seed fields. Mowing hay prior to 10	Lowest risk of gene flow out of the nine scenarios. Even in fields that bloom, hay is cut before seed is	The percent bloom at harvest will influence how much pollen could potentially be transported to feral populations. Mowing hay prior to 10 percent bloom

Table V-11. Relative Potential for Gene Flow Between Populations of Alfalfa (requires that viable seed is produced) (adapted from Van Deynze et al. 2008)

Pollen Donor	Pollen Acceptor		
	Seed Field	**Hay Field**	**Feral and Other Alfalfa**
	percent bloom and distance (350 to 600 feet) from seed fields can manage cross-pollination to below 0.01 percent	produced.	can reduce pollen availability.
Feral and other alfalfa	Feral populations need to be controlled near seed fields, or variety purity can be compromised. (Or the seed field edges can be harvested as a separate crop.)	Lowest risk of gene flow because hay is cut before seed is produced.	Gene flow between feral individuals that are close to each other is likely. Gene flow between feral populations depends on proximity, pollinators, flowering timing, and environmental stresses.

5.1 Conditions affecting the probability of Gene Flow

5.1.1 Conditions Increasing Probability of Gene Flow

As the above sections indicate, there are multiple factors involved in studying the impact of GT alfalfa. Factors have the potential to increase gene flow between crops include, but not limited to, the following:

- Feral alfalfa creates gene flow corridors: if feral alfalfa grows between fields of GT alfalfa and non-GT alfalfa, then it could provide a corridor for gene flow between these fields. It could act as a stepping stone for pollinators that would be more likely to travel between flowers that are closer together than between distant fields.
- A pest management strategy currently in practice is that of leaving a strip of uncut alfalfa during hay harvest, which can act as a reserve for both insect pests and beneficial pollinators. If these alfalfa strips are GT alfalfa, and are not ever cut, they would have the chance to flower and seed. This would result in a the risk of pollinator-mediated spreading of the GT trait to nearby established feral populations, and could mean that the chance of gene flow from hay fields to other fields can be just as great as the chance of gene flow from seed fields to other fields (Mueller 2005).
- Seed field proximity can increase gene flow between the fields. The seed fields are generally found in a compact geographic area, and with pollinators that have the potential to forage over miles (honey bees; for instance), this creates a potential for cross-fertilization in non-GT alfalfa seed fields (Hubbard 2008)
- As with any agricultural crop, there is the possibility of volunteer alfalfa growing in the field during other crop rotations. If these volunteer plants were GT, then normal glyphosate-based herbicide routines would not eradicate them, creating a possibility that the volunteer plants would flower, set seed, and be a source of pollen for gene flow (Altieri 2000). Also, alfalfa produces “hard seeds”, seeds whose hard coating prevents moisture from germinating the seed. It is possible that these seeds can remain dormant from one season to the next, and then germinate at a later time, creating the possibility of the presence of GT alfalfa even after alfalfa is no longer produced in a field (Hubbard 2008; Mallory-Smith and Zapiola 2008).

- Movement of honey bees from crop to crop could increase the chance of transferring pollen from one field to another.
- Release of too many bees to pollinate one field by farmers can lead to far dispersal of the bees. Bees will respond to the competition at that one field, and then will forage further to find nectar and pollen or to establish nests at alternate sites. This might happen before they visit any flowers of the target field, or they might visit the target field before traveling, increasing the risk of gene flow from the target field (which may be GT alfalfa) to other fields (possibly non-GT alfalfa) (Bosch and Kemp 2005).

5.1.2 Conditions Decreasing Probability of Gene Flow

FGI and Monsanto have developed stewardship programs to address concerns regarding gene flow (FGI 2007).[11] Seed growers undergo training and have to be licensed to grow GT alfalfa seed. Any farmer who purchases GT alfalfa seed for producing hay is required to sign a Monsanto Technology/Stewardship Agreement (MTA). The FGI Best Practices for seed growers is the primary mechanism for limiting gene flow. Features of the MTA and FGI Best Practices are as follows:

- GT alfalfa seed producers may not sell seed to any party other than FGI and growers may not save seed for any purpose.
- Beehives cannot be moved out of GT alfalfa until pollination is finished for the year. This prevents pollen being carried via hive between GT and non-GT alfalfa. Grower must indicate main pollinator species on the FGI Seed Grower Contract.
- Isolation through distance from other alfalfa fields is required. For pollination with leafcutter bees the distance must be greater than or equal to 900 feet, for Alkali bees greater than or equal to one mile, for honey bees greater than or equal to three miles.
- FGI reports seed field location and planting date to local seed certifying organizations, which GMO-sensitive farmers can refer to in order to certify isolation distances.
- Stand removal and volunteer management must be sufficient to allow seed certification inspectors to validate stand removal. Stand removal date and method must be reported to FGI and verified.
- Cleaning requirements for equipment are included in the FGI Best Practices.
- The Monsanto MTA requires hay growers to harvest at or before 10 percent bloom.
- The Monsanto MTA states that GT alfalfa hay growers are not allowed to produce seed off of hay production fields.

Additional factors that could decrease the potential for gene flow include:

- Barriers between fields – Types of barriers can include bodies of water, or other, more attractive plants for bee foraging in between fields. A border of plants at field edges has the benefit of being a buffer zone, as pollen would be deposited in the border

[11] The stewardship programs also address other concerns such as weediness potential and glyphosate resistant weed formation.

population before leaving a GT alfalfa field. If the border were also alfalfa, this would ensure that pollinators would not preferentially avoid the border area. However, the border would need to be treated as GT alfalfa, and if it starts out as non-GT alfalfa, then the spread of genes from that population to the GT alfalfa could adversely affect the cultivation of GT alfalfa seeds by reducing seed purity. If the border were not alfalfa, but a different plant, this would prevent bees from traveling far from the field, and fewer GT genes would be spread. However, this could be difficult if the border plant has different growing and management requirements from the alfalfa, or if it is an attractive plant to pollinators, which would discourage the alfalfa pollinators from pollinating the alfalfa, and could encourage distant bees to forage there, increasing long-distance pollen flow. Seeds produced by a non-alfalfa plant could also contaminate the purity of the alfalfa seed crop (Amand, et al. 2000; Rogan and Fitzpatrick 2004).

- Competition with existing plants is difficult for volunteer alfalfa plants that must establish themselves and compete for nutrients against adult plants.
- Size of fields (alfalfa populations) - The size of source GE alfalfa fields and sink non-GE alfalfa populations may impact the rate of gene flow. Studies comparing gene flow from large commercial fields to both small sink fields and large sink fields (FGI 2007) suggest that gene flow rates may be higher when source fields are much larger than sink populations. Gene flow rates between comparable source and sink fields at proscribed isolation distances are lower than gene flow from large plots to small plots. Studies in canola (Rieger, et al. 2002) revealed that in studies with large source fields of herbicide resistant canola (25-100 hectares), the gene flow pattern in canola does not follow the leptokurtic or fat tail distribution (i.e the extreme cases while rare are not as rare as expected) observed in studies evaluating small field sizes and instead had much higher variation in the distance of detectable hybridization. However, one major factor that could contribute to the pattern associated with this study is the pollination mode of canola, which includes both insect and wind pollination modes. As alfalfa relies on insect pollination only, differences in gene flow distance may not be directly comparable.

5.1.3 Modeling Gene Flow

GENESYS is a modeling program that allows the simulation of gene flow between crops, based on cropping systems and other parameters (Colbach et al. 2001a, 2001b). Given the available data and variables for entire cropping systems in a region, cropping systems that would minimize the potential of gene transmission and dispersal can be identified (along with those of high dispersal potential). This program has previously been used to evaluate the potential of transmission of herbicide-tolerant genes from rapeseed crops to rapeseed volunteers, and can be adapted and applied to other crops. It can also analyze the effect of cropping systems on the genetic evolution of the crop.

- Parameters that are used in the model include (Dzeroski et al. 2006):
- The field plan of the region, comprising cultivated fields and field-edges or borders consisting of spontaneous vegetation;
- The crop rotation of each field;

- The cultivation techniques applied to each crop (intercrop cultivation, soil tillage, sowing date and density, herbicide applications, cutting dates and seed loss at harvest);
- The type of the transgene, either a dominant allele A or recessive a, as well as the genotype of the crop varieties.

The more accurate and complete the parameters entered into the model are, the more accurate the results of the model will be. The model output is the level of GE seed present in conventional crops. The model can also identify key variables that are most influential on gene dispersal. This model could be adapted to predict alfalfa gene flow; however it may not be necessary because FGI has obtained actual gene flow data from commercial scale seed production (FGI 2007).

A Gene Flow Index (GFI) ranking scheme has been developed for sugar beet, oilseed rape, potato, perennial ryegrass, maize, wheat, and barley (Mullins 2005). This index could be used to put alfalfa in perspective to other GE crops.

Many studies have been conducted to examine the incidence of pollen-mediated gene flow in alfalfa. The key studies were summarized by Van Deynze et al. (2008) and are shown in table V-12 and figures V-8 to V-12. In the 1970s and 1980s, alfalfa scientists used pest resistance and flower color genes to evaluate genetic purity of dissimilar cultivar types produced at various isolation distances between seed fields and along contiguous field edges (Brown et al. 1980; Brown et al. 1986; etc.). The Association of Official Seed Certifying Agencies (AOSCA) alfalfa crop standards committee adopted the recommendations of the North American Alfalfa Improvement Conference Committee on Alfalfa Field Isolation (Brown et al., 1986) to set isolation distances for certified and foundation seed classes (AOSCA 2003). The AOSCA standards are consistent with established OECD seed certification standards with respect to isolation distance, field history requirements, and genetic purity tolerance for off-types, etc. In the 1990s, other naturally occurring genetic marker systems were used to study the risk of pollen-mediated gene flow between commercial hay, seed and feral plants (St. Amand et al. 2000) and since 2000, the Roundup Ready trait has been used to measure gene flow.

The most recent pollen-mediated gene flow studies (2000-2007) used the Roundup Ready gene as a high sensitivity pollen-marking tool under small, medium, and large field commercial seed and or hay production conditions. V-8 illustrates the various gene flow interface types (types of gene flow) where pollen movement can occur (also see table V-11). In order for true pollen-mediated gene flow to take place, an unbroken chain of six sequential events must occur as shown in figures V-9 and V-10 (Putnam 2006, Van Deynze et al. 2008).

In addition to pollen-mediated gene flow, seed-mediated gene flow could occur by in-field volunteers (soil seed bank and escapes of stand take-out), seed admixtures during transportation or seed processing, seed transport by water or wind (short distances), seeds in overly mature hay, equipment and animals.

Table V-12. Summary of U.S. Research on Gene Flow in Alfalfa

Location	Researcher Affiliation	Dates	Alfalfa Populations	Pollinators	Publications
Prosser, Washington	USDA/ARS and Kansas State University	1994	Seed to seed (within a field)	Leafcutter (*Megachile* spp.)	Amand et al. 2000
Prosser, Washington and Manhattan, Kansas*	USDA/ARS and Kansas State University	1995	Hay to seed and Seed to seed	Not indicated	Amand et al. 2000
Prosser, Washington	USDA/ARS and Kansas State University	Not indicated	Feral to feral	Not indicated	Amand et al. 2000
Canyon County, Idaho	Forage Genetics International (FGI)	2000, 2001, 2002	Seed to seed	Leafcutter bees (*Megachile rotundata*) (some honey bees observed)	Fitzpatrick et al., 2002; Fitzpatrick et al. 2003 ; Rogan and Fitzpatrick 2004
Touchet, Washington	University of CA Davis, FGI	2002	Hay (50 percent bloom) to seed	Leafcutter bees (*Megachile rotundata*)	Rogan and Fitzpatrick 2004; Teuber and Fitzpatrick 2007
Kings County, California (San Joaquin Valley)	University of CA Davis, FGI, Monsanto	2003	Seed to seed	Honey bee (*Apis mellifera*)	Teuber et al. 2004; Teuber et al. 2005; Teuber et al., 2007
San Joaquin Valley California	University of CA Davis, FGI	2006	Hay (20 percent bloom) to seed	Honey bee (*Apis mellifera*)	Teuber and Fitzpatrick 2007; Teuber et al. 2007
San Joaquin Valley California	University of CA Davis, FGI, USDA/ARS	2006, 2007	Seed to seed	Honey bees and leafcutter bees	Teuber et al. 2007; Van Deynze et al. 2008
Fruita, Colorado	Colorado State University	2006	Seed to feral	Honey bees and leafcutter bees plus 9 other bee species	Hammon et al. 2006
California, Colorado, Idaho, Montana, Nevada, Oregon, Texas, Washington, Wyoming	FGI	2006	Seed to seed	Leafcutter bees, alkali bees, and honey bees (used according to FGI Best Practices)	FGI 2007

* both locations had both seed and hay fields as pollen sources

Figure V-8 highlights the key references wherein data have been developed to address the risk of gene flow between each type of pollen flow interface. Gene flow was reviewed in the 2004 Petition appendix 5 for data available prior to April 16, 2004. Since the Petition, Teuber et al. (2005b) have developed an extensive literature review on honeybee pollination and gene flow in alfalfa. The following sections will present the new information since 2004 in combination with previous study data and discussion. As an aid to this discussion, a consolidated gene flow versus distance data illustration by crop setting and pollinator species is presented as figure V-11. Details for each study are found in the original references and numerous key original references will be provided to APHIS with this technical report.

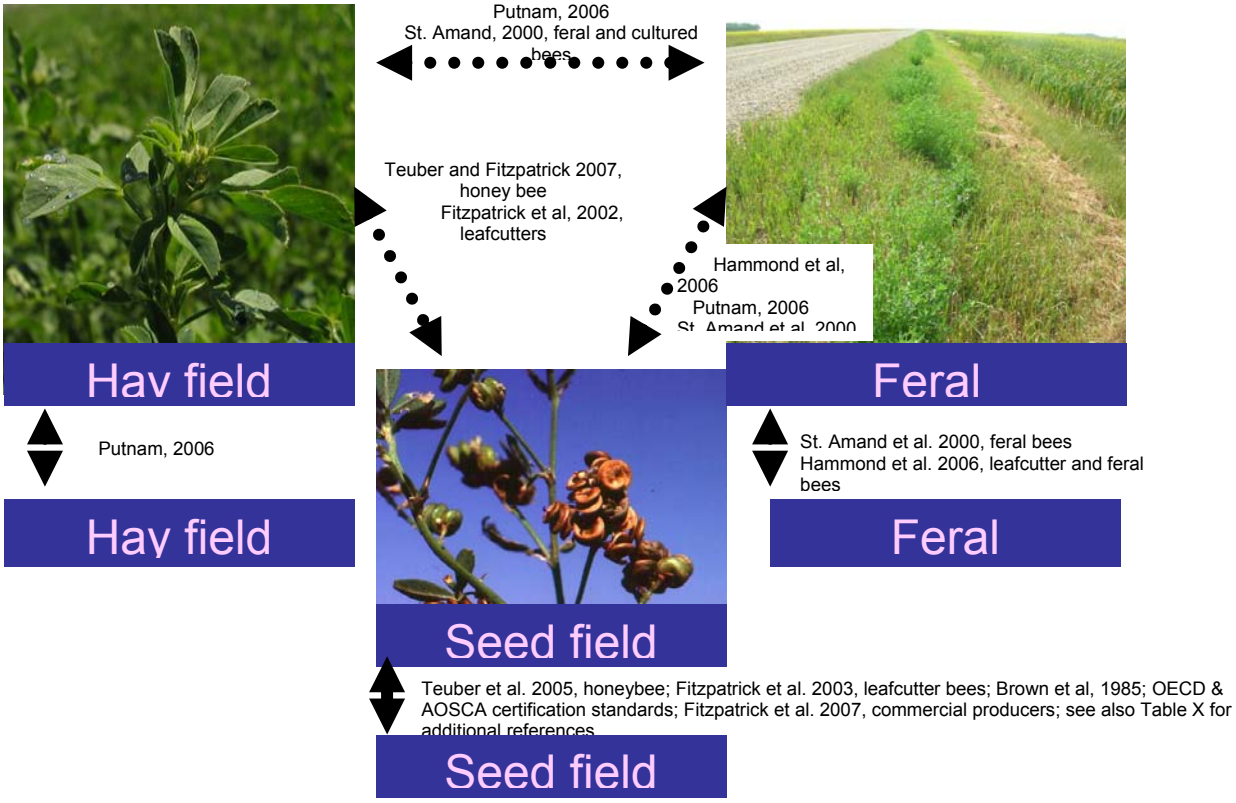

Figure V-8. Cultural practices influence the risk of gene flow among alfalfa populations. Each type of gene flow interface has been studied. Studies have been collectively reviewed and summarized by Van Deynze et al. (2008).

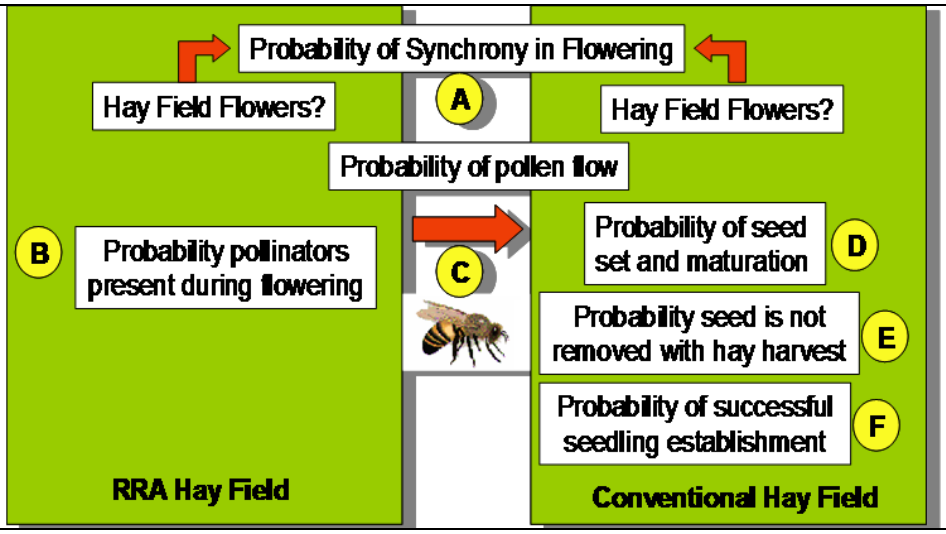

Figure V-9. Probability of hay-to-hay field gene flow in alfalfa.
Excerpted from Putnam 2006

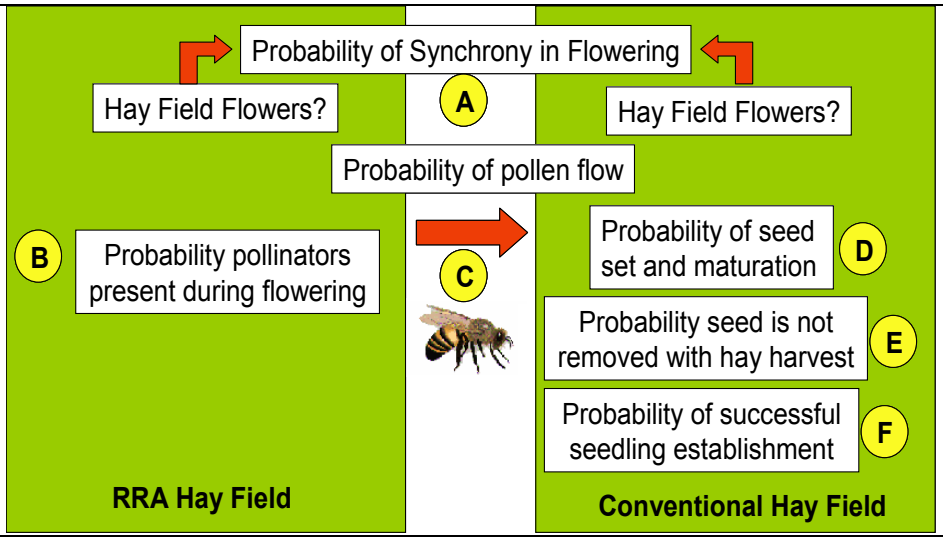

Figure V-10. Probability of Hay-to-Seed field gene flow in alfalfa.
Excerpted from Putnam 2006

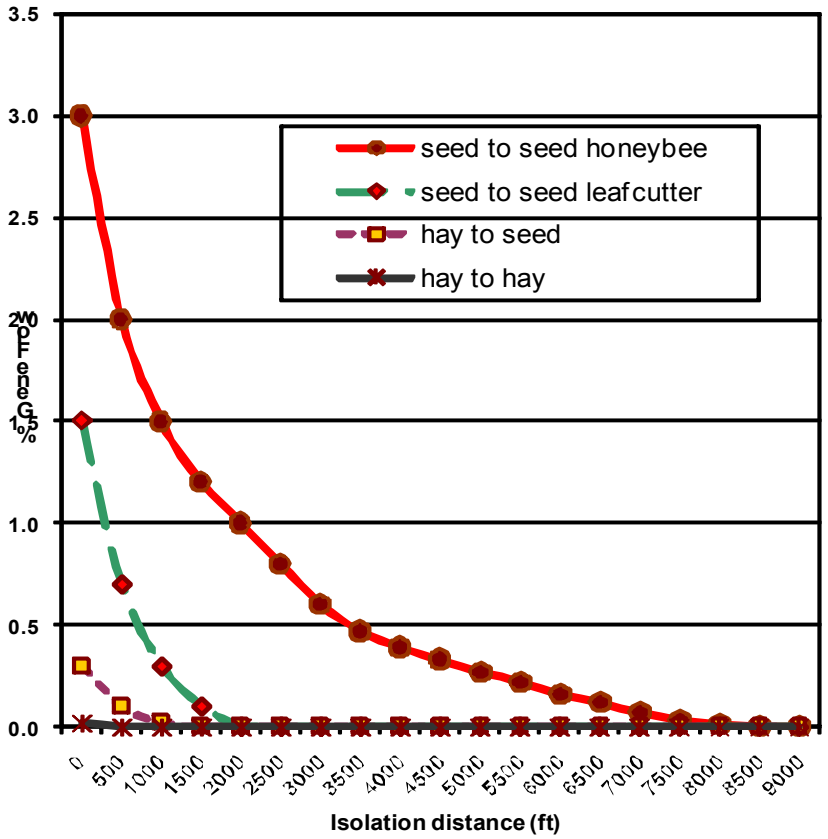

Figure V-11. A comparison of the relative gene flow potential for various production systems in alfalfa.

Excerpted from Van Deynze et al. 2008

By experimental design, most of these recent pollen-mediated gene flow studies estimated pollen-mediated gene flow under somewhat exaggerated and worst-case field conditions. From the standpoint of industry trait stewardship, gene flow mitigation and diverse market coexistence, worst-case studies are an important first-step in gaining an understanding of a novel trait production system. The results from these experiments were communicated to industry stakeholders and to the public. Through consultation with industry stakeholders, the data were used to design FGI's Best Practices Policies for Roundup Ready Trait Stewardship during Seed Production ("FGI Best Practices") in Fitzpatrick et al. (2007b). FGI Best Practices are a set of comprehensive process management requirements that encompass Roundup Ready trait stewardship requirements for licensees, mitigation of unintended gene flow and tools to foster peaceful market coexistence. FGI Best Practices were developed using identity preserved production models, foundation seed production standards and seed quality assurance principles. Data are presented in Fitzpatrick et al. (2007a and 2007b) that supports the effectiveness of the Monsanto trait stewardship program in combination with the FGI Best Practices policies in attaining successful market coexistence for Roundup Ready alfalfa. Recently, NAFA sponsored

a Roundup Ready seed producer Best Practices consensus protocol that was built upon the FGI Best Practices program foundation (2007).

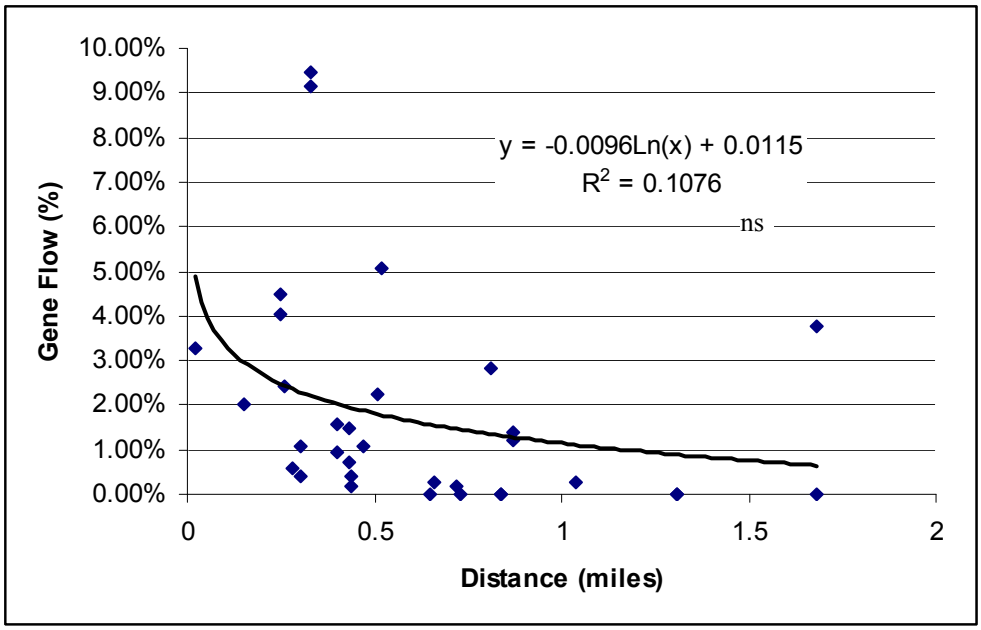

Figure V-12. Pollen-mediated seed-to-feral gene flow observed from three-year-old commercial "Roundup Ready" seed fields (900 acres overall) to small feral and unmanaged alfalfa clusters.

As for other crops with GM trait choices, mitigation and coexistence strategies were developed to allow for the production of high quality alfalfa seed (and hay) to serve diverse market sectors (organic, export, common, conventional and Roundup Ready) and preserve a grower's choice to plant conventional or Roundup Ready seeds. Independent third parties have lead in the industry's development of a broad-based coexistence consensus; examples are the University of California-Davis Seed Biotechnology Center (2005) and the National Alfalfa and Forage Alliance (2007).

Gene flow declines with increasing distance from the source. The amount of gene flow is pollinator species and cultural practice specific. Pollen-mediated gene flow is plotted for four specific types of cultivated alfalfa interface types: 1) seed field-to-seed field under honey bee pollination (Teuber et al. 2004); 2) seed field-to-seed field under leafcutter bee pollination (Fitzpatrick et al. 2003); 3) mature (20-50 percent flower stage) hay field-to-seed field data sets combined for leafcutter and honey bee pollinators (Teuber and Fitzpatrick 2007); and, 4) estimated hay field-to-hay field gene flow (Putnam 2006).

Gene flow was measured by evaluating 33 seed samples collected from 23 small clusters of feral or intentionally unmanaged alfalfa plants in western Colorado (raw data was excerpted from Hammon et al. 2006). The nearby Roundup Ready seed fields were commercially pollinated with cultured leafcutter bees, and, various species of native bees, alkali bees and honeybees were also intermittently present and pollinated the alfalfa. Median sampling distance and gene flow were 0.5 mile and 1 percent, respectively. There was no correlation (ns) between amount of gene flow and distance from the nearest field. The Roundup Ready seed production fields were

in the third consecutive seed production year. Within the survey zone, virtually no sources of conventional pollen were present to cross-pollinate the feral plants except for that from other small, dispersed patches of feral/unmanaged or forage field plants and forage fields cut at early maturity.

Key conclusions from alfalfa pollen-mediated gene flow studies are discussed in the following three sections.

5.2 Seed fields as the pollen-receiving environment

5.1 Hay-to-seed

Hay to seed gene flow is a possible source of presence of the GT trait in non-GT alfalfa seeds. The factors that influence hay to seed gene flow are as follows:

- Probability of synchronous flowering – Seed fields flower for about 5-6 weeks (Bosch and Kemp 2005). Hay fields that are harvested at 10 percent bloom only have flowers for about a week (Shroyer et al. 1984) and for most of that time the field is below 10 percent bloom.[12] There may be some overlap between flowering time among hay fields and seed fields, but it is likely to be limited to a few days (figure V-4).
- Availability of pollen from hay fields – Hay fields may be harvested any time between pre-bud and 100 percent bloom; however the majority of fields are harvested at or below 10 percent bloom.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bees are brought in for seed fields, but not for hay fields. Wild bees are present at relatively low levels compared to bees brought in for seed field pollination. There will likely be some bee activity in hay fields with bloom, especially if bee hobbyists have hives nearby.
- Distance between fields (alfalfa populations) – There are no required distances between hay fields, but seed fields are still required to be separated from hay fields (section 3.6).
- Probability of seed maturation – Although 100 percent of the flowers that are pollinated do not mature, in well cared for seed fields most of the pollinated flowers produce mature seed. This is in contrast to hay fields, which are mowed before seed is allowed to mature.
- Probability of seed germination. – Orloff et al. (1997) state that about 60 percent of seeds germinate when planted under good conditions (correct depth and soil conditions). When a hay farmer plants commercially produced seed the germination rate is indicated on the label. This assumes that the field has been properly prepared and the soil is free of herbicides and alfalfa autotoxic compounds.

"Hay into seed" field pollen-mediated gene flow potential is low to near zero with observance of routine AOSCA certified isolation distances (e.g., 165 ft) and or normal forage field cutting management practice (≤10 percent bloom cutting) during the seed field pollination period.

[12] Maturity timeline varies based on environmental conditions. However, Shroyer et al. (1984) present a figure with alfalfa progressing from bud to 1/10 bloom in one week.

Figure V-5 illustrates the environmental filters that act against effective gene flow into seed fields from hay field sources. If because of weather factors or mechanical problems, a neighboring GM forage field is harvested at a later stage of maturity (e.g., 20 to 50 percent bloom) during the peak seed-pollination period, the potential for gene flow into a nearby small seed field is very low (e.g., 0.3 percent for distances less than 165 ft). In field studies in 2002, in Washington, FGI detected 0.21 percent gene flow at 150 feet and 0.23 percent gene flow at 300 feet. The hay fields which were the source of pollen were mowed at 50 percent bloom (Teuber and Fitzpatrick 2007, Rogan and Fitzpatrick 2004). Figure V-13 presents this hay to seed in the same graph as the seed to seed data that FGI collected in the same time period in Idaho. A larger scale hay to seed gene flow study by the same researchers, but performed in the San Joaquin Valley of California, in 2006, yielded the data presented in table V-13. Hay was harvested at 20 percent bloom and seed fields had been stocked with honey bees. With greater isolation, it is exceptionally rare (e.g., for 350-600 ft isolation, mean flow was 0.01 percent) (Teuber and Fitzpatrick 2007). These values are at least ten-fold lower than predicted from worst-case seed-to-seed gene flow models. Therefore, current AOSCA isolation requirements for certified seed production (165 ft) are sufficient to mitigate gene flow from typical hay fields into conventional certified seed fields to low levels. Where feasible, highly GM-sensitive seed producers can lessen further the risk of pollen-mediated gene flow to their seed field from other/unknown varieties of hay by choosing to use larger size seed fields (>5 acres), stocking pollinator species that range shorter distances (i.e., leafcutter bees vs. others), harvesting the seed field border as a separate lot (St. Amand et al. 2000, Fitzpatrick et al. 2007a) and working to coexist with their neighbors who grow alfalfa for forage (e.g., ask them to their cut their hay early during mid-summer or to use non-GM varieties). Organic, export, common, or foundation class seed producers who are highly GM-sensitive can opt to work with local crop improvement organizations and use wider isolation or buffer zones with identity preserved practices for their non-GM seed fields. The AOSCA ASSP-2010 certification could be used to grow seed that is certified to be free from (highly likely to be below the level detection in standard tests) for GT alfalfa. Seed produced under lesser standards might have low-level presence. Common seed grown by individual, not in commercial production may also have LLP.

Table V-13. Hay to Seed Gene Flow (Teuber and Fitzpatrick 2007)

Distance between hay and seed field (feet)	Percentage gene flow
165	0.29
215	0.16
265	0.14
315	0.15
365	0.00
415	0.00
465	0.05
515	0.02
565	0.05
615	0.00

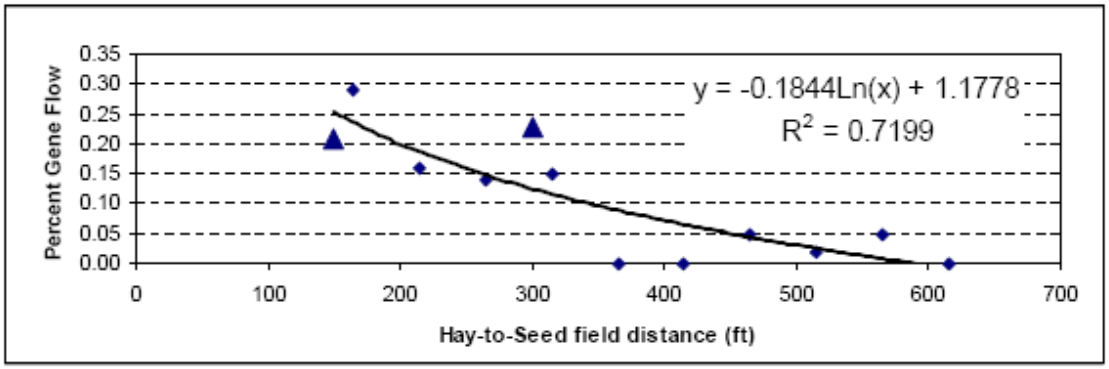

Figure V-13. Hay to seed gene flow (Teuber and Fitzpatrick 2007).

Observed gene flow (Y %) from hay field plots growing near replicated alfalfa seed field plots in 2000 (▲) and 2006. These trials simulated delayed harvest of hay fields growing near and beyond the AOSCA certified seed field isolation standard (i.e., 165 ft). In 2000, replicated hay plots in Washington were allowed to mature to 50% flower (5X the optimum hay cit stage), seed field plots were stocked with leafcutter bees and separated by 150 or 300 ft from the Roundup Ready ® hay field plot[2]. In 2006, replicated seed field plots at the University of California were allowed to mature to 20% flower (2X the optimum hay cut stage); seed field plots were stocked with honeybees and separated by 165 to 615 ft from the Roundup Ready hay field plot.

In summary, it is possible that successful gene flow from hay to seed production could occur but the probability is expected to be low as hay fields do not usually have more than 10 percent bloom, and that is only for a few days. In addition, certified and foundation seed fields are required to have specific distances between them and other alfalfa fields. The probability increases in cases where farmers grow uncertified seed which could be immediately adjacent to GT alfalfa hay fields.

5.1.1 Seed-to-seed

The factors that influence seed to seed gene flow are as follows:

- Probability of synchronous flowering – Seed fields flower for about 5-6 weeks (Bosch and Kemp 2005). The FGI Best Practices for seed production does not mention whether flowering is intentionally offset between seed fields. Because of the length of time alfalfa can flower, there will likely be some overlap between flowering times among seed fields.
- Availability of pollen – Seed fields are allowed to go to 100 percent bloom, so pollen is available.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bee activity is managed in seed fields. Wild bees are present, but at much lower levels than the bees that are brought in for pollination.
- Distance between fields (alfalfa populations) –FGI Best Practices requires 900 feet when leafcutter bees are used, 1 mile when alkali bees are used, and 3 miles when honeybees are the predominant pollinator species (FGI 2007).

- Probability of seed maturation – Although 100 percent of the flowers that are pollinated do not mature, in seed fields most of the pollinated flowers produce mature seed. This is in contrast to hay fields, which are mowed before seed is allowed to mature.
- Probability of seed germination. – Orloff et al. (1997) state that about 60 percent of seeds germinate when planted under good conditions (correct depth and soil conditions). When a hay farmer plants commercially produced seed the germination rate is indicated on the label. This assumes that the field has been properly prepared and the soil is free of herbicides and alfalfa autotoxic compounds.

"Seed into seed" field pollen-mediated gene flow is a common, measurable, natural occurrence for proximal seed fields of different cultivars. Conventional certified variety seed growers largely rely on physical isolation to minimize and mitigate pollen-mediated gene flow. The quantity of pollen-mediated gene flow between adjacent seed fields was extensively studied for the past three decades and the data used to develop current AOSCA and OECD seed isolation and production standards. The percentage of pollen-mediated gene flow into a seed field is dependent upon field isolation distance, bloom synchrony, cultural practices, in-crop volunteer management, gene frequency in the pollen, pollinator species present, density, available food resources, and shelter placement.

Under actual commercial conventional seed grower conditions, the use of pollinator-species specific isolation minimums and identity preserved production protocols have successfully mitigated GM gene flow to levels observed for other GM crop species and produced seeds of high genetic purity, i.e., seeds of greater than 99 percent genetic purity, and, AP level at zero to less than 0.2 percent (Fitzpatrick et al. 2007a and 2007b). The amount of actual gene flow under commercial-scale (large field) conditions is several times less than is predicted by research models developed using smaller-fields (Fitzpatrick et al. 2007a and 2007b).

This most recent data set developed using the Roundup Ready trait supports the findings of previous gene-flow research studies wherein conventional traits were used as pollen markers in large and small fields. Small fields or feral patches have a higher relative percentage of pollen in-flow than larger fields (AOSCA 2003; Brown et al. 1986; Fitzpatrick et al 2007a and 2007b; Hammon et al. 2006; Marble 1980 circa; St. Amand et al. 2000). Therefore, the predictive models developed using smaller field trap sizes (or isolated alfalfa feral trap patches) were very constructive in that they offer worst-case predictions for pollen-mediated gene flow, but they significantly overestimate pollen flow into larger more typical commercial fields. For small- to mid-scale seed-into-seed field settings, gene flow declines sharply with increasing distance from the source (see figure V-6 and supporting references).

Armand et al. (2000) examined gene flow from both alfalfa hay and seed fields to outside populations (trap plots), and the differences between small and large source populations. Their results indicate that pollen does not travel far within a field, with dispersal distances of 4 meters or less. This could indicate that the bees are being cleansed of marker pollen by visiting non-marker plants close by, as opposed to depositing marker pollen far away. At 1,000 meters (3,281 feet), up to 22.2 percent of the trap population seed was from outcrossing (possibly more as the 1,000 meter populations were the farthest examined). Also, a higher amount of gene flow was

found when the source population was a large field as opposed to a small study population, and the marker was exchanged between escaped alfalfa populations. A summary of Armand et al (2002) data is reproduced in figure V-14. It should be noted that the trap populations Armand et al. (2000) used were clonal and therefore likely to have a high degree of self-incompatibility. Self-incompatibility within the clonal trap population would lead to seeds being formed more frequently from outcrossing from the source field. The clonal traps may not represent how feral alfalfa would receive pollen, since feral populations would pollinate among themselves and thus be less receptive to distant pollen sources (Rogan and Fitzpatrick 2004). The clonal nature of the trap plots occurred because the researchers identified a unique marker gene in a single plant and bred several generations to develop homozygous plants for the marker band. GT alfalfa varieties and conventional varieties do not have as high degree of genetic similarity as the research line created by Armand et al. (2000).

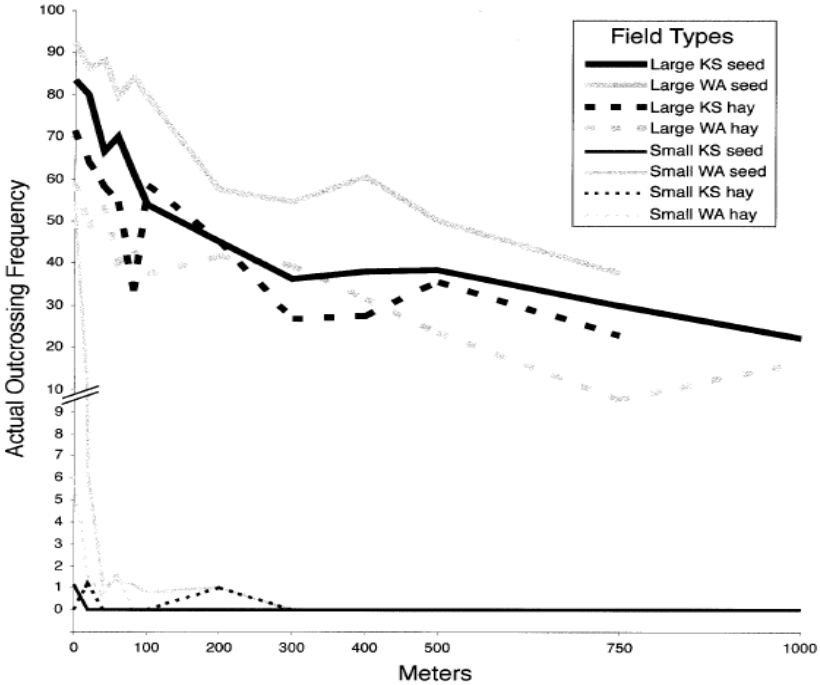

Figure V-14: Summary data from Armand et al. (2000).
Gene movement away from commercial-scale (large) seed and hay production fields and research-scale (small) seed and hay plots in Kansas and Washington State. Progeny from trap plots of cloned alfalfa were examined for a specific marker-gene and for apparent non-self events using RAPDs. Outcrossing from small plots was not detected beyond 200 m (note axis scale change)

FGI's 2000 to 2002 field studies using leafcutter bees, in Idaho, showed a range of 1.39 percent (1.72 percent is the 99.9 percent confidence level upper limit) at 500 feet and 0.0000 percent (0.01 percent CI) at ¾ of a mile (Fitzpatrick et al. 2002) . Table V-14 and figure V-15 present a summary of these field studies.

Table V-14. Summary of FGI Idaho Gene Flow Studies (Fitzpatrick et al. 2002)

Isolation distance	Year 2000	Year 2001 [a]	Year 2002 [a, b]	2000-2002 Overall Mean Observed gene flow (99.9% C.I. upper bound)
0 ft (Source Plot)	*Source* (1.0 A)	*Source* (1.6 A)	*Source* (1 A)	
500 ft	Reps 1-4: 0.03 A, N. [c]	-	-	**1.39%** (1.72%)
900 ft	-	Rep 1: 0.7 A, N. Rep 2: 1.6 A, N.E.	-	**0.28%** (0.34%)
1000 ft	Reps 1-4: 0.03 A, N. [c]	-	-	**0.32%** (0.45%)
1500 ft	Reps 1-4: 0.03 A, N. [c]	Rep 1: 1.6 A, W. Rep 2: 1.6 A, N.W.	Rep 1: 1 A, S.W. Rep 2: 1 A, S.E.	**0.08%** (0.13%)
2000 ft	Rep 1: 2.0 A, N.W. [a]	-	-	**0.00%** (0.05%)
2640 ft (1/2 mi)	-	-	Rep 1: 1 A, S.W. Rep 2: 1 A., S.E.	**0.003%** (0.02%)
3960 ft (3/4 mi)	-	-	Rep 1: 1 A, S.W. Rep 2: 1 A, S.E.	**0.0000%** (0.01%)
5280 ft (1 mi)	-	-	Rep 1: 1 A, N.W. Rep 2: 1 A, S.E.	**0.0000%** (0.01%)
Mean no. seed tested per trap distance	14,750	41,250	60,000	

Table V-14. Isolation distance between trap and source, number of replicates per distance, replicate plot size (acres), trap plot cardinal direction from source and, interplot land cover [a,b,c], the mean observed gene flow and the upper bound of true gene flow (i.e., the 99.9% confidence interval upper limit) are given.
Interplot land cover: [a] various crop species typical for the area (e.g., onions, corn, wheat, etc.); [b] roadways, or [c] fallow. "-" indicates distance not tested.

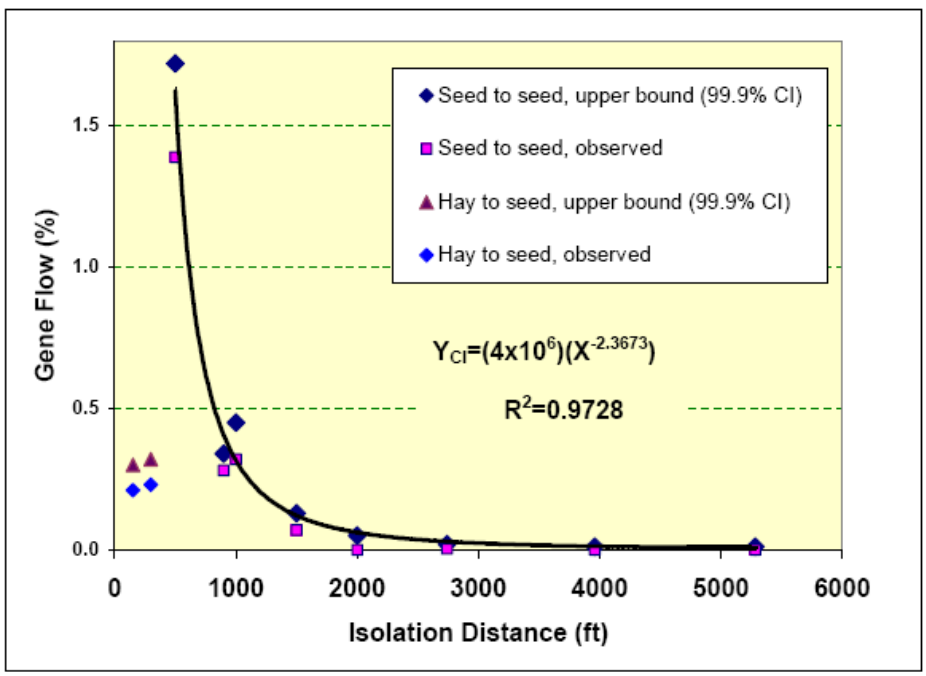

Figure V-15. Summary of GFI 2000-2002 gene flow studies (Rogan and Fitzpatrick 2004).
Three-year summary of alfalfa gene flow for seed production using leafcutter bees with 150 ft to 1 mile isolation distance from gene source plot. Values given are the observed and upper bound of true gene flow (99.9% confidence) based on all data collected during 2000, 2001, and 2002 field studies. The equation for, Y_{CI} = upper bound of 99% confidence interval at X (ft) isolation distance was calculated using seed production source plots only. USDA Notification Numbers: 00-053-07n (2000); 01-009-08n (2001); 02-020-09n (2002).

University of California—Davis, FGI and Monsanto studied seed to seed gene flow using honey bees (2003 growing season). At 900 feet gene flow was 1.49 percent, near 5,000 feet it was 0.2

percent, and at 2.53 miles is was ≤0.06 percent (Teuber et al. 2004). Gene flow was estimated by growing seeds from the trap plots and testing the seedlings for glyphosate tolerance (75,000 to 90,000 seedlings were tested per trap plot). Seed samples from the 2003 field study were also tested using the transgene protein detection strip test. The strip test results were in agreement with the large scale seedling growout assays (Teuber et al. 2007).

In 2006 and 2007, in the San Joaquin Valley of California, a mixed honey bee and leafcutter bee gene flow study was done (Teuber et al. 2007, Van Deynze et al. 2008). A summary of data from those studies is presented in table V-15.

Table V-15. Seed to Seed Gene Flow (Teuber et al. 2007)

Distance	Gene Flow (percent adventitious presence)
165 feet	2.3
900 feet	0.9
4,000 feet	0.6
1 mile	0.2
3 miles	0.03
5 miles	Not detected

Following 2005-2007, the alfalfa seed production firms of Dairyland and Cal/West seeds reported a number of instances where GT transgene presence was detected in non-GT alfalfa seed production fields in Montana, Wyoming, Idaho, and California. In 2006, Dairyland farmers reported 11 of 16 fields contained detectable levels of GT transgene; 9 fields in Montana and single fields in each Wyoming and Idaho. In the majority of fields, the level of GT transgene detected was below the 0.5 percent level of low-level presence (LLP) targeted by FGI for certified seed. However, in one field a level of 0.9 percent was observed. Additionally, a level of 0.2 percent LLP was detected in foundation seed fields. No report regarding the type of pollinator was supplied. However, the levels of LLP observed would suggest that long distance gene flow, likely due to feral bees (if honey bees were not used as pollinators) played a role in transgene gene flow to non-GT alfalfa seed production fields. Results reported by Dairyland are presented in table V-16.

Table V-16. Seed to Seed Gene Flow reported by Dairyland (Monsantos' J.A. Vol. II at (page 1018), *Monsanto* (No. 09-475)

State	Acres	Percent Roundup	Distance from GT alfalfa production
Montana	23	0.3	950 ft
Montana	35	0.9	1400 ft
Montana	26	0.2	1500 ft
Montana	43	0.2	1500 ft
Montana	15	0.3	1600 ft
Wyoming	26	0.3	1700 ft
Montana	16	0.4	2600 ft
Montana	25	0.4	4000 ft
Montana	25	0.3	4000 ft
Montana	22	Trace	1 mile
Idaho	2 (foundation)	0.2	1.5 miles

Data presented by Cal/West seeds reported LLP of the GT transgene in a single foundation seed field in California at a level of 0.01-0.03 percent. Additional testing of 3 seed fields in Washington, seeded from this particular seed lot, revealed 2 of 3 fields with a presence of 0.01 percent, a level acceptable for foundation seed for a non GE but not for a GE sensitive market. Reports of a second occurrence of LLP of GT transgenes was reported by Cal/West. Seed testing of a contracted alfalfa seed producer with fields in proximity (no distance detailed) demonstrated LLP at between 0.5 percent and 1.5 percent of the seed.

The winter issue of the Cal/West Seeds newsletter (Reich and Johnson 2010) reported:

- 12 percent of over 200 lots of non-GT-alfalfa seed tested positive for the GT transgene, compared to 3 percent in 2008 (no level given)
- During 2008, 6 of 6 research seed stock lots produced at Cal/West's Woodland, CA research farm tested positive to the GT transgene and that was attributed to hay-to-seed gene flow (no level given)
- Preliminary 2009 data indicate 3 of 10 seed stock lots have tested positive for the GT transgene (no level given)

It is unclear from the newsletter, which data overlap with the Supreme Court submission (Monsantos' J.A. Vol. II at (pages 672-673), *Monsanto* (No. 09-475)).

During the 2006 growing season, FGI conducted a study to validate their FGI Best Practices (FGI 2007). They tested 122 of their conventional commercially-grown seed lots. Of the 122 lots, 115 of the lots were grown in a region containing at least one GT alfalfa seed field at a known isolation distance and 32 of the lots had detectable gene flow. In the lots where gene flow was detected, it was at the low level of 0.004 to 0.180 percent. Experimental plots were also set up to capture gene flow that occurs at distances less than recommended by FGI Best Practices. The data from this study are presented in table V-17.

In summary, the largest data set collected under actual seed production conditions, using FGI Best Practices, found a range of gene flow from 0.00 to 0.18 percent. This is well below the FGI company goal of less than 0.5 percent adventitious presence.

Table V-17. Summary of FGI Commercial-scale Seed Production Gene Flow Study (FGI 2007)

Distance from source field (feet, x)	**Small** conventional fields with worse-case setting for gene flow			**Large** conventional fields grown under commercial-practice settings
	Predicted *gene flow (%) at < 1 mile isolation* [c]	***Predicted*** *gene flow (%) at > 1 mile isolation* [d]	**Observed** gene flow in test plots (%)[a or b]	**Observed** gene flow (%) in FGI commercial fields
50	*1.84*			
165	*1.80*		1.96 [b]	
600	*1.67*			
900	*1.58*		1.49 [a]	
1320	*1.46*		1.06 [b]	
1500	*1.40*			
2000	*1.25*			
2640	*1.06*		0.99 [b]	
2796	*1.01*		0.99 [a]	
3000	*0.95*			
3960	*0.66*		0.39 [b]	
4000	*0.65*			
4692	*0.44*		0.19 [a]	
5000	*0.35*			
5280 (1 mile)	*0.27*	*0.58*		0.09 [b]
6588	*0.00*	*0.42*	0.15 [a]	
6,600-7,920		*0.34*		0.01 [b]
8484		*0.20*	0.05 [a]	
10,119		*0.00*		
10,380		*0.00*	0.02 [a]	
12,276		*0.00*	0.07 [a]	
14,520-15,840		*0.00*		0.01 [e]
26,400		*0.00*		0.00 [e]
42,240-52,800		*0.00*		0.00 [e]
> 52,800		*0.00*		0.00 [e]

Analysis of honeybee-mediated gene-flow (%) between adjacent alfalfa seed fields predicted using two linear models developed from research trial data presented by Teuber et al. (2005) and data observed in 2003[a] and in 2006[b] experiments. "Small" (≤2.2 acre) or "large" conventional seed fields were separated from Roundup Ready alfalfa seed fields at a range of isolation distances (165 feet to more than 10 miles). In 2003 and 2006 experiments, each individual conventional trap plot was 11 or 150 times smaller than the Roundup Ready pollen source plot, respectively. Also in the experimental plots, isolation was compromised ("bridged") for all but shortest distance by other alfalfa trap seed plots stocked with honeybee hives at commercial density. Combined, these biases favored maximum Roundup Ready gene flow and allowed for measurement and modeling of pollen-mediated gene flow under "worse-case situations". In contrast, in the 2006 commercial-practice "large" seed fields true isolation was used and the conventional and Roundup Ready seed fields were approximately equal in size (nonworse-case). Gene flow potential using cultured honeybee pollination, commercial seed grower practices and 1 mile true isolation resulted in approximately three to five times less gene flow than predicted using worse-case models.

[a] 2003 Data published previously in Teuber et al., 2004 and 2005, % observed in bioassay test. These data values were used by Teuber et al. (2005) to calculate the linear regression models for honeybees, given below.

[b] 2006 Data developed by Teuber et al., unpublished, preliminary data; % estimated from laboratory test; these fields did not conform to Best Practices isolation by experimental design.

[c] Predicted values for <1 mile were calculated per Teuber et al. 2005 linear regression model: $y=-0.00030x + 1.8529$, $x^2 = 0.98$, where y is % gene flow and x is distance (ft.)

[d] Predicted values for >1 mile were calculated per linear regression model calculated using the data published by Teuber et al. 2005 data for distances >1 mile: $y= -0.00012x + 1.2143$, $r^2=0.72$, $p=0.02$ (significant fit).

[e] 2006 Data developed by FGI from commercial seed fields; these FGI-owned conventional seed fields were grown to conform to FGI Best Practices

5.1.2 Feral-to-seed

Many seed farmers control feral alfalfa that may be growing near their seed fields. The factors that influence feral to seed gene flow are as follows:

- Probability of synchronous flowering – Seed fields flower for about 5-6 weeks (Bosch and Kemp 2005). Feral alfalfa blooms as environmental growth conditions permit. There may be some overlap between flowering time among feral alfalfa and seed fields.
- Availability of pollen from feral alfalfa – Feral alfalfa, if not managed (mowed), and if environmental conditions permit, will flower. Environmental stresses such as drought and insects may greatly reduce flower formation compared to cultivated alfalfa.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bees are brought in for seed fields and may also visit feral alfalfa if the hives are close enough to the feral populations. Bees may prefer other flowering wild species growing with feral alfalfa, which could reduce bee visits to feral alfalfa. Wild bees are present at relatively low levels compared to bees brought in for seed field pollination. There will likely be some bee activity in feral alfalfa populations and those bees may also visit alfalfa seed fields.
- Distance between seed fields and feral alfalfa – If seed farmers properly manage feral populations, then there should be a sufficient distance between feral populations and seed fields to achieve the current goals of seed purity. Seed farmers will need to be aware of seeding practices in neighboring rangelands because falcata (yellow-flowered alfalfa) may become increasingly adopted for rangeland forage improvement. Falcata seed is available commercially (http://www.windriverseed.com/Website%20catalog.pdf).
- Probability of seed maturation – Feral alfalfa may produce seed if environmental conditions are favorable and the plants are not mowed. Environmental stresses such as drought and insects may greatly reduce seed maturation compared to cultivated alfalfa.
- Probability of seed germination – Orloff et al. (1997) state that about 60 percent of seeds germinate when planted under good conditions (correct depth and soil conditions). It can be expected that a smaller percentage of feral alfalfa will germinate because soil conditions are less optimal.

No studies that specifically addresses feral to seed gene flow were identified.

"Feral into seed" field pollen-mediated gene flow potential will be zero or very, very low and it can be reduced to zero or near zero when the GM-sensitive conventional seed producer mows feral alfalfa to prevent synchronous bloom or takes steps to eliminate feral alfalfa plants (see mitigation strategies discussed for seed-to-seed conditions, above). There is evidence that conventional seed producers already are managing against the presence of feral alfalfa in seed-growing counties of Idaho and California (Kendrick et al. 2005). Even if feral alfalfa were left unmanaged, factors of gene frequency (feral plants may be genetically conventional or GM in

type), factors of scale, asynchrony of bloom, low pollinator density in the feral areas and other environmental and agricultural filters in the cultured seed fields would make GM gene from feral plants into a seed field from feral plants extremely improbable or very low occurrence. As described in the previous section, the conventional seed field edge, if harvested separately as a border crop, may be used to further mitigate the potential for gene flow from feral alfalfa into seed fields. 2000).

5.2 Forage Fields as the Pollen-receiving Environment, i.e. Fields Grown for Hay, Haylage, Pasture or Greenchop

5.2.1 Seed-to-Hay

"Seed into hay" pollen-mediated gene flow is improbable because the pollen receiving hay field would not be grown to produce any viable seed. Pollen and pollinators would be present because of the proximity of the seed field. If viable cross-pollinated seed were to form on the forage field stems, it is highly unlikely that seeds in the forage mass still will contribute to gene flow because they rarely will establish new plants (Putnam, 2006, Cash, D., 2005, Personal Communication; and Cash, D., public comment 481 to USDA/APHIS Docket 04-085-1). The amount of pollen-mediated gene flow into a non-GM conventional or organic forage field can be reduced to zero when the GM-sensitive forage field is harvested prior to seed maturation. The factors that influence seed to hay gene flow are as follows:

- Probability of synchronous flowering – Seed fields flower for about 5-6 weeks (Bosch and Kemp 2005). Hay fields that are harvested at 10 percent bloom only have flowers for about a week (Shroyer et al. 1984) and for most of that time the field is at far below 10 percent bloom. There may be some overlap between flowering time among hay fields and seed fields, but it is likely to be limited to a few days (figure V-4).
- Availability of pollen – Seed fields have the highest amount of available pollen compared to the other two populations of alfalfa (hay and feral). Farmers manage field conditions (water and insect control) to maximize flower formation.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bees are brought in for seed fields, but not for hay fields. Wild bees are present at relatively low levels compared to bees brought in for seed field pollination. Seed farmers maximize pollinator activity within seed fields. Bees brought into seed fields do visit nearby hay fields. The attractiveness of forage fields to bees will be affected by the extent of flowering
- Distance between fields (alfalfa populations) – There are no required distances between hay fields, but seed fields are still required to be separated from hay fields (section 3.6).
- Probability of seed maturation – Mowing during early bloom basically eliminates the chances of seed set. If there are marginal areas of the field that escape mowing, those plants can be considered part of the escaped feral population. Hay fields that are intentionally left to set seed are considered seed fields. Sometimes farmers harvest a few crops of hay, then a crop of seed from the same field. Farmers growing GT alfalfa are

prohibited from this practice, unless they have a seed grower contract with FGI and follow FGI Best Practices.

- Probability of seed germination – If seed is not set, germination is irrelevant. In the case that some small amount of seed does set in a hay field, then germination is expected to be limited by the autotoxicity of alfalfa.

No studies that specifically address seed to hay gene flow were identified. Studies where trap plants were allowed to mature and set seed are not accurate representations of hay fields because standard production practice is to mow hay fields prior to seed set.

5.2.2 Feral-to-Hay

"Feral into hay" pollen-mediated gene flow also can be reduced to near zero with normal forage cutting management practice on the GM-sensitive hay field (Putnam 2006 and 2007). The amount of pollen-mediated gene flow into a GM-sensitive conventional or organic forage field from (likely infrequent) GM-feral plants can be reduced to zero when the conventional forage is harvested prior to seed maturation and or nearby feral plants are mowed prior to bloom or eliminated by non-glyphosate herbicides and or cultural methods. Feral plants have not typically been targeted for control and in most situations; glyphosate would not be the herbicide of first choice to control it (see Petition, section VII.F.6. and Petition appendix 3). The paucity of feral plants should limit the amount of Roundup Ready feral pollen available to the hay field in the local environment. There would be little or no selection pressure on future feral alfalfa populations toward glyphosate-tolerant genotypes in plants or pollen unless glyphosate is used to manage these plants. Even if feral alfalfa were left unmanaged, factors of scale, asynchrony, low pollinator density and other environmental and agricultural filters in the cultured fields would make it unlikely that gene flow would occur from feral plants into a hay field. The factors that influence feral to hay gene flow are as follows:

- Probability of synchronous flowering – Feral alfalfa blooms as environmental growth conditions permit. Hay fields that are harvested at 10 percent bloom only have flowers for about a week (Shroyer et al. 1984) and for most of that time the field is at far below 10 percent bloom. There may be some overlap between flowering time among hay fields and feral populations, but it is likely to be limited to a few days.
- Availability of pollen – Feral alfalfa, if not managed (mowed), and if environmental conditions permit, will flower. Environmental stresses such as drought and insects may greatly reduce flower formation compared to cultivated alfalfa.
- Pollinator activity on days of synchronous flowering and placement of beehives – Wild bees and hobbyist beehives are the sources of pollinators between hay and feral populations.
- Distance between feral populations and hay fields – Out of all the alfalfa populations, feral and hay populations are likely to be the closest the most often. Hay farmers do not typically control feral alfalfa, and much of the feral alfalfa near hay fields is escaped hay seed, which may not persist as a breeding population. (See section 3.8.6 for description of Kendrick et al., 2005)

- Probability of seed maturation – Mowing during early bloom basically eliminates the chances of seed set. If there are marginal areas of the field that escape mowing, those plants can be considered part of the escaped feral population. Hay fields that are intentionally left to set seed are considered seed fields. Feral populations should be managed near seed fields and hay fields that are occasionally harvested as seed fields.
- Probability of seed germination. – If seed is not set, germination is irrelevant. In the case that some small amount of seed does set in a hay field, then germination is expected to be limited by the autotoxicity of alfalfa.

5.2.3 Hay-to-Hay

"Hay into hay" pollen-mediated gene flow can be reduced to near zero with normal forage harvest management practices (Putnam, 2006 and 2007). The factors that influence hay to hay gene flow are as follows:

- Probability of synchronous flowering – Adjacent hay fields will bloom at the same time if the variety of alfalfa is similar, planting schedules are similar and the growing conditions are similar. Irrigation inputs, fertilization inputs, and pest management (weeds, insects, small mammals, and diseases) can influence the maturity of alfalfa hay.
- Availability of pollen – Hay fields may be harvested any time between pre-bud and 100 percent bloom; however the majority of fields are harvested at or below 10 percent bloom.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bees are brought in for seed fields, but not for hay fields. Wild bees are present at relatively low levels compared to bees brought in for seed field pollination. There will likely be some bee activity in hay fields with bloom, especially if bee hobbyists have hives nearby.
- Distance between hay fields (alfalfa populations) – There are no required distances between hay fields.
- Probability of seed maturation – Mowing during early bloom basically eliminates the chances of seed set. If there are marginal areas of the field that escape mowing, those plants can be considered part of the escaped feral population. Hay fields that are intentionally left to set seed are considered seed fields. Sometimes farmers harvest a few crops of hay, then a crop of seed from the same field. Farmers growing GT alfalfa are prohibited from this practice, unless they have a seed grower contract with FGI and follow FGI Best Practices.
- Probability of seed germination. – If seed is not set, germination is irrelevant. In the case that some small amount of seed does set in a hay field, then germination is expected to limited by the autotoxicity of alfalfa.

No studies that specifically address hay to hay gene flow were identified. However Putnam (2006) provided an estimate based on probabilities which are included in figure V-16. Gene flow between seed and hay fields is summarized in figure V-16 from Van Deynze et al. (2008).

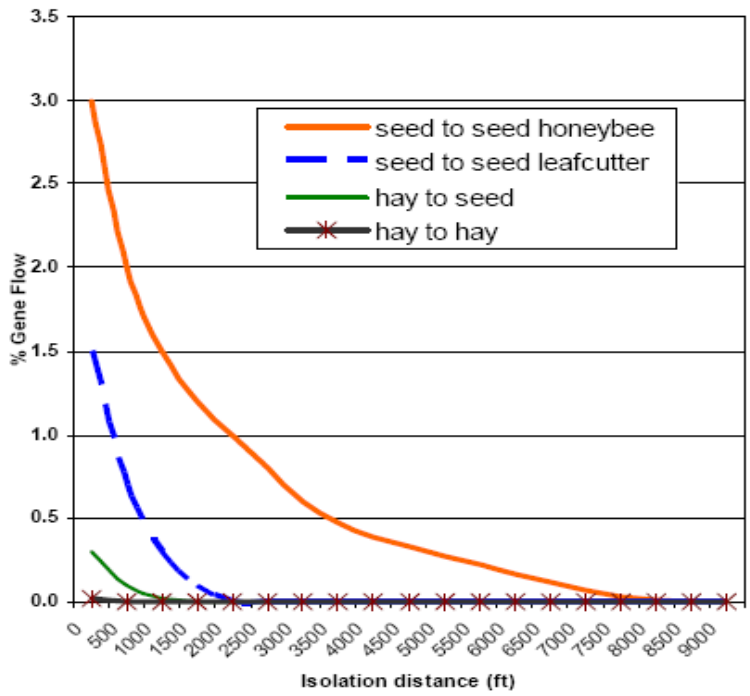

Figure V-16: Gene flow likelihood (Van Deynze et al., 2008)

5.3 Feral Alfalfa Plants as the Pollen-receiving Environment and Feral Plants as Bridges for GM (Roundup Ready) Gene Flow

5.3.1 Seed-to-Feral

The factors that influence seed to feral gene flow are as follows:

- Probability of synchronous flowering – Seed fields flower for about 5-6 weeks (Bosch and Kemp 2005). Feral alfalfa blooms as environmental growth conditions permit. There may be some overlap between flowering time among feral alfalfa and seed fields.
- Availability of pollen from seed fields – Seed fields have the highest amount of available pollen compared to the other two populations of alfalfa (hay and feral). Farmers manage field conditions (water and insect control) to maximize flower formation.
- Pollinator activity on days of synchronous flowering and placement of beehives – Bees are brought in for seed fields. Wild bees are present at relatively low levels compared to bees brought in for seed field pollination. There will likely be some bee activity in the feral populations, especially if bee hobbyists have hives nearby.
- Distance between seed fields and feral alfalfa – If seed farmers properly manage feral alfalfa, there should be very little feral alfalfa to receive pollen. Seed farmers will need to be aware of seeding practices in neighboring rangelands because falcata (yellow-flowered alfalfa) may become increasingly adopted for rangeland forage improvement. Most rangeland farmers will not be sensitive to the presence of GT alfalfa growing on their rangeland, however there may be organic beef and dairy farmers who will want their rangeland to be free of the presence of the GT trait.

- Probability of seed maturation – If genetic compatibility and the health of the plant are sufficient then feral seeds will mature. Because feral alfalfa is under environmental stresses, such as insect predation and drought that are managed in seed fields, feral alfalfa is likely to have a much lower seed yield than plants in seed fields. If feral alfalfa is mowed, then it will have a much reduced likelihood of maturing.
- Probability of seed germination. – If seed is not set, germination is irrelevant. Germination near parent plants is expected to be limited by the autotoxicity of alfalfa. Seeds of many plant species, including alfalfa, are spread through the feces of wildlife and other grazing animals (personal communication Gerald Schuman, retired USDA ARS, July 30, 2008).

Hammon et al. (2007) harvested feral alfalfa plants from 23 sites within two miles of GT alfalfa seed fields near Fruita, Colorado. The GT transgene was found at 83 percent of the collection sites out to a distance of 1.7 miles. No correlation was detected between gene flow rate and distance from seed fields. Qualitative evaluation of pollinators suggests feral bees play a role in gene movement. Figure V-17 shows the gene flow recorded by Hammon et al. (2007).

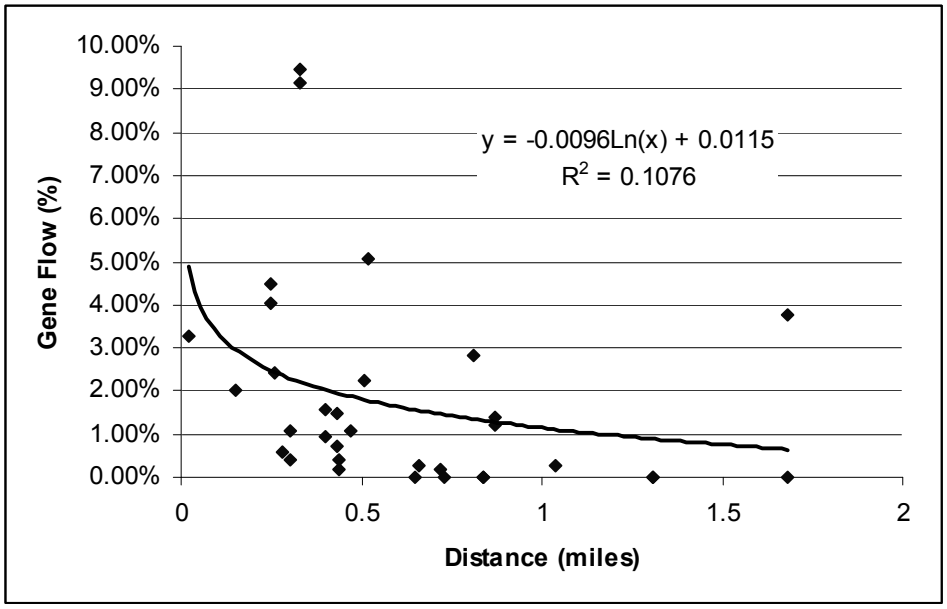

Figure V-17: Seed to feral gene flow (Hammon et al., 2006).

5.3.2 Feral-to-Feral

The factors that influence feral to feral gene flow are as follows:

- Probability of synchronous flowering – Feral alfalfa blooms as environmental growth conditions permit. There is evidence of naturalized alfalfa populations that are over 80 years old (Smith 1997), so it is clear that feral populations have some level of synchronous blooming even under rangeland grazing conditions. Very small feral

populations may have less synchronous blooming, unless the environment (including soil conditions) promotes synchronous blooming.

- Availability of pollen – Feral alfalfa, if not managed (mowed), and if environmental conditions permit, will flower. Environmental stresses such as drought and insects may greatly reduce flower formation compared to cultivated alfalfa.
- Pollinator activity on days of synchronous flowering and placement of beehives – Pollination within and between feral populations depends on wild pollinators, unless hobbyists' honey beehives are nearby. There may also be some bee activity from hives brought in for seed fields. The presence of bees for pollinating remote feral populations is much lower than for feral populations that are near seed fields and other farming operations that utilize managed beehives.
- Distance between feral populations – Feral populations may be so remote that they do not interbreed. Kendrick et al. (2005) surveyed feral alfalfa at 940 sites in six States. Feral alfalfa populations along roadsides were one to greater than 12 miles apart.[13]
- Probability of seed maturation – If genetic compatibility and the health of the plant are sufficient then feral seeds will mature. Because feral alfalfa is under environmental stresses, such as insect predation and drought that are managed in seed fields, feral alfalfa is likely to have a much lower seed yield than plants in seed fields. If feral alfalfa is mowed, then it will have a much reduced likelihood of maturing.
- Probability of seed germination. – If seed is not set, germination is irrelevant. Germination near parent plants is expected to be limited by the autotoxicity of alfalfa. Seeds of many plant species, including alfalfa, are spread through the feces of wildlife and other grazing animals (personal communication Gerald Schuman, retired USDA ARS, July 30, 2008).

St. Armand et al. (2000) placed feral trap plants along roadsides and in urban areas at least 800 meters from known alfalfa fields. Feral to feral gene flow was detected at 230 meters, but not in any of the urban sites. Table V-18 summarizes St. Armand et al. (2000) feral to feral gene flow data.

Table V-18. Feral-to-Feral Gene Flow (St. Armand et al. 2000)

Sample	Isolation Distance (meters)[a]	Total Seeds Tested	Outcrossing Frequency Percent
Escape #1	70	22	90.9
Escape #2	79	20	80.0
Escape #3	145	15	46.7
Escape #4	230	51	92.2
Urban #1	>800	0[b]	NA
Urban #2	>800	0[b]	NA
Urban #3	>800	0[b]	NA

[a] Distance to the nearest individual alfalfa plant or field.
[b] No seed set in urban areas.

[13] For a review of Kendrick et al. (2005) see the Technical Report Effects of Glyphosate-Tolerant Weeds in Non-Agricultural Ecosystems

If a gene is introduced to a population at a low frequency, then it will begin to move towards an equilibrium frequency in the population. In general, genes at low frequencies in populations undergoing random mating experience genetic drift, which can push an allele out of a population. (Genetic drift is the change in gene frequencies due to random chance. For example, if one plant out of 100 has the GT gene, then one percent of the population has the GT gene, but if that plant happens to be eaten by a deer, then the frequency of the gene in the population randomly changed to 0 percent). The GT trait is not expected to impart increased fitness in feral alfalfa. However, if the gene imparts a selective advantage, then natural selection will act to maintain the gene in the population. If there is high gene flow between populations, then even if a gene is pushed out of a population by a force such as drift, it could be reintroduced again at a later time (Scott 2007). The GT trait has not been shown to have a fitness cost and herbicide resistance transgenes have been shown to persist in *Brassica napus* and *Brassica rapa* hybrids under field conditions, even though the hybrids themselves had a fitness penalty (Mallory-Smith and Zapiola 2008). The frequency of the GT trait in feral alfalfa populations after introduction would be based on a balance between these factors.

5.3.3 Hay-to-Feral

The factors that influence hay to feral gene flow are as follows:

- Probability of synchronous flowering – Hay fields that are harvested at 10 percent bloom only have flowers for about a week (Shroyer et al. 1984) and for most of that time the field is at far below 10 percent bloom. Feral alfalfa blooms as environmental growth conditions permit. There may be some overlap between flowering time among feral alfalfa and hay fields.

- Availability of pollen – Hay fields may be harvested any time between pre-bud and 100 percent bloom; however the majority of fields are harvested at or below 10 percent bloom.

- Pollinator activity on days of synchronous flowering and placement of beehives – Wild bees and hobbyist beehives are the sources of pollinators between hay and feral populations.

- Distance between fields (alfalfa populations) – Out of all the alfalfa populations, feral and hay populations are likely to be the closest the most often. Hay farmers do not typically control feral alfalfa, and much of the feral alfalfa near hay fields is escaped hay seed, which may not persist as a breeding population. Kendrick et al. (2005) surveyed feral alfalfa populations in six States at 940 sites. In Idaho, feral and cultivated populations occurred within 2000 meters of each other in approximately three sites per county. In approximately half of those sites, the average distance was less than 20 meters. In Wisconsin, three-fourths of the sites where feral and cultivated populations occurred together had an average distance of less than 20 meters. In California, feral and cultivated populations occurred within 2000 meters of each other in approximately six

sites per county. In approximately half of those sites, the average distance was less than 20 meters.

- Probability of seed maturation – If the health of the plant is sufficient then feral seeds will mature. Because feral alfalfa is under environmental stresses, such as insect predation and drought that are managed in seed fields, feral alfalfa is likely to have a much lower seed yield than plants in seed fields. If feral alfalfa is mowed, then it will have a much reduced likelihood of maturing.

- Probability of seed germination – If seed is not set, germination is irrelevant. Germination near parent plants is expected to be limited by the autotoxicity of alfalfa. Seeds of many plant species, including alfalfa, are spread through the feces of wildlife and other grazing animals (personal communication Gerald Schuman, retired USDA ARS, July 30, 2008).

No studies that specifically address hay to feral gene flow were identified.

5.4 Feral Alfalfa as a Gene Flow Conduit

Due to several natural biological attributes mentioned above in section 1.1 and 1.2, feral alfalfa plants can arise due to natural dispersal from cultivation of all types of alfalfa, organic, conventional, and GT. Long-distance pollen and seed-mediated gene flow from GT commercial-scale seed and blooming hay production settings into feral alfalfa populations will occur (Hammon et al. 2006; Kendrick et al. 2005; St. Amand et al. 2000). In locations where feral alfalfa is of concern to GT-sensitive seed or hay producers, it can be controlled or reduced so that the economic impacts will be negligible (Kendrick et al. 2005; Petition appendix 3; Van Deynze et al. 2008). Large-scale feral or volunteer alfalfa patches can be a potential bridge for gene flow between managed seed or hay fields and within and among feral plant groups.
Recently, feral alfalfa is discussed by Van Deynze et al. (2008):

> "Feral plants are crop plants that grow and reproduce outside of cultivation. Feral alfalfa plants can sometimes be found on road edges, in fence lines and in abandoned fields. In the US, feral alfalfa populations have occurred through unintentional plantings of cultivated varieties ("escapes" from cultivation) or, in some cases, they originated from intentional planting of the abandoned fields, roadsides or marginal lands. Feral alfalfa occurs at very low density and scale relative to cultivated alfalfa grown for seed or hay. Biogeographic survey data from six states indicates that for most agricultural areas feral alfalfa plants do not occur or they are sparse (Kendrick et al. 2005). In a 2001/2002 multi-State survey, feral plants were found as dispersed plants or patches within 1.25 miles (2 km) of cultivated alfalfa at only 22 percent of the survey sites (Kendrick et al. 2005). Feral alfalfa plants are sometimes managed on roadsides by clipping, either with hay being harvested or simply left on the ground along with the other roadside vegetation. Feral plants are sometimes completely unmanaged and given adequate moisture and timely presence of pollinators, can flower and set seed. Feral plants are susceptible to the environmental (e.g. drought in the irrigated West) and insect (e.g. Lygus bugs in the West and potato leafhopper in the East) stresses common to the local

area. Although alfalfa was introduced to North America more than 200 years ago, it is not considered weedy, noxious or invasive in cultivated or feral settings."

The biogeographical survey data of Kendrick, et al. (2005) were used to assess the potential for gene flow from cultivated to feral alfalfa populations. In the survey, six states were selected representing major alfalfa production states in the United States. The states surveyed were California, Idaho, Pennsylvania, South Dakota, Washington and Wisconsin. A total of 940 roadside sites were surveyed (500 M^2/site). At approximately 22 percent of the sites, feral populations were located within 2000 meters of cultivated alfalfa. On average, alfalfa occupied <3 percent of the area surveyed. The results from this survey likely are representative of other states in the United States where alfalfa is produced for seed or forage. It is reasonable to assume that feral alfalfa populations also exist to a minor extent in other locations where alfalfa hay or seed is produced, or seeds are spilled during transportation or processing. The occurrence of feral alfalfa near seed-production sites was less than those observed where forage was produced; this may be because of the importance that seed producers place on maintaining isolation during seed production to ensure genetic purity of alfalfa varieties. Frequently, alfalfa exists outside of cultivation in abandoned and relegated fields or sown rangelands: i.e., the founder seeds for these populations were not recruited from feral or natural sowing and they were not transported by wildlife, but rather were initiated by human intentions and not since targeted for control.

Alfalfa is not considered invasive or weedy, so unless feral plants are actively humanly encouraged, it is unlikely that feral alfalfa populations or their role in pollinator or pollen-bridging would expand above current levels. This statement, that alfalfa "is not considered weedy, noxious or invasive in cultivated or feral settings" is additionally supported by statements submitted by several experts during the 2005 USDA public comment period [e.g., USDA docket 04-085-1, comments by Undersander #519 (Wisconsin), Doll #505 (Wisconsin), Murray #485 (Oklahoma), Beuselinck #501 (USDA-ARS Missouri), Whitesides # (Utah), Miller #502 (Wyoming), Howatt #510 (North Dakota), et al.].

The GT trait is not expected to enhance feral alfalfa fitness without selective pressure. Thus, without glyphosate application or chemical drift due to improper herbicide application, the trait should act as a neutral allele. Similarly, there is no expectation that expression of the glyphosate resistance trait without selection pressure would incur a fitness cost to feral alfalfa and thereby reduce its ability to persist in the environment. Some herbicide-resistant weed species have demonstrated costs associated with herbicide resistance without continued selection pressure (Neve 2007). However, this has been demonstrated for herbicide resistance to triazine and no studies to date have demonstrated a cost associated with expression of the glyphosate resistance transgene in the absence of selection. Transgenes could persist in populations that are not subject to selective pressure. Alleles with low frequency within a population can be quickly driven to fixation if selection pressure is strong. According to Ellstrand 2003, if gene flow to wild populations of a selectively neutral allele is repeated over multiple generations, wild populations will become increasingly crop-like, despite a lack of selection to maintain the neutral allele. If recurrent gene flow from cultivated to feral alfalfa were to occur for a significant

number of years, the frequency of the glyphosate resistance allele within a given population could increase compared to the present baseline.

Cultivated *M. sativa* and wild *M. falcata* hybrid swarms and backcross hybrids naturally occur as both diploid and tetraploid hybrids. Hybridization between these two types of alfalfa has been used in breeding programs to increase the hardiness of *M. sativa* cultivars (Rogan and Fitzpatrick 2004, Boe et al., 1998). Hybrids can be vigorous and fertile, and can thrive sympatrically with their *falcata* and *sativa* parents. Hybrid populations with *M. falcata* parentage inherit winter and drought hardiness, as well as the ability to exist in grass mixtures, making them more adaptable to resource poor environments and increasing the likelihood of feral populations (Bagavathiannan and Van Acker 2009). Unless properly managed, these feral populations could serve as bridges for long-distance gene flow, facilitating the low-level presence of novel traits, like glyphosate-tolerance, into the environment. As such, feral populations could potentially be a barrier to the coexistence of transgenic and non-transgenic alfalfa fields, and must be accounted for in landscape level gene flow models (Bagavathiannan and Van Acker 2009). The potential for gene flow and effects of hybridization between GT alfalfa J101 and J163 and *falcata* is unstudied. With respect to differences in weediness or persistence, there is no evidence that *sativa-falcata* hybrids with the GT trait will be significantly different than conventionally derived *sativa-falcata* hybrids without glyphosate selection pressure.

USDA's Conservation Reserve Program (CRP) encourages farmers to convert highly erodible cropland or other environmentally sensitive acreage to vegetative cover, such as tame or native grasses, wildlife plantings, trees, filterstrips, or riparian buffers (USDA 2010a). Alfalfa may be included in seed mixes for such covers (FAPRI 2007). Farmers receive an annual rental payment for the term of their multi-year contract. Haying and grazing of CRP acreage is authorized under certain conditions to improve the quality and performance of the CRP cover or to provide emergency relief to livestock producers due to certain natural disasters. There are two types of haying and grazing authorization: managed and emergency. Generally, CRP acreage may not be hayed or grazed during the Primary Nesting Season for certain wildlife. Managed haying and grazing is authorized no more frequently than one out of every three years after the CRP cover is fully established. Managed haying and grazing may be available less frequently in a particular area depending upon local conditions, resource capacity, and the conservation goals of the program (USDA 2010b). CRP land is a source of alfalfa that is only lightly managed and normally goes to full flower and seed. Alfalfa planted on CRP land would not meet the TUG required by GT alfalfa users, therefore, could not be intentionally planted on CRP land. Possible routes by which GT alfalfa could end up growing on CRP land include; low-level presence in alfalfa seed stocks used in CRP mixes, gene flow from neighboring GT alfalfa fields, and distribution by wildlife. Alfalfa on CRP land would behave like feral alfalfa, so the discussion above on feral alfalfa applies to alfalfa on CRP managed lands. For CRP lands with expiring contracts, either spraying or tilling are options for returning to crops. Low-level presence of GT alfalfa could affect the options for converting CRP land back to crops.

In California and Idaho, feral alfalfa was observed at fewer survey sites within counties where intensive alfalfa seed production occurs relative to those in counties where alfalfa is primarily grown for forage (Kendrick et al. 2005). This may indicate that professional, certified seed growers in California and Idaho are already controlling feral alfalfa as a means to ensure

certified seed crop genetic quality or at a minimum, producers in these counties are not actively encouraging alfalfa to grow outside of cultivation (e.g., non-cultivated areas are not irrigated). Also, these seed growers would see a benefit from mitigating feral, escaped, unmanaged alfalfa patches because such unmanaged plant patches act as a reservoir of alfalfa seed insect pests (e.g., Lygus) which are detrimental to seed yield and physical quality.

According to Mueller (2005), in California, "feral or volunteer alfalfa grows rampant along roads and ditch banks, in fallow fields, and as a weed in cropland throughout the State, [and that,] it can provide a bridge for pollen movement from production fields to other fields. A good stewardship program [for biotech alfalfa] includes grower education concerning control of volunteer plants using mechanical or chemical alternatives and preventing viable seed set in volunteers." To meet this objective, prior to the first commercial release of Roundup Ready alfalfa, FGI and Monsanto had incorporated feral and volunteer plant control education and seed field stand-take out requirements into the Roundup Ready alfalfa stewardship program.

Feral population densities are low. For example, in Colorado, Kansas and Washington when researchers conducted pollen-mediated gene flow into feral populations, the researchers opted to simulate (cultivate) higher than locally occurring feral plant densities: i.e., the researchers either opted to transplant artificial pollen-trap plant populations along a roadside (St. Amand et al., 2000) or the augmented the number of available "feral" seed collection sites by intentionally leaving a network of hay field patches non-harvested so that they would simulate feral alfalfa and form cross-pollinated seeds (Hammon, et al. 2006). This indicates that even near intensive seed production locations, the researchers did not find a sufficient number of escaped feral plants to conduct their desired study design.

Until the recent advent of GT alfalfa, feral, roadside, escaped, relegated fields and volunteer alfalfa plants have been largely ignored by non certified seed growers or encouraged to proliferate (e.g., planted intentionally along roadsides with occasional harvest during drought seasons). Due to autotoxicity associated with established stands of alfalfa (Bagavathiannan and Van Acker, 2009), dispersal of a glyphosate-resistant hybrid seed that occurs due to gene flow from GT-alfalfa into established feral populations would not likely contribute to a change in population structure. However, if hybrid seeds are dispersed beyond the range of autotoxicty or in less concentrated alfalfa populations, such as in mixed rangeland populations of alfalfa and other plant species, then self seeding of GT feral alfalfa could contribute to long-term persistence. Autotoxicity can extend up to a meter from mother plants and can depend on soil texture and water conditions; toxicity is greater in sandy loam versus silty clay (See Bagavathiannan and Van Acker, 2009). There have been no studies of gene flow rates within mixed rangeland populations of alfalfa. As noted above, many bee pollinators avoid collecting pollen or nectar from alfalfa flowers when other flowers (weeds or crops) are growing in the vicinity, thus gene flow in mixed stands could be variable and low. Gene flow rates would not be expected to be greater than rates measured for crop to feral studies (Hammon et al. 2006; Kendrick et al. 2005; St. Amand et al. 2000) where high ratios of crop pollen, relative to feral pollen, could contribute to directional gene flow. Dispersal of transgenic seeds into new habitats far from alfalfa fields (e.g. founder effect) could contribute to persistence of glyphosate resistance if hybrid transgenic seed successfully disperses, establishes, and matures (Mallory-

Smith and Zapiola 2008). Seeds which disperse beyond the range of autotoxicity have the potential to establish if appropriate conditions exist. As summarized by Bagavathiannan and Van Acker 2009, feral populations of alfalfa often have low flower abundance, which can contribute to competition among pollinators. Increased pollinator movement can lead to higher cross-pollination and increased seed set. However, isolated plants, while representing founder populations, can suffer from self-incompatibilty. Unless pollinators are able to migrate to isolated plants while carrying viable pollen, isolated feral alfalfa can suffer a substantial reduction in forage and seed yield. Both annual and perennial species can form feral populations in the wild. Unlike annual species, for which establishment in a plant community and persistence over many generations can be dependent on yearly seed production, perennial species such as feral GT alfalfa may be able to persist for several years despite seed production (Mallory-Smith and Zapiola, 2008).

Seed production experts (e.g., Brown et al. 1986), gene flow data (St. Amand et al. 2000, Fitzpatrick et al. 2007a, Marble 1980) and AOSCA foundation seed production standards (AOSCA 2003) support that the potential risk of gene flow is scale-dependent. Therefore, the relative size, density and proximity of feral patches to one another and to nearby blooming cultivated alfalfa (i.e., the size of any competing pollen source) will affect both the frequency of GT traits in feral populations and the likelihood that feral plants will act as bridges for GT trait gene flow. It is expected that over many sexual generations, the frequency of genotypes (conventional and or GM) in local feral populations will reach genetic equilibrium with locally grown commercial plantings if there is bloom synchrony and cross-pollination. The rate and extent for feral-to-feral population GM-trait gene flow will be affected by many environmental and agricultural filters (Putnam 2006). In the absence of glyphosate exposure, there is no adaptive benefit to feral plants containing the Roundup Ready gene versus their conventional counterparts. According to Van Deynze et al. (2008), "It is expected that only a minor portion of the seeds formed on the feral plants will result in true gene flow. Most seeds formed on feral plants will likely perish; as most would fail to successfully germinate, compete or establish outside of cultivation."

Feral alfalfa occurs at very low density and scale relative to cultivated alfalfa grown for seed or hay. Gene flow into small fields has been studied extensively; however, the reverse—gene flow out of small fields or feral patches into large seed fields has not. St. Amand et al. (2000) determined that gene flow is common between feral plants and that it likely occurs at short (0 to 4 m) and long distances (several miles). St. Amand et al. (2000) demonstrated that within an alfalfa seed field, pollen egress from a 1 meter diameter point source (similar in size to a patch of feral plants) could not be detected beyond 4 meters from the genetically marked pollen source plants. Dense patches of feral alfalfa, although not managed or protected from pests, could have similar bloom synchrony and comparable within patch gene flow dynamics to managed seed fields (but seed yield and quality would be much lower). St. Amand et al. (2000) found that, within seed fields:

> "Movement of the marker within the field occurred only over very short distances [and at low frequency]. Only 0.2 percent of the progeny 4-m away from the marker plants carried the marker gene and no progeny 6-m away were detected with the marker. Of those progeny carrying the GS marker, 97.4 percent occurred no more than 2-m from the source

> plants. Clearly, within-field gene movement only occurs over very short distances. It is likely that pollen is being cleansed [groomed] from the pollinators or is being covered with non-marker pollen after repeated visits to non-marker plants. This finding indicates that borders surrounding seed production fields may be useful in decreasing or limiting gene flow outside of a field."

Pollen cleansing or occlusion would be expected to occur on bees traveling long and or short distances among feral plants. Bee behavior or pollen dilution of conventional with GM pollen would be expected to constrain the rate and extent of Roundup Ready gene flow into and among feral plant populations.

6.0 Gene Flow Mitigation via Roundup Ready Licensing: Trait Stewardship Overview

A summary of the commercial trait stewardship program developed for GT alfalfa is presented as appendix V-2 and key elements are highlighted in this document when they pertain to mitigation of gene flow. All Roundup Ready forage producers are obligated to a core set of Monsanto trait stewardship license requirements and they are educated on the importance of their role in trait stewardship. Monsanto has an auditing program to ensure licensee compliance to the terms of the trait agreements.

Separate and in addition to the Monsanto requirements, all Roundup Ready seed producers are subject to an additional layer of licensing and contracting. Forage Genetics is the exclusive Monsanto licensee enabled to perform Roundup Ready alfalfa seed production. Through sublicensing agreements, Forage Genetics will contract with a small number of qualified, professional seed growers or seed companies to increase commercial quantities of Roundup Ready alfalfa varieties (e.g., approximately 76 alfalfa seed growers were contracted during 2006-07, exclusively in the United States). In addition to the Monsanto license requirements, each FGI Roundup Ready seed producer is contractually obligated to adhere to the FGI Best Practices. FGI has an auditing program to ensure licensee compliance to the terms of the FGI Best Practice trait agreement for seed producers. The field isolation at planting for Roundup Ready seed producers are pollinator species specific and they are five to 95 times the current isolation requirement for conventional certified seed production. Extreme isolation at field planting (3 miles) is observed for fields in California where much seed is exported and honeybees are the preferred pollinator species. The FGI/NAFA Best Practices isolation distances are illustrated in figure V-18.

At planting the location of all Roundup Ready alfalfa seed fields is reported to local crop improvement and the isolation distance to the nearest conventional alfalfa field is inspected. GM-sensitive seed producers may use this information to plan their field locations.

6.1 Gene Flow in Forage Crop Management Systems, Additional Comments and Background Information

6.1.1 Seed Admixtures

Separate from pollen-mediated gene flow, there exists the potential for a GT-sensitive hay (or seed) grower to inadvertently plant conventional seed containing low levels of biotech traits to establish their GT-sensitive forage field. As in other organic and GT-sensitive crop production settings, trait-sensitive producers can choose to purchase seed that is of known genetic origin (e.g., certified varieties) and or purchase seed that has been pre-planting tested for the presence of GT alfalfa. Seed sellers representing their product as organic or claiming other specialty qualities (e.g., "GM-free") should produce and source their products from identity preserved seed companies or growers.

6.1.2 Volunteers

Seedling recruitment from previous alfalfa seed or forage crops is also a possible source of rare seed-mediated gene flow into subsequent hay (or seed) fields. (Van Deynze et al. 2008):

Alfalfa grown for seed production occurs on approximately 0.5 percent of the total acreage planted to alfalfa. The acreage is small compared to that for hay; however, the likelihood of volunteer seedling-mediated gene flow is somewhat greater. Virtually all of the commercial alfalfa seed is produced under irrigation in the western US. Although there is no directly comparable data from the West looking at seed germination rate and hard seed content, some recent data monitoring the number of volunteers after alfalfa seed production in the field is useful. Reisen (unpublished) monitored volunteer seedlings following non-chemical fall termination of a dozen alfalfa seed production fields in Idaho. These fields were either fallowed or rotated to one of six rotational crops wherein alfalfa volunteer seedlings could be counted and controlled. The number of volunteer seedlings per acre were counted several times each year, and summarized by quarter, over the 3-year period (figure V-19). Arias (unpublished), likewise monitored volunteer seedling emergence in three Texas seed fields that had been fall-terminated using herbicide and cultivation. These three fields were rotated to corn for the next three years. Arias observed an average of 25 volunteers per acre during the first summer and zero thereafter (figure V-19). More aggressive stand take-out of seed fields and prudent management strategies for broadleaf plants limits the number of volunteer alfalfa plants.

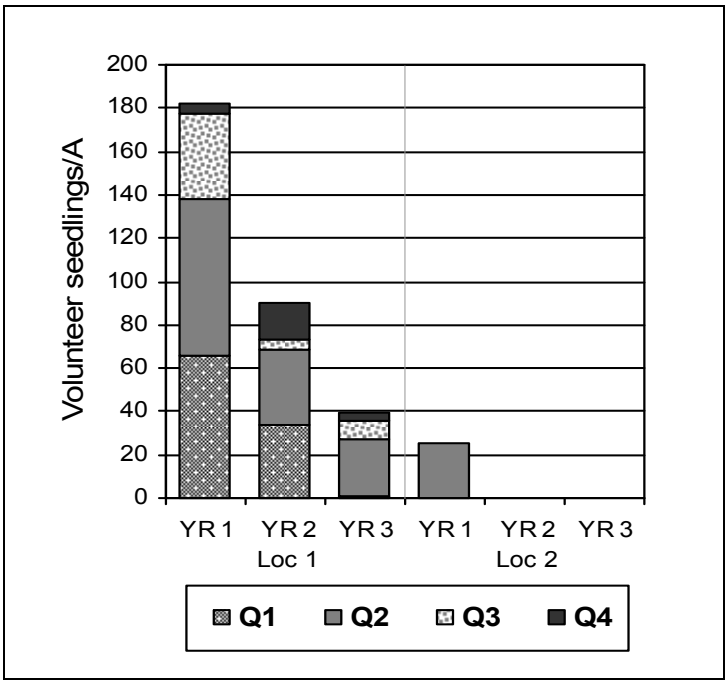

Figure V-19. Volunteer emergence by quarter in Idaho (Loc 1) and Texas (Loc 2) for three years after seed field take-out (mean count/A).
Figure excerpted from Van Deynze et al., 2008

6.2 Coexistence and Mitigation Strategies for Conventional, Organic, Export and Other GM-Sensitive Conventional Forage Producers

Seed and hay industry methods exist for successful coexistence of conventional, organic, and Roundup Ready forage (and seed) market segments. Coexistence of conventional and Roundup Ready alfalfa is desirable and possible (Bradford 2007; Putnam 2006; Van Deynze et al. 2008; NAFA 2007). Roundup Ready seed producers are required to take mitigation and stewardship steps to preserve conventional seed and hay markets. Similarly, Roundup Ready forage producers are obligated to follow trait stewardship and coexistence programs required by their Monsanto licensure (see section E.6., below). The following section describes GT (and conventional) trait avoidance measures that GM trait adverse forage growers can use to produce conventional forage to meet specifications for organic, export or other specialty market sectors.

In summary, the steps needed to produce hay for GE sensitive markets are: 1) purchase seed which has been tested and determined to be negative for the GT gene; 2) harvest hay prior to 10% bloom; 3) determine distances to neighboring fields that might be GT; 4) Control feral alfalfa in ditches; 5) If neighboring fields are GT and nearby, take steps to minimize probability of simultaneous flowering (e.g., stagger harvests), and do not place bees nearby; 6) Harvest and remove hay; 7) Identify and label hay destined for non-GE markets; and 8) Producers of non-GE alfalfa can test their hay to assure their buyers that the hay produced does not contain the GT gene. These test strips are ~$1 each, and are reliable at detecting the GT gene (Putnam 2007).

6.2.1 Field Establishment Using Conventional Varieties

According to Putnam (2006), "The most important steps to ensure hay trait purity are selecting non-GM varieties using certified seed from reputable companies, testing this seed for adventitious presence before planting, and maintaining product identity." It may be noted that in alfalfa, because it is a perennial crop, the seed choice decision is made only once during the multi-year life of the stand; therefore, as in all alfalfa production, selecting the appropriate variety and seed source is a long-lasting decision. Similar to other crops using the Roundup Ready trait, there are simple to use seed, leaf and hay trait testing kits for alfalfa (e.g., Strategic Diagnostics, Inc. and Envirologix, Inc.) and trait test service laboratories commercially available to the GM-sensitive buyers and sellers who wish to test for the presence of the Roundup Ready trait (e.g., BioDiagnostics, Inc., GeneScan, Inc. Mid-West Seed Services, Inc., and, various State's seed certification programs). The Roundup Ready seed test kits and protocols have been validated by the manufactures and by an independent third-party tester using field-grown seed samples (Teuber et al. 2007b). AP sensitive hay (or seed) growers should use seeds of planting stockseed grown under certification as well as an identity preserved system for additional assurance of high seed purity. Identity preserved production services are available from independent local third-party certifiers such as those accredited by the National Organic Program, Association of Official Seed Certification Agencies (AOSCA) and or OECD programs.

6.2.2 Management of Pollinators and Neighboring Cultivated or Feral Alfalfa

Pollinators serve no purpose for the production of forage, and, well-managed forage fields with few open blooms do not attract or support the nutritional needs of pollinating bees. GM-sensitive conventional or organic forage producers can mitigate the small potential for GM-trait cross-pollination by not placing or allowing others to place bee domiciles (hives) in or near their GM-sensitive conventional forage field and harvesting the forage prior to seed formation. This is the routine forage management practice so no special management will be needed by the non-GM forage producer.

In certain low-input forage production settings (e.g., southeast United States or northern Plains states), a very low number of conventional or organic hay producers allow honey producers to place hive boxes in their alfalfa forage fields. In general, honeybees demonstrate a strong behavioral preference *against* foraging for nectar in alfalfa (seed or hay fields) therefore flowering weeds or other crop species are more important sources of honeybee nectar and pollen (Arnett 2002; Pitts-Singer 2007; Teuber, et al. 2005b). Overall, honey production on alfalfa is a very uncommon forage grower practice in the United States and it results in very mature forage with very low forage quality or in some cases, the conventional field is grazed or is not harvested. Under the terms of the Monsanto Trait Stewardship License Agreement, forage producers are prohibited from placing bees in their Roundup Ready forage fields. A low frequency of *feral* solitary bee species may visit blooming alfalfa fields to collect pollen or nectar, but they would contribute to a very minor amount of pollen flow between forage, seed or feral alfalfa populations (Hammon et al. 2006).

Another exception within the conventional forage producer group is a small number of diversified farmers who, based on seasonal drought or immediate market prices (hay versus seed

price), they may sometimes opt not to cut forage but instead produce a summer "catch crop" of seed on the "forage" field. In this way, their alfalfa field is converted to a temporary, non-certified or "common" conventional seed production field. Catch crop seed producers were previously discussed in section VII, subsection B. The potential for gene flow in non-certified conventional or organic seed production is discussed as a part of the seed production system, below. Under the terms of the Monsanto Trait Stewardship License Agreement, Roundup Ready forage producers are prohibited from harvesting any seed in their Roundup Ready forage fields and in geographies where common conventional alfalfa seed production occurs, as a precaution to mitigate pollen availability and facilitate peaceful coexistence, the Monsanto licensee must harvest the Roundup Ready field for forage not later than 10 percent bloom.

Because feral alfalfa may act as a potential bridge (conduit) for pollinators between non-isolated fields or into feral populations of alfalfa (St. Amand et al. 2000), Putnam (2006) recommends that, "Prudent steps to prevent excess flowering or seed production during hay production will be beneficial to maintain coexistence of GM and non-GM alfalfa. […] Controlling feral alfalfa is a prudent measure to prevent movement of genes between hay fields." As discussed in the Petition, feral alfalfa is not common in most forage-growing geographies and where it is present and in close proximity, flowering can be controlled through clipping or feral plants eliminated by identity preserved forage producers, (appendix V-4, letters from Weed Control Experts).

6.2.3 Harvest Management

Optimal forage harvest management involves cutting at or before 10 percent bloom, typically occurring approximately one week after first flower. This harvest schedule greatly reduces the potential time in which there can be synchronous flowering of the conventional hay field and any neighboring Roundup Ready hay or seed production field. By managing their forage harvest dates and crop maturity, the non-GM conventional or organic forage producers can eliminate the potential for any seeds to form within their conventional forage field; whether the pollinations may have resulted from in-field or out-of-field pollen sources.

Sometimes forage producers struggle to cut their hay in a timely fashion due to rain, saturated soils, equipment breakdown, etc., or a forage field rapidly progresses to advanced maturity due to high temperature or drought stress. In delayed maturity harvest situations, viable seed may form within the hay canopy. For reasons explained in depth by Putnam (2006 and 2007), the risk of cross-pollination and dropped seed establishment in the AP sensitive conventional hay field is projected to be less than 2.5 seed per million.

6.2.4 Crop Rotation/field History

Alfalfa producers practice crop rotation between terminated alfalfa plantings to avoid a buildup of pests and maximize soil nitrogen credits to non-legume rotational crops. Conventional forage producers wishing to avoid GM traits may select fields without a prior history of GM variety use and or use pre-planting cultural and chemical controls and rotational crop choices appropriate to the elimination all alfalfa volunteers prior to planting the conventional alfalfa stand. Although "hard" alfalfa seeds may be viable for many years under dry laboratory conditions, in the field where moisture is present, extremely few or no hard seeds survive or successfully establish as volunteers within or outside of cultivation due to limited germling vigor, field biotic and abiotic decay processes (Undersander 2005, Bass et al. 1988). Even when abundant mature seeds have

been grown and dropped in the field (e.g., within or adjacent to a seed production field or its area of water drainage), two to four growing seasons without alfalfa are sufficient to mitigate remaining hard seed volunteers for the production of certified or foundation class alfalfa seeds, respectively (AOSCA 2003). Therefore, non-GM forage (or seed) producers can utilize traditional, reasonable crop rotation cycles and volunteer controls to mitigate the small potential for in-field volunteers from a previous alfalfa planting or mitigation of seeds that may have been moved by natural or irrigation water, wind or animals, etc.

6.2.5 Identity Preservation of Conventional, Organic or GM-sensitive Hay Lots

Post-harvest mechanical co-mixing of forage lots is a separate, potential source of determinate (non viable) gene (detectible trait) flow between harvested hay lots, i.e., trait flow that is independent from seeds or living plant material. Identity preserved hay growers such as those producing conventional, export, weed-free, non-GM, and or organic hays can adapt their existing market-required hay lot identification processes to segregate, label, test, and sell hay lots by cutting date, field and farm identification number. The available Roundup Ready trait hay test kits and hay lot sampling protocols have been validated by third-party testers using harvested hay field samples (Putnam 2007; Woodward et al. 2006a, 2006b). As stated by Daniel Putnam (2006), "Organic growers already have a process by which they identify and document organic hay, so no greater paperwork burden would be generally required to deal with this situation". However GT trait testing, if not already done, would add an additional cost to the identity preservation process. For conventional hays, quality tested hay lots are likewise routinely identified and segregated (Putnam 2004). According to Putnam (2006), prior to the advent of GT alfalfa, "Export and organic markets already require paperwork that identifies the lot, seller, and origin of hay, so a GT or non-GT label may be relatively simple for these marketers."

6.2.6 Equipment Sanitation

Contaminated equipment can be a source of physical co-mixing between dissimilar hay products (e.g., organic, conventional or GM trait), cross contamination between planting seed lots, or it might transfer soil/stems that contains weed and or alfalfa seeds. Equipment sanitation is an effective, simple precaution that is highly effective and recognized as critical in all identity preserved production systems. Planters, cultivators and forage harvesters, processors, etc. may be cleaned before or after use to avoid unwanted residual debris or seed. In some situations, it is possible to designate that a piece of equipment may only be used in a preplanned sequence or for certain types of products (e.g., organic fields only), so that the need for cleaning between product types is reduced or eliminated. It should be noted that equipment sanitation is useful to prevent the unintended dissemination of weeds, foreign materials and pests between fields so equipment sanitation is recommended and will remain important to product integrity irrespective of GM-trait sensitivity.

6.2.7 Mitigation of Negative Impacts Through Prudent Record Keeping, Planning and Contractual Agreements

Complete and correct field and harvest records and advanced planning are recognized as critical to producing crops with consistent and predictable attributes. Producers of organic crops must maintain records and develop an organic crop production plan that satisfies National Organic Program Standards. Certifiers are available to document that minimum production standards

were fulfilled. Similar use of records, planning and independent certification will assist those forage (or seed) growers who wish to avoid Roundup Ready or other GM traits in fields, seeds or equipment.

Contract stipulations regarding tolerance limits to GM traits: To manage potential economic negative impacts of unwanted traits in hay, non-GM, organic or other conventional hay producers may use the effective gene/trait mitigation and avoidance practices discussed above which are based on sound science and successful co-existence models adopted in previous GM crop market channels and in use for specialty market alfalfa hays such as organic, certified weed-free or forage quality tested hay lots [see USDA-AMS Livestock and Seed Program (http://www.ams.usda.gov/lsmnpubs/hsum.htm) Web site for examples of by-feature hay lot identification strategies]. In addition, some of the potential negative impacts on sensitive producers may be avoided by careful consideration of voluntary contract specifications prior to signature on the agreement. Buyers and sellers alike, must read, understand and manage the GM specific language that is offered to/by them in voluntary buyer-seller contracts. As in other crops where GM traits are in commercial use, forage (or seed) producers who agree to absolute zero-presence GM contracts could individually experience more negative impacts than producers using contracts with verifiable testing standards for GM trait presence/absence. During the initial deregulation period for Roundup Ready alfalfa (June 14, 2005 to May 12, 2007), several conventional alfalfa seed and hay producers implemented contracts that stated a greater-than-zero tolerance level (low-level presence threshold) for conventional alfalfa products [e.g., less than 0.5-1 percent trait threshold in conventional seed (McCaslin 2007)]. Contract verbiage recommendations for GM-sensitive alfalfa forage contracts have been published by William Woodward, Extension Faculty, Washington State University (Woodward 2006a and 2006b). Woodward has provided examples of recommended contract statements to adopt and statements to be avoided in conventional (specifically export) hay contracts. Woodward's hay industry guidance recommendations, are congruent with statements regarding the adventitious presence contract and labeling guidance positions of American Seed Trade Association (ASTA), Council for Agriculture Science and Technology (CAST) and the USDA National Organic Standards Program. Testing tools and statistical interpretation for GM trait tolerances are available on-line to help guide and inform members of the GM-sensitive hay (and seed) industries (e.g., see Woodward 2006a, CAST and ISTA Web sites).

6.3 Coexistence Strategies for Producers of GT Alfalfa Forage

All purchasers of Monsanto's Roundup Ready treated alfalfa seed are obligated to adhere to all terms of the Monsanto Trait Stewardship Agreement (MTA) which is a limited use license for the patented seeds. These important coexistence and trait stewardship program obligations and limitations are presented in the 2008 Monsanto Technology Use Guide (TUG) (Monsanto, 2007).

Alfalfa hay is planted in every State in the United States. Intermingled alfalfa hay and alfalfa seed production only occurs in portions of a few Western states which have the specific climate required for successful commercial production (figure V-1 and figure V-2, compare areas of overlap). Certified alfalfa seed production requires minimum isolation from alfalfa (hay/seed/feral) of any other variety (AOSCA 2003). The isolation is inspected and verified by State seed certification organizations or, if organic, the planned buffer zone is verified by an

accredited organic certifier. Although non-certified seed is not officially regulated or inspected, in areas where "common" seed production is intermingled with Roundup Ready forage production fields, the Roundup Ready alfalfa forage producer is required to harvest at or before 10 percent bloom (Monsanto Technology Use Guide and Monsanto Technology/Stewardship Agreement 2008).

6.4 Overview of Roundup Ready Alfalfa Seed Crop Management and Seed Industry Systems

All Roundup Ready alfalfa varieties are patent protected, AOSCA registered proprietary cultivars that are phenotypically and genetically identifiable due to the presence of the Roundup Ready gene. Trait licensing requirements and the presence of the unique gene insertion will offer previously unavailable tools for intellectual property protection (e.g., genetic fingerprinting of parent lines) and marketplace stewardship opportunities in alfalfa.

Forage Genetics is the exclusive Monsanto seed-producer-licensee for Roundup Ready alfalfa cultivars. Seed increase on any Roundup Ready alfalfa cultivar requires a special seed grower contract issued through Forage Genetics or one of its sub-licensees (to-date, December 16, 2007, there is only one sub licensee and the number is expected to remain small due to the small number of candidates).

All Roundup Ready seeds must be grown and sold by variety name (no common seed production is authorized). Each FGI Roundup Ready alfalfa seed grower and seed company is obligated to the terms of the Monsanto Trait Stewardship Agreement and Forage Genetics seed producer contract terms that require implementation of FGI Best Practices for Roundup Ready Trait Stewardship.

To maintain variety type, FGI Best Practices and official variety registration descriptions require seed growers to apply one application of Roundup agricultural herbicide per growing season and in accordance with State and Federal product registrations. There are separate labels for Roundup agricultural herbicides in forage versus seed production settings: one or more Roundup agricultural herbicide formulation is labeled in 50 or 13 states, for forage or seed, respectively. Roundup Ready cultivar seed production may only be legally grown in states where a Roundup agricultural herbicide label has been approved for seed crop production. As a part of the seed trait stewardship program and as a means to mitigate gene flow to occasional common conventional or potential organic seed producers, FGI and Monsanto have chosen not to contract for or to enable legal seed production of Roundup Ready alfalfa in some states where common seed production is most typical and or certified alfalfa variety production is very atypical (e.g., Kansas, South Dakota, North Dakota, Minnesota, and Nebraska). An outcome of the NAFA Coexistence workshop was that the steering committee agreed to facilitate the development of a binding Best Practices for Roundup Ready seed production document which was supported by all three of the genetic supplier member companies (Peaceful Coexistence: Creating a Strategy for Harmony Among GM, Organic and Conventional Alfalfa Producers, October 10, 2007, Denver, CO). These three current NAFA members represent approximately 90 percent of the U.S. proprietary cultivar conventional genetic supply capacity and 100 percent of the Roundup

Ready seed producer companies. Terms of the Best Practices document will be publicly available information and it will be available from Forage Genetics and or the other Roundup Ready Seed Producers. Along with the genetic suppliers, State seed certification organizations will play a key role in coexistence monitoring and management. This agreement will help ensure mechanisms for peaceful coexistence among conventional, organic and GM alfalfa seed producers and choice for growers to use organic, conventional and GM varieties. The Best Practices also stipulates that all Roundup Ready seed producers will respect local "conventional only" seed zones should they develop.

6.5 Overview of Alfalfa Industry Coexistence Systems Development

During 2000-2005, APHIS regulated studies were conducted using either introduced leafcutter bees as the primary pollinators (McCaslin et al. 2001, Fitzpatrick et al. 2002, Fitzpatrick et al. 2003, Teuber and Fitzpatrick 2007) or introduced honeybees (Teuber et al. 2004 and 2005b). In all gene flow studies to date, in addition to the stocked pollinator, native and feral non-native bees were present at ambient levels so their potential contribution to gene flow under commercial conditions has been accounted for in the available data sets. In the 2006 growing season while Roundup Ready alfalfa was not APHIS regulated, FGI and public scientists conducted additional gene flow research studies (Teuber and Fitzpatrick 2007; Hammon et al. 2006; Fitzpatrick et al. 2007b; Teuber et al. 2005a and 2007).

During 2006, FGI continued to implement and evaluate efficacy of its Best Practices for Trait Stewardship in Seed Production. Over 120 conventional FGI commercial seed lot samples were evaluated to ascertain the actual amount of gene flow between commercial-scale, Roundup Ready and conventional seed fields under leafcutter bee, honeybee or typical mixed-species pollination systems throughout key regions of the western United States These commercial seed lot data validated that the FGI Best Practices are effective in mitigating adventitious presence of Roundup Ready alfalfa pollen-flow to very low or non-detectable amounts: AP percentages were, in all lots, not detected or at levels less than would have been projected using the worst-case research trial study predictions (Fitzpatrick et al. 2007a and 2007b), thereby validating that the commercial seed trait stewardship program was achieving a key objective—mitigation of gene flow to conventional and organic seed producers.

As required by Roundup Ready seed production contracts and NAFA Best Practices for seed production (NAFA 2007), Roundup Ready seed producers will continue to report the locations of all Roundup Ready seed fields to their local crop improvement organizations. These independent third-party agents will maintain Roundup Ready seed field location records and field histories. GM-sensitive seed producers, therefore, have an available mechanism to obtain GM alfalfa seed field location information and request professional services to assist them in seed field isolation planning. As stated above, where feasible, many GM-sensitive seed producers can lessen further the risk of pollen-mediated gene in-flow from other/unknown varieties of seed (or hay) by choosing to use larger size seed fields (e.g., >5 acres), stocking pollinator species that range shorter distances (i.e., leafcutter bees vs. others), harvesting the seed field border as a separate lot (St. Amand et al. 2000), and working to coexist with their neighbors who grow alfalfa for forage and seed. Organic, export, common or foundation class seed producers who are highly GM sensitive can opt to use additional isolation or buffer zones and identity preserved practices for

their non-GM seed fields (e.g. follow the most conservative AOSCA foundation seed class standards which require use of 900 ft or greater isolation to any different alfalfa variety whether grown for hay or seed purpose, etc.). In addition, groups of local seed growers can use informal or formal conventional-only seed production zones. NAFA Best Practices stipulates that Roundup Ready seed producers will not contract for Roundup Ready seed production in such grower organized zones.

Commercial seed lot data validate that implementation of the Best Practices system is a very effective industry tool in the management of conventional seed lot genetic quality (Fitzpatrick et al. 2007a and 2007b). Adventitious presence of the Roundup Ready trait in conventional seed lots has been infrequent and in all cases well below the 0.5 percent industry consensus AP tolerance for domestic alfalfa and below values predicted using worst-case research models. The large-scale commercial validation also helps put in perspective the minimum incremental risk associated with potential real world concerns about pollination from wild pollinators, extraordinary pollen flow by wind-driven pollinator movement, and low-level presence through physical mixtures of seed in harvesting and/or seed processing.

The data of St. Amand et al. (2000) and Fitzpatrick et al. (2007a) supports that a conventional border (or buffer) area is a sound gene flow mitigation strategy for producers of highly GM sensitive seed crops (the border could be of the same conventional variety as in the field center). The outside edge could be harvested separately and tested, and if no GM trait is detected in the border seed, it could be bulked with the main field lot. It should be noted, however, that if, as St. Amand et al. suggest, a conventional cultivar were used to border a GM containing seed field or research plot, the genetically different variety border would violate seed certification isolation standards (i.e., the genetically dissimilar border would preclude variety certification of the main field seed crop). Moreover, even for non-certified seeds lots, especially small experimental lots, the encompassing conventional pollen would negatively affect GM seed crop's trait percentage and genetic purity.

During the development of field-isolation standards, AOSCA and other certifying agencies have considered data from fields and plots with little or no isolation (e.g., <100 ft.). Data sets developed using conventional traits indicated that although there is a very minor amount of gene flow between seed fields (Brown et al. 1986) or within seed fields (St. Amand et al. 2000) bulking of seeds throughout the field help mitigate the percentage of whole-field off-types to industry and AOSCA accepted levels (<1 percent off-types).

Seed growers who are highly GM sensitive may opt not to apply the 10 percent rule or any other exceptions to isolation in the production of their seed crop whether or not it is officially certified as to varietal purity. Specifically, the AOSCA certified class seed standards (2003) have a "10 percent rule exception" as follows:

1) "Minimum distance from a different variety or fields of the same variety that do not meet the varietal purity requirements for certification, shall be 600 and 300 feet for fields over 5 acres; and 900 and 450 feet for fields 5 acres or less that produce the Foundation and Registered seed classes, respectively.

2) "Isolation requirements for the certified class are based on the size of the certified field and the percentage of the field within 165 feet of another variety of alfalfa. If 10 percent or less of the certified field is within the 165-foot isolation zone, no isolation is required – only a definite separation. If more than 10 percent of the field is within the isolation zone, that part of the field must not be harvested as certified seed. The isolation zone is that area calculated by multiplying the length of the common border(s) with other varieties of alfalfa by the average width of the certified alfalfa field falling within the 165-foot isolation distance requirement.
3) "In those cases where a portion of the field meets isolation requirements, then a clear line of demarcation shall be established between the certified and non-certified portion of the field."

The U.S. alfalfa industry has taken key steps toward coexistence and product differentiation. Some examples are:

- *LLP tolerance, greater than zero:* During 2005-2007 growing seasons, certain alfalfa seed companies with GM-trait production sensitivities began to issue seed grower contracts with stated LLP tolerances, e.g., "<1 percent GM trait" (McCaslin 2007), thereby, demonstrating that mainstream conventional seed companies are (were) shifting toward market coexistence and GM-trait tolerance in conventional seeds. Roundup Ready trait detection methods and commercial test services are readily available to help seed and hay producers, buyers and sellers support their company's quality assurance programs. A stated position of the American Seed Trade Association (ASTA 2007) is that zero tolerance to gene flow of a commercially adopted trait (e.g., Roundup Ready alfalfa 2005-2007) is not an attainable or realistic goal for most producers in large scale U.S. production agriculture. There is an alfalfa industry consensus that a tolerance value greater than zero for GM traits should be developed and implemented (e.g., NAFA 2007). A 0.5 percent LLP tolerance for domestic use, GM seed, has been proposed by several NAFA members to be an attainable and effective tolerance goal (NAFA, Denver, CO, October 10, 2007).

- *Offering sale of GM trait alfalfa varieties:* Although not necessarily producing the Roundup Ready seeds or breeding the varieties themselves, all four of the major U.S. seed genetic suppliers and seed production companies (Forage Genetics, Pioneer Hi-Bred, Dairyland Seeds and Cal/West Seeds) were Monsanto licensed and sold one or more Roundup Ready alfalfa varieties alongside of their own conventional and or organic seed product offerings. Roundup Ready alfalfa was sold by more than 20 seed brands all of which continued to offer conventional cultivar products.

- *Rapid market penetration:* During the 2005-2007 period of deregulation, approximately 300,000 and 18,000 acres of Roundup Ready alfalfa forage and seed, respectively, were grown with no substantiated cases of conventional forage or seed market disruption (McCaslin 2007).

- *Value-added trait opportunity for domestic seed growers:* Roundup Ready alfalfa seed is only produced (and consumed) in the United States. Therefore, U.S. seed producers of

Roundup Ready alfalfa seed have had an exclusive value-added market opportunity wherein there is no foreign competition by lower-cost seed producers.

- *Seed industry cooperation toward peaceful coexistence:* The FGI Best Practices are effective in mitigating gene flow to conventional seed production to a low level (Fitzpatrick et al. 2007b). Monsanto, FGI and Roundup Ready alfalfa licensees have implemented comprehensive trait stewardship policies and have worked proactively with State seed certification organizations to develop industry tools for coexistence and mitigate GM-trait gene flow to low levels. Two examples of such industry cooperation are the California Alfalfa Industry Stakeholders Meeting hosted by the University of California-Davis Seed Biotechnology Center, on January 27, 2005 (UCDSBC 2005) and the National Alfalfa and Forage Alliance's workshop on peaceful coexistence strategies held on October 10, 2007 in Denver, CO (NAFA 2007). The NAFA coexistence workshop resulted in the joint authorship and development of five alfalfa industry consensus documents that are available from the authors or from NAFA (http://www.alfalfa.org) with the following topics

    1. Gene flow in Alfalfa: Biology, Mitigation and Potential Impact upon Production for GM-sensitive Markets (White Paper); Authors: A. Van Deynze, S. Fitzpatrick, B. Hammond, M. McCaslin, L. Teuber, D. Undersander and D. Putnam (Van Deynze et al. 2008)
    2. Best Management Practices for Roundup Ready Alfalfa Seed Production (NAFA 2008a)
    3. Coexistence for Alfalfa Hay Export Markets (NAFA 2008b)
    4. Coexistence for Alfalfa Seed Export Markets (NAFA 2008c)
    5. Coexistence for Organic Alfalfa Seed & Hay Markets (NAFA 2008d)

- *Independent monitoring and oversight of FGI seed stewardship practices:* FGI has used third-party inspection services to document its compliance with its Best Practices seed field isolation distance policies (e.g., Lowry, 2007). Crop Improvement Organizations have used this GM seed field location information to assist GM-sensitive seed producers in seed field isolation planning and their identity preserved process inspections.

- *Validation of best practices for seed production*: FGI and NAFA Best Practices isolation requirements and stewardship during seed production have been evaluated and validated. Use of the Best Practices resulted in 0.00 to 0.18 percent of the Roundup Ready trait in conventional seed lots (Fitzpatrick et al., 2007a and 2007b). NAFA BMPs were tested in commercial production, in limited third-party testing for low-level presence of the GT gene summer, 2009. Data collected from over 1,000 seed lots in 10 Western states indicated that the NAFA BMP system was working on a commercial scale. (Lowry 2010). In addition GT alfalfa seed production contracts are limited to the following 11 states: Arizona, California, Colorado, Idaho, Montana, Nevada, Oregon, Texas, Utah, Washington, and Wyoming. GT alfalfa see producers are also required to respect any non-GE seed production zones designated as such by a consensus of local seed growers

(NAFA 2008a). GT alfalfa seed growers are also required to undergo stewardship training (NAFA, 2008a).

- *Seed company communication and low-level presence:* In specific conventional seed lots, when detectable AP has been publicly claimed to have occurred outside of FGI contracted conventional seed lots (e.g., informal comments quoted from a 2006 Idaho Eastern Oregon Alfalfa Seed Grower meeting), if a percentage LLP has been publicly claimed it has been at a very low-level, i.e., "trace to 0.9 percent"—levels below the 1 percent contract tolerance in all relevant cases (McCaslin, 2007 declaration) and not in conflict with the up to 1 percent off-types allowed by varietal purity standards. FGI has worked cooperatively with other seed companies on a case-by-case basis to successfully resolve seed field proximity challenges (McCaslin, 2007).

- *Growth of organic market sector:* For the past several years, the number of alfalfa forage acres certified as organic has increased (2005-2007) and likely flourished in-part due to the publicity surrounding the commercial use of the first GM-trait in alfalfa (Putnam 2006; Putnam 2007). Organic and identity preserved non-GM forage and seed products are new premium-priced, differentiated, and specialty market opportunities available to U.S. alfalfa producers. Demand for organic alfalfa is expected to grow for the next several years so that supply is sufficient to meet the growing market size of organic dairy and livestock producers.

- *Increased awareness and education regarding seed quality issues and seed certification*: Especially for GM-sensitive market sectors like organic and export, there has been increased education and discussion about the importance of and value in using identity preserved production methods, planting certified variety seeds and benefits of enhanced quality control. In 2010, AOSCA announced a new certification program where member agencies will offer new inspection and certification services that exceed traditional seed certification standards and that are customized specifically for the identity preserved conventional, organic, export and GM alfalfa seed markets. For more information on this program see sections IV.B.4 and appendix V 4.12. The gene flow information has and is being used by State, national and international seed certification programs to review and if needed update certified seed production standards. Individual seed companies are also using the information to review current company policies. Information to support coexistence of diverse market sectors has been made available in numerous public, government, court, scientific society, seed grower, hay grower, industry stakeholder and industry-wide presentations and much information is readily accessible to the public on searchable internet Web sites or by contacting a State forage extension specialist.

6.5.1 Effectiveness of Current Alfalfa Gene Flow Mitigation Strategies

The alfalfa seed industry has recently implemented two complementary programs that together enable mutual coexistence between conventional and GT alfalfa seed producers. The 2010 Alfalfa Seed Stewardship Program (ASSP-2010) is an identity preserved process-based certificate offered by State seed certification agencies. It was developed by the Association of Official Seed Certifying Agencies (AOSCA) designed to serve GE-trait sensitive conventional seed producers (e.g., export). In 2008, the Best Management Practices for GT alfalfa Seed Production (BMPs) was adopted by the National Alfalfa & Forage Alliance (NAFA). These

BMPs are required coexistence protocols that apply only to GT alfalfa seed-producing companies (i.e., no new requirements are imposed upon external conventional seed producers). These market-driven, science-based programs were developed with the involvement of alfalfa industry stakeholders over a 5-year period (2005 to 2010) using all available market and gene flow data. An array of stakeholders were involved that represented diverse segments of the alfalfa seed and hay industries: scientists, seed certifiers, breeders, exporters, marketers, producers, growers and organic. (see table V-19) These new programs are independent from and more stringent than AOSCA or OECD Seed Certification Programs. Forage Genetics and Pioneer Hi-Bred International (the only companies producing GT alfalfa seed), have collectively reported to inspectors that in 2009 greater than 97 percent of their conventional seed lots were produced without detection of the GT alfalfa trait (>500 lots tested with <0.00 percent GT alfalfa). If detected, AP was less than 0.5 percent (overall lot average <0.1 percent).

Table V-19. Effectiveness of current alfalfa gene flow mitigation strategies

	No Program (e.g., common seed)	USDA National Organic Program Certification	Certified Seed	Roundup Ready Alfalfa (GT) Seed	AOSCA AASP-2010 Identity Preserved, Certified Seed
Market	U.S. domestic conventional (baseline)	Organic forage planting	U.S domestic conventional & GT alfalfa seed	U.S. domestic GT alfalfa seed	U.S. conventional seed for export
Purity Standard or Objective	n/a	No official purity standards; process-based requirements	≤1% off types	≤ 0.5% GE in *neighboring* conventional seed production	Non-detect GE
Spatial isolation from other seed field	n/a	Customized farm plan; not uniform mitigation standard	165 ft	900 ft to 3 miles GT alfalfa seed field planting (pollinator specific)	≥5 miles
Program conforms to:	n/a	USDA-AMS National Organic Program	Federal Seed Act	Industry consensus and GT alfalfa seed co. contracts	AOSCA I. P. Program
Program monitored by:	n/a	Local Organic Certifying Agency	State Seed Certifying Agency	State Seed Certifying Agency	State Seed Certifying Agency
Program obligations fulfilled by:	n/a	Organic, conventional grower	Seed company and seed grower	GT alfalfa seed company and seed grower	GE-sensitive seed company and conventional seed grower

Table V-19. Effectiveness of current alfalfa gene flow mitigation strategies

No Program (e.g., common seed)		USDA National Organic Program Certification	Certified Seed	Roundup Ready Alfalfa (GT) Seed	AOSCA AASP-2010 Identity Preserved, Certified Seed
Growers using the program:	Conventional only	Conventional only	Both, conventional and GT alfalfa	All GT alfalfa, only	Conventional only

Source: Fitzpatrick and Lowry 2010.

6.6 Coexistence and Mitigation Strategies for Conventional, Organic and Other GM-Sensitive Alfalfa Seed Producers

Key references are the NAFA and Van Deynze (2008) describing coexistence and mitigation of gene flow for organic and export producers.

6.6.1 Isolation from Unknown or Dissimilar Alfalfa Varieties and Field History (Physical and Temporal Separation)

GM-sensitive conventional and organic alfalfa seed producers wishing to produce a genetically pure seed crop may utilize AOSCA (2003), State seed certification program rules, and/or National Organic Program standards to physically and/or temporally genetically isolate their GM-trait sensitive production field. AOSCA programs stipulate standards for an appropriate physical isolation distance from other alfalfa pollen sources and NOP standards require that a buffer zone should be observed. Seed certification rules require a minimum crop rotation period and in-crop volunteer control (e.g., two or more years of field history without alfalfa cultivation). Typically, when genetic purity is desired, in-crop volunteers are controlled to ensure seed quality. Where a GM-trait sensitive conventional alfalfa seed field is grown in proximity to any other alfalfa, at a minimum, the AOSCA isolation requirements for certified seed production should be observed. The requirements are designed to ensure high genetic purity and minimize off-types (AOSCA 2003). The seed producer may wish to utilize seed or organic certification inspection services to document that the grower's desired isolation and other available crop identity preserved protocols have been observed. The certified seed program rules, the Federal Seed Act and National Organic Program Standards all allow for a low frequency of genetic off-types in certified seeds; zero tolerance is not required. Individual conventional GM trait sensitive seed producers may follow isolation or other protocols more stringent than program certification requirements so that unintended traits may be further avoided. Some State seed certification programs offer inspection services for enhanced, grower-defined identity-preserved programs intended to produce seeds of specialty quality (e.g., foundation generation or certified stockseed, low linoleic acid soy grains, certified organic, certified low-level GM products). The AOSCA AASP-2010 identity preserved certification program is designed specifically to enhance market coexistence opportunities and facilitate the segregated production of Roundup Ready and certified no-LLP conventional alfalfa seeds lots (AOSCA 2010a).

In conformance with the NAFA and the FGI Best Practices for Seed Production Stewardship, Roundup Ready alfalfa seed production is currently planted with a minimum isolation distance of 900 ft, 1 mile and 3 miles from existing conventional seed production, for leafcutter bee, alkali bee and honey bee pollination, respectively (figure V-8). Field location and field size are reported to local State seed certifying agencies that inspect to determine that the minimum isolation requirement is met. This isolation requirement is 5-95 times the current isolation requirement for conventional certified seed production. These science-based FGI isolation guidelines for Roundup Ready alfalfa seed production were established through open discussion among stakeholders, in forums organized by State crop improvement organizations (UCDSBC 2005, Lowry 2007, NAFA 2007).

6.6.2 Stockseed Genetic Purity

Conventional (and organic) alfalfa seed producers wishing to avoid GM-traits, inferior seed germination or vigor, weed seeds or organic program prohibited methods should plant high quality seeds of a known variety sourced from a reputable seed supplier. Prior to planting, the GM-sensitive conventional seed producer can require that an identity preserved seed lot sample be tested for the presence of the Roundup Ready (or other GM) trait. Similar to other crops using the Roundup Ready trait, there are simple to use seed, leaf and hay trait testing kits for alfalfa (e.g., Strategic Diagnostics, Inc. and Envirologix, Inc.), and trait test service laboratories commercially available to the non-GM buyers and sellers who wish to test for the presence of the Roundup Ready trait (e.g., GeneScan, Inc.; Mid-West Seed Services, Inc., and, various State's seed certification programs). The Roundup Ready seed test kits and protocols have been validated by the manufactures and or by a third-party tester using field seed samples (Teuber et al. 2007b). Most of the U.S. seed crop is grown by variety name under contract with an alfalfa seed company who provides and defines the genetic quality of the planting stockseed. Therefore, because most alfalfa seed growers produce seed under a contract, most individual alfalfa seed growers do not self-determine which seed lot to use; the variety developer or seed company provides the grower with the seedstock. Seed companies that contract and provide stockseed to seed growers by variety name routinely test the stockseed for germination, purity, and key variety characteristics and non-GM conventional producers have and will test seed lot samples for the presence of GM traits. The commercial issues associated with adventitious presence of GM traits in seed have been extensively discussed with the global seed industry for more than a decade; the alfalfa seed industry has and will build coexistence and trait stewardship models using the experience gained in other crops enhanced using modern breeding methods (e.g., UCDSBC 2005, NAFA 2007).

Professional plant breeders and seed certification inspectors routinely monitor foundation seed stocks for off-types (e.g. flower color, plant stature, etc.) that are not characteristic for the described variety and breeders use simple conventional breeding approaches to proactively eliminate and manage against undesired off-types. These routine procedures for seedstock maintenance and quality control can effectively manage the rare occurrence low levels of biotech traits in conventional seedstock. In the event that conventional seedstock or breeder germplasm lines were suspected of containing adventitious presence of a biotech trait, low-level presence can be excluded in parental lines using simple Roundup Ready trait/protein detection screening methods prior to pollination of the breeder generation. Tissues from candidate parent plants can

be pooled or individually tested using the lateral flow strips designed specifically for fresh leaf tissues available from Strategic Diagnostics, Inc. and Envirologix, Inc. Any individual plants found to be trait positive would be excluded from the conventional parents prior to pollination of the breeder seed. Breeder seeds would then be grown using foundation or greater isolation to produce foundation seeds. Foundation seeds would be planted to produce commercial certified class seeds using certified class or greater isolation.

6.6.3 Management of Cultured Pollinators

Most conventional seed producers control the bee species, bee domicile field placement, bee stocking date(s) and bee stocking rate(s). Seed producers can mitigate (reduce) the potential for cross-pollination with outside fields through prudent cultured bee management, and, where summer temperature is not too high, they can opt for the use of leafcutter bees instead of other bee species because leafcutter bees tend to forage at shorter distance from their domicile. The majority of alfalfa seed producers add either leafcutter bees or honeybees to the seed field to synchronously and efficiently pollinate the crop. Conventional alfalfa seed has historically been grown using either cultured honeybees or leafcutter bees, in the Desert Southwest and Pacific Northwest, respectively. In the northern, central and southern Plains where a small proportion of the alfalfa seed is produced (and seed yield per acre is very low), the type of pollinators used is more variable: typically seed producers in these marginal production areas rely on feral and native bees. In certain niche geographies where suitable soil beds exist (e.g., southern Washington), natural and managed colonies of the ground-nesting alkali bee may contribute significantly to commercial alfalfa pollination (estimated to contribute 20 percent pollination for alfalfa fields proximal to an alkali bee bed); alkali bee pollination is often augmented by adding cultured leafcutter bees. Non-GM conventional seed producers can take further steps to mitigate cross-pollination with unknown alfalfa pollen by stocking the field with bees (to synchronously and quickly pollinate the field and make it less attractive to feral bees). They should stock freshly emerged young leafcutter bees and maintain them in the same field for the full pollination cycle (i.e., to keep them oriented to forage near the home hive).

6.7 Coexistence Strategies for Roundup Ready Alfalfa Seed Producers

Roundup Ready alfalfa seed growers and seed producing companies ("producers") must be licensed by Forage Genetics and by Monsanto. The Roundup Ready seed producers, therefore, are obligated to follow all of the trait stewardship terms in their contracts and license agreements. Key features of the licensing agreements that will be important to seed marketplace coexistence are the requirements for implementation of all of the following:

- Seed field location and planting date; inspected by crop improvement organization
- Extraordinary, pollinator species-specific seed field isolation at planting (extraordinary pollinator-specific isolation will be 900 ft, 1 mile or 3 miles minimum for leafcutter, alkali or honeybees, respectively); inspected by crop improvement organization
- Seed field stand termination date; inspected by crop improvement organization

- Education and compliance auditing: FGI and Monsanto have implemented grower education and third-party auditing procedures to help insure awareness and compliance with license agreements.
- Authorized seed transfers only: All Roundup Ready seeds transferred only to the contracting company (no saved seeds, no transfer or sale to third parties). Any seed transfer requires licensure and associated stewardship.
- Product labeling/differentiation: Roundup Ready seed may not be legally sold without its variety name or into unlicensed or common seed channels. Processed Roundup Ready alfalfa seeds will be differentiated from conventional or common alfalfa in that they will be coated with a distinct purple colorant and transferred in packages labeled "Roundup Ready alfalfa".
- Best Practices policies: Rigorous policies are science-based and market stakeholder-driven, and as such, the company (and or NAFA) may revise its Best Practices as new information is developed or as new coexistence consensus agreements or strategies are developed by the alfalfa industry (e.g., NAFA 2007).

6.8 "Non-GM" alfalfa markets

The Roundup Ready technology would offer the technology-adopting alfalfa producers a new tool for broad-spectrum weed control throughout the life of the alfalfa stand. However, because the Roundup Ready varieties are genetically engineered ("GM") certain market sectors (e.g., organic) and individual producers who object to GM traits *per se* will not want to use them and they will take steps to strictly avoid any detectible levels of GM in their products or on their farms. This has occurred in other crops. The stated GM sensitivity of individual customers or markets varies from those having "zero tolerance", "none detected", "not more than *X* percent GM", etc., and it varies among individual contracts and by importing country tolerance limits, if applicable. For purposes of the Roundup Ready market impacts analysis, "GM sensitive" is defined hereafter as the producers/customers that require that less than 0.5 percent GM trait is present. Although certain buyer-seller agreements require it, zero presence is not statistically provable, it is not required to meet USDA National Organic Program (NOP) Standards, and it is unlikely to be feasible after large-scale commercialization of GM varieties (ASTA 2007; see statements made by Mike Gumina in NASDA-PEW 2006).

The organic and a portion of the U.S. agricultural export market channels are now averse and categorically closed (or nearly so) to essentially all traces of GM products, regardless of the crop, the commodity type, the trait or its regulatory approval status. In most of these markets there is no stated tolerance to GM trait presence, but international trade has continued in these crops. There have been GM AP tolerances—with conditions, established for food products in Australia (1%), Brazil (1%), the European Union (E.U.) (0.9%), Japan (5%) and Korea (3%) [see statements by M. Zumwickle (NASDA-PEW 2006)]. Various tolerance levels may be agreed to in buyer-seller contracts.

The development of identity preservation systems where growers produce a crop to meet certain market segments (i.e organic, conventional) is not specific to the Roundup Ready trait. The alfalfa market GM-sensitivity is highly subjective as is the case for certain other sales attributes in some cases (Putnam 2005). In other crop species—and in alfalfa following the June, 2005, deregulation action for Roundup Ready alfalfa, market segmentation has occurred to differentiate organic, conventional and non-GM products. Identity preserved market segments have grown even within conventional crop markets. United States market coexistence has also developed so as to serve the economic, trade, environmental and social interests of diverse alfalfa market sectors (NASDA-PEW 2006, UCDSBC 2005, NAFA 2007).

Organic forage and other specialty crop markets are demonstrating an increasingly rapid growth rate (e.g., 17 percent per year) despite, or perhaps in-part, because of increased organic food consumer awareness of biotechnology use in crops (Putnam 2006). Based on the development of coexistence strategies and overall strong organic market trends, although individual organic hay producers may need to take new steps to avoid the unwanted presence/detection of Roundup Ready or other future GM alfalfa varieties, the introduction of Roundup Ready alfalfa and the likely public discussion surrounding it, may result in increased organic market demand and expanded opportunities for existing and new organic hay growers.

7.0 Summary of Findings

Alfalfa is a perennial crop, pollinated by alfalfa leafcutter bees (*Megachile rotundata*), honey bees (*Apis mellifera*), and alkali bees (*Nomia melanderi*), and it is cultivated primarily for hay (forage) or seed. Because it is pollinated by insects, pollen and gene flow between different alfalfa fields is possible. This is an issue when a proportion of cultivated alfalfa is GT alfalfa, and growers of non-GT alfalfa wish to prevent the entry of GT traits in their seed stocks and crops.

For hay farmers, the quality of the hay is dependent on nutrient content, which is linked to the maturity of the plants at harvest, both of which are predicted by the amount of bloom present at harvest. The nutrient content of hay is tested before sale price is determined. The earlier the harvest, the better quality the hay is. It is often recommended to harvest hay when it is at one-tenth bloom. It is important to know the level of bloom present in alfalfa hay fields in order to gauge the possibility of pollen flow between the fields or from hay fields to seed fields. Alfalfa hay production occurs mainly in the West and Northwest United States, and while California produces the most hay in the country by weight, South Dakota has the most hay acreage.

Seed farmers are concerned with the purity of their seed stock, and follow State and Federal-mandated standards in order to produce seed of certified purity. Isolation distances between fields and threshold amounts of allowable off-types of seed vary by State, but in general, seed stock must be 99 percent of the variety or varieties stated on the label. Alfalfa seed production also occurs mainly in the West and Northwest United States, and California produces the most seed in the country, in both pounds and acreage.

Pollination of alfalfa is highly dependent on the species of pollinator used. Honey bees have been known to forage over 6 miles from their nests, while alfalfa leafcutter bees typically only forage 300-600 feet from their nests. Female alfalfa leafcutter and alkali bees are the most effective alfalfa pollinators, pollinating 81 and 78 percent of visited flowers, respectively. Honey bees only pollinate 22 percent of visited flowers. Alfalfa seed farmers manipulate bee colonies in order to ensure pollination of their fields, and release of pollinators influences the duration of flowering in alfalfa fields. The more bees released at one time, the quicker the flowers stop blooming, and flower availability and number of bees can impact pollen transport. Other factors that affect bee activity and therefore affect pollination include weather, season, time of day, hive placement, plant competition, strength of the colony and disease.

Given proper adherence to FGI Best Practices and Monsanto's MTA the risk of cross-fertilization is below FGI's goal of less than 0.5 percent presence. Gene flow between and into seed fields is of higher concern than gene flow into hay fields. This is primarily because hay fields are mowed before seed is set, so even if pollen with GT trait arrives at a non-GT hay field, propagation of seed is unlikely. Large-scale feral or volunteer alfalfa patches can be a potential bridge for gene flow between managed seed or hay fields and within and among feral plant groups. It is important to note that many bee pollinators avoid collecting pollen or nectar from alfalfa flowers when other flowers (weeds or crops) are growing in the vicinity. Feral alfalfa is a concern if it is not managed near seed fields. Feral alfalfa near GT alfalfa hay fields may

receive GT trait, but the trait's survival in the feral population depends on whether there is selection pressure to maintain the trait and chance. The GT trait is not expected to enhance feral alfalfa fitness. Rangeland alfalfa (falcata) populations may be growing as ranchers intentionally seed falcata into rangeland to increase forage quality and soil nitrogen. Hybrids can be vigorous and fertile, and can thrive sympatrically with their *falcata* and *sativa* parents. Hybrid populations with *M. falcata* parentage inherit winter and drought hardiness, as well as the ability to exist in grass mixtures, making them more adaptable to resource poor environments and increasing the likelihood of feral populations (Bagavathiannan and Van Acker 2009). Unless properly managed, these feral populations could serve as bridges for long-distance gene flow.

Appendix V-1. References

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Appendix V-2. Stewardship Program for Gene Flow Management in Roundup Ready® Alfalfa Production

Monsanto and Forage Genetics International (FGI) have jointly developed Roundup Ready® alfalfa for commercial forage production in the United States. Prior to and after the introduction of Roundup Ready alfalfa (GT alfalfa) to U.S. alfalfa producers, Monsanto and FGI developed a stewardship plan that included securing regulatory approvals in key U.S. alfalfa hay export markets, implementation of hay and seed production practices designed to facilitate co-existence of both Roundup Ready and conventional alfalfa hay and seed [1,2,3] and a commitment to continued dialogue with alfalfa forage and seed producers.

1.0 Key Elements of Traits Stewardship

1.1 Licensing, contracting and pesticide registration

Under terms of the Monsanto Technology/Stewardship Agreement (MTA) GT alfalfa may only be used to produce forage for feed uses. Seed production of GT alfalfa requires a specific contract from FGI or an FGI authorized FGI seed contractor. FGI is the sole Monsanto licensed seed producer. Although Roundup herbicide is broadly registered for GT alfalfa forage production purposes in the United States, Roundup herbicide is labeled for seed production purposes in only certain states. Unregistered pesticide use and unlicensed commercial harvest, sale or uses of patent protected seed are violations of State and Federal laws.

1.2 Seed channel management

All GT alfalfa seed is packaged with a unique purple seed coating and "Roundup Ready alfalfa" seed-bag labeling. GT alfalfa seed will only be sold by variety name. All seed purchasers, dealers, distributors, producers and processors of Roundup Ready seed must be licensed by Monsanto and agree to trait stewardship appropriate to their activities.

1.3 Industry tools

To support buyer-seller agreements that may stipulate conditions for biotech trait status, simple to use trait detection strips are available for alfalfa seed and hay product use (e.g., Strategic Diagnostics, Inc., Envirologix, Inc.).

1.4 Mitigation of Pollen-Mediation GMNE Flow

Gene flow between populations is a natural occurrence in all crops. Alfalfa requires cross-pollination by certain bee species; it is not wind pollinated. In alfalfa, the potential for Roundup Ready gene flow between fields is significantly constrained (limited) by crop and pollinator

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biology, typical alfalfa grower practices, and numerous additional grower practices required in the Monsanto license for forage growers and Forage Genetics best practices for contracted GT alfalfa seed growers as follows.

1.4.1 Hay-to-hay field gene flow

In alfalfa, forage producers cut hay at regular intervals prior to or near early bloom for optimum forage quality and yield. Pollinators are not attracted to non-flowering, vegetative alfalfa fields. The potential for pollen-mediated hay-to-hay field gene flow has been thoroughly discussed in a recent University of California publication [4] wherein it is stated that, "…the likelihood of AP [adventitious presence] occurring between hay fields becomes infinitely small, likely to be far less than 0.001 percent of field biomass even under high estimates. …The combination of frequent harvests, lack of significant flowering, lack of significant seed production, and the highly competitive and allelopathic nature of alfalfa that prevents ready germination of alfalfa seeds in existing fields should prevent most if not all gene transfer".

1.4.2 Hay-to-seed field gene flow

Certified alfalfa seed must be produced using production methods and isolation defined and inspected by State seed certification organizations or an accredited organic certifier. Non-certified seed is not officially regulated. Seed producers and regulators recognize that genetic purity of the seed is related to physical isolation between the seed field and outside sources of pollen. For reasons explained above, due to sparse and short-term flowering, Roundup Ready forage fields are an unlikely source of pollen or pollinators. Hay-to-seed gene flow research has been conducted and data are publicly available [1,2]. Bees were applied to pollinate the seed fields in these studies, and, during the midsummer pollination period, the hay field plots were intentionally allowed to flower more extensively than is typical for forage: i.e., 50 percent bloom in 2000[1,2] and 20 percent bloom in 2006. Therefore, the hay field gene sources were representative of delayed or poorly managed hay fields growing close to a seed field. Under these conditions, gene flow into the seed field was 0.2% at 150-300 ft and, it was 0.00 to 0.05% at distances greater than 350 ft. In contrast, forage producers who plant Roundup Ready alfalfa seed are expected to cut hay aggressively to manage for optimum forage quality and minimize the number of open flowers on the standing hay. In the western United States where hay and seed are both produced, licensees must identify the exact field location where Roundup Ready alfalfa seed will be planted. GT alfalfa forage growers will be monitored for compliance to license terms. If conventional seed producers use recommended seed field isolation distances from neighboring alfalfa fields and certified identity preserved best management practices (such as organic certification), it is expected that the resulting seed will contain little or no adventitious presence of traits from hay fields.

1.4.3 Seed-to-seed field gene flow

Certified alfalfa seed must be produced using production methods and isolation defined and inspected by State seed certification organizations or an accredited organic certifier. Non-certified seed is not officially regulated. Forage Genetics requires that all contracted GT alfalfa seed growers and conditioners follow FGI best practices policies for Roundup Ready trait

stewardship. Additionally, the companies who produce Roundup Ready seeds in the National Alfalfa & Forage Alliance (NAFA) agreed jointly to adopt, as a minimum, best management practices for GT alfalfa seed production in the United States. These production practices meet or exceed AOSCA standards for the seed production of Foundation Class alfalfa seed production. Compliance to the guidelines is required under a separate and binding agreement (NAFA 2008a). These best practices have been implemented to maintain high seed quality in FGI seeds; are based on industry experience and gene flow research data; and, they are effective strategies for assuring seed and hay industry market coexistence. It is well documented that isolation is one of the effective measures to mitigate pollen flow to very low levels[1,2,5,6] and that bee species differ in their potential to effect gene flow[1, 2, 5, 6, 7]. FGI and its contractors use process-based best practices that are modeled on the principles of AOSCA Foundation Class alfalfa seed production standards and crop identity preservation. Likewise, other producers of conventional and or specialty quality alfalfa seeds independently implement various production strategies to ensure their company's product quality claims. Research data are available to the industry so that other seed producers can use the information to manage seed quality[2, 5, 6].

NAFA and FGI Best Practices for GT alfalfa seed producers include: (1) All GT alfalfa seed is produced exclusively in the United States using AOSCA and/or OECD certification standards. (2) At planting, all GT alfalfa seed fields respect a foundation isolation distance (or greater) to existing conventional seed fields: GT alfalfa eligible fields have 900 ft, 1 mile or 3 mile isolation for leafcutter bees, alkali bees or honeybees, respectively. (3) The GT alfalfa Seed Contractor shall report GPS coordinates of all established and planned GT alfalfa seed production fields to local State seed certification officials as early as possible. State officials will confirm minimum isolation and establish a State pinning map for GT alfalfa seed production. (4) The GT alfalfa Seed Contractor will limit GT alfalfa seed production contracts to the following states: Arizona, California, Colorado, Idaho, Montana, Nevada, Oregon, Texas, Utah, Washington and Wyoming. (5) The GT alfalfa Seed Contractor will also respect any non-GE alfalfa seed production zone designated as such by a consensus of local seed growers. (6) Seed growers must follow NAFA and FGI's strict equipment sanitation, pollinator management and seed handling requirements, and (7) the planting date, location, field size, variety name and termination date for all GT alfalfa seed production fields are reported to and inspected by the State's certifying organizations to facilitate the industry's management of field history and seed field isolation information. FGI data on the effectiveness of the Best Practices in limiting gene flow from GT alfalfa seed to non GT alfalfa is described in appendix V, section 3.4.2.

1.5 References

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