



The Science Source for Food,
Agricultural, and Environmental Issues

Gene Flow in Alfalfa:

*Biology, Mitigation, and Potential Impact
on Production*





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Agricultural, and Environmental Issues

Council for Agricultural Science and Technology

4420 West Lincoln Way, Ames, IA 50014-3447, USA

Telephone: (515) 292-2125; Fax: (515) 292-4512

E-mail: cast@cast-science.org

CAST Website: www.cast-science.org

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Gene Flow in Alfalfa: Biology, Mitigation, and Potential Impact on Production

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Task Force Members

Authors

Allen E. Van Deynze (Chair), Seed Biotechnology Center, University of California–Davis

Sharie Fitzpatrick, Forage Genetics International, West Salem, Wisconsin

Bob Hammon, Agronomy and Entomology, Tri River Area Extension, Grand Junction, Colorado

Mark H. McCaslin, Forage Genetics International, Prior Lake, Minnesota

Daniel H. Putnam, Department of Plant Sciences, University of California–Davis

Larry R. Teuber, Foundation Seed Program, Department of Plant Sciences, University of California–Davis

Daniel J. Undersander, Department of Agronomy, University of Wisconsin–Madison

Reviewers

Edward Charles Brummer, Crop and Soil Sciences, University of Georgia, Athens

A. Bryan Endres, Department of Agricultural and Consumer Economics, University of Illinois, Urbana–Champaign

Allan S. Felsot, Entomology & Environmental Toxicology, Washington State University–Tri-Cities, Richland

Heathcliffe Riday, U.S. Dairy Forage Research Center, USDA–ARS, Madison, Wisconsin

Zeng-yu Wang, Forage Improvement Division, Samuel Roberts Noble Foundation, Ardmore, Oklahoma

CAST Liaison

John Havlin, Department of Soil Science, North Carolina State University, Raleigh

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Foreword

In January 2008, the National Concerns Committee and the CAST Board of Directors authorized CAST to complete a special publication on the topic of gene flow in alfalfa.

Dr. Allen Van Deynze, University of California-Davis, served as Project Chair. Six additional scientists participated as authors; five subject specialists selected by CAST served as reviewers; one member of the CAST Board of Directors acted as project liaison.

The authors prepared an initial draft of this document and reviewed and revised all subsequent drafts based on reviewers' comments. The CAST Executive Committee and Editorial and Publications Committee reviewed the final draft, and all task force members reviewed the galley proofs. The CAST staff provided editorial and structural suggestions and published the document. The task force authors are responsible for the publication's scientific content.

On behalf of CAST, we thank the chairperson, authors, and reviewers who gave of their time and expertise to prepare this publication as a contribution by the scientific community to public understanding of

the issue. We also thank the employers of the scientists who made the time of these individuals available to CAST, thus making the project a reality.

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Dr. Kassim Al-Khatib
President

John M. Bonner
Executive Vice President

Linda M. Chimenti
Managing Scientific Editor

Note

This publication is an outcome of the National Alfalfa & Forage Alliance-sponsored workshop, *Peaceful Coexistence: Creating a Strategy for Harmony among GM, Organic, and Conventional Alfalfa Producers*, Denver, Colorado, October 10, 2007.

Executive Summary

U.S. alfalfa (*Medicago sativa* L.) hay and seed growers produce for various markets and should continue to have the choice to do so. Growers also need the ability to adopt new technologies that enable them to address changing global market situations and remain competitive. These technologies can be production, agronomic, or quality related. At the same time, it is essential that growers work together to ensure that differing market requirements can be met. As in all biological systems, 100% purity (or 0% impurities) of any constituent is very difficult to achieve and may not be possible economically. This is especially true for field-scale agriculture. As a result, growers and agricultural societies have adopted process-based strategies such as the Certified Seed and National Organic Programs that tolerate a low and acceptable threshold of impurities in the final product, whether pesticide, weed seed, or varietal seed. These systems have been recognized and adopted worldwide.

This paper describes the biology and agronomic practices in alfalfa that should be considered in developing coexistence strategies to allow growers to have choices, specifically between *genetically engineered*¹ (GE), conventional, and GE-sensitive markets in the United States. These strategies are based on science and are a direct extension of well-established coexistence principles routinely used by seed growers. The management practices currently being used by growers of Roundup Ready alfalfa (RRA) seed are presented as an example of strategies developed by the alfalfa seed industry that allow coexistence of different production systems.

The majority of the domestic market is not sensitive to GE alfalfa, but portions of the domestic hay and seed markets and much of the export hay and seed markets are sensitive to the *adventitious presence* (AP) of GE traits. A successful coexistence strategy for GE, conventional, and organic alfalfa recognizes and addresses potential *gene flow* between hay and seed.

Gene flow is described as the exchange of genes from

one population to another. There are various modes of opportunity for pollen- and seed-mediated gene flow in alfalfa. Understanding the relative magnitude of risk associated with each mode can help in the development of rational mitigation strategies that minimize potential gene flow from GE alfalfa to conventional alfalfa hay and seed production. In terms of acres and value, hay-to-hay is the most prevalent interface, yet scientific evidence shows that this situation is manageable and provides the least opportunity for gene flow. Alfalfa is not sexually compatible with any other wild or cultivated plant species in the United States (McGregor 1976).

Unlike the majority of biotech² crops grown today, the primary commodity for alfalfa is forage hay, not seed. The fact that the production of viable seed is required for gene flow has large implications for potential gene flow in alfalfa. Although the potential for seed-mediated gene flow does exist, best management practices in the cleaning and management of seed harvesting and processing equipment are effective in managing *admixtures* between GE and conventional alfalfa seed. Coupled with cultural and rotational practices to manage volunteer seedlings, seed-mediated gene flow in alfalfa likely will be very low.

Pollen-mediated gene flow requires the presence of pollinators, proximity, and synchrony in flowering of source and recipient fields/plants, and viable seed production in the recipient fields/plants. Routine cutting management schedules in hay production limit flowering synchrony and nearly eliminate the potential for viable seed production, both of which are required for effective pollen-mediated gene flow. These factors, when combined with the other biological barriers described in this paper, decrease the likelihood of gene flow in hay to near zero.

There are fewer barriers to gene flow into alfalfa seed production fields because the seed fields flower for an extended time, introduced pollinators are used to maximize seed production, and the harvest of viable

¹ Italicized terms (except genus and species names) are defined in Appendix B: Glossary.

² For purposes of this paper, the term biotechnology-derived (also termed genetically modified [GM] or genetically engineered [GE]) is shortened to “biotech.”

seed is the desired outcome. Although the potential for pollen-mediated gene flow from *feral* alfalfa or hay-to-seed production exists, it commonly is controlled through various management practices and is limited by the low relative pollen abundance of the source versus recipient seed production fields during flowering. The primary mechanism for pollen-mediated gene flow in alfalfa is from one seed field to another.

Gene flow is managed proactively in conventional commercial seed production to optimize *varietal genetic purity*. The key mitigation strategy used to manage gene flow commercially is the planned spatial isolation of one seed production field from another. Results from several gene flow experiments have provided pollinator-specific, science-based isolation guidelines designed to minimize gene flow from GE alfalfa to conventional varieties. These isolation distances have been adopted by the industry for RRA seed production and serve as a model for future bio-

tech crops in alfalfa. The National Alfalfa and Forage Alliance (NAFA) Best Management Practices protocol also calls for regular testing of AP in conventional seed lots using publicly available test kits to assess the adequacy of stewardship standards (NAFA 2008). This testing ensures a dynamic process to monitor and, if necessary, to adjust the isolation requirements for GE alfalfa seed production.

Understanding potential gene flow in alfalfa hay and seed production is an important first step in developing management strategies designed to mitigate gene flow. The authors conclude that sufficient scientific data are available to design these strategies and, as outlined in this document, those strategies will be successful in managing gene flow from GE to conventional alfalfa hay and seed production. Regular testing will be needed, however, to monitor the effectiveness of these strategies, and adjustments should be made if and when appropriate.

Introduction

United States alfalfa (*Medicago sativa* L.) hay and seed growers produce for various markets and should continue to have the choice to do so. Growers also need the ability to adopt new technologies that enable them to address changing global market situations and remain competitive. These technologies can be related to production, agronomic, or quality factors. At the same time, it is essential that growers work together to ensure that differing market requirements can be met. As in all biological systems, 100% purity (or 0% impurities) of any constituent is very difficult to achieve and may not be possible economically, especially for field-scale agriculture. As a result, growers and agricultural societies have adopted process-based strategies such as the Certified Seed (AOSCA 2003) and National Organic Programs (USDA 2005) that tolerate a low and acceptable threshold of impurities of the final product, whether pesticide, weed seed, or varietal seed. These systems have been recognized and adopted worldwide for a century (AOSCA 2008; ISF 2008).

The rapid and sustained adoption of biotechnology, mainly in field crops modified for herbicide, insect, and disease resistance, clearly indicates an economic advantage. For example, 31% of U.S. cropland is cultivated with biotech crops (James 2005; USDA–NASS 2007b). Similarly, certified organic acres have increased by an average of 25% each year from 1995 to 2005, making up 0.51% of U.S. cropland in 2005 (USDA–NASS 2007b). This paper describes the biology and agronomic practices in alfalfa that should be considered in developing coexistence strategies allowing growers to have choices, specifically between genetically engineered (GE), conventional, and GE-sensitive markets in the United States.

Alfalfa has been improved for cultivation using conventional breeding, and new traits are being introduced using biotechnology. In many agricultural production sectors, there has been rapid adoption of varieties improved through the use of novel genetic technologies. Other market sectors reject all varieties, feeds, and foods containing even trace amounts of such genetic modifications. Mechanisms for producers of conventional, organic, and biotechnology-derived

varieties to coexist in the marketplace have developed rapidly in global seed and grain markets. Unlike the majority of biotech crops grown today, the primary commodity for alfalfa is forage hay, not seed. This fact has important implications for the chance of adventitious presence (AP) in the commodity.

A critical component of coexistence for alfalfa will be the careful management of genetic purity during seed and/or forage production activities. Genetic purity is affected by seed or pollen transfer that may result in gene flow between alfalfa growing in hay fields, seed fields, or growing feral outside of cultivation. Genetic purity is attained by mitigating genetic mixing (i.e., using agricultural practices to constrain the natural flow of genes between populations). Seed certification rules require, and professional seed producers currently apply, core principles necessary for successful mitigation of gene flow among alfalfa populations (AOSCA 2003). Virtually all U.S. seed production of proprietary alfalfa varieties and most of the seed production of public varieties are carried out using Association of Official Seed Certifying Agencies (AOSCA) restrictions that make the seed eligible for certification by state seed certifying agencies. There are no such restrictions for common seed production.

Typically, gene flow during seed production is controlled using spatial isolation from other alfalfa plants, crop rotation to eliminate previously grown alfalfa varieties, and thorough equipment cleaning between seed lots of different varieties to avoid admixture (AOSCA 2003; Sundstrom et al. 2003). Forage producers concerned with genetic purity and/or organic status select seeds of known origin, variety, and purity. Although gene flow is technically possible in all scenarios, its likelihood and impact are dependent on numerous, well-understood principles (CAST 2007; Putnam 2006).

The introduction and sensitivity of biotech traits in alfalfa has caused scientists, seed companies, AOSCA, the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA–APHIS), and seed producers to reexamine gene flow and its potential effect on AP of a biotech trait in nonbiotechnology-derived forage or seed. The low tolerance to biotech

traits is sometimes driven largely by the ability to detect biotech traits at levels not achievable for most nonbiotech traits.

In agriculture, the basis of coexistence is the adoption of practical low thresholds for AP. As described in the Federal Seed Act and elsewhere, hay seed has not been recognized historically as a pure substance, and certain genetic, species, and inert impurities are tolerated. In contrast, a tolerance for GE traits has not been determined by some organic or other GE-sensitive market sectors, such as seed importers or exporters (Federal 1998). For example, the European

Union (EU) has adopted a 0.9% threshold for AP for approved biotech traits in organically and conventionally derived products, and Japan has adopted a 5% threshold for AP in food. The EU, however, does not accept even trace amounts of GE in seed (ESA and EuropaBio 2007).

In this paper, the authors summarize recent research on gene flow and place it in the context of alfalfa biology, use, and U.S. cultural practices. This paper also explores key strategies for managing gene flow to mitigate AP in hay and seed produced for AP-sensitive markets.

Background and Demographics

Alfalfa is an introduced, cultivated species in North America and the fourth largest U.S. crop by land area. 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 (Peters and Linscott 1988), the use of which precludes the seeds' legal use for food/sprouting purposes. Therefore, seed products entering the planting and sprouting seed channels are kept as distinct.

Alfalfa grows in three forms:

1. Alfalfa cultivated for commercial forage production—dry hay, silage, *greenchop* or *haylage* (collectively referred to as hay);
2. Alfalfa cultivated for commercial seed production (seed); and
3. Alfalfa outside of cultivation occurring as feral plants in the landscape (feral).

For this paper, unmanaged alfalfa used for pasture and grazing will be considered feral because of its similarity. As happens for conventional genes, there is the potential for transgenes to flow to and from each alfalfa population interface (i.e., seed, hay, and feral). Each form of alfalfa has several unique features that are important in understanding the potential for gene flow into or out of the population. Although alfalfa grown in pastures and harvested by grazing animals is included in the “hay” category, there are unique features associated with the sometimes-less-intensive harvest management with these systems. Alfalfa grazing is extensive in other countries, but it is not practiced widely in the United States (Lacefield et al. 1997).

Alfalfa hay and seed production differ from each other in demographics, total acreage, production systems, and geographic location. In 2007, there were approximately 23.5 million acres of alfalfa hay and haylage and an estimated fewer than 100,000 acres (0.45%) of alfalfa seed production in the United States

(cumulative data from USDA–NASS 2007c). The annual crop values for hay and seed were approximately \$8.9 billion and \$80 million, respectively (USDA–NASS 2007a). Based on acres and value, hay-to-hay interface is the most prevalent, yet scientific evidence shows that this situation is manageable and provides the least opportunity for gene flow (see Hay-to-Hay section). Correspondingly, seed-to-seed, hay-to-seed, and feral interfaces likely make up the minority (<1%) of potential gene flow situations (Putnam 2006). The production systems of hay and seed differ dramatically as well—a fact that impacts gene flow and its mitigation.

Alfalfa hay is grown across the continental United States and, in general, is grown wherever alfalfa-consuming livestock are fed. In contrast, alfalfa grown for seed is not scattered uniformly throughout the country. The main locations for seed production are California, Idaho, Oregon, Washington, and several other western states (USDA–NASS 2007c). Even within these states, the production regions for seed typically are limited and concentrated in locales where the climate is well suited to seed production.

For example, California has approximately 28,000 total acres of certified alfalfa seed production, but the majority of those fields are in two large counties: Imperial County has 475,000 acres of crop production overall, of which 168,000 are hay and 14,000 are seed; and Fresno County has 3.3 million acres of crop production overall, of which 300,000 are hay and 14,000 are seed (cumulative from county reports 2004–2006, USDA–NASS 2007d). Therefore, even in those counties where seed is important, the seed-to-seed or hay-to-seed interactions comprise a minority of situations and are confined to specific fields in specific areas.

Alfalfa seed production is a highly coordinated undertaking (Rincker et al. 1988). Most seed growers produce seed by contract, and the seed field locations are planned carefully in advance to obtain the appropriate previous cropping history and physical isolation from other alfalfa seed and hay fields to ensure seed purity and quality (Rincker et al. 1988). In Imperial County, hay-to-seed interactions are somewhat likely,

which must be taken into account when placing seed fields. The AOSCA and the Federal Seed Act require that certified alfalfa seed production practices ensure a minimum 95–99% genetic varietal purity (AOSCA

2003; Federal 1998). The level of genetic purity is specific to the generation or class of certified seed. GE-sensitive markets may require higher levels of genetic purity.

Alfalfa Biology

Alfalfa is an *herbaceous*, perennial forage legume species. By virtue of its broad environmental adaptation, forage production of the crop occurs in all growing regions of the United States. There are no sexually cross-compatible wild or cultivated species in North America (McGregor 1976).

Growth and Flowering

An alfalfa plant starts its initial growth from a seed during establishment, but after each harvest or winter it regrows from buds arising from either stubble stems or the crown. The vegetative growth interval (i.e., harvest schedule) during most times of the year is 22 to 40 days. Harvest for forage typically is done two to eight times per year, depending on location and seasonal climate. Most alfalfa in the United States is managed to limit growth to the juvenile (vegetative) state for optimizing forage biomass 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 (Blank, Orloff, and Putnam 2001).

Alfalfa fields planted with GE alfalfa and harvested by grazing will be high-input by definition, and likely intensively managed by rotational grazing. Lacefield and colleagues (1997) determined that timing and extent of harvest in grazing can be variable. The predominance of hay versus seed acres and commonly used harvest timing for hay production suggests that at any one time, more than 99% of U.S. alfalfa (compare total hay and seed acres, USDA–NASS 2007a) is likely to be without flowers (vegetative) or in an early stage of flower development. It is therefore atypical, sporadic, and rare that managed hay fields sustain flowers or, subsequent to flowering, produce any viable seed.

In seed fields, flowering and seed production are promoted, and in most seed 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 by short days or cool weather (i.e., late summer through midspring).

Once flowering ensues, alfalfa flowers indeterminately, and its duration depends on moisture, temperature, light, and several other factors (McGregor 1976).

Pollination

Alfalfa is predominantly cross-pollinated, and the flowers are dependent on bees for *cross-pollination*. Wind cross-pollination in alfalfa does not occur (Viands, Sun, and Barnes 1988). 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 cultured leafcutter bees (*Megachile rotundata* F.) in the Pacific Northwest and with cultured honeybees (*Apis mellifera* L.) in California (30% of production, USDA–NASS 2007c). Some growers in niche areas of southern Washington use cultured alkali bees (*Nomia melanderi* C.), and certain seed producers use a blend of cultured species for pollination. Wild honeybees and native bees—including *Bombus* spp., *Osmia* spp., *Agapostomen* spp., and native *Megachile* spp.—can be found visiting alfalfa in varying numbers. Other insect pollinators have not been shown to be effective for alfalfa (Hammon, Rinderle, and Franklin 2006; McGregor 1976).

Seed Formation

After pollination of the flowers and fertilization, alfalfa seed embryos require 4 to 6 additional weeks of adequate growing conditions to develop into viable seed (McGregor 1976). Rainfall, low temperatures, or snow during the ripening time will cause decreased viable seed production and poor seed quality (e.g., decreases in seedling vigor and decreased percentage of germination because of fungal pathogen infection of the seed, or seed sprouting prematurely and dying while still in the pod) (Rincker et al. 1988).

Genetic differences between varieties for maturity also will affect seed development. Alfalfa seed is borne in a coiled, leguminous pod and is nonshattering. Natural, nonmechanized seed dispersal is very local, and alfalfa seed is too dense and smooth for effective

wind dispersal. In situations where animals feed on alfalfa containing ripe seed pods, seed dispersal by animals also is possible, but seed decay during digestion and/or ensiling may decrease that likelihood (Blackshaw and Rode 1991).

Hard Seed

It is typical for a proportion of alfalfa seeds in any seed lot to exhibit postharvest latency (dormancy) related to “hard seed.” A hard, water-impervious seedcoat temporarily prevents water uptake and delays germination until the seedcoat is weathered, aged, or abraded. In contrast to seeds in moist soils, seeds kept dry in storage may remain viable for decades. Alfalfa does not exhibit true physiological seed dormancy, and the majority of seeds imbibe water and germinate readily in most situations. In alfalfa, hard seed is defined as the percentage of seed that does not imbibe water during a standard 72-hour germination test. This hard seed, however, commonly has been observed to germinate in field conditions. Undersander and colleagues (1993) examined the rate and extent of germination of alfalfa seed lots varying in hard seed content and found no correlation between the laboratory hard seed rating (percentage of hard seed) and germination in the field.

Autotoxicity

Alfalfa plants and alfalfa debris produce compounds that elicit an *autotoxic* reaction to germinating alfalfa seeds. The autotoxic reaction and interplant competition severely limit germination and seedling vigor of alfalfa sown or dropped into existing or newly terminated alfalfa stands. Cultivated fields do not self-seed successfully. Attempts to thicken existing alfalfa stands by deliberately interplanting new seed into them typically fail, which is why most agronomists do not recommend the practice (Canevari et al. 2000). Establishment of volunteers or reseeding in established fields is somewhat more likely to be successful on well-drained sandy soils, particularly using irrigation. Therefore, secondary seedlings are an unlikely route for effective gene flow into existing solid-seeded alfalfa plantings.

Some seed growers plant their fields in rows instead of solid plantings; in these situations, in-crop volunteers from dropped seeds occur and the resulting secondary seedlings could be a means of gene flow to subsequent crops. To maintain required varietal and species purity, however, 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 growers must rotate to a different crop for at least one full year following removal of established alfalfa fields.

Longevity

Alfalfa is a short-lived perennial. Fields grown for hay production typically are maintained for 3 to 6 years, longer in some areas. Commercial production of the alfalfa seed crop is confined exclusively to the western regions of the United States where late season (postpollination) rain is unlikely; irrigation is managed carefully; and specialized alfalfa seed growers, equipment, and infrastructure are available (Rincker et al. 1988). To assure varietal integrity, commercial seed production contracts typically require that stands be terminated after 3 years.

Alfalfa is terminated effectively using a variety of mechanical, cultural, and/or chemical methods. Glyphosate, although fairly effective in the control of unwanted conventional alfalfa, typically is not used or is used in combination with other stand-termination practices. Glyphosate will not control RRA; other herbicides and cultural practices, however, remain effective (Van Deynze et al. 2004). After cultivated stands are terminated, both seed and hay farmers rotate the field to a different crop species for one or more years, during which alfalfa volunteers can be controlled, if necessary.

Feral Alfalfa

Feral plants are crop plants that grow and reproduce outside of cultivation. Feral alfalfa plants sometimes can be found on road edges, in fence lines, and in abandoned fields. In the United States, feral alfalfa populations have occurred through unintentional plantings of cultivated varieties (“escapes” from cultivation) or, in certain instances, feral populations originated from intentional planting of the abandoned fields, roadsides, or marginal lands. Feral alfalfa occurs at very low plant density and scale relative to cultivated alfalfa grown for seed or hay.

Biogeographic survey data from five states (California, Idaho, Pennsylvania, South Dakota, and Wisconsin) indicate that for most agricultural areas, feral alfalfa plants do not occur or are sparse (Kendrick et al. 2005). In a 2001–2002 multistate survey, feral plants were found as dispersed plants or patches

within 1.25 miles of cultivated alfalfa at 22% of the survey sites. Feral alfalfa plants sometimes are managed on roadsides by routine mowing, either with hay being harvested or simply left on the ground along with other roadside vegetation. One exceptional feral population of yellow-flowered alfalfa has become naturalized in a remote rangeland in South Dakota (Boe et al. 2004).

Feral plants sometimes are completely unmanaged

and, given adequate moisture and timely presence of pollinators, can flower and set seed. Feral plants are susceptible to environmental stresses (e.g., drought in the irrigated West) and biotic stresses (e.g., *Lygus* bugs in the West and potato leafhoppers in the East) common to the local area. Despite the occurrence of feral alfalfa and its 200-year history in North America, it is not considered weedy, noxious, or invasive in cultivated or feral settings (CFIA 2005).

Pollen-mediated Gene Flow in Alfalfa

Gene flow is described as the exchange of genes from one population to another. It is the natural mechanism that changes population genetic frequency over time and is responsible for the wealth of present biological diversity. Gene flow is a neutral concept: it could be considered helpful, deleterious, annoying, or unimportant, depending on the situation and which genes are involved. For true gene flow to occur there must be both the formation of cross-pollinated seeds and the subsequent establishment of the seedling. Although introduced and/or naturally occurring pollinator bees can carry pollen long distances, true gene flow does not occur until the pollen results in a viable, growing offspring.

Pollen-mediated gene flow generally is dependent on the physical isolation distance between two populations, the degree to which flowering is synchronous, gene frequency, the density of pollinators available for

pollination, and the prevalent species of pollinating bee (Cane 2008). The primary mechanism for pollen-mediated gene flow in alfalfa is from one seed field to another (Figure 1).

There are nine combinations of gene source and gene-receiving populations for pollen-mediated gene flow possible in alfalfa in the United States (Table 1). Pollen flow from any source to commercial hay or commercial seed potentially could result in effective gene flow and AP in a commercial crop. Discussion of each scenario follows.

Table 1. Potential scenarios for pollen-mediated gene flow in alfalfa

	To:	Hay	Seed	Feral
From:				
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

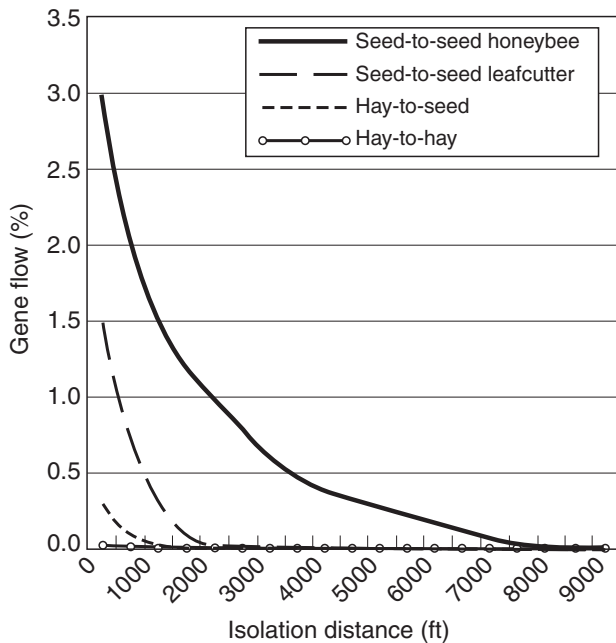


Figure 1. A comparison of the relative gene flow potential for various production systems. (Summarized from Fitzpatrick, Reisen, and McCaslin 2003; Putnam 2006; Teuber et al. 2004, 2007).

Hay-to-Hay

For gene transfer to occur between one hay field and another, several steps must be completed, each of which has a certain probability (Figure 2). Pollen flow requires insect pollinators and is insufficient by itself for gene transfer. If gene flow is to have an impact on hay production, it must result in fertilization of a flower; production and dehiscence of a viable seed; germination; and establishment of a plant contributing to the biomass of the surrounding hay crop (CFIA 2005). Although gene transfer from one alfalfa hay field to another is possible theoretically, a range of environmental barriers make hay-to-hay gene movement a very low-probability event. These barriers include

1. grower practices to harvest alfalfa in a vegetative to early flower stage (before significant flowering) for high-quality forage;
2. scarcity of appropriate pollinators;
3. frequent and complete removal of all above-ground biomass, preventing seed set; and

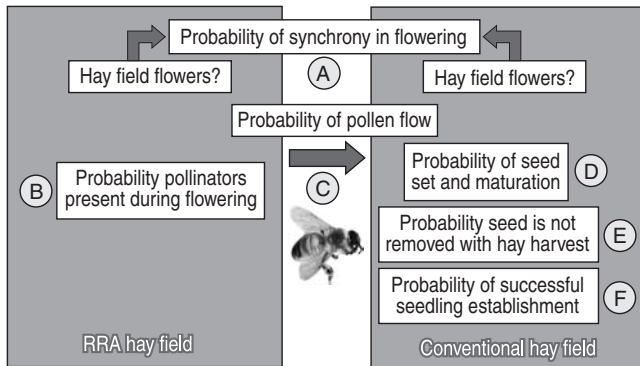


Figure 2. Probability of hay-to-hay gene flow (Putnam 2006). RRA refers to Roundup Ready alfalfa.

- demonstrated inability of the rare seed that is set in hay fields actually to germinate, grow, and compete with existing plants to result in a viable plant that contributes to the dry matter of the forage crop.

The risk of gene flow into AP-sensitive hay fields can be decreased to near zero by harvesting the hay field before ripe seed is formed (i.e., the probability of "D" in Figure 2 is near zero). Alfalfa hay normally is harvested at or before first flower, 6 to 9 weeks before the ripe seed stage, making hay-to-hay gene flow highly unlikely (Putnam 2006). Growers who wish to avoid gene flow (e.g., those who produce hay for markets that reject GE crops) should pay attention to flowering habits (avoiding simultaneous flowering) and harvest schedules, and disallow or remove commercial beekeepers' hives. Although the hay harvest date can be delayed a week or more by wet weather or equipment failure, harvesting before the ripe seed stage is possible in all but the most extreme circumstances (See Pollination and Autotoxicity sections).

If neighbors do not plant GE varieties for hay production, the risk of GE trait gene flow is decreased further because gene flow decreases exponentially with distance between fields (Fitzpatrick, Reisen, and McCaslin 2003; Teuber et al. 2004, 2007). Tests are available commercially to monitor presence or absence of the Roundup Ready trait in harvested hay and fresh leaf tissue. Protein-based test strips are used widely to confirm *no-detect* AP in hay that is sold (Teuber et al. 2007; Woodward, Putnam, and Reisen 2006).

Seed-to-Hay

The potential for pollen-mediated gene flow from an alfalfa seed production field to a hay field is governed

by most of the environmental barriers described previously for hay-to-hay situations. Although the seed field will bloom and pollinators will be present, other factors related to management of the hay crop also apply. Because of the increased presence of pollen from fully flowering seed fields in the vicinity and the presence of pollinators for the seed field, growers of AP-sensitive hay who are interested in strictly limiting the possibility of gene flow may adjust their hay-cutting schedules to limit flowering duration and avoid allowing the hay to ripen seeds.

Again, prudent cutting management of an AP-sensitive hay field is an effective method for decreasing the risk of seed-to-hay gene flow to near zero. If neighbors do not plant GE varieties for seed production, the risk of GE trait gene flow is decreased further. As in the hay-to-hay scenario, the most important mitigation for gene flow in seed-to-hay is to begin with certified seed or GE-free seed (Teuber et al. 2007).

Feral-to-Hay

The environmental barriers described for hay-to-hay gene flow also apply to feral-to-hay. If feral plants are sparse and dispersed, there will be very little pollen available from them compared with the amount from local cultivated sources. Although unmanaged feral alfalfa plants may flower throughout an extended period of time, these plants typically are less fecund than plants grown in cultivation, limiting their capability as a pollen source (Hammon, Rinderle, and Franklin 2006).

Hay growers sensitive to AP may opt to decrease the small potential for gene flow further by carefully managing cutting schedules to limit bloom and avoid *ripe seed set* (see Seed-to-Hay section). In addition, it is prudent to control or clip back neighboring feral alfalfa plants to prevent synchronous flowering. Clipping back also limits the potential for feral plants to act as conduits for bees or as population bridges for gene flow from more distant commercial seed or hay fields that might contain GE traits.

Proper harvest management of the conventional hay field, however, will decrease gene flow risk from this scenario to near zero because of the interruption of the reproductive process necessitated by frequent hay harvests. The low density and fecundity of feral plants, combined with typical cutting management of hay fields, severely limits the potential for gene flow from feral plants to hay fields even from unmanaged or lightly managed pastures and natural populations.

Hay-to-Seed

Potential hay-to-seed, pollen-mediated gene flow is governed by several primary factors:

1. degree of flowering within the hay field;
2. duration of flowering and thereby the abundance of pollen;
3. activity, species, and abundance of pollinators; and
4. distance between fields (Figure 3).

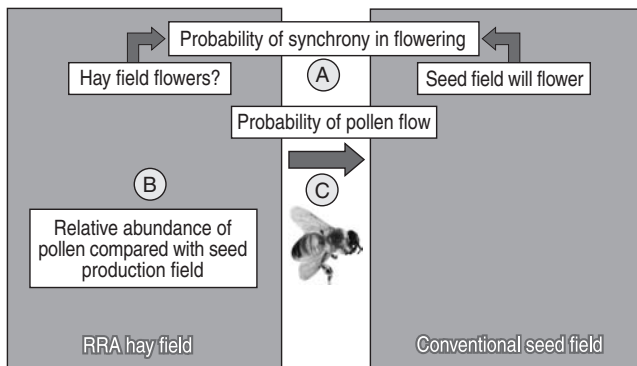


Figure 3. Probability of hay-to-seed gene flow (Putnam 2006). RRA refers to Roundup Ready alfalfa.

If the seed producer or his/her neighbors do not plant GE varieties for hay production, the risk of GE trait gene flow is decreased further because there is little or no risk of the gene being present in the pollen on pollinating bees.

Recent research shows that hay-to-seed pollen-mediated gene flow is very low with observance of routine AOSCA certified isolation distances (e.g., 165 feet [ft]), given normal cutting management of the neighboring hay field during the seed field pollination period. Cutting at or before 10% bloom is typical hay field management. If, because of weather factors or mechanical problems, a neighboring alfalfa hay field is harvested at a later stage of maturity (e.g., 20 to 50% bloom) during the peak seed pollination period, the potential for gene flow into a nearby seed field increases, but is still low (e.g., less than 0.5% for seed produced less than 165 ft from the flowering GE hay field). Longer isolation distances result in gene flow of near zero (e.g., at 350 to 600 ft from the edge of a hay field, the mean gene flow to the seed crop was 0.01%) (Teuber et al. 2007).

Prolific, long-term flowering in all or part of a neighboring hay field will increase the opportunity for pollen-mediated gene flow into the seed crop to approach that expected for the seed-to-seed scenario. In

typical hay-seed field situations, however, the observed gene flow values were at least 10-fold lower than predicted from worst-case seed-to-seed gene flow models (Fitzpatrick, Reisen, and McCaslin 2003; Teuber et al. 2007). Therefore, for most seed producers, the current AOSCA isolation requirement for certified seed production (>165 ft) is sufficient to mitigate nearly all gene flow anticipated from a typical hay field into a conventional seed field (>99% varietal purity is possible). A very low level of gene flow should be expected, however, between closely situated fields.

It would be prudent for producers of GE-sensitive seed with very low or no tolerance for GE traits to use greater isolation distances from all hay fields of unknown variety, from hay fields known to be planted to GE varieties, and from hay fields not under the seed grower's direct management. In some circumstances, the closest hay field and the GE-sensitive seed field may be operated by the same producer who can coordinate the harvest schedule for the hay field(s) to avoid bloom during the pollination period.

Where feasible, producers of GE-sensitive seed can decrease further the risk of pollen-mediated gene flow to the seed field from other varieties of hay in several ways:

1. choose to use larger seed fields (e.g., >5 acres);
2. stock pollinator species that range shorter distances (e.g., leafcutter bees vs. others);
3. harvest the seed field border as a separate lot (Fitzpatrick et al. 2007a; Rincker et al. 1988; St. Amand, Skinner, and Peaden 2000); and
4. work to coexist with neighbors who grow alfalfa for forage (e.g., ask neighbors to cut their hay early during midsummer or to use non-GE varieties).

Some negative consequences of AP can be mitigated through forward planning, forming relationships with multiple customers (some of whom may not be GE-sensitive), and prudent seed sale contracting. Contracts are a voluntary agreement between one seller and one buyer. Before signing the contract, a seed grower considering any GE-sensitive contract should understand the contract stipulations fully and agree in advance to the GE trait evaluation method. Woodward (2006) has presented several examples of GE-sensitive contract wording for seed grower consideration. Seed growers producing for GE-sensitive markets can work with local seed certification agencies and use increased spatial isolation, buffer zones, border lot segregation, or other identity preserved (IP) practices for their GE-sensitive seed fields.

Seed-to-Seed

Because pollen-mediated gene flow is a seed production phenomenon, the seed-to-seed interface has fewer environmental barriers limiting gene flow than the other eight scenarios. This type of field interface, however, also represents less than 1% of alfalfa acreage interactions (see Background and Demographics section), and it is the single most rigorously managed of the nine possible pollen-mediated gene flow scenarios (Table 1). For example, most professional seed growers and/or the seed companies for which they produce seed use third-party seed crop inspection services offered by local seed certification agencies to assure the integrity of the seed production process.

In seed fields, steps are taken to maximize pollination, which makes gene flow between neighboring fields more likely. The fields will have synchrony in flowering, pollinators will be present and some bees will visit flowers in both seed fields, and both fields will advance to, and be harvested at, the ripe seed stage. It is widely recognized that seed-to-seed pollen-mediated gene flow is a common, measurable, natural occurrence for proximal seed production fields of different cultivars (Rincker et al. 1988). This is the reason that seed production scientists and seed companies for decades have promoted using spatial isolation to mitigate gene flow and maintain seed varietal purity. Official seed certification agencies currently ensure the integrity of these practices for the production of certified seed.

Official Certified Seed Field Isolation Standards

To manage genetic purity of conventional and GE varieties, certified seed growers largely rely on physical isolation to minimize and mitigate gene flow. The quantity of gene flow between adjacent seed fields has been studied extensively (Brown et al. 1986; Fitzpatrick, Reisen, and McCaslin 2003; Hammon, Rinderle, and Franklin 2006; St. Amand, Skinner, and Peadar 2000; Teuber et al. 2004) for the past three decades. The data have been used to develop the current AOSCA and Organization for Economic Cooperation and Development (OECD) seed isolation and production standards to meet Federal Seed Act requirements (i.e., >99.0 and >99.9% varietal purity for certified and foundation class seeds, respectively) (AOSCA 2003; Federal 1998; OECD 2005).

Current isolation standards for U.S. alfalfa seed production are 165 ft isolation from other alfalfa for certified seed and, for foundation class seeds, 600 or 900 ft for large (≥ 5 acres) or small (< 5 acres) fields,

respectively (AOSCA 2003). These isolation standards have yielded high-quality seed for markets worldwide for the past 90 years. It is important to note that AOSCA standards or any international seed isolation standards are not designed to achieve zero genetic impurity seeds.

Although AOSCA currently has an isolation standard for alfalfa that is uniform across all pollinator species, it is recognized that the honeybee, leafcutter bee, and alkali bee have different foraging ranges and somewhat different gene flow potential. Beginning in 2000, scientists at Forage Genetics International (FGI) and the University of California–Davis began a series of experiments using the Roundup Ready gene as a highly sensitive gene marker to measure separately the gene flow potential using leafcutter bee and honeybee pollinators (Fitzpatrick, Reisen, and McCaslin 2003; Teuber et al. 2004).

Leafcutter Bee Research Using the Roundup Ready Trait

A 3-year study by FGI scientists used leafcutter bees and various sizes of pollen source and trap plots (Fitzpatrick, Reisen, and McCaslin 2003). All plots were less than 2 acres, smaller than typical commercial-scale certified seed fields (> 40 acres). The results were consistent throughout years and indicated that although gene flow can be detected more than 1,500 ft from the pollen source, it is decreased to less than 0.5% at 1,000 ft, less than 0.2% at distances greater than 1,500 ft, and was not detected at 2,000 ft of true isolation (Figure 4) (Fitzpatrick, Reisen, and McCaslin

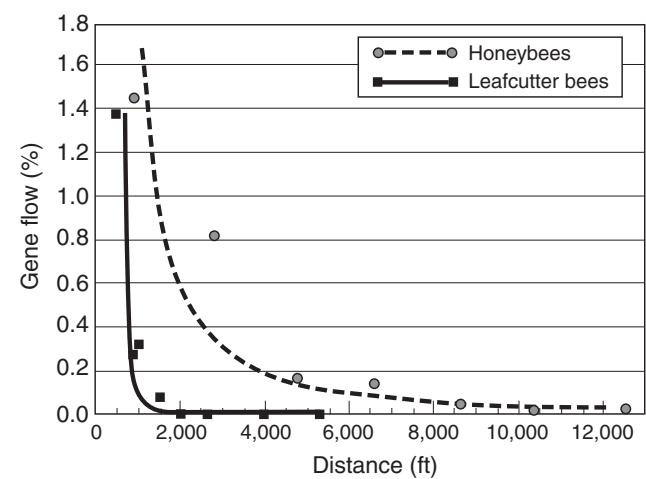


Figure 4. Pollen mediated gene flow in seed production using leafcutter bees during 2000–2002 (Fitzpatrick, Reisen, and McCaslin 2003) or honeybees as pollinators in 2003 (Teuber et al. 2004).

2003). The percentage of gene flow observed was less in large, commercial-scale fields, likely resulting from the effects of field size and whole-field seed lot bulking (Fitzpatrick et al. 2007a).

Honeybee Research Using the Roundup Ready Trait

2003 Study

Preliminary studies conducted by University of California scientists in 2003 using honeybees as pollinators evaluated movement of the Roundup Ready gene into herbicide-susceptible trap crops west and east of a 6-acre Roundup Ready source plot at distances up to 2.5 miles (Teuber et al. 2004; Van Deynze et al. 2004). Researchers observed a significant decrease in gene flow with increasing distance from the source plot (Figure 4). At 900 ft, gene flow was less than 1.5%, and it decreased to less than 0.2% approaching 5,000 ft. Gene flow continued to decline to a distance of 2.5 miles where it was detected at a very low frequency (<0.03%). There was no significant interaction between gene flow and directional orientation of the trap plots.

2006–2007 Study, Honeybee and Leafcutter Bee Species Blend

A larger, 2-year honeybee-pollinated gene flow study took place in the San Joaquin Valley of California, and the first year's preliminary findings were reported recently (Teuber et al. 2007). The pollen source field was a commercial, Roundup Ready cultivar seed field (240 acres). The source field was isolated from all other seed production by 3 miles in all directions, with the exception of the conventional seed fields within the study area. Three conventional cultivar seed fields within the study area were planted as pollen traps at 1 mile (240 acres), 3 miles (40 acres), and 5 miles (100 acres). All commercial seed production was pollinated by a combination of honeybees and leafcutter bees.

A second set of four 1.8-acre *bridged isolation* traps were planted along one field edge of the study area with the nearest at 165 ft, and 900-ft *fallow* spaces between the other three traps. The isolation between these small trap fields was bridged to the source field. At the distal end of the small bridged traps, the nearest commercial (240 acre) trap field was grown at 1 mile (one corner of the large field had isolation bridged by the four 1.8-acre traps); the two other commercial trap fields had true isolation. Equal size (1.8-acre) study areas were sampled intensely within each of the three commercial (large) and four small trap areas.

Outcrossing was detected by protein detection test strips developed for the Roundup Ready trait.

Among the small bridged traps, AP averaged 2.3% at 165 ft and decreased rapidly to 0.9% at 900 ft and 0.6% at approximately 4,000 ft. At 1 mile, AP was less than 0.2%; at 3 miles, AP was less than 0.03%; and at 5 miles from the source plot, no AP was detected. Additional evaluations of seedlings from these test areas are still in progress for 2006 and 2007 seed crop years. Early measurements indicate that the amount of gene flow observed in these test fields is somewhat less than or in close agreement with the 2003 study that used 11 smaller-sized (0.54-acre) trap plots (Teuber et al. 2004).

Feral-to-Seed

As in hay-to-seed gene flow, feral-to-seed gene flow will be governed by several primary variables:

1. Will feral plants flower?
2. If so, what is the relative abundance and duration of synchrony between feral and commercial pollen sources?
3. What is the potential for gene flow as a function of gene frequency, isolation distance, and predominant pollinator species?

The risk of feral-to-seed field gene flow is almost always mitigated by seed growers who eliminate or clip feral alfalfa plants in the vicinity of seed production fields. In California and Idaho, feral alfalfa was observed at fewer survey sites within the counties where intensive alfalfa seed production occurs than at sites in counties where alfalfa is grown primarily for forage (Kendrick et al. 2005). This finding supports the observation that, to a large extent, professional seed growers in California and Idaho already are controlling feral alfalfa as a means to ensure genetic purity of the harvested seed crop.

Even if dispersed feral alfalfa plants are in the vicinity and left unmanaged, the relative abundance of pollen available for gene flow is very low compared with that from cultivated sources. Virtually all alfalfa seed production in the western states is irrigated, and seed production fields are sprayed regularly to control *Lygus* (*Lygus* spp.) bugs and other insect pests hindering seed production. Feral plants do not have the benefit of irrigation or insect control and have poor agronomic fitness compared with their cultivated counterparts. Furthermore, the amount of pollen from a handful of weakened feral plant flowers will be negligible compared with the pollen produced within

the seed production field itself—where plants are at a much higher density, flowers have full synchrony, and plants are managed specifically to optimize seed production and pollination.

Unless the GE trait in alfalfa confers a natural selective fitness advantage, there is no reason to believe GE traits will be over-represented in feral alfalfa populations. This dynamic will need to be examined on a trait-by-trait basis if additional GM traits are commercialized in alfalfa. The potential feral-to-seed gene flow is very low based on the relative fitness and relative low abundance of pollen from feral plants.

As in seed-to-seed gene flow, potential feral-to-seed gene flow will be inversely proportional to isolation distance between the feral plants and the seed production field. St. Amand, Skinner, and Peaden (2000) and Strickler and Freitas (1999) showed that within seed production fields, greater numbers of bees were foraging at distances within 82 ft of the nests than at distances out to 328 ft. Placement of bee nests inside seed fields and away from feral alfalfa will encourage a concentration of bee foraging (pollination) within the intended seed field and away from feral plants (Rincker et al. 1988). Location of bee sites within a seed field is not always possible, especially with alkali bees whose nesting areas can be as far as 4,000 ft from the seed field (Cane 2004).

Producers that are GE-sensitive and are growing seed in proximity to large groups of unmanaged feral alfalfa populations may opt to harvest the conventional seed field edge(s) as a separate seed lot and test that lot for AP before combining it with the main lot intended for GE-sensitive markets. Field edges, if harvested separately as a border crop, may be helpful to further mitigate gene flow potential from feral alfalfa of unknown genetic origin or GE trait status (Fitzpatrick et al. 2007a; St. Amand, Skinner, and Peaden 2000). In some locales, alfalfa is sown intermittently into roadsides or marginal lands as one component of a species mixture. In areas where AP-sensitive seed production occurs, this practice could be modified so that only conventional alfalfa seed is planted, alfalfa is omitted, or a different forage legume is substituted.

The combination of environmental filters and mitigation opportunities suggests that the risk of feral-to-seed field gene flow is very low and can be decreased to near zero if seed producers remove or mow feral alfalfa plants in the vicinity of seed production fields. Seed growers producing GE-sensitive seed can decrease AP risk further by increasing the distance for which feral plants are managed and/or by initially segregating and testing the seed from the

edges of the seed field.

Application of Gene Flow Data to Stewardship in Seed Production

The gene flow studies presented in this paper and the AOSCA standards were used to design two seed industry gene flow mitigation and stewardship plans: the FGI Best Practices for Stewardship in Roundup Ready Seed Production in 2004 (Fitzpatrick et al. 2007b) and the NAFA Best Management Practices for Roundup Ready Seed Production (NAFA 2008). These protocols are similar because both require the same, above-AOSCA isolation standard distances to proactively mitigate AP of GE traits in conventional seed production. Although the protocols may need to be refined for specific traits, the same concepts and strategies are applicable to other IP traits in alfalfa.

In the Pacific Northwest, the overwhelming amount of seed production is for domestic markets, and leafcutter bees are used as primary pollinators. In this region, a 900-ft isolation distance (the same isolation requirement for foundation class seed) between biotech and conventional alfalfa seed production is used to manage seed purity and AP to <0.5%, a common industry standard for AP tolerance in conventional seed of other crop species. In specific fields in niche areas of the West (e.g., two counties in south-central Washington), alkali bees are used alone or in combination with leafcutter bees to pollinate commercial seed fields. The Best Practices isolation minimum for alkali bee-pollinated fields is 1 mile.

In California, more than 60% of seed production is for AP-sensitive export markets, and honeybees are used as primary pollinators. In this area, a 3-mile isolation distance is being used to manage AP to a nondetectable level. This isolation standard is more than 95 times the standard isolation requirement of 165 ft for conventional certified seed. This honeybee isolation distance was adopted by a California seed industry stakeholder group convened by the University of California Seed Biotech Center in 2005 (University 2005). The common ground for the alfalfa industry consensus-building initiative is that an effective GE-alfalfa seed production coexistence and trait stewardship strategy needs to be science-based, market-sensitive, and pollinator-specific. The NAFA Best Management Practices protocol has been adopted and implemented by all three NAFA member companies that currently produce, or intend to develop or produce, GE alfalfa seeds.

The NAFA Best Management Practices isolation

standards for GE/RRA seed fields are based on the smaller-scale gene flow experiments where each trap field was less than 2 acres (Fitzpatrick, Reisen, and McCaslin 2003; Teuber et al. 2004). Because foraging behavior of bees is affected by the attractiveness of the food source, the size of fields affects pollinator dispersion dynamics and potential gene flow between adjacent seed fields. Small fields or feral patches have a higher relative percentage of gene in-flow than larger fields (AOSCA 2003; Brown et al. 1986; Fitzpatrick et al. 2007b; Hammon, Rinderle, and Franklin 2006; Rincker et al. 1988; St. Amand, Skinner, and Peaden 2000). Therefore, the gene flow models developed using the smaller-size traps or isolated alfalfa feral trap patches were anticipated to offer worst-case model projections for pollen-mediated gene flow potential among large commercial-scale fields.

The efficacy of the Best Practices for mitigating seed-to-seed pollen-mediated gene flow has been evaluated. In separate surveys, Fitzpatrick and colleagues collectively examined more than 300 conventional commercial seed lots produced in eight western states in 2006 or 2007 (Fitzpatrick et al. 2007a, b). The percentage of AP was determined from 3,000 seeds using pooling procedures and statistics by Remund and colleagues (2001), then was plotted against the isolation distance to the nearest Roundup Ready seed field. For all three pollinator species and pollinator species blends, and for all samples in the surveys, the observed commercial AP was four to five times lower than predicted by the smaller field research experiments. For all seed lots analyzed by FGI and grown using the Best Practices minimum isolation, gene flow was mitigated sufficiently to meet a target threshold of 0.5%; the AP was 0.00 to 0.18% in these samples (Fitzpatrick et al. 2007b).

Commercial test kits for RRA seed have been validated by the manufacturers and by a third party (Teuber et al. 2007) and are available to monitor gene flow. There is an industry consensus that third-party verification of AP in random conventional seed lots will be a useful mechanism for validating and monitoring the industry's GE trait coexistence and stewardship gene flow mitigation protocols. This mechanism also will be useful for recommending changes to Best Practices, when and if required (NAFA 2008).

The establishment of verifiable isolation standards for GE alfalfa seed production is a critical component for managing gene flow and AP in GE-sensitive conventional alfalfa seed production (AOSCA 2008). Other factors include bee management, equipment cleaning, crop rotation, and GE seed field location reporting to local seed certification agencies. All these

relevant factors are outlined in detail in the NAFA Best Management Practices for Roundup Ready Seed Production (NAFA 2008). This document and protocol are intended to be used as an industry standard for the production of GE alfalfa seed in the United States. Producers of GE-sensitive seed can use the information to help them implement field and seed production plans. All Roundup Ready seed producers are required to report all Roundup Ready seed field locations to their local seed certification agencies. These independent third-party agents will maintain GE seed field location records and field histories.

To assist in seed field isolation planning, GE-sensitive seed producers can choose to use seed certification services to obtain the isolation distances between their prospective or current production location and the nearest RRA seed production field. When feasible, GE-sensitive seed producers can decrease further the risk of pollen-mediated gene flow to the seed field from other/unknown varieties of seed by several methods:

1. using larger seed fields (e.g., >5 acres);
2. stocking pollinator species that range shorter distances (e.g., leafcutter bees vs. others);
3. harvesting the seed field border as a separate lot or planting a sexually incompatible species as a border (Brown et al. 1986; Fitzpatrick et al. 2007a; St. Amand, Skinner, and Peaden 2000); and
4. working to coexist with their neighbors who grow alfalfa for seed (e.g., asking them to position their bee domiciles away from common field edges or to consider growing seed of non-GE varieties).

As in hay-to-seed gene flow situations, some negative consequences of unintended GE trait presence can be mitigated through good communication with neighbors and customers, prudent seed sale contracting, work with local seed certification agencies to coordinate spatial isolation and buffer zones, and border lot segregation or other well-established IP practices for GE-sensitive seed fields.

Hay-to-Feral

Hay-to-feral gene flow will be subject to the same environmental barriers present for hay-to-seed gene flow: synchrony in flowering, presence of pollinators, and isolation distance between hay fields and feral plants. Two other factors need to be considered: feral plant fecundity and relative abundance of local

feral pollen relative to pollen from the neighboring hay field.

Feral plants typically receive no benefits from agricultural inputs that enhance cultivated alfalfa forage and/or seed production (e.g., artificial breeding selection, irrigation, application of insecticides, etc.). Therefore, feral plants frequently are less fecund as a result of being unprotected from biotic or *abiotic* stress compared with their cultivated counterparts. A probable exception may be a locally adapted, naturalized, feral population such as that reported growing on an isolated South Dakota rangeland (Boe et al. 2004). This agronomic disadvantage greatly decreases the amount of seed produced per feral plant relative to those under seed field cultivation.

When scattered feral plants are growing near synchronously blooming hay fields and pollinators are active, the size of the cultivated pollen pool will be much larger than the feral pool. Therefore, the proportion of feral seeds tracing to the incoming cultivated pollen potentially will be biased upward until genetic equilibrium between the populations is achieved.

Insect feeding, foraging by wildlife, or mowing will decrease the number of feral flowers along roadsides and ditches. Any seed produced on feral plants would need adequate moisture, soil cover, fertility, a frost-free period after germination, and minimal competition from other plants to establish and survive under feral conditions. Most seeds formed on feral plants likely would perish because they would fail to germinate successfully, to compete, or to establish outside of cultivation (see Autotoxicity and Hard Seed sections).

Despite these limitations, some natural feral populations persist in the wild (Boe et al. 2004). In natural environments with large populations, gene frequencies for traits stabilize or remain in “equilibrium” without selection. In practice, most populations have some selection pressure for specific traits that changes from season to season. For example, in one season there may be insect pressure that allows plants with resistance to increase in frequency, but in the following season drought may result in selection for different plants that can tolerate moisture stress. For herbicide resistance, the frequency of genes remains similar (in equilibrium) unless sprayed with the specific herbicide. Therefore, if there were a 0.5% gene flow to a population, that frequency would remain low unless selected for specifically or naturally.

Because cultivated alfalfa is reseeded routinely, pollen-mediated gene flow is controlled by seed stock purity and coexistence principles discussed in the following sections (Falconer and MacKay 1996). Pas-

tures seeded with alfalfa mixtures are unmanaged except for reseeding and grazing. Gene flow in this instance is a consequence of the same parameters as feral with grazing pressure, although flowering may be more synchronous in the year of stand establishment than for feral plant populations.

Hay-to-feral alfalfa gene flow was investigated by St. Amand, Skinner, and Peaden (2000). Using conventional genetic traits to differentiate self- from cross-pollinations, these researchers measured cross-pollinations onto a single, genetically identical clone of transplanted “simulated feral” trap plots at two locations. Although the researchers reported that the hay pollen source plots were harvested three times at the Kansas location and five times at the Washington location, their report did not state the maximum stage of flowering of the hay plots during the pollination time for the trap plants. For example, because alfalfa can be cut four to five times in Kansas, cutting only three times may result in greater-than-average flowering, thus overestimating gene flow (see Figure 3). The authors reported the percentage of gene flow in each trap, but they did not report the gross number of cross-pollinated seeds produced in each trap and the duration of bloom synchrony. The trap plots may be considered to be atypical and not representative of true feral plants for several reasons.

- The trap plant groups (10.76 ft²) were placed in a single path, at regular intervals along one edge of the study field.
- It is likely, but not stated, that after being transplanted, the trap plants were cared for by the researchers (e.g., hand watered, protected from competition).
- Because all trap plants were of the same genotype (clone), they were unlikely to (self) pollinate with their nearest neighbors, which resulted in bias and selection against short-distance gene flow and toward long-distance gene flow (i.e., that from the cultivated hay fields).

For these reasons, these data should be somewhat narrowly interpreted and considered a worst-case scenario for hay-to-feral gene flow potential. Given the stated conditions for this study, the amount of gene flow was a function of the distance from the hay field source and the size of the gene source plot. At 2,640 ft, gene flow averaged 0% and 18% with small (ca. 40 ft²) and large (ca. 6.0 acre) hay source plots, respectively. This result reflects the relative pollen and pollinator abundance in the local environment based on source plot size. Because there was measurable gene flow at the 2,640-ft trap plant distance, St. Amand, Skinner,

and Peaden (2000) stated that gene flow would probably be detected at a further distance.

Seed-to-Feral

Seed-to-feral gene flow will be subject to environmental barriers similar to those for seed-to-seed gene flow: synchrony in flowering and isolation distance between seed production fields and the fecundity of feral plants forming seeds. Most seeds formed on feral plants will not result in true gene flow because most would fail to germinate, compete, or establish outside of cultivation successfully.

Gene flow from seed production fields to nearby feral or simulated feral alfalfa has been documented in two studies: St. Amand, Skinner, and Peaden (2000) and Hammon, Rinderle, and Franklin (2006). In contrast to the earlier study in 2000, the 2006 study by Hammon, Rinderle, and Franklin was conducted using typical, commercial-scale, RRA seed fields as the gene source (900 acres overall) and typical, unmanaged feral plants as traps (23 sites overall). For the year in which data were gathered and for 2 prior years, there was a high concentration of spatially isolated Roundup Ready seed production in the area (900 acres of seed was grown). The study found evidence of pollen-mediated gene flow on the feral alfalfa traps up to 1.7 miles from the nearest RRA seed production field; the study found no correlation between percentage of gene flow and the distance to the nearest source field.

This finding supports the observation by others that bees within seed fields tend to forage in a localized patch with a large nutritional reward before returning to the nest/hive (Cane 2004; Rincker et al. 1988). Seed with AP of the Roundup Ready trait was collected from 19 of 23 feral alfalfa sites. The closest collection without Roundup Ready seed present was 0.65 miles from the nearest seed production field. Based on the observations of pollinating bee activities and emergence dates, the Hammon, Rinderle, and Franklin (2006) study suggested that long-distance transport of pollen was done by honeybees rather than alkali bees, although many taxa of bees were collected in seed fields and from feral plants.

Using the limited plot design discussed in the previous section (see Hay-to-Feral section), St. Amand, Skinner, and Peaden (2000) found gene flow from seed production source plots into transplanted trap plants out to 3,280 ft and to 2,640 ft at one location in eastern Washington and one in central Kansas, respectively. These trap plants were transplanted to the edge of cultivated areas; for this and other reasons, the trap

plants used in this study may be atypical of feral alfalfa plants in general (see Hay-to-Feral section). The amount of gene flow was a function of distance from the seed field source and size of the source plot. At 2,640 ft, gene flow averaged approximately 0% and 37% with small (ca. 40 ft²) and large (0.3 acre) source plots, respectively.

The percentages for gene flow into these unique single-genotype traps were much higher than those documented for commercial fields by Hammon, Rinderle, and Franklin (2006), and they were much higher than observed gene flow at that distance in seed-to-seed research plots (Figure 4) where seed production was encouraged. The very high percentage of gene flow observed by St. Amand, Skinner, and Peaden (2000) likely was due in part to the small number of plants (and seeds) from a single genotype available for sampling per trap ($n = 15$ plants, 3.3-ft radius of the plot) and the lack of any pollen or seed dilution effect that would be typical in true feral populations or very small seed fields. Because of self-incompatibility and a single genotype available for pollination, the St. Amand, Skinner, and Peaden (2000) feral alfalfa experiments overestimate the distance and level of outcrossing in alfalfa.

Consequently, data collected in feral studies are useful to guide the potential distance of gene flow, but are limited in determining the amount of gene flow. This is further biased because, by definition, small feral populations or single plants lack the statistical power to make objective conclusions on frequency of gene flow. This does not negate the fact that gene flow can be mediated by feral plants.

Unlike seed-to-seed gene flow, seed-to-feral gene flow is the movement of genes from a much more abundant source of pollen and pollinators to a receiving population that is much less abundant and conducive to seed production. Also, when feral plants occur in dispersed small patches, in the context of gene flow studies, they are sometimes called “sentinel” plants because they stand alone. The “scouting” bees will have a higher probability of visiting these free-standing plants, which helps explain the higher rate and greater distance of gene flow observed in seed-to-feral compared with seed-to-seed scenarios.

The impact of seed-to-feral gene flow on GE-sensitive commercial producers needs to be examined in combination with feral-to-hay and feral-to-seed gene flow. The low relative abundance of pollen and pollinators and the high degree of environmental stress on feral plants compared with plants within commercial seed production fields will help decrease the likelihood and commercial importance of seed-to-

feral and subsequent feral-to-seed or feral-to-hay gene flow risks to near zero.

Alfalfa seed growers can limit gene movement from GE trait seed production fields to feral alfalfa by managing the feral alfalfa plants, routinely mowing roadsides, and controlling alfalfa sowing and vegetation in areas such as fencerows, ditches, or irrigation canals. In general, professional alfalfa seed growers find it beneficial to control feral vegetation in waste areas so that the weeds do not multiply and do not harbor or attract untreated pests. It takes only a single management act on a feral alfalfa plant to eliminate it or its potential to form feral seed, or to disrupt the synchrony needed for successful GE trait gene flow from a commercial seed field.

Seed production of feral alfalfa also will be limited by flower- and seed-feeding insects such as Lygus bugs, thrips (*Frankiniella* spp.), and seed chalcid (*Bruchophagus roddi* G.). Gene flow from seed production fields to feral alfalfa is, and will be, mitigated with management by RRA seed growers who are required by contract to control feral alfalfa in the vicinity of their seed production fields (Fitzpatrick et al. 2007b). Mitigation (i.e., coexistence) strategies for intentional management of pollen-mediated gene flow from GE seed (or hay) fields, coupled with the low likelihood of effective pollen-mediated gene flow from feral plants to commercial alfalfa hay or seed fields, limit the risk of feral alfalfa plants being an effective bridge between commercial GE and non-GE hay or seed production fields.

Feral-to-Feral

Potential pollen-mediated gene flow between feral alfalfa populations will depend on distance between feral plants (e.g., feral plant density and dispersion of the population), synchrony of flowering, pollinator presence, gene frequency, and damage to developing seeds or flowers caused by insect pests and local abiotic stress. Only a minor portion of the seeds formed on the feral plants will result in true gene flow. Of the few seeds formed on feral alfalfa, most are likely to perish because most will fail to germinate successfully (see Autotoxicity and Hard Seed sections), compete, or establish outside of cultivation.

The paucity of feral plants will decrease and act to slow the rate of feral-to-feral gene flow. St. Amand, Skinner, and Peadar (2000) reported gene flow between isolated feral alfalfa plants at distances of up to 750 ft. Thus, paucity of feral plants will decrease feral-to-feral gene flow. The authors also noted that individual trap plants located in urban areas failed to set seed, probably because of lack of pollinators. For unmanaged naturalized feral populations growing in dryland conditions with adequate moisture for plant survival and reproduction, as reported by Boe and colleagues (2004), gene flow within the population will be limited primarily by the dearth of pollinators and suitability of conditions for successful secondary seedling establishment.

Seed-mediated Gene Flow in Alfalfa

Alfalfa seed is too dense and smooth for effective wind or water dispersal; therefore, natural seed dispersal generally is local and is not considered a significant mechanism for gene flow. There are three potential avenues for seed-mediated gene flow:

1. admixture of GE and non-GE seed in seed harvest, seed processing, and/or seed planting;
2. volunteer seedlings from a GE seed production field emerging and establishing in a subsequent non-GE seed production field; and
3. animal-mediated gene flow from grazing on alfalfa containing ripe pods with mature GE seed.

Seed Admixture

Careful cleaning of seed harvest and processing equipment is a key quality control component of a conventional seed IP program or of a trait stewardship program for commercialization of GE traits in crops. The NAFA Best Management Practices document outlines required equipment cleaning precautions to mitigate the potential risk of inadvertent seed admixture (NAFA 2008). Routine monitoring for AP of the GE trait in non-GE seed, as described in the NAFA document, will be used to monitor and verify successful AP management, including aspects of both isolation distance and seed harvest/processing quality control procedures.

Before planting or seed lot processing, GE-sensitive seed production companies or growers can avoid the use of seed admixtures by testing seeds for the AP of the GE trait using validated seed test methods (Teuber et al. 2007). Furthermore, as with most GE crops, RRA varieties will be sold only in clearly labeled packages. Seeds will be coated with an obvious purple-colored coating not used for any other alfalfa. Therefore, the on-farm admixture of Roundup Ready and conventional seeds will be obvious and easy to avoid during planting operations.

The AOSCA currently is offering the Alfalfa Seed Stewardship Production Program, a voluntary, fee-

based, IP program of process certification for the production of alfalfa seed destined for GE-sensitive markets. As part of this program, the IP process certification includes the testing and third-party verification of genetic origin and no-detect GE-trait status of planting seed stock and the observance of a minimum stated isolation distance from GE-alfalfa seed production (AOSCA 2008).

Volunteer Seedlings

Volunteer alfalfa can germinate from viable, hard remnant seeds left in or on the soil by a previous alfalfa crop cycle. Alfalfa grown for hay represents more than 99% of U.S. alfalfa acreage (compare seed and hay acreage, USDA–NASS 2007a), so the potential for volunteer seed-mediated gene flow within hay fields will be considered first.

The potential for seedling volunteers to arise from a previous hay crop seed bank is exceptionally small or nonexistent because it is determined by the same parameters as hay-to-hay gene flow. Namely, a mature viable seed pollinated from a GE field must fall to the ground and germinate in the new stand. The potential for gene flow via remnant seed volunteers to a subsequent alfalfa hay crop planted in the same field is even more remote because the remnant planting seeds would be 3 to 6 years old by the time the stand is terminated (see Hard Seed and Autotoxicity sections). Both events are further circumvented by crop rotation. Most hay land is rotated to a different species for at least 2 years before replanting alfalfa for hay. Therefore, hard remnant seeds will decompose during approximately 7 years of seed aging, cropping activities, and continuous exposure to the biota in the soil.

Hard seed content for different seed lots of a single variety can be as high as 69% and as low as 14%, depending on where the seed was produced and the weather conditions during seed maturation (Bass et al. 1988). Undersander and colleagues (1993) planted various seed lots of the same variety in which hard seed varied from 11% to 44%. At three locations in

the Midwest, emergence was measured on a regular basis. These data showed that differences in hard seed content in commercial seed lots did not delay germination significantly, and that—*independent of hard seed percentage*—virtually all viable seed germinated within the first 90 days. All viable hard seed germinated in the seeding year with no seed germinating the next year, even in the absence of any competition.

Although the acreage for alfalfa seed production is small compared with that for hay, the likelihood of volunteer seedling-mediated gene flow is somewhat greater. Virtually all commercial alfalfa seed is produced under irrigation in the western United States.

Although there are no data from the West that directly compare seed germination rate and hard seed content, some recent data monitoring the number of volunteers after alfalfa seed production in the field are useful. Reisen monitored volunteer seedlings following nonchemical fall termination of twelve alfalfa seed production fields in Idaho (Reisen, P. 2008. Personal communication). These fields either were 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 was counted several times each year and summarized by quarter for the 3-year period (Figure 5, Location 1).

Arias also monitored volunteer seedling emergence in three Texas seed fields that had been fall-terminated using herbicide and cultivation (Arias, J. 2008. Personal communication). These fields were rotated to corn for the next 3 years. Arias observed an average of 25 volunteers per acre during the first summer and zero thereafter (Figure 5, Location 2). More aggressive stand take-out of seed fields and prudent management strategies for broadleaf plants in the subsequent crops were demonstrated to be very effective in mitigating nearly all alfalfa seed produc-

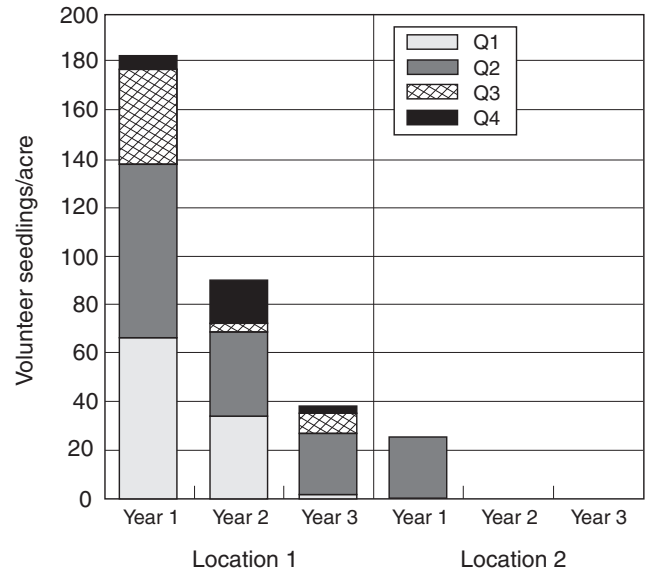


Figure 5. Volunteer emergence by quarter in Idaho (Location 1) and Texas (Location 2) for 3 years after seed field take-out (mean count/acre). (P. Reisen and J. Arias, unpublished)

tion volunteers in subsequent crops.

The AOSCA seed certification standards require a minimum 1-, 3-, or 4-year rotation period between termination and reestablishment of certified, registered, or foundation class alfalfa seed production fields, respectively (AOSCA 2003). The Idaho Crop Improvement Association has a 3-year rotation requirement for conventional alfalfa seed following RRA seed production. Based on the very low number of volunteers counted late in the third year, a 3-year requirement seems to be conservatively appropriate for the production of seed for GE-sensitive markets. As with isolation distance, a seed producer can always elect to adopt more conservative crop rotation standards based on the intended market and market seed quality demands.

Animal Grazing

Will and Tackenberg (2008) describe mechanisms for animal-mediated seed dispersal and models that can be used to predict the likelihood of such dispersal. Anecdotal evidence demonstrates that at least some ripe alfalfa seed consumed by grazing animals can pass through the gastrointestinal tract, be excreted in manure, and be capable of germination. But because ensiling and digestion processes significantly decrease weed seed viability for numerous species (Blackshaw and Rode 1991), it is likely that a large portion of the alfalfa seeds present in overripe hay also may be rendered nonviable before consumption or excretion.

Based on commonly used alfalfa seed and hay production practices, grazing of domestic animals on

cultivated alfalfa containing ripe seed pods and mature seed will be rare. Domestic animal grazing on rangeland containing alfalfa as a component is more likely, especially if the grazing interval is sufficient to allow the production of ripe alfalfa seed.

Wild animals grazing on alfalfa plants in commercial seed production can be discouraged but not absolutely prevented; thus, they represent another possible mechanism for animal-mediated seed dispersal in alfalfa. Wild or domestic animals also may graze on feral alfalfa plants containing ripe seed. In comparison with many other seeds and grains potentially present in the wildlife grazing environment, alfalfa seed may be less attractive because it is of lower caloric and nutritional quality.

Summary

Although there have been instances in which low-level presence of *regulated* materials has resulted in market disruptions (e.g., Starlink corn, LL601 and LL604 rice), there is no evidence of significant market disruption associated with the commercialization of deregulated biotech traits in the United States. Concurrent with increased U.S. farmer adoption of biotech traits in corn, soybean, and cotton there have been increases in U.S. grain/fiber export (USDA–FAS 2007) and organic production (USDA–NASS 2007a, b, c). Although only 3 to 5% of the U.S. alfalfa hay production is sold to GE-sensitive markets (Putnam 2006), production for these markets has significant economic importance in specific regions of the United States. Approximately 33% of U.S. alfalfa seed production is exported, primarily to GE-sensitive markets. A thorough understanding of gene flow in alfalfa is critical to establishing stewardship programs that enable coexistence between alfalfa growers producing GE alfalfa hay or seed and growers producing these products for GE-sensitive markets.

Understanding the relative importance of gene flow between and within feral plants, hay, and seed production fields helps to identify key biological, agricultural, and environmental barriers to gene

flow and to formulate logical mitigation strategies for managing the AP of GE traits in non-GE alfalfa seed and hay. Synchrony in flowering, presence of pollinators, isolation distance, and relative abundance of pollen between pollen source and pollen recipient plants are typical biological barriers, most of which are amenable to management in hay and/or seed production systems.

In general, it seems that NAFA Best Management Practices in hay and certified alfalfa seed production, coupled with the pollinator-specific isolation guidelines outlined in the NAFA Best Management Practices document, are adequate for managing AP to tolerance levels appropriate for most markets. These types of management practices are employed successfully by producers of certified seed in most crops, including alfalfa, to ensure genetic purity of seed stocks. Increased isolation distances in seed production—including production in non-GE seed production zones—use of border areas, crop rotation, use of certified seed, careful selection of the introduced pollinator, and routine elimination of neighboring feral alfalfa plants are tools that can be applied to decrease further the risk of gene flow in the production of seed for GE-sensitive markets.

Appendix A: Abbreviations and Acronyms

AOSCA	Association of Official Seed Certifying Agencies	RRA	Roundup Ready alfalfa
AP	adventitious presence	spp.	species (plural)
EU	European Union	USDA	U.S. Department of Agriculture
FGI	Forage Genetics International	USDA–APHIS	U.S. Department of Agriculture–Animal and Plant Health Inspection Service
ft	feet	USDA–FAS	U.S. Department of Agriculture–Foreign Agricultural Service
GE	genetically engineered	USDA–NASS	U.S. Department of Agriculture–National Agricultural Statistics Service
GM	genetically modified		
IP	identity preserved		
NAFA	National Alfalfa & Forage Alliance		
OECD	Organization for Economic Cooperation and Development		

Appendix B: Glossary

Abiotic. Nonliving elements (such as soil or climate) that impact the growth or performance of a plant or crop.

Admixtures. In seed production, usually the mixture of seeds into a seed lot.

Adventitious presence. Unintended low level occurrence of seed or plant materials in a crop or crop products.

Autotoxic/Autotoxicity. A substance that poisons the system within which it is formed.

Bridged isolation. Spatial separation of a field that has one or more sexually compatible plants between it and a source of genes/pollen.

Fallow/Fallowed. Land left unseeded after plowing for a period of time to recover natural fertility.

Feral. Animals or plants that live or grow in the wild after having been domestically reared or cultivated.

Gene flow. The exchange of genes from one population to another.

Genetically engineered. An organism that has its genetic constitution modified by insertion of foreign or endogenous genes.

Greenchop. Fresh forages, wilted to 35–50% moisture, that are made of grass and alfalfa mixtures and used to feed livestock.

Haylage. Hay that has been ensiled.

Herbaceous. Plants or plant parts that are fleshy and wither after each growing season, as opposed to plants such as trees that grow woody stems and are persistent.

No-detect. Not detectable using specific sampling and statistical procedures.

Photoperiod. The daily cycle of light and darkness that affects the behavior and physiological functions of organisms

Pollination. The transfer of pollen grains from the male floral structure (anther) of a plant to the female floral structure (stigma) of the same or different plant, leading to fertilization and seed formation.

Regulated. Crop plants or traits that are restricted in use and are controlled by government agencies such as the U. S. Department of Agriculture, the U.S. Food and Drug Administration, or the U.S. Environmental Protection Agency.

Ripe seed set. Crop stage at which new seeds have reached physiological maturity.

Varietal genetic purity. The proportion of seeds having the same genetic identity as described in its registered variety description.

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