

# MARKET SENSITIVITY AND METHODS TO ENSURE TOLERANCE OF BIOTECH AND NON-BIOTECH ALFALFA PRODUCTION SYSTEMS

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## ABSTRACT

Roundup-Ready® alfalfa was released in June of 2005, the first trait to be commercialized in alfalfa (*Medicago sativa* L.) using biotechnology. Traditional alfalfa varieties will likely be grown in close proximity to these new biotech varieties. While most users will probably not be sensitive to the presence of these varieties, other markets (particularly export and organic) will request hay that is free of the biotech traits. The acreage estimated to be sensitive to this trait is estimated to be less than 3-5% in the US, but some regions, markets, and growers will be more sensitive than others. Methods to assure identity and preservation of non-biotech alfalfa include understanding the potential for gene flow, reducing feral alfalfa, and improved methods to maintain commercial identity, including the use of test strips which confirm the absence or presence of RR hay. The potential for gene flow from alfalfa hay field to hay field differs significantly from gene flow in seed production due to a series of 'environmental filters' under forage production which are likely to prevent significant gene movement. Successful coexistence of biotech and non-biotech alfalfa hay for sensitive markets should be feasible with a higher degree of awareness of these issues and several simple management steps.

**Key Words:** biotechnology, varieties, organic farming, exports, organic hay, weed control, roundup-ready, economics, markets

## INTRODUCTION

During the past decade, biotech-origin traits have been successfully commercialized in corn, cotton, and soybean, particularly the Roundup-Ready™ (RR)<sup>2</sup> trait. On June 14<sup>th</sup>, 2005, the RR trait was de-regulated in alfalfa (USDA, 2005), allowing biotech alfalfa varieties to be sold which are tolerant to the herbicide glyphosate (Roundup®). This act enabled not only the commercialization of the first biotech varieties in alfalfa, but also provided a new previously-unavailable weed management system for this crop. The first commercial fields were planted in 2005, with the first major biotech forage products to be utilized in 2006.

Alfalfa (*Medicago sativa*) in recent years has overtaken wheat as the third most important crop economically in the United States, exceeded only by corn and soybean (USDA-

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<sup>2</sup> Mention of a product does not imply endorsement of that product nor disparagement of similar products which may serve the same function.

NASS, 2004). Over 22 million acres are harvested each year, with the largest alfalfa producing states being in the West and upper Midwest, particularly CA, ID, IA, NE, MN, SD and WI. Alfalfa is primarily used for dairy production, but is incorporated into beef, sheep, and horse grazing and hay systems as well.

If adopted by growers, the RR technology may cause profound changes in the way that alfalfa growers approach weed control as well as the varieties that they grow (Doll, 2003; Orloff et al., 2003; Van Dynze et al., 2004). Although research on the issues surrounding this technology is on-going, the weed control efficacy of Roundup is well known, and several comparative studies have been published (Van Dynze et al., 2004).

However, the release of the first commercial biotech trait in alfalfa also presents several challenges. From a grower's perspective, the questions with this technology are related to crop management, economics, weed management, stewardship of the trait, environmental issues, and utilization or marketing of the hay. Since biotech and non-biotech alfalfa varieties will likely be grown in close proximity, growers of both types will need to understand the issues associated with stewardship of the trait and the potential impacts of these coexisting systems on markets, crop profitability, and risk. This paper provides an overview of these issues, and suggests steps to assure stewardship of both biotech and non-biotech alfalfa varieties in hay production and marketing systems.

## **UNDERSTANDING SENSITIVITIES OF ALFALFA MARKETS AND CONSUMERS**

Biotech traits have been used in animal feeding systems for more than a decade now. Over 100 digestion and feeding studies examining the effects of feeding GE crops to various food-producing animal species (e.g., beef cattle, swine, sheep, fish, dairy cows, water buffalo, and chickens) have shown that DNA or proteins from biotech crops are not detected in milk, meat, eggs, or other products from animals that consume these crops (Cullor and Rossitto, 2004, Van Dynze et al., 2004, Van Eenennaam, 2005). The National Academy of Sciences has concluded that biotechnology is no more likely to produce unintended consequences for human food risk than conventional methods, and actually may hold some advantages, since the genetic changes are more defined than conventional crossing (NAS, 2004). The USDA uses the standard of 'substantial equivalence' in judging the safety in utilization of biotech crops, and has determined that Roundup Ready alfalfa is safe to enter the US feed market (USDA, 2005).

Nevertheless, some markets may be sensitive to the presence of biotech alfalfa, and will wish to maintain crops which are considered 'free' of biotech traits. Thus understanding the utilization patterns of alfalfa and the markets or uses for alfalfa which might be sensitive to the purchase or use of RR alfalfa products is important to the issue of co-existence of biotech and non-biotech systems.

### **How is alfalfa used?**

While it is difficult to know with a large degree of certainty the utilization and disappearance of alfalfa in the United States, there are three major consuming markets for

alfalfa hay. These are: alfalfa for dairies, alfalfa for beef, and alfalfa for horses. More minor, but still important uses for alfalfa forage include alfalfa hay for export, alfalfa for small ruminants (sheep, goats), alfalfa meal for processed feeds, and alfalfa pellets for pets and rabbits. Alfalfa sprouts for human consumption is a by-product of the seed industry, and not related to forage production, the major use of alfalfa.

This discussion is limited to the markets for alfalfa hay, not seed, which presents separate and important issues with regards to biotech traits (Mueller, 2004). Alfalfa seed is a more localized issue, with a scope of less than 200,000 acres in several western states, whereas alfalfa forage impacts over 22 million acres of cropland in the US. Of these uses for alfalfa forage, dairy is clearly the most important, and must be considered the ‘driving force’ for alfalfa production in the United States.

### **Alfalfa for use on dairies.**

The use of alfalfa for dairy production is by far the largest consumer of alfalfa in the nation. In California, we estimate this amount to be about 75% of the production, and it could be higher, although no agency keeps close statistics on utilization of hay or forage. The other major forage for dairies is corn silage, but alfalfa is often considered the ‘staple’ of dairy production in the US.

Utilization of alfalfa for dairies can be divided into on-farm use raised by dairies and fed on-farm, vs. hay raised independently and sold to dairies; these systems have clear differences in terms of marketability and acceptance. On-farm feeding is more common in Eastern and Midwestern US states, whereas alfalfa hay is mostly sold as a cash crop in western states. For on-farm use, marketing issues are confined largely to the views of the dairy or producer, as well as to the marketing of milk and meat. For cash hay producers, the market signals are purely through price and willingness to buy. For a cash hay producer, the worst fate is being stuck with hay that simply will not sell—a common occurrence for hay discounted for quality traits, not considering biotech traits.

It is anticipated that the dairy industry will not, with some notable exceptions, be highly sensitive to the presence of a biotech trait in alfalfa. This is due to the fact that the dairy industry has already absorbed at least 6-8 GMO traits which are currently components of the milk or milk-product production system. *Bovine Somatotropin* (BST), a naturally-occurring hormone produced via biotech methods, has been used for over a decade on many dairies. This was followed by the introduction of genetically engineered crops (both Roundup-Resistant and crops with the *Bacillus thuringiensis* (Bt) gene), particularly corn, soybean, canola meal and cottonseed, all of which feature prominently in dairy feed rations. Additionally, most cheese is now produced with biotech-based rennet, produced in fermentation vats since 1990 rather than by sacrificing calves for rennet. While some have raised concerns about the inclusion of these biotech traits in our milk supply, the predominance of these biotech traits in the dairy system has by-and-large not proved to be a major marketing issue.

The exception to this is organic dairy producers. Organic dairies have rejected biotech crops as a component of certification for organic status (USDA). Organic milk is a small

but growing component of US milk production. The number of certified organic milk cows reportedly increased by 277% from 1997 to 2001, but still constitute less than 1% of the total US milk production (Miller, 2005).

Organic dairy producers will demand hay which originates not only from biotech-free alfalfa crops, but from fields that have been approved for organic production according to the certification standards to which they subscribe. Since organic alfalfa precludes pesticides, the RR trait would be useless anyway, since Roundup is also precluded. Therefore, organic dairy producers will demand alfalfa from certified fields that do not contain biotech varieties.

Thus, although specific dairies will demand biotech-free alfalfa, at this point in time, the vast majority (e.g. 95 to 99%) of dairies in the United States are not likely to be highly sensitive to the inclusion of a biotech trait (RR alfalfa) in rations.

### **Alfalfa for Beef-Sheep-Goats.**

The quantity of alfalfa disappearance into the nation's beef industry is also not known, but a larger percentage of non-alfalfa forage (grass hay, grazed forages and corn silage) is fed to beef cattle than to dairy cattle. Of these consumers, less than 1% of beef production is organic (NCBA, 2005), but the National Organic Trade Association projects growth to be 30% per year through 2008.

However, for the same reasons cited in the above paragraphs, it is highly unlikely that the vast majority of beef producers will be sensitive to the use of biotech alfalfa in rations. The beef industry, similar to dairy, has already absorbed a number of GE-based traits in the feed ration, the most prominently biotech corn and soybean. The food safety concerns of the public with beef are generally more focused on specific disease issues (e.g. bovine spongiform encephalopathy or Mad Cow), or with the use of antibiotics, steroids or growth hormones in beef production, rather than whether the feed was produced using genetically modified crops.

However, the categories of organic beef and 'natural' beef will likely entail a demand for hay which is free of biotech traits. Grass-fed beef is another specialty category for discerning consumers, but the presence of biotech alfalfa will not be important to this market for obvious reasons. While organic beef requires the use of non-biotech feeds, conventional beef producers are not likely to be highly sensitive to the presence of biotech alfalfa.

Sheep and goat enterprises are tied to ethnic or specialty uses, and thus may be somewhat more sensitive than beef to the use of Biotech alfalfa, but the quantity of utilization by this class of livestock in the United States is very small.

### **Alfalfa for the Horse Market.**

Alfalfa and alfalfa-grass mixtures are the most important hay crop for the US horse industry (Shewmaker et al., 2005), and weed-free bright green alfalfa or grass-alfalfa mixes are highly sought by horse owners. There are probably 6-7 million horses in the

US, the biggest states being Texas and California. Rodiek (2004) estimated that there were of 650,000 horses in California. Horses are probably a significant consumer of the nation's alfalfa crop, but again, exact data on the disappearance of alfalfa into this market does not exist. It may be on the order of 5-15% in California. If each horse in California consumed 5 lbs of alfalfa/day (a flake or two) this would consume about 8% of our production each year (Rodiek, 2004), but I should emphasize that the exact dimensions of this market are not known.

Horse owners do not really consist of an 'industry' in the same way as dairy and beef producers, but is made up of thousands of individual buyers, each with their own concepts of animal nutrition. The use of professional nutritionists is rare, so scientific approaches to feeding of horses are not common. Horse owners are largely urban, not farmers in the traditional sense. Additionally, most horses are largely pets rather than income-producing enterprises. Thus, individual prejudices are much more likely to enter into hay buying decisions for the horse markets than for dairy or beef (Rodiek, 2004; Lawrence, 1998). We should also keep in mind that alfalfa is n't the only hay of choice—timothy and grass hays are also desired by this market segment.

The horse market is widely known amongst growers for its individualistic and idiosyncratic approach to purchasing hay. While some buyers prefer pure alfalfa, others insist upon grass-alfalfa mixes or pure grass hay. Color is a major factor, complaints are frequent, and this market is full of stories of diverse buyers, each of whom has their own concepts of the 'best' hay. Many horse owners are not aware of the methods used to produce hay, and may not be aware of the presence of biotech alfalfa. Due to the strong subjectivity of the horse market, it is clear that some individual horse owners will resist the feeding of RR alfalfa due to their own preferences and opinions about biotech, but this is not likely to be universal.

The converse may also be the case, since each year a sizeable number of horses are sickened or die due to the presence of poisonous weeds in hay (Pushner, 2004), which could be alleviated by RR alfalfa. There have as yet been no documented side-effects due to the consumption of biotech feeds, though biotech feeds have been consumed in large quantities for at least 5 years. This fact may favor pure, weed-free RR alfalfa compared with weedy hay for some horse owners who buy alfalfa hay. However, since the range of viewpoints on the feeding of horses is wide, there will undoubtedly be some horse owners who will reject biotech alfalfa.

### **Alfalfa for Exports.**

Some alfalfa hay is exported to foreign countries, mostly from the western states of WA, CA, OR, ID, UT, and NV (western ports account for >99% of the exports from the US). In these states, exports may be about 4.5% of production (Table 1). On a national basis, exports account for about 1% of the production. However, in certain regions export percentage is much higher, such as in central Washington, and Imperial Valley of California. The percentage of alfalfa grown for export in these areas is much higher than nationally, or even within those states. There is some dispute about the numbers. Some experts estimate exports from Washington state to be as high as 34% of the state's

production for alfalfa (T. Woodward, WSU Cooperative Extension, pers. Comm. ), and in California perhaps 1.5% of the state's production (Seth Hoyt, CDFR, pers. Comm.,2005). Coarse hays, such as sudangrass, and grass-seed straw are more important in some areas for hay exports than alfalfa.

It should also be kept in mind that, though a haygrower may export only a portion of their hay (e.g. one or two cuttings), the market acceptance (or lack of acceptance) of the export market in these regions will impact the whole acreage, since hay for export is one component of a multi-use market strategy for that grower. For example, it is estimated that over 80% of the growers in the Columbia Basin of Washington will export at least one of their harvests each year. Therefore, the acreage which might be sensitive to biotech alfalfa due to exports is larger than indicated by the production actually exported.

It is clear there will be resistance to biotech traits in alfalfa grown for export. The vast majority of exported hay goes to Japan (Woodward, 2004). Exporters have in some cases insisted on written contracts with producers stating that they will guarantee biotech-free alfalfa for the Japanese markets because most importers have asked for biotech-free alfalfa. Thus, initially, growers who wish to sell into the hay export market must be careful to keep hay for that market separate and distinct from biotech-based alfalfa varieties.

1997	1998	1999	2000	2001	2002	2003
<b>Alfalfa Production (Metric Tons, 6 Western States)</b>						
17,585,823	17,868,807	17,678,337	17,362,701	17,475,169	18,852,902	18,478,311
<b>Alfalfa Exports US (Metric Tons)</b>						
841,748	790,769	827,303	853,268	782,137	782,137	839,532
<b>Percentage of Alfalfa Production Exported (6 States total)</b>						
4.79%	4.43%	4.68%	4.91%	4.48%	4.15%	4.54%
<b>Percentage of Alfalfa Exported (US)</b>						
1.06%	0.96%	0.98%	1.06%	0.97%	1.06%	1.10%

Official approval of the Japanese government for importation of RR alfalfa is pending, and some believe should be completed by Spring, 2006. However, official approval only partly addresses the market sensitivity issue, since some importers are likely to continue to insist upon non-GE hay. The initial requirements for the commercialization of RR alfalfa in 2005 include restrictions that prohibit exportation of the hay by growers. Additionally, seed sales have been restricted in regions such as Imperial Valley and Central Washington where exports are important. The Washington State Haygrowers Association has requested a delay in the release of RR alfalfa in that state until Japanese

buyers have indicated they would accept that hay (see website). Similar requests have been made in the Imperial Valley.

### **Are Hay Markets Rational?**

Although an economist might argue that markets are truly rational reflections of supply and demand, there are numerous examples of irrationality entering into marketing of hay in terms of quality preference. Consumer preference for factors which are not supported by scientific principles are common for the horse market, the export market, and to a lesser degree for the dairy market. If one accepts the business axiom 'the customer is always right', growers must respond to these factors or risk losing their markets.

Alfalfa does not receive government subsidies, and follows free market conditions to a greater degree than most agricultural commodities. Market quality standards are highly unregulated. Although markets reward genuinely higher quality hay as judged by lab analysis (Putnam, 2004), there are numerous examples of highly subjective and non-scientific factors entering into the sales process for alfalfa hay. With horse buyers, fears about 'something in the hay' affecting health of animals, and prejudices about alfalfa hay commonly enter into negotiations or willingness to buy. With exporters, perception about 'quality' may be entirely contrary to known scientific principles of forage quality factors. A case in point is the demand for 'long seed heads' in timothy hay for export—such features are entirely contrary to known scientific attributes of forage quality (hay with few seed heads is likely to be higher in quality). In California and other locations, dairy buyers and hay sellers commonly argue over a few tenths of a point TDN, ADF, NDF or CP value, when it is abundantly clear that the process of forage testing cannot deliver this degree of precision (Putnam, 2004). Some markets will commonly gravitate toward physical factors (such as bright green color) which are poor predictors of quality from an animal feeding perspective.

This reality of subjective factors (or 'irrational' factors) impacting markets is pertinent to the biotech question. The current concern about biotech traits in crops is linked in vague ways with a whole range of concerns about food safety, from Mad Cow Disease to hormones, to pesticide residues and nitrates, as well as the 'corporatization' of the food supply and environmental impacts, even though these are all distinct issues. This is the case in Japan, but also Europe and US where these concerns are often lumped together in the same discussion. Markets may be affected in the short run by these artificially linked factors, due to the desire simply to have only 'safe' food and feed. We should also not forget the natural inclination to reject certain products due to simple protectionism and price bargaining.

### **Summary of market sensitivities for alfalfa.**

In summary, the majority of users of alfalfa are not likely to be sensitive to the presence of the Roundup Ready trait in alfalfa for either on-farm use or hay for market sale. A minority of the users of alfalfa (probably 3-5%) are likely to be sensitive to biotech alfalfa, specifically the organic sector (dairy and beef), a small portion of the horse market, and a majority of growers who participate in alfalfa hay exports.

However, it is also clear that it is important to protect these segments of the market that may be sensitive to a biotech trait. An organic grower and an export grower who wishes to maintain crops without GE traits has as much right to maintain their production methods and prevent loss of markets as do other growers who wish to try new GE traits on their farms. Thus, all growers need to be aware of the sensitivity of their specific markets to this trait, and discover methods to allow coexistence of both biotech and non-biotech traits within their region without unduly upsetting market patterns.

### **IMPORTANCE OF ALFALFA FOR CROP ROTATIONS AND ORGANIC SYSTEMS**

Some have raised objections to the release of RR alfalfa due to the potential for losing its soil benefits for organic systems (Pridham, 2004). Alfalfa ‘the queen of forage crops’ is highly valued not only for its high yield and high quality as an animal feed, but for its N<sub>2</sub> fixation, soil benefits, crop rotation, and wildlife habitat (Russelle, 2004, Putnam, 2001). While Roundup Ready alfalfa varieties have not been fully tested for their N<sub>2</sub> fixation ability, there is no reason to suspect that these RR varieties will be any different than conventional varieties in this respect. Early results on their yield potential indicate similar yields to conventional varieties, and the plants are well-nodulated and similar in quality to conventional lines (Putnam et al., 2004). Roundup-Ready varieties should provide some of the same benefits to rotations as do conventional varieties, and growers will continue to have a choice to choose RR and conventional varieties for this purpose.

It is possible that the RR trait may open up other options for rotations, overseeding, and minimum tillage practices not previously available to growers, which could reduce fuel or nitrogen costs (Doll, 2003). Proponents of organic farming have objected to the release of RR alfalfa (Pridham, 2004) due to the potential for losing its soil benefits for organic systems. However, biotech crops are prohibited in organic systems, and RR alfalfa would not be used in any event, since the only value of these varieties is to use a different herbicide—banned in organic systems in any case. The objection to GMOs on the part of organic and export-based growers is primarily based upon philosophical objections to biotechnology, or customer-based negative perceptions of the trait. The presence of a GE trait in alfalfa should have a neutral effect on the soil benefits of alfalfa, and, although it’s too early to tell, may enhance some of these properties if new tillage practices or production systems are enabled. Some weed scientists have predicted that RR alfalfa stands may last longer since weeds can be effectively controlled, but that remains to be seen.

### **WILL THE INTRODUCTION OF RR ALFALFA INCREASE HERBICIDE USE OR WATER CONTAMINATION?**

Most California growers currently use herbicides during stand establishment or for maintenance of weed-free stands. If RR alfalfa is adopted, it would likely represent the replacement of currently-used herbicides in most cases in fields planted with RR alfalfa. Therefore, it is not anticipated that the introduction of this GE crop would increase herbicide in total, but where adopted, will cause a shift of herbicide use to glyphosate. It

should be emphasized that, even where growers are not concerned about their markets, they may not adapt the technology due to cost factors or weed management factors; it should not be considered a panacea (Van Dynze et al., 2004).

Where glyphosate (Roundup) replaces other herbicides that are more highly soluble and subject to runoff, the introduction of this technology may reduce the overall impact of herbicides on the environment. Several winter-applied herbicides currently used in California have been detected in wells in the Central Valley and are of strong concern to regulators (Prichard, 2002). Growers who use these herbicides are subject to severe restrictions for use based upon soil type and groundwater characteristics. It is anticipated that glyphosate presents a lower risk to the environment compared with some of these herbicides (DPR website, State of California).

## **METHODOLOGIES FOR MAINTAINING MARKET IDENTITY**

Alfalfa hay growers who wish to sell into markets sensitive to biotech traits (such as hay for export or organic production) may be concerned with methods to ensure co-existence of these genetically diverse systems of biotech-origin and non-biotech origin alfalfa varieties. The stewardship of purity of both non-biotech and biotech traits within a region will depend upon a range of practices, beginning with seed production and purity.

### **1) Select Certified Varieties for Seed Purity and Quality**

The first step in stewardship of both biotech and biotech-free alfalfa is choice of pure seed. It is difficult to overemphasize the importance of variety and seed choice for those hay growers who are concerned with the purity of their alfalfa stands and/or who are selling into sensitive markets. This is likely to be the most crucial step in assuring trait purity in a hay product.

Alfalfa seed is produced on small acreage in western states of the Pacific Northwest, California, and a few other Western states. Seed production is an exacting enterprise, and seed growers take pride in their abilities to manage a complex system to produce high quality seed (Mueller, 1996). Industry standards for isolation have been developed, and Crop Inspection and Certification Services are available in each seed-producing state to assure seed purity which includes variety identity, seed quality, and lack of contamination with weeds or foreign matter. The incorporation of biotech traits in alfalfa seed production has been considered in detail elsewhere (Mueller, 2004).

Choice of certified seed should be combined with as the assurances, reputation, and standards of the seed company. Thus the first step in the process of stewardship is selection of certified varieties backed by a company with high standards for seed production.

### **2) Understand the potential for Gene Flow**

Adventitious Presence (AP) is the presence of an unwanted trait (such as a biotech trait) in a variety meant to be free of that trait. Alfalfa is a cross-pollinated crop that requires insect pollinators (usually honeybees or leafcutter bees) for seed production. Successful

seed production requires the transfer of pollen from one plant to another. Thus, adequate isolation of alfalfa seed fields is required to assure that very little pollen transfers from one crop to another during seed production.

The potential for gene flow in alfalfa harvested for hay is not the same as the potential for gene flow in alfalfa seed crops due to difference in production methods. However, the **upper limits** for the possibility of gene flow in hay fields can be understood by examining what happens during seed production (Mueller, 2004, Ricker et al., 1988). Studies in seed fields in Idaho, using leafcutter bees as pollinators, showed gene flow (AP) from a RR alfalfa seed crop to a non-biotech crop to be about 1.5% at 500 feet, less than 0.5% at 900 feet (274 meters), and zero at 2,000 feet (610 meters) (Fitzpatrick et al., 2002). Honeybees generally have longer flights than leafcutter bees and a study in California using honeybees as pollinators showed gene flow (AP) to be 1.5% at 500 feet, below 1% at 2500 feet (0.8 km), but sporadic gene flow (<0.03%) could be detected up to 2.5 miles away (Teuber et al., 2004; Van Dynze et al., 2004). These estimates are under conditions to maximize pollination and seed production, conditions which do not generally occur in alfalfa hay fields.

### **3) Understand the limits to alfalfa gene transfer from hay fields to neighboring hay fields**

Will the Roundup-Ready gene (or any biotech trait) be transferred from alfalfa hay field to another hay field? The short answer is: very rarely, and only with great difficulty under most conditions. Although gene transfer from alfalfa to other alfalfa hay fields is theoretically possible, there are a range of environmental barriers to such transfer, which would make significant movement from a biotech alfalfa crop to a neighboring non-biotech alfalfa field very unlikely. Additionally, there are steps that growers can take to limit traits from moving from field to field.

The potential for a trait to be successfully transferred from one alfalfa hay field to another, sufficient to cause Adventitious Presence (AP) in hay is dependent upon a series of steps (Figure 1). These steps are significantly different for hay than for seed crops. Alfalfa seed fields are managed to maximize flower production, pollen transfer, and seed maturation and seed yield. However, alfalfa forage production fields are managed to maximize biomass production of high quality forage for animal production. Seed production in hay fields in most parts of California is minimal, although some flowering does occur. The vast majority of alfalfa fields in the California are harvested with only a small amount of flowering and zero seed production.

In seed fields, for adventitious presence to be transferred from one field to another, pollen has to be transferred, resulting in seed that is harvested with the AP trait. However, for AP to occur in hay fields, this must occur, along with several additional steps—that seed must 1) remain in the field, 2) fall to the ground and germinate, and 3) that seedling must survive to become a component of the new hay crop.

Each of these steps (Figure 1) have a certain probability of occurring. While the exact

probabilities of each of these steps are not known with great certitude, an analysis of the potential for unwanted AP in hay begins with an estimation of the probability of these steps occurring in practice.

- a. **Probability of Flowering.** In order for gene transfer to occur, flowering must be simultaneous between two fields. Historically, it was recommended to harvest alfalfa at 10% bloom. However, many alfalfa fields harvested for dairy production are harvested in the bud or vegetative stages for high quality, when only occasional flowers are present. Nevertheless, we should assume that some flowering will be present at harvest of each hay crop, even if only a few flowers in a field. In most cases this will range from an occasional flower to 10% flowering, but under hot midsummer conditions, or with delayed harvests, the percentage of plants flowering tends to be higher. Flowering is particularly high during summer months, and under desert conditions, such as the Imperial Valley, with a correspondingly higher risk for cross-pollination.
- b. **Probability of Pollinators.** For pollen to flow between fields, pollinators must be present. These include honeybees (*Aphis mellifera* L.) Alkalai bees (*Nomia melanderi* Cockerell) and leafcutter bees (*Megachile rotundata* Fabricus). Alfalfa is not a preferred nectar or pollen source for many pollinators. If other flowering plants are available, these are frequently preferred, so alfalfa seed growers must place hives and manage pollination carefully in seed fields to accomplish cross pollination (Rincker et al., 1988). Conditions of plentiful and active pollinators may occur in some situations, but are not necessarily common in alfalfa hay fields.
- c. **Probability of Pollen Movement between fields.** If flowering is occurring, and pollinators are present, the probability that pollen will move from one field to another must be considered. Pollen movement is NOT the same as gene transfer (Mueller, 2004), which requires the additional steps of fertilization and seed production (e and f). Most pollen movement occurs within fields given the close proximity of neighboring plants to the pollinators, and transfer from field to field is a small percentage of pollen movement. The most effective pollination is accomplished within 90 meter radius of a hive (Rincker et al., 1988). The probability of pollen movement will depend upon factors such as wind direction, pollinator activity, and feral alfalfa which may act as a 'bridge' between fields (Figure 1). Control of feral alfalfa around RR fields may be an important step to control the unwanted movement of pollen around these fields. Since the vast majority of pollen movement is within fields, only some fraction of pollen movement would have the potential to cause gene movement from field to field.
- d. **Neighboring Field must 'nick' with the first field.** The probability of pollen flow depends upon two fields flowering substantially at the same time. Cutting schedule, variety, and other factors may influence the 'nicking' or convergence of flowering time between two fields. Since pollen has a very short life (hours or days), two fields must flower at roughly the same time to allow gene transfer.

- e. **Probability of Pollination, Fertilization of flower.** Once pollinators are determined to have transferred pollen from one field to another, they must land on and trip flowers to deliver the pollen grains. Alfalfa requires floret tripping by insects, delivery of pollen from another plant, the pollen must embed on the stigma, grow a pollen tube through the style, resulting in fertilization of ovule (Viands et al., 1988). This process takes 24-32 hours. The fertilization of the ovule would result in gene transfer, but not all pollen transferred results in gene transfer, due to failure to fertilize the ovule or death of pollen cells.
- f. **Probability of Seed Maturation and Production.** For gene transfer to be accomplished in hay crops, several additional steps are required after pollen flow. Fertilized embryos must then mature into seed in order for gene transfer to have an impact upon the reproduction of the plant. Seed maturation generally occurs several months after mid bloom flowering, which occurs about 35-50 days after regrowth or emergence (Rickner, et al., 1988). Since forage harvests generally occur 28-32 days after regrowth in most areas, seed maturation from cross-pollinated flowers is rare, and not a normal consequence of forage production. However, seed production can occur on a very small number of plants under the following conditions: occasional unharvested stems which are allowed to continue growing past harvest in a field, very late harvested hay, and hay grown under intense heat conditions, such as the Imperial Valley of California. Flowering and seed maturation is more rapid under hot and droughty conditions than under well watered conditions. However full seed maturation in alfalfa hay fields should be considered unusual in hay production, and occurs infrequently, and only under specific conditions.
- g. **Probability that Seed Falls to the Ground and Germinates.** Seed production itself is not sufficient to accomplish gene transfer from forage crop to forage crop. Most of the occasional seeds produced in hay fields are removed at harvest. However, for AP seed to produce 'adventitious presence' in a neighboring hay crop, it must fall to the ground, germinate and produce a plant. With the normal practice of harvesting every 28 days, all above-ground plant parts to about 2" height are generally removed. This usually results in the removal of whatever flowers and seeds are produced—and the product is fed to livestock. However, the probability that the small amount seed that remains might germinate must be considered. This seed must have sufficient moisture and coverage on the soil surface to be able to germinate in an already-established alfalfa stand. Growers in many areas have tried deliberately overseeding alfalfa into existing alfalfa stands using grain drills, a practice that is not widely recommended (Canevari et al., 2001). Usually it requires some tillage (harrowing) in the existing stand to provide the seeds with a favorable environment for germination. However, even with tillage more often than not, the result is failure. Surface-seeding onto unprepared ground in existing alfalfa stands is even less likely to succeed. Growers who have tried this have often been frustrated by lack of germination due to competition, poor seedbed, and allelopathy of the existing plants in the stand.

Given the general lack of success in deliberately seeding alfalfa into existing stands, it is not likely that the few AP seeds falling on the hard soil surface would result in plants.

- h. **Probability of Survival of New AP Recruits.** A germinating seedling in an existing alfalfa stand must then survive the intense competition from neighboring alfalfa plants and weeds sufficient to become established. In order for AP presence to be detected, it must contribute to the forage yield of the crop. Alfalfa is a very weak seedling for several months of its early growth, and does not survive well under intense competition from either weeds or existing alfalfa plants. Growers who have attempted to ‘overseed’ alfalfa into existing alfalfa stands have frequently been disappointed at not only germination, but the survival of the plants that do somehow manage to germinate and grow (Canevari et al., 2001).

The combination of frequent harvests, lack of significant flowering, lack of seed production, poor soil conditions for germinating seedlings, the highly competitive and allelopathic nature of alfalfa which prevents ready germination of alfalfa seeds in existing fields would prevent virtually all gene transfer from occurring between alfalfa hay fields.

**Estimating the limits to gene flow in alfalfa hay.** In alfalfa **seed** fields the gene flow is estimated at approximately 1.5% at 500-900 feet for both honeybees and leafcutter bees according to field studies. This is in situations where flowering is encouraged, pollinators are placed, and seed maturation is accomplished (Teuber et al., 2004; Van Dynze et al., 2004). This may be considered an ‘upper limit’ for alfalfa hay fields, to which the ‘environmental filters’ listed above should be applied. Thus, the probability of gene movement in hay fields would be reduced significantly from this number if little simultaneous flowering occurred, pollinators were few, the crop is harvested before significant flowering, seeds are removed during harvest, and the conditions for germination and survival of the few volunteer seedlings are not favorable. Although these probabilities are not known with precision, it is clear that, the probabilities for gene transfer in hay fields under normal conditions would be very small, likely far less than 0.01% AP from this source under most conditions. The potential may be higher in the very hot regions, such as the Imperial Valley, where alfalfa flowers profusely at 28 days, or in situations where alfalfa is harvested very late. However, the highest probability of AP presence in hay fields is likely to originate from AP presence in seed, not transfer from field to field. Thus, excess care should be taken in purchase of certified seed for those growers interested in sensitive markets.

#### **4) Control Feral Alfalfa**

Feral (wild) alfalfa is alfalfa that was not intentionally planted and typically occurs along ditch banks or roadsides and remains unharvested. Feral alfalfa is common feature of agricultural regions where alfalfa is grown. Its origin is not known, but could be from older plantings by highway departments, spilled seed, small amounts of seed from hay, or movement by birds. Since feral alfalfa is more likely to flower and set seed, and feral alfalfa may act as a ‘bridge’ for pollinators between distant fields, control of feral alfalfa is a prudent method to prevent movement of genes between hay fields.

### **5) Identification of Biotech-free Alfalfa Hay**

The coexistence of biotech and non-biotech alfalfa will require a higher level of awareness of crop identity for products destined for sensitive markets. This may require some simple identification steps for hay lots to assure that hay lots are not mixed, but also careful filing of records of during stand establishment (seed tags) that indicates that conventional varieties were sown. This is currently practiced on commercial hay farms anyway—but the presence of biotech alfalfa will increase the need for crop identity for those sensitive markets.

The introduction of the first biotech trait in alfalfa may actually sharpen or increase the demand for biotech-free hay, perhaps even leading to greater market identification and value for organic or biotech-free hay. This remains to be seen, but there may be incentives for this greater identification to the grower. Organic growers have a process by which they identify and document organic hay anyway, and so this does not entail any greater paperwork on their part, other than to specify that the alfalfa is free of biotech traits. Larger exporters generally have methods of identification, and since contracts frequently require biotech-free crops for export, this will likely become a component of market identification. Test kits (discussed below) should enable further confirmation of lots and identification of fields as to their origin.

### **6) Prevent Mixing of Hay Lots.**

A ‘lot’ for forage quality testing purposes is defined as a stack from the same field, same cutting, less than 200 tons, with identification as to farm and field and cutting (Putnam, 2002). There is no reason not to utilize this definition for biotech or ‘biotech free’ identification.

It is common sense that those wishing to sell into sensitive markets must take steps to prevent the mixing of haystacks, to maintain identity, and to assure customers of that identity through the harvesting, storage and transport process. The use of colored baling twine may be helpful in assisting growers in market identification, but growers may also want to develop their own methods of preserving identification.

### **7) Understand the sensitivities and tolerances of the Market**

Given that the food crops in Japan require labeling if over 5% GE presence in **food crops** (over 0.9% in Europe), these very small probabilities (e.g. less than 0.01%) of Adventitious Presence in hay (**feed crops**) should not present difficulties for buyers in terms of market identity. Europe has adapted a tolerance of 0.5% for AP in foods. Logically, the tolerance for feed crops should not be greater than for food crops. According to USDA, unintentional presence of biotech traits will not result in loss of organic status. For organic producers, the very small potential estimated above for AP in alfalfa hay should not ordinarily present significant worries for their markets for feed crops. This level of risk of AP originating from neighboring hay crops is likely to be on the same order of magnitude to the risk of using any non-organic practice (spraying, fertilizing) in non-organic fields which are near organic fields. In both cases, some caution should be used to prevent influence between neighboring fields, but organic

growers have coexisted with non-organic growers for decades, and methods have been developed to prevent unwanted influence of non-organic practices on organic fields or visa-versa (keep in mind that there is also risk from organic practices, if weeds are not controlled in neighboring fields).

There is no reason to think that coexistence of GE crops in regions where non-GE crops are desired cannot be successfully managed under most situations. Exceptions to this may be situations where seed production is occurring in close proximity to hay production, and flowering and seed production is early and prolific due to excessive heat, such as the Imperial Valley of California. Growers should be aware of the conditions which facilitate gene movement (abundant flowering, seed production, good germination conditions), and take steps to limit the possibility of gene flow and market identification to prevent mixing of seed or hay products..

### **8) Testing for Adventitious Presence in hay.**

Testing of hay lots is the final step to confirm that hay lots either contain, or do not contain, biotech traits. Procedures to measure the presence of AP in shipments of grain, for the purposes of market identification of biotech traits in crops have been developed, and now are routine for corn, soybean, and other crops. 'Test kits' have been developed by several companies for grain handlers, marketers and farmers to manage GMO traits in markets (e.g. Envirologix Co. <http://www.envirologix.com/> and Strategic Diagnostics (<http://www.sdix.com/>), and are now (November, 2005) available for alfalfa hay and fresh leaves.

These simple tests (Figure 2) can be performed in a lab during normal lab testing for quality, in a clean room, or even in the field. These are commercially available at this point in time for measuring AP presence in hay. These test strips detect the presence of the protein marker produced by the insertion of the Roundup-resistance gene. Fresh leaves, unground hay, and ground hay tests (for testing of routine cored hay samples by commercial laboratories) should be available. These are currently under testing by UC and others. These tests should enable growers to confirm the lack of presence of biotech traits in their hays destined for sensitive markets, and provide assurances to buyers as to the stewardship of biotech-free hay for organic, export, or other markets. The recommendations for use of these test strips, sampling and analysis procedures should likely be completed by early 2006.

Those testing for any substance, such as an unwanted gene product, in a food product must wrestle with the issue of tolerance. There are analytical tolerances (limits of detection), and practical tolerances (due to the possibility of contamination of samples in grinders, the efficiency with which the protein is extracted from samples, and other technical factors), and commercial tolerances (what is accepted by markets). Given that the threshold for tolerance of a GMO trait in food products and grain is approximately 5%, it is not likely that tolerances for feed products to be fed to animals will be higher than this, but different markets may have differing views. A tolerance of zero is impractical for testing of hay production systems, since just dust on the fingers of the

sampler, tiny flecks in a grinder, or just analytical variation could result in a false positive reading for otherwise biotech-free alfalfa hay.

## SUMMARY

The majority of markets for alfalfa are not likely to be highly sensitive to the presence of a biotech trait, with the notable exception of organic producers, export producers, and some horse owners. Both organic and export markets will demand biotech-free alfalfa, at least currently. I estimate the percentage of alfalfa production sensitive to the presence of a biotech trait to be less than 3-5%, given current utilization patterns, although that may change as the trait is introduced. Methods to enable the coexistence of biotech and biotech-free alfalfa production and marketing in regions where both types will be grown are necessary. The possibilities of genetic transfer between biotech and non-biotech alfalfa hay fields are very small—orders of magnitude smaller than the possibility of genetic transfer between seed fields. Environmental ‘filters’ which prevent significant cross-pollination, seed production, seed germination, and survival of volunteer plants in hay fields makes gene transfer highly improbable. Hay fields are managed to minimize flowering, seed production and volunteer plants whereas the opposite is true for seed-producing crops. Exceptions to this may include very late harvested hay, and excessive flowering due to conditions of excessive heat such as the Imperial Valley of California. Growers can lower the possibility of adventitious presence of a biotech trait even further by selecting certified seed from a reputable company, harvesting before significant bloom, and being especially aware of conditions that enhance flowering (heat, drought, late cutting) and preventing seedling recruitment and survival. Control of feral alfalfa in areas near GE fields may improve the containment of pollen movement. Practices which enable preservation of identity of crop origin through careful hay lot identification and management, along with testing of hay lots will improve confidence of buyers and sellers of non-biotech alfalfa into sensitive markets. If growers follow good management procedures, biotech and non-biotech alfalfa hay crops should be able to coexist in the same region or on same farm while maintaining trait purity and identity for use in markets sensitive to biotech traits.

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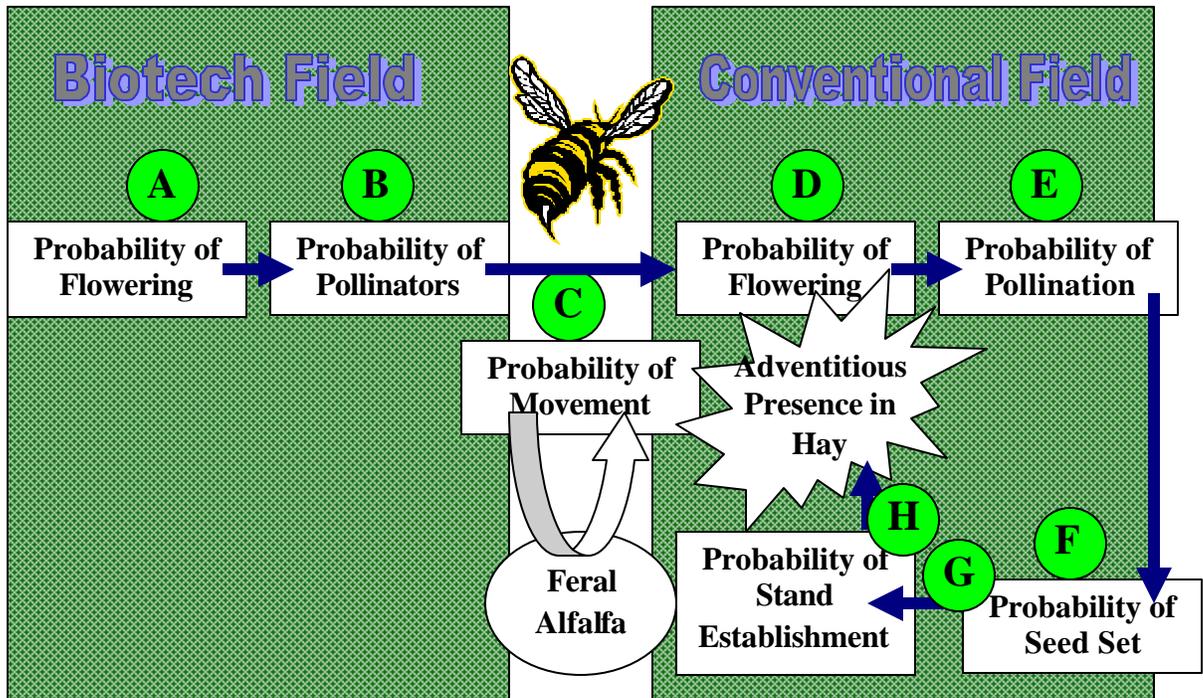
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**Figure 1.** Steps necessary for gene flow to occur between biotech and conventional alfalfa forage production fields sufficient to cause adventitious presence (AP) in hay.

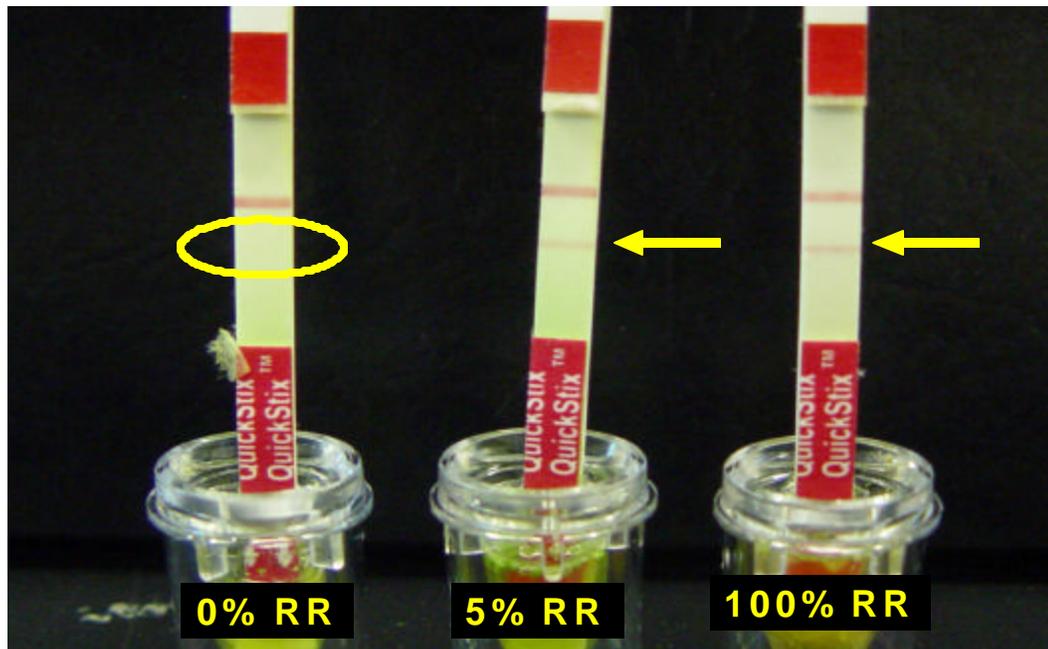


Figure 2. Test strips have the potential to detect adventitious presence (AP) of Roundup-Ready (RR) alfalfa in hay and could assist in confirming non-biotech hay for sensitive markets. Arrows show positive reading for 100% RR and 5% RR samples (right), and circle shows negative reading for control sample (left). Test strips are currently available commercially for alfalfa hay from Enviroligix (Portland, ME, [www.enviroligix.com](http://www.enviroligix.com)) and Strategic Diagnostics Inc. (Newark, DE, [www.sdix.com](http://www.sdix.com)).