Management Guidelines:

Coexistence and Market Assurance for Production of Non-Genetically Engineered (Non-GE) Alfalfa Hay and Forage in a Biotech Era

Daniel H. Putnam, Tim Woodward, Peter Reisen, Steve Orloff[1]

*(in press-**Crop, Forage and Turfgrass Management, 2016,** peer-reviewed)*

ABSTRACT

The introduction of Genetically-Engineered (GE) alfalfa requires a mechanism for producers to successfully grow and market alfalfa hay destined for GE-sensitive markets such as organic and export. A process of coexistence includes elements of respect for diverse agricultural systems, improved communication, scientific knowledge, and market clarity. A definition for 'Non-GE Alfalfa Forage' is proposed, along with suggested production protocols. These protocols include securing non GE-detect seed, steps to reduce the probability of gene flow in hay fields, equipment sanitation, hay lot identification, and hay testing for low-level presence. The largest risk for low-level presence in hay is likely to originate from unwanted GE presence in the planting seed. Secondary risks include accidental mixing of hays during harvest or storage, followed by gene flow between forage fields. The tolerance for low-level presence in non-GE hay must meet specific market sensitivities. Promoting absolute zero GE hay (e.g. 'GMO Free) is a practical and analytical impossibility, creates difficulties for farmers, and makes no sense for a non-toxic unwanted market factor. Regulatory-based tolerances, driven largely by countries that do not permit a GE trait may require non-GE determination to a limit of detection of approximately 0.1%. Market-based tolerance thresholds may differ greatly depending upon the sensitivity of markets. For market purposes, a definition of non-GE alfalfa as having a low-level presence of less than 0.9% of dry matter is suggested. Coexistence strategies for alfalfa forage require an understanding of the sources of low-level presence, market tolerances of diverse markets, and market assurance processes.

INTRODUCTION

Genetically-engineered (GE) traits in crop plants have been commercialized across many crops including corn (*Zea mays* L), cotton (*Gossypium hirsutum* L.), canola (*Brassica napus* L.), soybean (*Glycine max (L.) Merr.*), and sugarbeet (*Beta vulgaris* L.) with very high rates of adoption in North America. The predominant traits are the glyphosate-

[1] D.H. Putnam, Extension Forage Specialist, Department of Plant Sciences MS #1, One Shields Ave., University of California, Davis, CA 95616. Email: dhputnam@ucdavis.edu ; T. Woodward, Professor, Columbia Basin College, Pasco, WA (wwoodward@columbiabasin.edu) ; P. Reisen, Forage Genetics International, Nampa, ID (preisen@foragegenetics.com), S. Orloff, UCCE, Siskiyou County, Yreka, CA (sborloff@ucanr.edu)

tolerant trait (or so-called Roundup-Ready™ (RR) trait)[2], which enables broadcast applications of glyphosate for weed control, and the Bt trait, which confers protection from lepidopterous insect pests.

However, not all growers wish to adopt GE crops due to their personal preference or the preference of the markets for their crop. Organic growers are required to use non-GE crops. Additionally, export markets may prefer or require non-GE crop products either due to regulatory barriers or market preference. There may also be other markets (e.g. some retail markets) that demand non-GE hay products. Additionally, some growers object to GE crops out of concern about the technology or philosophical opposition to genetic engineering.

It should be pointed out that the vast majority of the markets for alfalfa hay and forage in the US are not sensitive to GE presence, since those markets are dominated by dairy and beef, both of which have widely adopted GE crops as feedstuff (Van Eenennaam and Young, 2014). Research has shown the RR trait in feeds to be safe for animal production. Currently over 9 billion animals are fed GE crops annually, accounting for 70 - 90% of the consumption of GE-crop biomass (Flachowsky et al, 2005; Combs and Hartnell, 2008; Van Eenennaam and Young, 2014).

Nevertheless, there exists a diversity of views about how to farm, as well as a diversity of market sensitivities to the presence of GE crops in farm products. To support growers' choice requires a commitment to coexistence between GE-adapting and GE-rejecting growers, which may require technical understanding as well as steps to promote cooperation. Grower organizations have promoted the concept of 'coexistence' to protect the rights of farmers to continue to farm in the manner of their choosing, or to successfully meet market demands (USDA, 2012; Putnam et al., 2012; McCaslin and Van Deynze, 2012; Putnam, 2014, see NAFA website). Additionally, mechanisms that encourage communication and coexistence may be needed as farmers adjust to these technologies (Christiansen, 2011).

DEFINING COEXISTENCE

A recent USDA committee defined coexistence as *'the concurrent cultivation of conventional, organic, Identity Preserved (IP), and Genetically Engineered (GE) crops consistent with underlying consumer preferences and farmer choices'* (USDA Task Force, 2012).

Further, we define successful coexistence as *'The ability of diverse systems (GE, organic, non-GE) to thrive without undue influence of neighbors or resorting to extraordinary protection measures."*

In line with this coexistence goal, the objective of this paper is to provide guidelines and coping strategies so that growers can continue to successfully produce non-GE alfalfa forage in an environment where neighbors may be adopting GE-crops. Specifically, the purpose is 1) to offer a definition for "Non-GE Alfalfa Forage", 2) to describe and discuss the market factors which may determine tolerance of low-level presence in non-GE alfalfa, 4) to describe the primary risks of low-level presence in alfalfa hay, 5) to describe a protocol for production of Non-GE alfalfa that would reduce

[2] Mention of a product does not imply endorsement of that product nor disparagement of similar products which may serve the same function.

the risk of low-level presence to satisfy markets, 6) to discuss the proper use of sampling and testing to determine the GE status of hay. Although Roundup-Ready alfalfa and reduced-lignin alfalfa are currently the only GE traits approved in this crop, this discussion may apply to all GE traits that may be commercialized in the future.

ADVENT OF GENETICALLY ENGINEERED ALFALFA

Alfalfa is the fourth-ranked crop in economic value in the USA (USDA-NASS), behind corn, soybean, and wheat, at 16.7 to 22.4 million acres and $7.7 to $11 billion in value annually (2009-2013, USDA-NASS). Alfalfa is the most important forage crop for dairy farming in the US, an enterprise worth over $40 billion in 2014 (NASS).

The RR trait was developed by inserting an *Agrobacterium*-derived gene for the CP4 EPSPS protein, so that EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) enzyme necessary for plant growth is not injured by the herbicide glyphosate. This was the first GE trait commercialized in alfalfa (*Medicago sativa* L.), first deregulated and approved for commercial use in 2005 (USDA-APHIS, 2005). Plantings of Roundup-Ready alfalfa were subsequently halted in 2007 due to a lawsuit when a federal judge ruled that USDA-APHIS must complete an Environmental Impact Study (EIS). After completion of the EIS the trait was once again deregulated in 2011 after USDA-APHIS concluded that RR alfalfa “will not have a different impact on the physical and biological environment than conventional alfalfa” (USDA-APHIS, 2011). A more complete description of the process of adoption of RR alfalfa and a discussion of the advantages and disadvantages of the trait can be found in Putnam & Orloff, 2013 and Van Dynze et al., 2004.

In November 2014 a second GE trait in alfalfa, the ‘HarvXtra’ trait with reduced lignin, was de-regulated by USDA-APHIS with a FONSI (Finding of No Significant Impact--USDA-APHIS, 2014). Thus, there are now two GE traits that are commercialized in alfalfa.

It is not known whether GE traits in alfalfa will come to dominate planted acreage as they have with other crops. GE crops occupied 93%, 94% and 96% of corn, soybean, and cotton acreage, respectively, in 2014 (USDA-ERS, 2015). Since 2011, there has been significant adoption (e.g. over 50%) of RR alfalfa in some regions but little in others, but adoption is necessarily slow in this crop due to its perennial nature. New plantings to replace existing alfalfa fields typically occur every 3-4 years, and in some regions every 5-8 years or longer, so maximum adoption rates are likely to be between about 12 and 20% per year. It is estimated that adoption by growers nationwide may have been in the 30% range in 2014, with major differences between eastern and western states (source: communications from seed companies, Cooperative Extension sources).

Concerns of GE-sensitive Growers. Whatever the eventual level of adoption of GE traits in alfalfa is, the presence of a commercially available GE trait raises concerns by those who grow alfalfa for GE-sensitive markets. Specifically, there are concerns about the potential for gene flow from bee-mediated pollen transfer from field to field, or other sources of unwanted low-level presence of a gene which would serve to negatively affect organic or ‘GE-sensitive’ alfalfa fields or hay lots–potentially with loss of certification or other market consequences.

GE-Sensitive Markets. 'GE-sensitive' markets are those markets that specifically reject genetically engineered crops. Farmers in these markets may be harmed through rejection of their product if that product contains GE material or has too-high a level of unwanted presence of the gene to be tolerated by that market. This does not include those who simply choose not to grow GE crops. There are many farmers who do not grow RR (GE) alfalfa because they grow crop mixtures where it has less utility, they do not use herbicides, or they have effective weed control programs without it. These would be described as conventional growers for non-sensitive markets.

DEFINING NON-GE ALFALFA FORAGE

For the purposes of market assurance, a Non-GE alfalfa forage can be defined as:

> *"Alfalfa or Alfalfa-Grass Mixtures that have been produced implementing **Recommendations for Non-GE Management Practices** and the hay has been determined to be 'Non-Detect' using an appropriate sensitivity threshold and level of detection for a specific market."*

The ***Recommendations for Non-GE Management Practices*** are provide below.

Most conventional markets do not require defining 'non-GE status' since those markets are not considered sensitive to the trait. However, a definition of 'Non-GE Alfalfa Hay" is likely to be of use to those producing for sensitive markets.

A Process-based Approach. The management practices suggested here are a 'process-based' approach similar to that taken for other purposes, such as certified organic production or certified seed production. Typically, this will be hay described as having a low-level presence less than an amount described for specific market purposes. Note that this definition does not include the concept of 'GE-Free' hay.

The Impossibility of a GE-Free Designation. Some governments have not approved specific GE traits, and in so doing, have intended a zero tolerance for the importation of that trait in an agricultural product. Likewise, some buyers or consumers wish to have 'GE-Free' crops. However, to analytically and practically declare an agricultural product as 'GE-Free', containing none of a GE trait, is a technical and practical impossibility. In order to be 100% assured that a hay mass is 'GE-Free', every last gram of that mass must be tested, leaving none for its intended use.

For example a single stem or a few pounds in a 200 ton hay crop (consisting of 400,000 lbs and billions of stems) would constitute 'low-level presence' in a technical sense–but it's highly unlikely that any sampling or analytical method would detect this amount, since this level of low-level presence is likely to be much lower than the capability of any sampling or detection method to detect it. There are sampling, sample handling, and analytical protocols that determine the limits of detection for small amounts of a trait.

Non-GE Defined within a Production Protocol and Limits of Testing. Thus, declaration of 'Non-GE' status is made within a definition of the threshold of tolerance, implementation of a protocol to prevent unwanted gene presence, and recognition of the analytical limits of detection and the sampling method. A declaration of 'Non-GE' status hay may include other stewardship methods, such as care in labeling, management of inventory, and steps to prevent contamination in the field or during handling. Recognition of the role of testing and sampling variation must also be considered.

DETERMINING LOW-LEVEL PRESENCE TOLERANCES FOR NON-GE HAY

The primary GE-sensitive markets for alfalfa hay are export (Figure 1) and organic (Figure 2) which have grown to approximately 3.5% and 1.5%, respectively, of US alfalfa production in recent years. These markets differ significantly in their sensitivity to GE traits, since some export markets do accept GE alfalfa. Organic markets do not. Some sensitivity to the GE trait (preference for non-GE) may also come from horse hay producers or other markets. The total extent of GE-sensitive markets for alfalfa in the US is unknown but was estimated by Putnam (2006) at 3-5% of hay acreage. The importance of producing non-GE hay may be especially important in high export states such as Washington and California.

In 2014 China began using DNA-based PCR testing to detect presence of the RR trait in conventional alfalfa exported to China. U.S. exporters had been using less sensitive protein-based ELISA test strips for GE detection. China's new testing regime detected the RR trait in hay that was GE non-detect using the ELISA test. In late 2014 there was significant rejection of alfalfa hay shipments to China due to low-level presence of the RR alfalfa gene, resulting in millions of dollars of losses to exporters and reported overall loss of market strength (White, 2014). Alfalfa hay exporters in the U.S. are now using third party testing labs for detection of low-level presence using the PCR method. However, despite these problems, alfalfa exports to China increased by 22% in 2014 from 2013, and increased again in 2015 (Putnam et al, 2015). These events served to emphasize the importance of producing and assuring non-GE alfalfa for sensitive markets.

GE Sensitivity varies by market. It's important to emphasize that the low-level presence tolerance thresholds may range from non-detect by laboratory analysis to simply written or oral assurances of non-GE status. There are two basic types of threshold tolerance in practice: regulatory and market-based.

Regulatory Tolerances. Requirements originate when a GE trait is not approved in a country, and thus a trait cannot be imported or grown. Essentially this is a zero tolerance, but it is circumscribed by the limits to testing. The requirements for non-GE assurances differ by country, but could be enforced via testing requirements or written statements of non-GE status depending on the degree of acceptance. In some countries, any detection is considered to be cause for rejection – thus testing by PCR, with a Limit of Detection (LOD) of approximately 0.1% may be required, such as currently the case with hay and seed exports to China. This does not pertain to US markets, or to Japan, Korea, or Taiwan, where the RR alfalfa trait is approved, but there are still market sensitivities. Mideast export markets generally do not allow GE hay, but generally do not have a regulatory infrastructure or a defined threshold of tolerance.

Market Tolerances. Market-based thresholds of tolerance of low-level presence are based solely upon customer preference, which includes organic certification. US organic markets must adhere to a process-based market (not regulatory) requirement for organic certification, which requires non-GE hay for organic animal production. However, there is no defined threshold for low-level presence for organic; tolerance levels of 0.9 - 1.5% have been proposed. For human food markets, GE presence (of approved traits) up to 0.9% is tolerated for food products imported into the EU, and above that amount, the product must be labelled as containing a GE product. Non-approved traits fall under the regulatory threshold (above) not a market threshold. There are currently no uniform labeling requirements for food products in the US, although this may change (see CAST, 2014). In Japan food must be labeled if over 5% of it contains a GE trait.

In terms of markets, the degree of sensitivity of a small amount of unwanted low-level presence is likely to differ between individual buyers. In the case of exports, some growers must sign contracts assuring non-GE status of hay crops, and organic buyers must source organic hay, part of which is defined by the non-GE status of the crop. However, in neither case is a tolerance threshold fully described. Some exports of RR alfalfa have occurred in the last 3 years to Japan and Korea. Additionally, for some markets, the degree of sensitivity may change over time, for example if China approves the RR alfalfa trait, sensitivity to GE alfalfa in that country will likely diminish or change from regulatory to a market-based sensitivity.

Market Tolerances to low-level presence cannot be derived scientifically. A tolerance standard based upon animal or human health considerations for low-level presence of the RR gene in alfalfa is not possible. This is because peer-reviewed research has confirmed the safety of the current traits contained in alfalfa (USDA-APHIS, 2011; van Eenennaam, 2014, USDA-APHIS, 2014). Therefore, standards can be determined only by market factors, unlike some contaminant factors in hays, such as toxic weeds or nitrates (Puschner, 2009), which may have negative effects on livestock health at a given concentration, and for which a level of safety can be derived from toxicity studies.

The market tolerance threshold for a GE crop is similar to other unwanted market-determined contaminants in agricultural products, such as a small amount of barley present in a wheat crop, or the amount of forage grass allowed in an otherwise pure alfalfa hay product. Zero tolerances are rare in agricultural products, even for toxic factors, and they are not practical for non-toxic factors. Market threshold tolerances for unwanted product 'contaminants' are determined by industry habit, the degree of impact on product utilization, and a practical understanding of agricultural practices. This includes low-level presence of an otherwise safe gene product (e.g. RR alfalfa) in a hay product, which is produced for a GE-sensitive market.

Proposed Tolerances. Thus, we propose low-level presence tolerances as defined and described in Table 1. Tolerance levels for non-approved traits in a country (regulatory tolerance levels) are based upon practical limits of detection and sampling utilizing PCR technology. Proposed tolerance levels for marketing purposes are based upon the current tolerances of the most sensitive markets (European market for food products). Additionally, for market tolerances, successful production of seed below low-level presence of 0.9% appears to be practical, and this is likely to be the most important source of low-level presence in hay production.

Detection of the Low Lignin GE trait. Table 1 shows that for the RR trait, either protein-based test strips or PCR can be used for detection, depending on the desired low-level presence tolerance level. HarvXtra is a GE trait that relies on suppression of one of the genes in the lignin biosynthetic pathway, thus there is no novel GE protein produced, and only genetic-based PCR methods can be used for detection. Forage Genetics International, the developer of the low lignin trait, has indicated that the HarvXtra trait will be sold combined with RR trait in all HarvXtra varieties (a stacked trait), with trait purity of about 90% of both traits. In bags of commercial HarvXtra seed there will be about 10% of the seed that contain the HarvExtra trait but not the RR trait, but this is not the case in hay fields where glyphosate sprays during production will eliminate those nulls. Thus detection of the RR trait using PCR or Elisa strips at low levels in stacked HarvExtra/RR hay fields should predict the level of GE presence accurately. However, if detection of the HarvExtra trait is desired, PCR techniques must be used at any level.

SOURCES OF UNWANTED GENE PRESENCE IN ALFALFA HAY

There are four potential sources of an unwanted presence of a GE trait in alfalfa hay. Each of these sources of low-level presence represents different levels of risk.

I. Risk of low-level presence in the Seed Source.

The most important source of low-level presence is likely to be the planting of seed that already has some level of the gene present. Seed companies have reported that they produce zero low-level presence in most conventional seed, but do have low-level presence in some seed lots, generally <0.5%, which is sufficient for most non-sensitive, and market-based requirements. However, these levels are less likely to satisfy regulatory-based tolerance thresholds. Low-level presence of GE traits in conventional seed may trace to the pollination period if the conventional seed crop was grown in proximity to a RR alfalfa field, or it could have occurred during harvesting, seed cleaning and processing. Alfalfa is an obligate outcrossing plant, and insect-mediated cross-pollination (and gene flow) is necessary for full seed production (van Dynze et al., 2008). Unless there is selection pressure, which shifts the genetic makeup in hay fields (not likely), whatever low-level presence exists in seed will be expressed in the hay crop. Thus the most important first step in assuring non-GE hay is the selection of non-detect seed at planting.

II. Risk of Equipment Movement Between Fields

Since balers move from field to field, partial bales or foliage may be present in the baler and it is sometimes combined with forage from other fields. If GE alfalfa is present in balers or carried by swathers or rakes, this is another source of movement into conventional GE-sensitive fields.

III. Risk of Misidentification after Harvest

After harvest, if inventories are not managed carefully, bales, partial stacks, or entire stacks can be mistakenly included in non-GE alfalfa hay lots, resulting in unwanted gene presence.

IV. Risk of Gene Flow in Hay

Lastly, gene flow between neighboring fields is a risk of low-level presence in otherwise non-GE alfalfa forage. While much has been made of this source of low-level presence, in practice, it may be the lowest source of low-level presence.

UNDERSTANDING AND MINIMIZING RISKS OF GENE FLOW IN HAY

Gene flow in alfalfa as it relates to biotech traits has been thoroughly reviewed elsewhere (Van Dynze et al., 2008). To date, we are not aware of any field research quantifying the potential for gene flow between alfalfa hay fields. However, the **upper limits** for the possibility of gene flow in hay fields can be understood by examining evidence from seed experiments. A field experiment was conducted on the UC Davis campus in 2008-2010 to examine hay-to-seed gene flow risk. This study was conducted with four replicate 'receptor' seed fields (conventional), and a single GE 'source' field (100% RR) managed as a hay crop. Total sizes of the source fields were equal to the receptor fields, and the receptor seed fields were oriented in four blocks in an 'X' pattern to account for wind effects for movement of pollinators. Receptor fields began at 165 feet from the source field, and were 30' wide and 400 feet long. Active pollinators (honeybees) were placed close to the middle of the trial, and the source hay field was allowed to reach 50% bloom before each hay harvest. There were 5 hay harvests per year. The receptor seed fields were brought to seed maturity, and seed harvested at regular intervals to detect the effect of distance on gene movement.

Upper Limits of Gene Flow in Hay. Gene transfer between these closely spaced plots were in the range of 0.15% to 0.25% at the closest distance, and decayed exponentially from that point to near zero levels at 300-600 feet (90-200 m, Figure 3). These numbers are approximately the same or greater than the levels of low-level presence reported by seed companies between commercial hay fields and commercial seed production fields, where size of field may impact percentages (alfalfa seed companies, pers. Comm.).

This research quantifies gene flow from hay to seed fields, but there is a range of environmental barriers to such transfer between neighboring hay fields. These barriers would make significant movement from a biotech crop to a neighboring non-biotech alfalfa field much less likely than that in Figure 2.

Determining Probability of Hay-Hay Gene Flow. The potential for a trait to be transferred from one alfalfa hay field to another, sufficient to cause detectable low-level presence in hay is dependent upon a series of steps, each of which has a probability of occurring (Figure 4). While the exact probability of each step is not known with great certitude, an analysis of the potential for unwanted low-level presence in hay begins with an estimation of the probability of these steps occurring in practice.

- **Probability of Simultaneous Flowering.** In order for gene transfer to occur, flowering must be simultaneous between two fields (Figure 4, A and D). Most alfalfa fields harvested for dairy production are harvested in the bud or vegetative stages before any appreciable bloom has occurred to attain high quality, but depending upon the intended market, some fields may have 10% or greater flowering. Since pollen has a very short life (hours or days), two fields must flower at roughly the same time to allow gene transfer. Flowering is greater under hot midsummer conditions, and with delayed harvests.

- **Probability of Pollinators.** For pollen to flow between fields, pollinators must be present and active. These include honeybees (*Aphis mellifera* L.) Alkalai bees (*Nomia melanderi* Cockerell) and leafcutter bees (*Megachile rotundata* Frabricus). Alfalfa is not a preferred nectar or pollen source for pollinators, and other flowering plants are usually preferred, so alfalfa seed growers must place hives and manage pollination carefully in seed fields to accomplish cross pollination (Rincker et al., 1988). Pollinators are typically not as plentiful in hay fields as in seed fields, since bee hives are deliberately placed near seed fields, not hay fields.
- **Distance-Probability of Pollen Movement.** The probability that pollen will move from one field to another is primarily a function of distance (Figure 3, Van Dyneze et al., 2008). Pollen movement is NOT the same as gene transfer (Mueller, 2004), which requires the additional steps of fertilization and seed production (E and F, Figure 4). Most pollen movement occurs within fields given the close proximity of neighboring plants to the pollinators. The most effective pollination is accomplished within a 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 4).
- **Probability of Fertilization.** Once pollinators 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, and fertilize the ovule (Viands et al., 1988). This process takes 24-32 hours. Fertilization of the ovule completes gene transfer, but not all pollen transferred results in gene transfer, due to failure to fertilize the ovule or death of pollen cells.
- **Probability of Seed Maturation and Production**. For low-level presence to occur to a significant degree in <u>hay</u> crops, several additional steps are required. Fertilized embryos must then mature into seed. Flowering typically occurs about 35 days after regrowth, and seed maturation generally occurs an additional 20-40 days after mid bloom flowering (so 60-70 days total, Rickner, et al., 1988). Therefore, seed maturation is not a normal consequence of forage production, since forage harvests are generally spaced 28-35 days apart. However, seed production is feasible on a very small number of plants with occasional unharvested stems, very late harvested hay, hay grown under intense heat conditions, and on plants located on field edges and ditches that may not be harvested (feral alfalfa). However seed maturation is not a usual outcome of hay production, and occurs only under specific conditions.
- **Probability that Seed Falls to the Ground and Germinates**. For low-level presence to occur in a neighboring hay crop, any seed potentially produced must fall to the ground, germinate and produce a plant. The seed must have sufficient moisture and coverage on the soil surface to be able to germinate and emerge in an already-established alfalfa stand. Deliberate efforts at overseeding alfalfa to increase the density of existing alfalfa stands using grain drills are typically unsuccessful (Canevari et al., 2001) due to

competition, an inadequate seedbed, and allelopathy of the existing plants in the stand. Thus seed that falls onto an unprepared seedbed with competing established alfalfa plants would have a lower level of success.

- **Probability of Survival of New AP Recruits.** A germinating seedling in an existing alfalfa stand must then survive intense competition from neighboring alfalfa plants and weeds sufficient to become established and contribute to yield. In order for gene presence to be detected, the forage containing that gene must contribute to the forage yield of the crop. Alfalfa is a very weak seedling during 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 and productivity of the plants that do somehow manage to germinate and grow (Canevari et al., 2001, Canevari and Putnam, 2007).

Environmental filters limit gene flow in alfalfa hay. The above probabilities, to the extent that they are less than 100%, serve as ‘environmental filters’ that limit gene flow in hay crops. Thus, if little simultaneous flowering occurs, pollinators are few, the crop is harvested before significant flowering, the few seeds produced are removed during harvest, and the conditions for seed germination and survival are limited, gene flow would be greatly reduced in hay-hay compared with seed-seed or hay-seed gene flow. Further, if a probability of an event is zero, this effectively halts any chance of gene flow – for example if zero flowering occurs and all hay is harvested and removed, then gene flow between hay crops becomes zero.

The observed levels of low-level presence in close-proximity hay-to-seed gene flow of about 0.25% observed in Dr. Teuber’s experiment (Figure 3) represents a ‘worse case scenario’ for gene flow between hay crops. Observed levels of hay-seed gene flow reported by seed companies have been at this level or below (3 seed companies, pers. Comm.). In hay-hay gene flow, this amount is then reduced significantly due to these environmental filters. For example, if 1% of the stems in an alfalfa hay field sets seed due to a few plants that remain unharvested (actually 1% is a very large number of remaining stems in practice), and of those, 1% of the seeds germinate and survive in the stand, the contribution to low-level presence would be predicted to be 0.000025% at the closest distance (0.25% at 165 feet x 0.01 x 0.01 = 0.000025%). The gene flow would be diluted more by larger distances within fields and between fields, since this estimate is at 165 feet. Gene flow may not be zero between neighboring alfalfa hay fields, but is likely to be much lower than the detection capability using normal sampling and analysis methods, which are normally in the 0.1% and above range depending upon level of tolerance (Table 1). The potential for gene flow is likely to be higher in the very hot regions, such as the deserts of California and Arizona, where alfalfa flowers profusely at 28 days, or in situations where alfalfa is harvested very late. Typically, though, 4-6 weeks after flowering is required to produce viable seed.

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 the 0.1% low-level presence important for sensitive markets under most conditions.

The highest probability of low level presence in hay fields is likely to originate from low level presence in seed, not transfer from hay field to hay field. Thus, excess care should be taken to purchase certified seed for those growers interested in sensitive markets.

TESTING FOR DETECTION OF PRESENCE IN HAY

There is strong interest in the testing of hay for the presence of an unwanted GE product at a low level. However, testing should be considered within the context of the 'process-based' series of steps that reduced unwanted low-level presence.

Field Trial. A trial was conducted in 2005-2006 in Meridian, Idaho to determine the ability to detect low-level presence in hay harvested from seed that was deliberately spiked with low-level presence in the seed. Roundup Ready seed was added on a weight basis to non-RR seed at the rates of 0, 1%, 5% and 10%, and planted at the rate of 25 lbs/acre in a farmer's field on 1 acre plots side-by-side in 2006 (Figure 6). Hay was harvested from each of these blocks in 2006 from cutting 2 and cutting 3, and stacked in separate blocks. A standard hay-coring device (Penn State Sampler) was used to sample each stack using the standardized sampling method (Putnam and Orloff, 2002) using 20 composited cores per sample per replication, creating 5 separate replications of 20-cores each per stack for each of 2 cuttings. Samples were brought to Davis, CA, split utilizing a riffle-splitting device (Fischer Labs), and ½ of each sample was ground utilizing a 1 mil laboratory cyclone grinder. Each sample was then again split, samples coded and sent to each laboratory as a double-blind test of the ability to detect the RR trait in the hay samples.

Test Strips Identify GE and non-GE Hay. Results can be seen in Table 2. All of the samples (5 replications each x 2 cuts x 3 laboratory measurements x 2 laboratory test strips X ground and unground = 120 samples for each level) for the zero GE plots resulted in non-detect in the sample (Table 2). All of the samples at 5 and 10% resulted in a positive detection (Table 2). At the 1% level of GE in the seed, over 90% of the observations resulted in a positive reading, but the readings were often faint (indicated by the operator with an *). The same results were found for the second cutting, and for unground samples (data not shown). It should be noted that these test strips were designed to meet a 5% detection level, but were largely (but not always) successful at a 1% level. Currently, ELISA test strips are available to detect low-level presence at 0.1% level (Envirologix website), but have not been widely tested at that level of sensitivity. Currently, it is recommended to use PCR for lower levels of detection in alfalfa hay destined for highly sensitive markets such as the regulated market in China.

These results serve to confirm that 1) low-level presence in the hay can be detected in the hay when planted at the 1,5, and 10% level using standard ELISA methodology, 2) that zero levels of low-level presence resulted in non-detect results, and 3) that standard hay sampling methods are capable of detecting low-level presence at these levels provided the trait is evenly distributed throughout the hay mass.

PROTOCOL FOR PRODUCING NON-GE ALFALFA

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

I. Select Certified Cultivars for Seed Purity and Quality

It is difficult to overemphasize the importance of cultivar and seed choice for those hay growers who are selling into sensitive markets and are concerned with the purity of their alfalfa stands. This is likely the most crucial step to assure trait purity in a hay product. Alfalfa seed is produced on small acreage in 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, Mueller, 2008). 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 (CCIA, 2015). The incorporation of biotech traits in alfalfa seed production has been considered in detail elsewhere (Mueller, 2004). Thus, the first step in the process of stewardship is selection of certified varieties backed by a company with high standards for seed production.

Recommendation: Request non-GE alfalfa seed which has been determined by a lab test to be non-detect below the level of tolerance demanded for your market (Table 1). **Note:** PCR testing is required for China currently. All major seed production companies have reported availability of non-GE alfalfa seed confirmed through PCR testing to meet low-level presence tolerances described in Table 1.

II. Reduce Possibility of Gene Flow

Reducing the possibility of excessive gene flow involves understanding the distances of GE-sensitive fields to GE-containing fields, and reducing the possibility of simultaneous flowering. Assure that flowering of your GE-sensitive field does not occur at the same time as a neighbor’s GE-containing field through harvest management. Harvesting before excessive flowering is a key management factor useful for reducing risks of gene flow. Feral (wild) alfalfa may occur along field edges, ditch banks or roadsides and remains unharvested. Its origin is not known, but could be from older plantings by highway departments, spilled seed, or transferred by hay trucks or moved 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. As described earlier, hay to hay gene flow requires that each of a sequence of events is required for effect gene flow, eliminating any one of those factors will eliminate hay to hay gene flow–the easiest of which is probably to harvest before ripe seed set.

Recommendation: Determine distances to GE-fields, harvest before excessive flowering, or certainly before ripe seed is formed, prevent synchronous flowering, and remove feral alfalfa from areas in close proximity to GE-sensitive alfalfa fields to reduce the possibility of gene flow. **Note:** Several farmers are currently successfully producing GE and non-GE hay on closely situated fields (Simon, 2011).

III. Prevent Inadvertent Transfer of Hay During Harvest.

In regions where both GE and non-GE alfalfa is grown, it is possible for equipment to move hay from field to field, especially with balers (where partial bales may be contained in a baler), but also with rakes or swathers.
Recommendation: Clean balers and equipment when moving between fields or alternatively reject the first few bales which may contain unwanted genes. **Note:** Organic growers already must follow this practice when moving from non-organic fields.

IV. Identification of Non-GE Alfalfa Hay/Prevent Mixing of Lots.

The coexistence of GE and non-GE 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 keeping records during stand establishment (seed tags) that indicate that conventional varieties were sown. A 'lot' in the hay industry 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).
Recommendations: Prevent the mixing of hay lots, maintain identity, and assure customers of that identity through record keeping for the planting, harvesting, storage and transport process for either GE-containing or Non-GE alfalfa hay. **Note:** This is commonly practiced on commercial hay farms (especially organic and export).

V. Understand the Sensitivities and Tolerances of the Market

Non-GE hay must be produced to meet market demand, and as discussed above, the threshold of market or regulatory sensitivity must be determined (Table 1). A practical solution to coexistence requires a respect for sensitive markets, a determination of the level of tolerance, and recognition that a zero threshold of tolerance is both impossible to confirm scientifically and difficult to obtain practically. Given that the food crops in some of the most sensitive markets are 0.9% low-level presence in foods (Europe) and 5% in Japan, a market-based threshold of 0.9% may serve the purposes for most markets, with the exception of regulatory, which currently requires non-detect at a limit of detection of approximately 0.1% (Table 1).
Recommendation: Determine the level of non-detect tolerance of specific markets (organic, export market, or export regulatory). **Note:** Although there is no market tolerance for regulated GE traits in China, the level of detection based on their proscribed methods is approximately 0.1%.

VI. Testing to Confirm non-GE status in hay.

Testing of hay lots is the final step to confirm that hay lots either contain, or do not contain, biotech traits. Testing may or may not be required for specific markets and in some cases a written or oral assurance of non-GE status may be adequate. ELISA test strips (Figure 4) have been developed (e.g. http://envirologix.com) that identify the presence of the CP4 EPSPS protein (product of the RR gene) in alfalfa hay, seed, and other crops. Additionally, Polymerase Chain Reaction (PCR) techniques have been developed that identify the gene at lower levels. Protocols for fresh leaf material, unground hay, and ground hay tests (for testing of routine cored hay samples by commercial laboratories) are available. Reported limits to detection and scientific evidence should be used to determine which method is adequate to meet the needs of specific markets (e.g. 0.9% or 0.1%).

Regardless of method, sampling procedures are important to detection, and the detection of a gene may be limited by sampling method and type of low-level presence (whether randomly dispersed throughout a hay lot or just a few segments of a bale or an occasional bale). See Putnam (2014) for a full discussion of sampling methods for GE traits.

Recommendation. Testing for the presence or absence of a GE trait should be used in combination with process-based protocols (1-5) above. The limits of detection of each specific method, and the limitations of sampling should be considered when interpreting laboratory GE tests. Note: Due to the limitations of sampling and analysis at low-level tolerance levels, a combination of process-based and testing protocols are likely necessary.

SUMMARY

Coexistence strategies are a necessary and important component of successful production of both GE- and non-GE alfalfa hay, consistent with consumer preferences and a farmer's right to farm. The majority of markets for alfalfa in the US are not likely to be sensitive to the presence of a biotech trait, but both organic and export markets currently demand non-biotech alfalfa. 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. A number of farmers have produced both GE-sensitive (organic or export) and GE alfalfa hay on nearby farms or adjacent fields successfully. Communication and cooperation between farmers are obvious components of any coexistence strategy within a region. Common-sense steps for the production of non-GE alfalfa include primarily securing of non-detect (tested) seed, preventing accidental mixing of hay lots, and taking steps to prevent gene-flow between alfalfa fields. Market assurance can be further assured by hay lot sampling and testing using a method (PCR or ELISA strips) appropriate for a given level of market or regulatory tolerance, but the limits of testing and sampling must be considered.

Degree of sensitivities of different markets are likely to differ – thus Non-GE alfalfa produced for sensitive market purposes may have detection limits of 0.9%, whereas Non-GE alfalfa produced for regions where the trait is not approved may have a limit of 0.1% detection, such as importation into countries which have not approved the trait. The latter is based upon limits of detection of the most sensitive methods available (PCR). Coexistence strategies for alfalfa hay require an understanding of the market tolerances of diverse markets, the mechanisms for unwanted gene presence, and market assurance processes.

Acknowledgements: The authors are grateful for the assistance of Dr. Larry Teuber, UC Davis (now deceased) who provided the data for the hay to seed gene flow. We are grateful to Chris DeBen, Marianna Campana, Rachael Grande Soto, Tamar Cohen Davidyan, and Raissa Curry Pires Da Silva, UC Davis, for assistance on the test strip evaluations, to Dr. Emilio Laca for assistance in analysis of gene flow data, and to Bill Matthews, UC Davis for the hay export data graphs.

REFERENCES

CCIA, 2015. California Crop Improvement Association. University of California, Davis. http://ccia.ucdavis.edu/Certification_Programs/

Combs, D. K. and G. F. Hartnell. 2008. Alfalfa containing the glyphosate-tolerant trait has no effect on feed intake, milk composition, or milk production of dairy cattle. J. Dairy Sci. 91:673-678.

Council for Agricultural Science and Technology (CAST) 2014. The Potential Impacts of Mandatory Labelling for Genetically Engineered Food in the United States. Issue Paper 54. CAST, Ames, IA.

Canevari, M., D.H. Putnam, W. T. Lanini, R.F. Long, S.B. Orloff, B.A. Reed, R.V. Vargas. 2001 Overseeding and Companion Cropping in Alfalfa. University of California Agriculture and Natural Resources Publication 21594. http://anrcatalog.ucdavis.edu

Canevari, M. and D.H. Putnam. 2007. Managing Depleted Alfalfa Stands: Overseeding and Other Options. IN Irrigated Alfalfa Management for Mediterranean and Desert Zones. Publication 8301. UC ANR. 12/2007. http://alfalfa.ucdavis.edu/IrrigatedAlfalfa/pdfs/UCAlfalfa8301DepletedStands_free.pdf

Cullor, J., and P. Rossitto. 2004. Can Genetically-Enhanced Traits be detected in Milk or Meat? IN: Proceedings, 2004 National Alfalfa Symposium, 13-15 December, 2005, San Diego, CA. UC Cooperative Extension, University of California, Davis 95616 http://alfalfa.ucdavis.edu

Doll, J. 2003. Future of Roundup Ready Alfalfa. Proc. Wisconsin Fertilizer AgLime and Pest Management Conference. Vol. 42

Flachowsky, G., A. Chesson, and K. Aulrich. 2005. Animal nutrition with feeds from genetically modified plants. Arch. Anim. Nutra. 59: 1-40.

Lawrence, Laurie. 1998. Evaluating hay for horses: Myths and Realities. IN California Alfalfa Symposium, 13-15 December, Reno, NV. http://ucanr.org/alf_symp/1998/98-128.pdf

Miller, M. 2005. Organic Dairy Profile. Agricultural Marketing Resource Center, Iowa State University. On line: http://www.agmrc.org/agmrc/commodity/livestock/dairy/organicdairyprofile.htm

McCaslin, M., A. Van Deynze. 2012. Coexistence for Organic Alfalfa Seed and Hay Markets. National Alfalfa & Forage Alliance. www.alfalfa.org/pdf/oOrganic.pdf

Mueller, S. 1996. Alfalfa Seed Production in the Western US: A commitment to Quality. IN Proceedings, California Alfalfa Symposium, 7-8 December, 1996, Modesto, CA. UC Cooperative Extension, University of California, Davis 95616 http://alfalfa.ucdavis.edu

Mueller, S. 2004. Seed Production for Genetically Enhanced Alfalfa. IN Proceedings, 2004 National Alfalfa Symposium, 13-15 December, 2005, San Diego, CA. UC Cooperative Extension, University of California, Davis 95616 http://alfalfa.ucdavis.edu

Mueller, 2008. Alfalfa Seed production in California. University of California Division of Agriculture and natural Resources. Publication 8308. http://alfalfa.ucdavis.edu/IrrigatedAlfalfa/pdfs/UCAlfalfa8308SeedProd_free.pdf

National Academy of Sciences (NAS). 2004. Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. National Academy of Sciences, Washington, D.C. http://books.nap.edu/catalog/10977.html?onpi_newsdoc07272004

NCBA, 2005. Retailers, Consumers Hungry for Organic Beef. National Cattlemen's Beef Association Website (Routers) http://www.beefusa.org/NEWSRetailersConsumersHungryforOrganicBeef10813.aspx

Orloff, S.B., R. Vargas, M. Canevari, K. Hembree. 2003. Progress in Roundup Ready Alfalfa In: Proceedings, California Alfalfa Symposium, 18-19 December, 2003, Monterey, CA, UC Cooperative Extension. http://alfalfa.ucdavis.edu

Prichard, T., 2002. Total Maximum Daily Loads (TMDLs) in Alfalfa – The Implications for Alfalfa Irrigation Management. Western Alfalfa Symposium, Reno, NV Dec. 12 – 13, 2002. University of California Cooperative Extension.

Pridham, J. 2004. The Impact of Roundup Ready Alfalfa on Organic Systems. Website: http://www.organicagcentre.ca/DOCs/wc_sp_pridham.pdf

Putnam, D.H., 2002. Recommended Principles for Proper hay Sampling. http://alfalfa.ucdavis.edu/+producing/forage_quality/hay_sampling/HAYSAMPLINGSTEPS.htm

Putnam, D. H. 2004. Marketing Issues Associated with Bio-tech traits in Alfalfa. IN Proceedings, 2004 Northwest Alfalfa Seed Growers Conference, 19-20 January, Reno, NV. Northwest Alfalfa Seed Growers Assoc., Kennewick, WA. See http://alfalfa.ucdavis.edu

Putnam, D.H., S.B. Orloff, 2013. Benefits and Risks of Adapting Genetically Engineered Crops: The Roundup Ready Alfalfa Story. IN Cool Forages-Advanced management of temperate forages.S. Bittman and D. Hunt. Pacific Field Corn Association.: 71-76.

Putnam, D.H. 2004. Forage Quality, Testing and Markets, where are we going? IN Proceedings, 2004 National Alfalfa Symposium, 13-15 December, 2005, San Diego, CA. UC Cooperative Extension, University of California, Davis 95616 http://alfalfa.ucdavis.edu

Putnam, D.H. 2014. Sampling Considerations for the Detection of Genetically Engineered Traits in Alfalfa Hay. IN Proceedings, 2014 California Alfalfa Forage and Grain symposium, Long Beach, CA, 10-12 December, 2014. UC Cooperative Extension, UC Davis. http://alfalfa.ucdavis.edu/+symposium/proceedings/2014/14CAS08_Putnam_SamplingDetection.pdf

Putnam, D. H. J. Liu, L. Gibbs, S. Orloff, H. Carlson, D. Kirby, K. Taggard, C. Rivera and L. Teuber, 2004. California Alfalfa Variety Trials: Yield and Fall Dormancy Results and Roundup Ready Yield and Quality Results. Agronomy Progress Report No. 289. UC Agricultural Experiment Station and UC Cooperative Extension. http://alfalfa.ucdavis.edu

Putnam, D.H., P. Peisen, J. Plourd, C. Gauntt, S. Norberg. 2012. Coexistence for Alfalfa Hay Export Markets. National Alfalfa & Forage Alliance. www.alfalfa.org/pdf/ExportHay.pdf

Putnam, D.H., B. Matthews, D.A. Sumner. 2015. Alfalfa and Grass Hay Exports Decline after 7 Years of Dramatic Growth. IN. Alfalfa & Forage News Blog. April 28, 2915. University of California Cooperative Extension. http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=17566

Puschner, B. 2004. Toxic Weeds and the Effects on Livestock. IN National Alfalfa Symposium13-15 December, 2004, San Diego, CA. UC Cooperative Extension, University of California, Davis. 95616 http://alfalfa.ucdavis.edu

Rincker, C. M. V.L. Marble, D.E. Brown, C. A. Johansen. 1988. Seed Production Practices. IN: Alfalfa and Alfalfa Improvement. A.A. Hanson, D.K. Barnes, R. R. Hill (eds.). Monograph No. 29 American Society of Agronomy, Inc.

Shewmaker, G.E., D. Undersander, L. M. Lawrence, G.D. Lacefield. 2005. Alfalfa, the High Quality Hay for Horses. Alfalfa Alliance. Kennewick, WA. www.alfalfa.org

Simon, W. What are the Key Elements to Implement Coexistence between GE and Non-GE Alfalfa? IN Proceedings, 2011 Western Alfalfa & forage Conference, Las Vegas, NV 11-13, December, 2011. UC Cooperative Extension, UC Davis http://alfalfa.ucdavis.edu/+symposium/proceedings/2011/11-205.pdf

Teuber, L., A. Van Deynze, S. Mueller, M. McCaslin, S. Fitzpatrick, and G. Rogan. 2004. Gene flow in alfalfa under honey bee (Apis mellifera) pollination. Proceedings 39th North American Alfalfa Improvement Conference, July 18-21, 2004, Quebec City, Canada. http://www.naaic.org/Meetings/National/index.html

USDA-APHIS, 2005. Animal and Plant Health Inspection Service. Determination of Nonregulated Status for Alfalfa Genetically Engineered for Tolerance to the Herbicide Glyphosate. Federal Register. Vol. 70. No. 122. 7 June, 2005

USDA-APHIS, 2014. Animal and Plant Health Inspection Service. Determination of Nonregulated Status for Reduced lignin Alfalfa KK179. November 10, 2014. http://www.aphis.usda.gov/brs/fedregister/BRS_20141110a.pdf

USDA Advisory Committee on Biotechnology and 21st Century Agriculture (AC21). Enhancing Coexistence: A Report of the AC21 to the Secretary of Agriculture. November 19, 2012. http://www.usda.gov/documents/ac21_report-enhancing-coexistence.pdf

USDA-ERS. Economic Research Service. 2014. Genetically engineered varieties of corn, upland cotton, and soybeans, by state and for the United States, 2000-14. http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx

Van Eenennaam, A.L., 2005. Do Genetically Engineered (GE) Crops Impact Animal Health and Food Products? **In**: Proceedings, 35th California Alfalfa and Forage Symposium, 12-14 December, 2005, Visalia, CA, UC Cooperative Extension, Agronomy Research and Extension Center, Plant Sciences Department, University of California, Davis 95616.

Van Deynze, A., S. Fitzpatrick, B. Hammon, M.H. McCaslin, D.H. Putnam, L.R. Teuber, and D. J. Undersander, 2008. Gene Flow in Alfalfa: Biology, Mitigation, and Potential Impact on Production. Council for Agricultural Science and Technology. Ames, IA.

Van Deynze, A., D.H. Putnam, S. Orloff, T. Lanini, M. Canevari, R. Vargas, K. Hembree, S. Mueller, L. Teuber. 2004. Roundup Ready Alfalfa: An Emerging Technology. Publication 8153 University of California Division of Agriculture and Natural Resources. http://anrcatalog.ucdavis.edu/pdf/8153.pdf

Viands, D.R., P. Sun, and D.K. Barnes. 1988. Pollination Control: Mechanical and Sterility. IN: Alfalfa and Alfalfa Improvement. A.A. Hanson, D.K. Barnes, R. R. Hill (eds.). Monograph No. 29 American Society of Agronomy, Inc.

Wheat, D. 2014. GMO Test Slows Hay Exports to China. Capital Press. September 25, 2014. http://www.capitalpress.com/20140925/gmo-test-slows-hay-exports-to-china

Woodward, T. 2005. Issues with GMO in the Marketplace. Washington State Cooperative Extension. Web: http://www.wa-hay.org/publications/Issues%20With%20GMO%20Alfalfa%20in%20the%20Marketplace.pdf

Table 1. Tolerance levels for low-level GE presence in alfalfa hay grown for markets of varying sensitivity.

Name of Crop Product	Type of Market	Non-GE Management Protocol Followed?	Tolerance Level for Non-Detect (% of hay mass)	Method used to confirm
GE-Alfalfa Hay (Roundup Ready (RR) or HarvXtra (RL) Alfalfa)	Non GE-Sensitive	No	100%	Not necessary, but ELISA test strips can be used to confirm RR. PCR must be used to confirm RL
Conventional	Non GE-Sensitive (may include GE traits or not)	No	N/A	Not necessary
Conventional Non-GE Alfalfa Hay (ND≤0.9%)	Market-based or Organic Sensitivity (GE trait is not desired by buyer or non-GE required for market certification)	Yes	≤0.9%	ELISA Test Strips or quantitative PCR*
Conventional Non-GE Alfalfa Hay (ND≤0.1)	Regulatory Sensitivity (GE trait is not legally permitted in country)	Yes	≤0.1%	PCR*

*Confirmation of sensitivity of ELISA strips and Polymerase Chain Reaction (PCR) at different levels should be confirmed at desired level of detection. There may be ELISA tests with sensitivity at 0.1% and below available in the market, but generally PCR is preferred.

Table 2. Detection of the CP4-EPSPS gene in alfalfa hay harvested from seed grown with various known levels of Roundup-Ready Alfalfa. Two commercial test strips were used (Envirologix and RUR). Five replicates of composited 20-core samples were used, and three different labs (Washington, Idaho, and California) independently measured split blind samples. Strips had an advertised detection limit of 5%. Asterisks (*) indicate that the signal was faintly detected by the operator. These are results from ground samples cut 1. Similar results were found in cut 2, and in unground samples from both cuts (four sets total).

		Test Strip					
		EL6285	RUR	EL6285	RUR	EL6285	RUR
		Assay Operator					
Treatment	Replication	ID	ID	WA	WA	CA	CA
Zero %	1	-	-	-	-	-	-
Zero %	2	-	-	-	-	-	-
Zero %	3	-	-	-	-	-	-
Zero %	4	-	-	-	-	-	-
Zero %	5	-	-	-	-	-	-
One %	1	+	+	+**	+*	+**	+*
One %	2	+*	+*	+***	+**	+***	+**
One %	3	+**	+*	+***	+*	+***	+*
One %	4	+	+	+*	+**	+*	+**
One %	5	+*	+*	+***	+**	+***	+**
Five %	1	+	+	+	+	+	+
Five %	2	+	+	+	+	+	+
Five %	3	+	+	+	+	+	+
Five %	4	+	+	+	+	+	+
Five %	5	+	+	+	+	+	+
Ten %	1	+	+	+	+	+	+
Ten %	2	+	+	+	+	+	+
Ten %	3	+	+	+	+	+	+
Ten %	4	+	+	+	+	+	+
Ten %	5	+	+	+	+	+	+

Figure Captions:

Figure 1. Exports of alfalfa and grassy hay from US. The drop in 2014 was partially due to port strike, lower demand by UAE, changes in US dollar, and the GE sensitivity of alfalfa in China. In spite of this export reduction, alfalfa exports to China increased by 30% in 2014 vs. 2013. (Source: US Department of Commerce, National Ag. Statistics Services).

Figure 2. Acreage of organic alfalfa, 2000-2011. Percentage of US acreage is shown for each year. (source: US Economic Research Service).

Figure 3. Gene flow from hay to seed, UC Davis, 2008. Roundup ready hay harvested at 50% bloom was grown 165 feet from seed-producing fields, which were allowed to produce fully mature seed. This represents a starting point to estimate hay-to-hay gene flow (L. Teuber, UC Davis data).

Figure 4. Steps necessary for gene flow to occur between biotech and conventional alfalfa forage production fields sufficient to cause adventitious presence (AP) in hay.

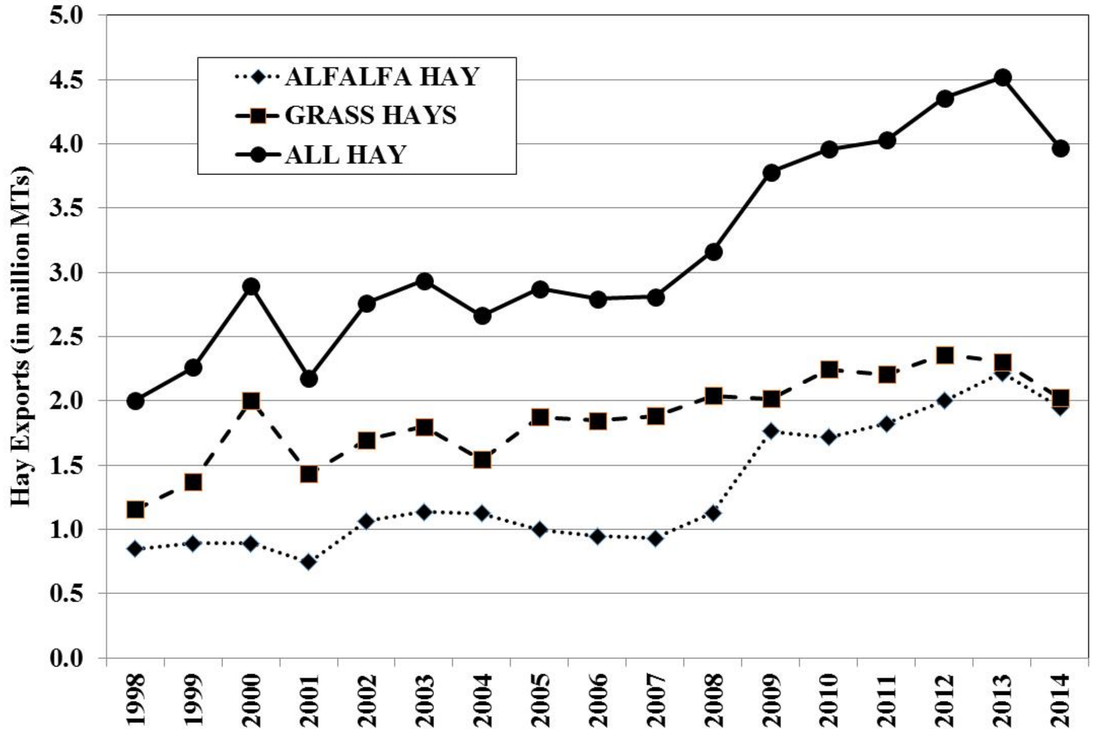
Figure 5. Test strips have the potential to detect adventitious presence (AP) of Roundup-Ready (RR) alfalfa in hay and could assist in confirming non-biotech hay for sensitive markets. Arrows show positive reading for 100% RR and 5% RR samples (right), and circle shows negative reading for control sample (left). However, the limits of detection determined by the method must be considered.

Figure 6. Field Study on low level gene presence with varying levels of low level presence (0, 1%,5%,10%), planted in Meridian, ID, 2005-2006. Hay was sampled to determine presence or absence of the CP4 ESPS (Roundup Ready) gene.

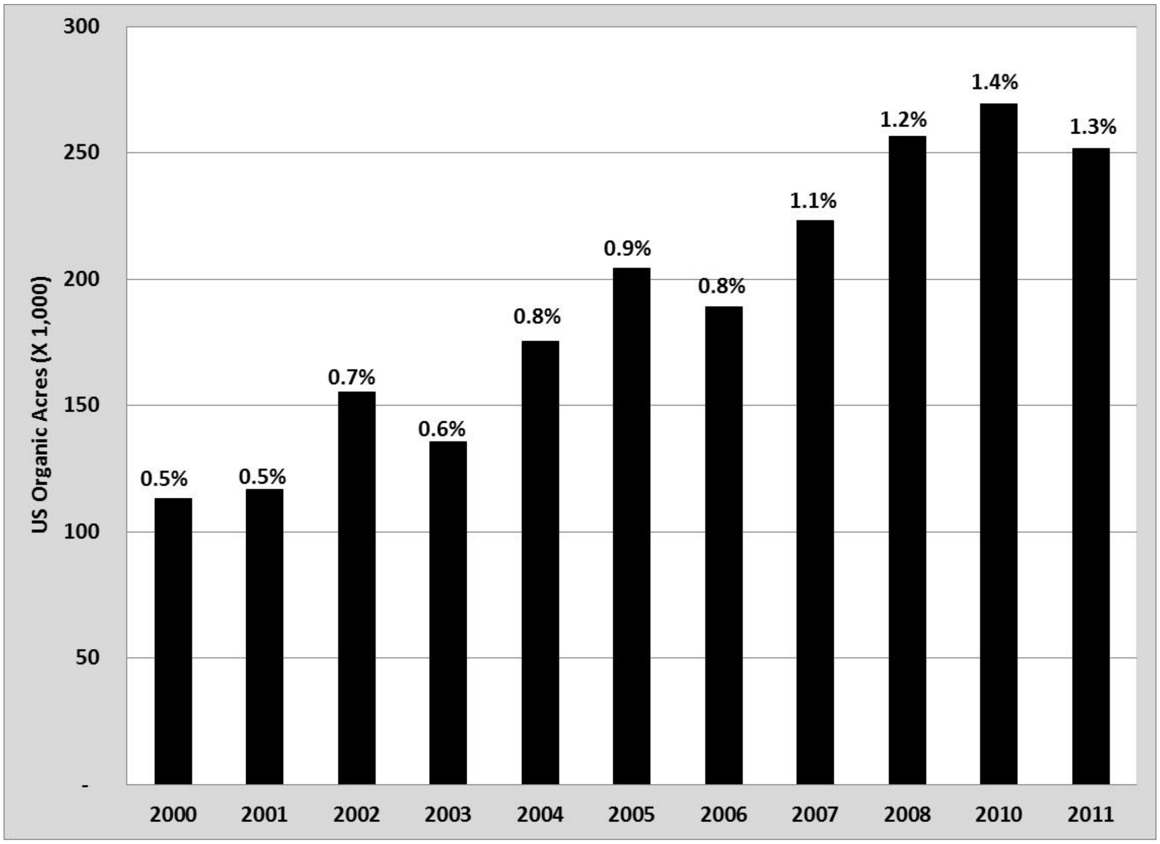
Table Captions:

Table 1. Tolerance Levels for Low Level Presence (LLP) of a GE trait in alfalfa hay grown for markets of varying sensitivity.

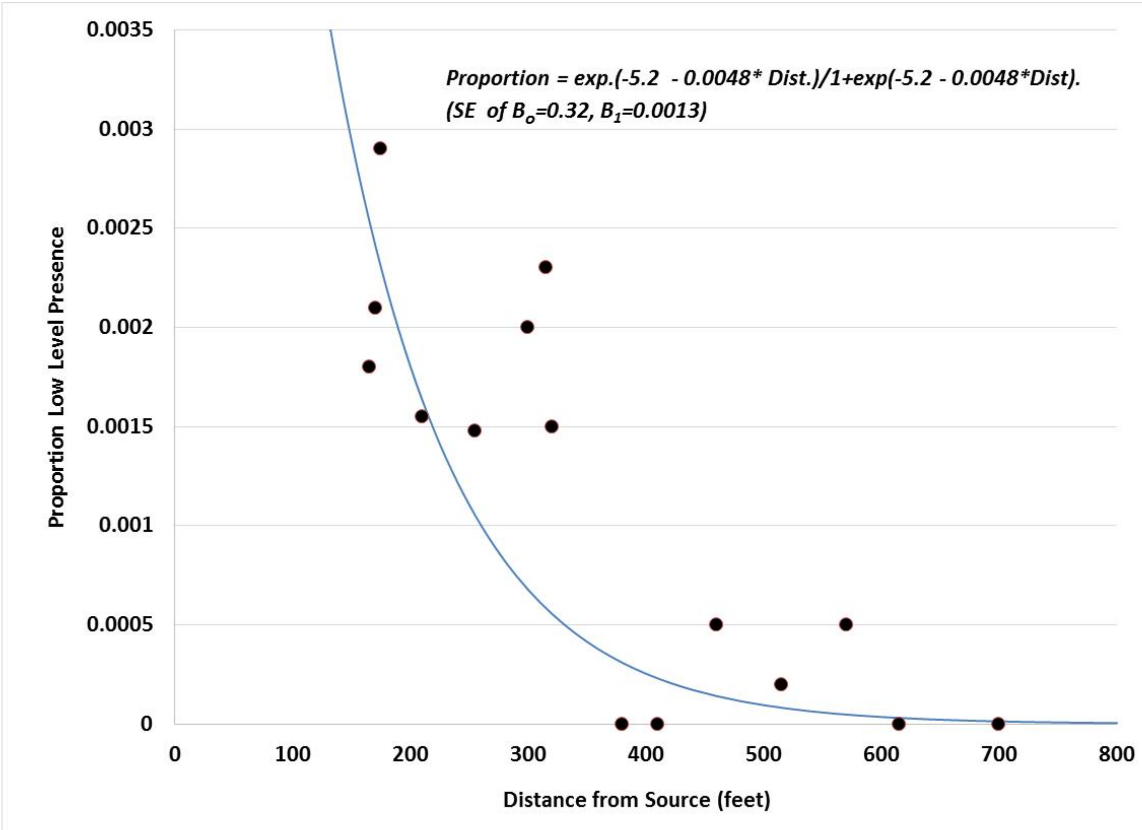
Table 2. Detection of the CP4-EPSPS gene in alfalfa hay harvested from seed grown with various known levels of Roundup-Ready Alfalfa. Two commercial test strips were used (Envirologix and RUR). Five replicates per cut were used, and three different labs (Washington, Idaho, and California) independently measured split blind samples. Strips had an advertised detection limit of 5%. Asterisks (*) indicate that the signal was faintly detected by the operator. These are results from ground samples cut 1. Similar results were found in cut 2, and in unground samples from both cuts that were measured (four sets total).



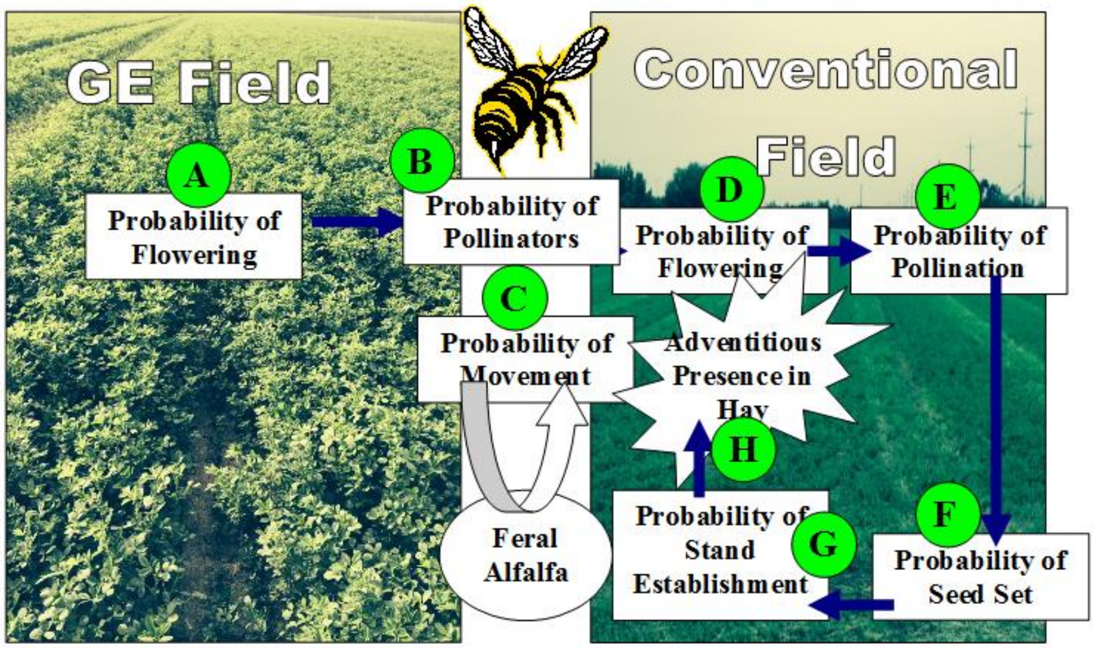
ALFALFA HAY
GRASS HAYS
ALL HAY
Hay Exports (in million MTs)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

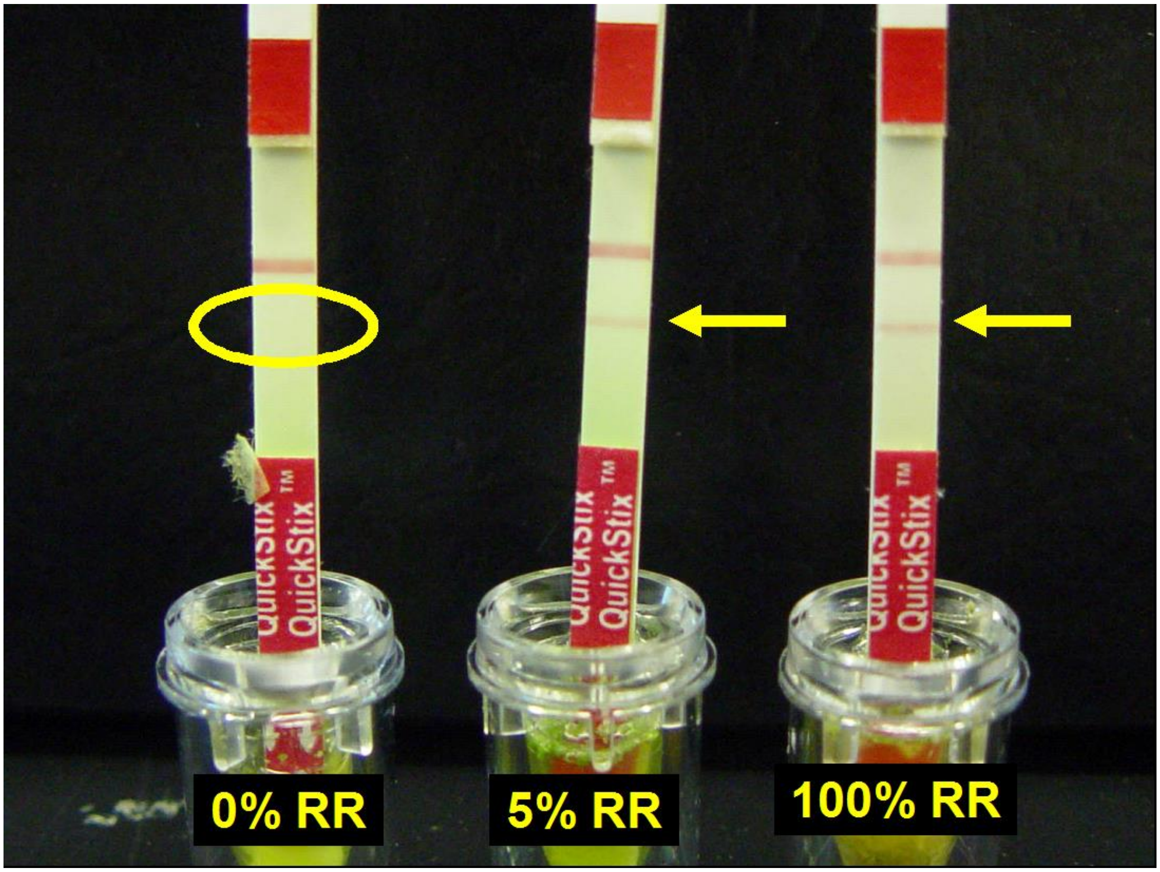


US Organic Acres (X 1,000)
300
250
200
150
100
50
-
0.5%
0.5%
0.7%
0.6%
0.8%
0.9%
0.8%
1.1%
1.2%
1.4%
1.3%
2000
2001
2002
2003
2004
2005
2006
2007
2008
2010
2011



Proportion = exp.(-5.2 - 0.0048* Dist.)/1+exp(-5.2 - 0.0048*Dist).
(SE of Bo=0.32, B1=0.0013)
Proportion Low Level Presence
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
0
100
200
300
400
500
600
700
800
Distance from Source (feet)


GE Field
Conventional Field
A
Probability of Flowering
B
Probability of Pollinators
C
Probability of Movement
Feral Alfalfa
D
Probability of Flowering
E
Probability of Pollination
Adventitious Presence in Hay
H
F
Probability of Stand Establishment
G
Probability of Seed Set


QuickStix
QuickStix ™
QuickStix
QuickStix ™
QuickStix
QuickStix ™
0% RR
5% RR
100% RR