CHAPTER 18

Benefits and Risks of Adapting Genetically-Engineered Crops: The Roundup Ready Alfalfa Story

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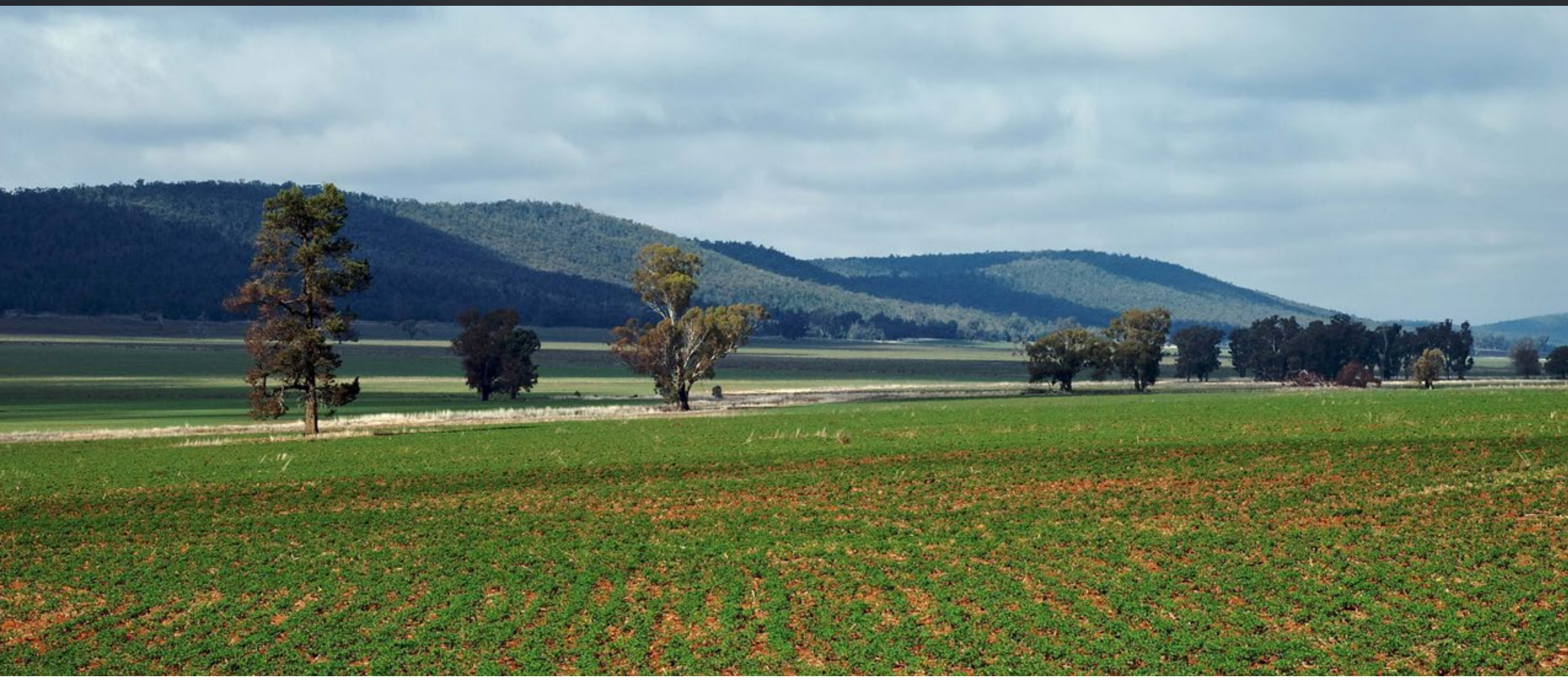

Alfalfa (*Medicago sativa* L.) is one of the oldest domesticated crop species, having had impacts on ancient and European civilizations dating back to before the fourth millennium, BC (Russelle 2001). While selection of superior lines undoubtedly occurred over these thousands of years, scientific improvement did not begin in earnest until the early 20th Century. Adaptation of alfalfa to cold and wet continental regions and to long-season irrigated desert conditions were among the major breeding accomplishments. New strains with improved resistance to bacterial wilt, phytophthora, nematodes, and aphids were also major traditional plant breeding successes, exploiting the tremendous genetic diversity of this polyploid crop. Currently, hundreds of commercial varieties are available with tolerances to winter injury, grazing and salt, and with documented genetic resistance to 14 disease and insect pests (NAFA 2012). In fact, alfalfa has more documented genetic pest resistance characteristics than any other major crop.

Introducing genetically engineered alfalfa

The era of genetically-engineered (GE) alfalfa varieties began in 1997, when the first alfalfa constructs incorporating the CP4 gene (Monsanto Company) for glyphosate tolerance (RR) were developed at Montana State University. RR alfalfa (also known as Roundup Ready, or RR alfalfa) varieties were subsequently developed under a cooperative agreement between Forage Genetics International and Monsanto, and released in 2005. The RR technology enables alfalfa to tolerate glyphosate, a broad-spectrum post emergence herbicide. In the USA in 2011, 94% of soybean, 73% of cotton and 72% of corn acres contained the RR gene, totaling 60 million ha (148 million ac) (USDA-ERS 2011).

However, the introduction of RR alfalfa varieties has raised a series of technical, legal, environmental and public perception questions related to the risks of GE crops. Here we review the RR alfalfa story and discuss it in the context of the potential impacts of future GE varieties in alfalfa and other forage species.

The roundup-ready alfalfa saga

After the first RR alfalfa constructs, breeders continued to develop varieties through backcrossing methods, with testing of the first lines in 1999-2005. A petition was submitted in April of 2004 to USDA-APHIS (the US regulatory agency for biotech crops) and the required Environmental Assessment conducted. Alfalfa with the RR trait was granted non-regulated status in June 2005 and Forage Genetics, Int'l began selling seed. Shortly after, a lawsuit was filed against USDA-APHIS claiming that the agency had failed to address important environmental issues. The lawsuit was filed by the Center for Food Safety (Oakland, CA), several environmental groups and some independent farmers. In 2007, the 9th Circuit Court in San Francisco ordered a halt to any further plantings, and required USDA-APHIS to begin a more complete Environmental Impact Statement. Supplemental court orders in 2007 placed restrictions on how existing plantings could be grown, stored, labeled and distributed. USDA-APHIS issued a draft Environmental Impact Statement in December 2009, and held public meetings in 2010. However, after a 2010 lawsuit by Monsanto, the Supreme Court of the USA overturned the 9th Circuit prohibition on further plantings, ruling that the 9th Circuit 'abused its discretion' by issuing the 2007 injunction.

This case had implications beyond alfalfa, including a closely-related RR sugarbeet case. There were also implications for the National Environmental Policy Act which governs all environmental decisions. In the meantime, USDA-APHIS completed the Environmental Impact Statement and published their findings in December 2010 (USDA-APHIS 2010). During the comment phase, more than 244,000 comments, many highly charged with rhetoric about genetically modified organisms (GMOs) were provided to USDA, indicating a high degree of public interest in the case. In January 2011, APHIS made a final decision to grant non-regulated status to RR alfalfa allowing plantings to resume in February 2011. Essentially, APHIS concluded that: 'RR alfalfa plants will not have a different impact on the physical and biological environment than conventional alfalfa' (USDA-APHIS, Record of Decision, Jan. 2011). Further lawsuits were filed in March 2011, and some are still pending.

Figure 1. Field plots illustrating the effectiveness of Glyphosate Tolerant alfalfa. Weedy control plots are shown interspersed with glyphosate-treated plots.

Despite the 2011 decision by APHIS that RR alfalfa is environmentally safe, there remains concern by some alfalfa growers and seed companies, by the organic community and by hay and seed exporters, that the potential of gene flow may affect production methods or markets. There also remains concern about buildup of resistant weeds after repeated use of glyphosate. The sections below will address the 'pros and cons' of RR alfalfa, stewardship of the RR trait by industry, and the overall need for coexistence of diverse cropping systems.

What were the key environmental issues raised in the lawsuits?

The environmental issues raised by the initial lawsuits that led to the Environmental Impact Statement review were that APHIS failed to consider: 1) the inadvertent transmission of genes from RR alfalfa to organic and conventional alfalfa (also known as Adventitious Presence or AP), which could make it difficult or impossible to produce organic or non-GE alfalfa hence affecting 'the human environment' (9th Circuit language); 2) that the introduction of RR alfalfa, in addition to other RR crops, could lead to increased spread of glyphosate-resistant weeds; 3) whether total herbicide use or glyphosate use would increase, with possible impacts on the environment.

Benefits of the glyphosate tolerance technology in alfalfa

Effective weed management

The greatest advantage of the RR technology in alfalfa is the potential for enhanced weed management compared with current methods (Fig. 1). This was confirmed in weed management studies (Van Dynze et al. 2004) and a survey of 113 alfalfa producers who have grown RR alfalfa (Putnam and Orloff 2011). Better weed control, simplicity, and flexibility of weed management were the key advantages cited by farmers and by university researchers. Weed management is a critical component for alfalfa producers, particularly for stand establishment, and weed scientists and farmers generally agree that glyphosate is the most effective broad spectrum herbicide available. This herbicide controls most weeds (grass and broadleaf) under a range of field conditions, and crop and weed stages. Also, glyphosate molecules adsorb to soils, rendering them inactive and allowing seeding.

Glyphosate alone or with soil residual herbicides is particularly effective for controlling many problematic weeds that are not adequately controlled with conventional herbicides or cultural methods (Orloff and Putnam 2011) such as dodder (*Cuscuta* spp.), a parasitic weed of alfalfa and troublesome perennial weeds with extensive root systems or storage organs such as rhizomes, stolons or tubers such as quackgrass (*Elytrigia repens*) and Bermudagrass (*Cynodon dactylon*) (Orloff and Putnam 2009).

Crop safety

The RR technology is safer on alfalfa, especially seedlings, than most selective herbicides (Fig. 2). We have observed 0.3 to 1.0 t/ha (0.14 to 0.45 T/ac) yield increase in spring plantings with RR alfalfa compared with conventional alfalfa (Orloff and Putnam 2011). Benefits are lower but still important with fall plantings.

Flexibility in weed management

Since glyphosate controls weeds of varying sizes better than other herbicides, the application flexibility is much greater. Also, it is less sensitive to environmental limitations, such as heat or cold. Nonetheless, timely weed control minimizes early-season weed competition and the risk of weed escapes.

Forage quality, animal production, animal and human safety and health

By reducing weeds better than other herbicides, the RR alfalfa technology results in higher forage quality (improved protein, higher digestibility) and better animal performance, particularly for high-producing milk cows. This translates to economic value for both commercial hay growers and livestock producers. Additionally, the technology reduces the risk of animals being poisoned by weeds containing high-nitrate such as lambsquarters (*Chenopodium alba*) or toxins such as common groundsel (Senecio vulgaris), or weeds which injure animals' mouths, such as foxtail (*Hordeum jubatum* or *Setaria glaucans*). RR alfalfa helps growers respond to the demand for certified weed-free hay to prevent horses and other animals transporting invasive weeds into national parks or other pristine regions. The health benefit for farmers is that glyphosate has low human toxicity, especially compared to paraquat which is the common alternative.

Persistence

We know of no evidence that the RR gene innately offers superior persistence. However, improved weed management during stand establishment can confer enhanced stand persistence since weed competition during seedling development reduces plant density and vigor throughout the life of the stand. Conversely, crop injury by conventional herbicides may reduce alfalfa vigor and increase susceptibility to other stresses such as plant pathogens.

Water quality/environmental benefits

RR alfalfa is advantageous for ground water as glyphosate is less mobile in soils than water soluble soil-residual herbicides, especially those used for control of winter weeds such as hexazinone and diuron. This has been an important benefit to the environment, reducing herbicide impacts on water quality.

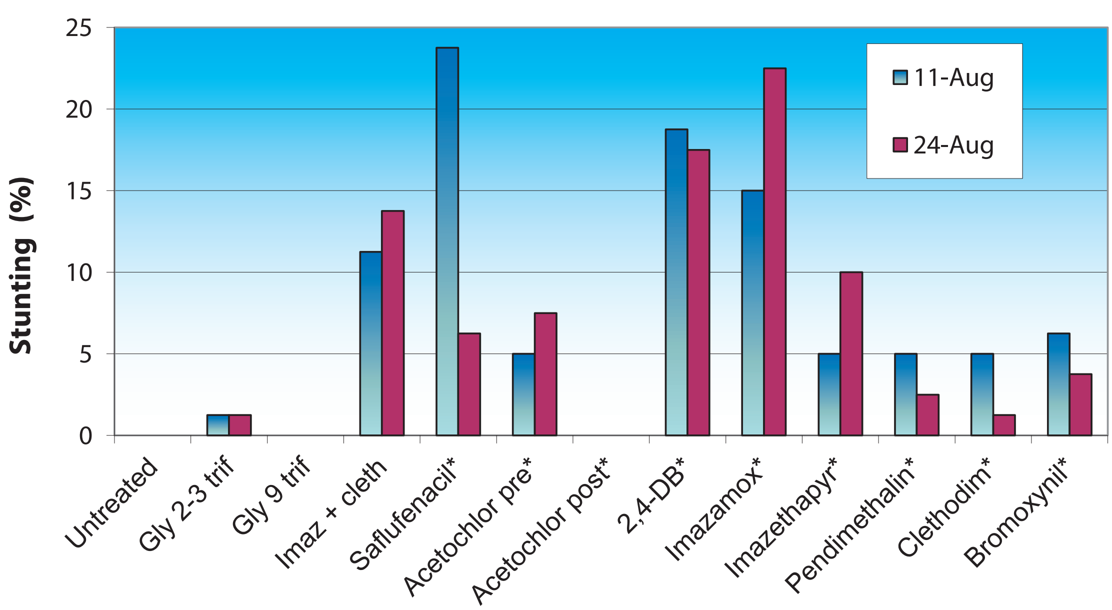

Figure 2. Crop injury of RR alfalfa during stand establishment was very small when unsprayed or sprayed with glyphosate (left three bars) compared with many of the common alfalfa herbicides (data from Tulelake, CA, 2006).

Economics
The same range of fall dormancy levels, yield, quality, and pest resistance characteristics are available in RR alfalfa as for conventional varieties. Economic comparisons have shown benefits for RR alfalfa due both to the low cost of glyphosate compared with other herbicides and to the lack of alfalfa phytotoxicity. However, the additional 'technology fee' (currently about $6/kg or $3/lb of seed) is a major up-front cost which must be weighed against anticipated losses due to weeds. It is interesting that according to multistate research, growers can lower seeding rates to offset the additional cost of RR seed (Hall et al. 2010). RR alfalfa has less value for alfalfa-grass mixtures or where there is very low weed pressure.

Limitations and risks of the glyphosate tolerance technology

Weed control limitations, weed shifts and weed resistance
As a polar molecule, glyphosate is readily adsorbed onto negatively charged soil particles rendering it ineffective so weeds may readily emerge after an application. Thus, repeated glyphosate applications may be needed or it may be necessary to combine glyphosate with a soil-active residual herbicide.

While very broad spectrum, there is a range in weed susceptibility to glyphosate. Moderately resistant weeds can be controlled as seedlings while weeds that are quite resistant to glyphosate may require a herbicide tank mix for complete control (Van Dynze et al. 2004). If glyphosate applications are repeated, there can be a shift towards glyphosate-tolerant weed species.

Note that this is distinct from a change in the genetic makeup of a normally susceptible weed species causing a shift towards this species. The continual use of a single class of herbicides, over time, selects for resistant individuals that survive and reproduce, causing a genetic shift towards resistance. While currently there are just 22 glyphosate-resistant biotypes compared with a total of 379 herbicide resistant biotypes (WSSA 2012), glyphosate resistance is of particular concern since it is so widely used.

Evolution of weed resistance is delayed by weed management that reduces selection pressure including: not relying solely on glyphosate (or any single herbicide), employing non-chemical practices (crop rotation and tillage), and alternating or tank-mixing herbicides with different mechanisms of action. These practices have been developed in detail for RR alfalfa (Orloff et al. 2008).

Unwanted gene flow during seed or hay production
A key risk with RR technology (and the core of the legal case against RR alfalfa in 2007) is the possibility of gene flow from RR alfalfa to other cropping systems. A complete review of alfalfa gene flow was provided in Van Dynze et al. (2008) and in documents published by National Alfalfa & Forage Alliance (NAFA 2012). To understand the gene flow issue, it is important to distinguish between seed and hay production, a nuance that has been often overlooked in the public discussion and even in the legal documents related to RR alfalfa.

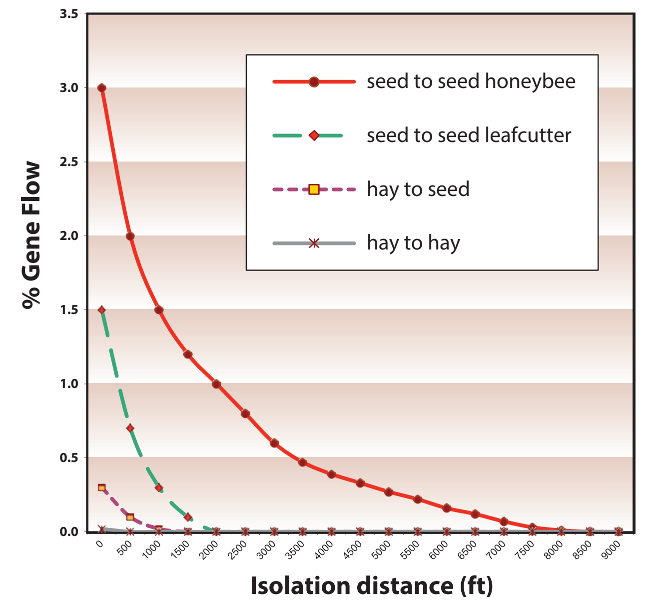

Figure 3. Gene flow estimates are greater for seed-to-seed or hay-to-seed gene flow than for hay-to-hay gene flow, and are dependent upon distances between fields. Hay fields constitute more than 99% of the landscape situations in the US. (Figure adapted from Van Dynze et al. 2008) (for meters multiply by 0.3).

Gene flow during seed production
Alfalfa is an obligate outcrossing crop, requiring insect pollinators to transfer pollen (gene flow) between plants for seed set. Therefore alfalfa seed growers must ensure that pollinators are present and that seed fields are sufficiently isolated from other alfalfa varieties. Transfer of an unwanted trait by pollen, referred to as adventitious presence (AP), has always been an issue for seed certification. In fact, gene flow of GE traits can be easily measured.

The risk of gene flow in alfalfa seed production at the closest isolation distances used by seed certifiers (50 m or 165 ft) depends on pollinator type: about 2.5-3% for honeybees and 1.5% for leafcutter bees, and this percentage approaches zero with increasing distance between fields (Fig. 3). However, since much alfalfa seed is grown on small fields and farms, gene flow is a concern. Major seed producing companies have instituted Best Management Practices (BMPs) to mitigate gene flow for RR and other AP-sensitive seed production (NAFA 2012), setting a much greater isolation distance (5 km or 3 miles for honeybees). The California Crop Improvement Association has provided 'pinning maps' to determine distances for GE sensitive and non-GE sensitive seed production (Teuber et al. 2008). In

2010, the National Seed Certification Association (AOSCA) developed a quality certification program called 'Alfalfa Seed Stewardship Program' for those requiring certified non-GE, non-RR seed. Additionally, growers have developed 'Grower Opportunity Zones' through NAFA to allow seed growers to elect (with an 80% majority) to grow only non-RR or only RR alfalfa to further reduce risk of gene flow. The Imperial Valley of California, the largest alfalfa seed-producing region in the world, has elected to grow only non-RR alfalfa, since much of their seed and hay markets are sensitive to RR alfalfa (due to high exports).

The alfalfa industry, through NAFA and other farm organizations, has taken these steps driven not by government regulation, legal fiat, nor by companies, but by seed growers and industry stakeholders. This represents a 'bottom up' approach to coexistence (Christiansen, 2011; NAFA 2012).

Gene flow during hay production

The risk of gene flow during hay production is much lower than during seed production (Fig. 3). Environmental and management barriers which largely prevent gene flow include the fact that most alfalfa is harvested before 10% flowering, that seed is typically not allowed to mature in hay fields, and that the entire above-ground crop is removed. In order to contaminate a hay field, GE seed would need to fall to the ground, germinate and mature in an existing untilled field.

There have been no measurements of gene flow from hay field to hay field to our knowledge, nor evidence to date of contamination from a RR hay field to a non-RR hay field. However, one can extrapolate the gene flow risk from seed gene flow data. Gene flow from a flowering *hay* field to a *seed* field has been measured at 0.25% of seed lots at the closest distance (50 m or 165 feet), and declines to trace amounts within 300 feet (L. Teuber, pers. comm.). If 1% of the crop is unharvested (estimated maximum area skipped) before seed is set, then the amount of genetic contamination at 50 m is reduced from 0.25% to 0.0025%, and if 10% of seed from the skipped plants germinates and produce seed in the field, the risk is lowered further to 0.00025%. With wider field separation or if pollinators are not plentiful, the risk is even lower.

Growers can take further steps to control gene flow, including controlling unharvested alfalfa on the edges of fields or ditches and harvesting to prevent simultaneous flowering of RR and non-RR fields (Putnam, 2006). Although the risk from gene flow in hay fields is not zero, it is low. The more likely sources of contamination on farms are 1) purchased seed which has not been tested to be non-GE, and 2) accidental mixing of hay in balers or during stacking and shipping. Inexpensive readily-available test strips for both hay and seed crops in the field can assure growers and buyers of the non-RR status of hay or seed destined for sensitive markets.

The risk of gene flow from a neighbor appears to be of the same order of magnitude as the risks from drift of wind-blown pesticide or weed seeds, and is usually highly dependent upon farming practice. However, the risk of inadvertent AP must be understood in the context of level of market tolerance.

Market sensitivity, market risks

There are segments of the alfalfa industry that reject the use of GE crops because of market demands. These are termed 'GE sensitive' growers and markets, as distinct from growers who simply don't wish to use the technology. For sensitive markets, namely export markets and organic hay producers, non-GE status is important. Since alfalfa is foremost a crop fed to dairy cattle, and greater than 95% of the US dairy market currently utilizes GE products, the vast majority of the alfalfa market is <u>not</u> sensitive to RR alfalfa.

Although activist groups have portrayed the RR alfalfa story as a battle between organic growers vs. Monsanto, in fact the largest 'GE sensitive' market is clearly exports, which has grown tremendously during recent decades (Putnam et al. 2012). Currently over 4% of US alfalfa production and 14% of western-grown hays are exported. In areas like the Columbia Basin and Imperial Valley, 1/4 to 1/3 of alfalfa hay is exported. These growers are obviously concerned about losing markets due to GE contamination. The contamination threshold differs among importing countries, and some rejection is logistical (difficult to segregate lots), or regulatory (countries don't yet have regulatory approval). Imports of RR alfalfa are allowed into Japan, a major buyer, but some Japanese importers still reject RR alfalfa. Nearly 15% of US seed production is exported, and many of those markets are quite sensitive to AP presence in seed.

Organic markets consume about 2% of US alfalfa hay production, but demand for organic milk has risen significantly in the past 10 years, and is likely to rise in the future. Thus alfalfa growers are concerned about the possibility of losing organic certification from gene flow or other types of contamination while organic dairy producers are concerned about availability of non-GE alfalfa. Organic certification is a 'process-based' certification, and the current steps taken by organic growers (procurement of non-GE seed, cleaning of equipment, segregation and identification of hay lots) may suffice in most cases to satisfy organic milk producers of the non-GE status of hay. Additionally, the routine use of inexpensive test strips may provide additional assurance to organic hay buyers (Putnam et al. 2012).

Thresholds

There are no market standards for a small amount of unwanted genes in an otherwise non-GE crop at this time.

Figure 4. On the Simon Farm in Fairfield, ID, USA, conventional, organic, and RR alfalfa are all grown in close proximity. Care is taken to clean equipment, keep stacks separate, and prevent excess flowering to prevent Adventitious Presence (AP) in organic hay. Alfalfa hay and seed farmers have responded to the introduction of RR alfalfa by developing methods to promote coexistence of different farming systems, and to protect sensitive export and organic markets.

Also, there are no testing requirements for presence of GE crops in organic or export hays although some buyers request it. Japan requires foods with greater than 5% GE, and the European Union food with greater than 0.9% GE to be labeled so it seems that feeds will have similar standards. It is perhaps ironic that, unlike toxins, market sensitivities to RR alfalfa cannot be set by objective criteria since no harm has been associated with feeding RR alfalfa. Discussions on thresholds with hay and dairy producers are now underway (Benbrook 2011). However, since reasonable steps are available to assure buyers or certifiers of the non-GE status, growers should be able to continue to produce organic hay in spite of the introduction of RR alfalfa. Similarly, most buyers have accepted written assurances of non-GE status provided by hay growers and exporters.

The need for coexistence and stewardship

USDA-APHIS and industry groups (e.g. NAFA) have acknowledged the ongoing GE issue, and have promoted the concept of ‘coexistence’ of farmers with ‘GE sensitive production’ alongside neighbors who grow GE crops. Considerable progress has been made, particularly in the alfalfa seed industry, where the risk of gene flow is greatest. There are available technical tools of isolation, management and testing, but the most important component is an ethic of cooperation between neighbors to respect diverse cropping systems and needs (Christiansen 2011). Farmers themselves have demonstrated interest in the coexistence concept: some have even grown both organic alfalfa, RR alfalfa, and conventional alfalfa on the same farm (Fig. 4; W.E. Simon 2011).

Implications for the future of GE constructs in forages

It is clear that the RR alfalfa story has important implications for future genetic improvements in alfalfa, and perhaps other crops. Innovative alfalfa GE traits under development include low lignin, pest resistance, improved protein quality, bloat resistance, biofuel traits, improved rooting patterns and tolerance to several abiotic stresses, and specialized traits such as production of plant pharmaceuticals. Although the issues related to weed resistance are unique to RR alfalfa, the lessons learned will lead the way for further innovations in GE alfalfa.

Summary

The glyphosate tolerant (RR) trait in alfalfa has been found to have a range of pros and cons for growers. The key benefits are the use of an effective, broad spectrum weed management system, lack of herbicide injury, potential for higher quality, increased animal safety, and potential environmental benefits, especially for water quality. The key negatives are lack of residual weed control, the possibility of weed shifts and weed resistance, the potential for unwanted gene flow, and market limitations primarily for export and organic markets. During the process of introduction of RR alfalfa, the need for assurance for non-GE or GE-sensitive markets (especially export and organic) was apparent, and the promotion of ‘coexistence’ or respect for diverse cropping systems has been critical to the success of both non-GE and GE-adapting growers. It is interesting that since 2005 (the year RR alfalfa was introduced) export hay and seed, organic hay, as well as the adaptation of RR alfalfa have all grown by leaps and bounds. Farmers and industry members have demonstrated the ability of diverse systems to coexist, often on the same farm, along with the widely-held belief in the ‘right to farm’ according to farmer preference. Promotion of the ethics of cooperation and respect between diverse systems along with technical tools such as pinning maps, best management practices and certifications have assisted in promoting coexistence between GE and GE-sensitive growers. The introduction of RR alfalfa has provided important lessons on the process of introducing new biotech technologies that may occur in the future.

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