

Roundup Ready Alfalfa Update and New Biotech Traits

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INTRODUCTION

Roundup Ready® alfalfa (RRA) varieties were originally deregulated by the USDA during the period from June, 2005, to May, 2007¹. Separately, FDA and EPA also completed their respective safety assessments of RRA feed/food and Roundup® (glyphosate) herbicide for over-the-top use on RRA. Approximately six months after farmers began to plant RRA, a federal lawsuit was filed by the Center for Food Safety that challenged the USDA's deregulation procedure; specifically, it cited USDA's failure to prepare an Environmental Impact Statement (EIS) prior to deregulation. On May 3, 2007, Judge Breyer issued a permanent injunction that regulated the production of RRA pending completion of an EIS process. During the 22-month commercial period, seed sales of RRA varieties exceeded all expectations with an estimated 300,000 acres of Roundup Ready alfalfa forage production planted and a 98 percent customer satisfaction rating. The new technology was rapidly adopted by forage growers—especially those in regions where weed control has historically been difficult and costly.

The technical justification for the injunction was that USDA's Animal and Plant Health Inspection Service (APHIS) had failed to meet its obligations under the National Environmental Policy Act (NEPA). Judge Charles R. Breyer disagreed with APHIS' 2005 deregulation process: that is, he found that the Environmental Assessment (EA) prepared by APHIS in 2005 had not sufficiently examined the full range of environmental impacts as required by NEPA. Furthermore, he determined that an Environmental Impact Statement (EIS) must be conducted to address the potential impacts of gene flow to genetically engineered (GE) trait sensitive markets (organic and export), plus, it should address the impacts of the herbicide glyphosate use in conjunction with RRA. The judge's ruling did not imply that Roundup Ready alfalfa might be harmful for food or feed nor did he express disagreement with the deregulation determination *per se*. Rather, what was at issue was that the USDA's decision documents did not demonstrate that it took a sufficiently *hard look* at the impacts on the broader *human environment as required by NEPA*. For example, one such potential impact on

¹ **Roundup Ready® Alfalfa is currently not for sale or distribution.** Additional information may be found at: <http://www.aphis.usda.gov/biotechnology/alfalfa.shtml> and <http://www.roundupreadyalfalfa.com>. Monsanto Company is a member of BIO's Excellence through Stewardship (SM) (ETS) Initiative. This product has been commercialized in compliance with the ETS and Monsanto Product Launch Stewardship policies, after meeting applicable regulatory requirements in key export markets with functioning regulatory systems. Any crop or material produced from this product can only be exported to, or used, processed or sold in countries where all necessary regulatory approvals have been granted. It is a violation of national and international law to move material containing biotech traits across boundaries into nations where import is not permitted. Monsanto encourages growers to talk to their grain handler to confirm their buying position for this product. ALWAYS READ AND FOLLOW PESTICIDE LABEL DIRECTIONS. Roundup Ready® crops contain genes that confer tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides. Roundup® agricultural herbicides will kill crops that are not tolerant to glyphosate. Roundup® and Roundup Ready® are registered trademarks of Monsanto Technology LLC. ©2008 Monsanto Company.

humans might be to eliminate a grower's choice to produce organic or purely conventional alfalfa.

As of December 3, 2008, USDA-APHIS is in the process of drafting its EIS (Meier, 2008; current status updates from USDA-APHIS may be found at <http://www.aphis.usda.gov/biotechnology/alfalfa.shtml>). Based on the Court proceedings, it is likely that five principle points of the Environmental Impact Statement will be:

1. Will the deregulation of Roundup Ready alfalfa lead to the transmission of the GE-trait to organic and conventional alfalfa?
2. What is the possible extent of such transmission?
3. How can concerned farmers protect their crops from acquiring the GE gene?
4. Will RRA contribute to the development of glyphosate-resistant weeds, especially when considered in conjunction with other existing Roundup Ready crops?
5. If so, how can farmers address such weeds?

The focus of this presentation will be to address points #1 through #3, i.e., issues specifically related to gene flow. Points #4 and #5—broad issues related to weed population shifts and herbicide resistance are discussed elsewhere (See Duke and Powles, 2008; Orloff et al. *in press*).

U.S. ALFLAFA HAY AND SEED MARKETS

In 2006, 21.4 million acres of alfalfa hay and 3.3 million acres of alfalfa haylage were harvested in the United States (NASS)². The majority of hay produced is consumed domestically with over three-fourths being consumed on the farm where it was produced. Putnam (2005) has estimated that three to five percent of the U.S. alfalfa hay production is sold to GE-sensitive markets (organic or export).

USDA-NASS statistics for alfalfa seed production for 2002 to 2006 ranged from 110 to 122 thousand acres annually with seed total production ranging from 58 to 72 million pounds. Seven western states account for approximately 95 percent of the total U.S. seed production. Just like for seeds of other field crops, professional alfalfa seed growers and seed companies typically utilize State and or OECD Seed Certification Programs. By following U.S. Federal Seed Law and official state seed standards, conventional seed producers routinely limit varietal off-types including GE adventitious presence (AP) to less than one percent. Approximately 70 percent of U.S. seed production is sold domestically and the remaining 30 percent is sold to export markets. The vast majority of U.S. alfalfa seed exports are of the non-dormant (winter-active) variety types produced in concentrated areas of Southern California.

² The National Ag Statistics Service (NASS) collects and publishes data on planted and harvested acres of alfalfa hay, by state. The NASS data also includes an estimate of the % of all hay produced that is sold as organic. The USDA Foreign Ag Service (FAS) collects and publishes data on alfalfa hay and seed exports, by export country.

Roundup Ready alfalfa seed may only be produced under a specific RRA seed company contract (license). The contract requires that both parties abide by many industry-adopted consensus procedures demonstrated as being effective in the avoidance of unintended gene flow (see next section). Since some of the key non-dormant seed export markets do not yet have approvals granted or regulatory procedures enacted, the requirement for RRA seed production isolation distances have been designed to be proactive and rigorous. Isolation requirements have been set to manage adventitious presence of GE-traits in conventional seed to non-detectable levels (zero to near zero). For example, in Southern California where much of the non-dormant export seed is grown under honeybee pollination, the required minimum isolation practices (≥ 3 miles) allow the production of export-quality conventional seeds. This isolation distance is science-based and market-driven; it was developed during a 2005 California alfalfa stakeholder consensus meeting led by the University of California (UCSBC 2005).

Due to serious pest pressures, the use of the organic method of alfalfa seed production in the U.S is exceedingly rare (estimated at less than one-tenth of one percent ($<0.01\%$)). Therefore, most organic certified alfalfa seeds planted in the U.S. are imported from countries with lesser pest pressures (e.g., Canada). Under certain USDA National Organic Program Standard defined circumstances, non-organic seeds are also allowed for the establishment of organic forage fields and pastures. Sprouting seeds are likewise typically imported from non-treated sources because Federal food safety laws preclude the use of many conventional practices (pesticides, desiccants, manuring, sewage-water irrigation, etc.).

GENE FLOW AND STEWARDSHIP

In 2007, to help develop a coexistence strategy, the National Alfalfa & Forage Alliance (NAFA) held a forum entitled, “Peaceful Coexistence: Creating a Strategy for Harmony Among GM, Organic and Conventional Alfalfa Producers” (NAFA, 2008). In attendance were greater than 70 alfalfa stakeholders representing Hay and Seed Growers, Industry Hay and Seed Companies, Exporters, Organic Industry Representatives, University and USDA Research Scientists. The consensus of the attendees was that coexistence is possible provided that trait stewardship programs are in place to enable GE sensitive seed and hay markets. Additionally, stakeholders at the NAFA meeting were in agreement that an adventitious presence (AP) tolerance greater than zero should be established. The forum resulted in the development of a coordinated series of five documents examining potential sources of gene flow and supporting divergent-market coexistence strategies (see <http://www.alfalfa.org/CSCoexistenceDocs.html>; and, CAST, 2008).

In order to manage gene flow from biotech to non-biotech alfalfa it is first necessary to understand potential sources for gene flow. An exhaustive, peer-reviewed scientific report, “Gene Flow in Alfalfa: Biology, Mitigation, and Potential Impact on Production”, has been published by the Council for Agricultural Science and Technology (CAST, 2008). This report is the scientific foundation upon which the industry sponsored coexistence programs and best practices for RRA seed production were structured.

Alongside of the NAFA Best Management Practices for RRA Seed Production which applies to all RRA seed producers, The Association of Official Seed Certifying Agencies (AOSCA), has developed the Alfalfa Seed Stewardship Program. The new AOSCA Program provides a platform by which seed certification agencies can assist alfalfa seed producers in producing seed that will meet the needs of GE-sensitive seed customers. Details of the two complementary programs were recently presented and compared (Moore, 2008).

Table 1. Potential scenarios for pollen-mediated gene flow between conventional (Conv.) and Roundup Ready Alfalfa (RRA) (Table excerpted from CAST, 2008)

↓From To→	Conv. Hay	Conv. Seed	Conv. Feral
RRA Hay	Hay-to-Hay	Hay-to-Seed	Hay-to-Feral
RRA Seed	Seed-to-Hay	Seed-to-Seed	Seed-to-Feral
RRA Feral	Feral-to-Hay	Feral-to-Seed	Feral-to-Feral

Hay to Hay gene flow

The many biological barriers to gene flow—asynchrony of flowering, absence of pollinators, inability of plants to produce viable seed and for that seed to successfully germinate into a viable plant—reduces the probability of successful gene flow between adjacent hay fields to near zero (Putnam, 2006). For organic or AP-sensitive export market hay producers, the purchasing of non-RRA planting seed and timely harvesting of each hay crop before the ripe seed stage will help to ensure near 100% RRA-free hay.

Gene flow to or from Feral alfalfa

Feral plants are crop plants that are occasionally found on roadways, ditches, fence lines and abandoned fields. A multi-state survey found feral plants within 2000 meters of cultivated alfalfa at 22% of the survey sites (Kendrick et al., 2005). While it is reasonable to assume that over time RRA plants may contribute to the feral plant population, there is no evidence to show they will become a disproportionate part of the feral population. Additionally, the same biological barriers that limit hay-to-hay gene flow also exist for feral-to-hay gene flow. Add to this to the facts that feral plants have neither the benefit of irrigation or control of abundant insects detrimental to seed development, the potential

for feral-to-seed field pollen-mediated gene flow is very low (Hammon et al., 2006; CAST, 2008).

Hay to Seed gene flow

Experiments were conducted to measure the potential for pollen flow from late-cut RRA forage plots (20 to 50 percent bloom) to adjacent, actively-pollinated conventional seed plots. These results showed that current isolation requirements of 165 feet for Certified seed production are adequate to manage potential RRA pollen flow to very low levels under honeybee or leafcutter bee pollination of the seed crop (Teuber and Fitzpatrick, 2007).

Seed to Seed gene flow

Certain bee pollinators transfer pollen from plant to plant within an alfalfa seed production field and can occasionally stray to neighboring seed production fields. To meet Federal or international standards of 99% genetic purity for certified seed production, minimum isolation distances between certified alfalfa seed production fields and any neighboring alfalfa seed (or hay) fields are required. To test if current standards are adequate for RRA seed fields, multiple experiments using the Roundup Ready gene as a pollen-marker under leafcutter bee and or honeybee pollination have been completed (See literature review in CAST, 2008).

In production areas using leafcutter bees as pollinators, a 900 ft isolation distance between RRA and conventional alfalfa seed fields managed AP to $\leq 0.5\%$ (a *de facto* domestic industry standard for AP tolerance in conventional seed of other crop species). The 900 ft NAFA Best Management Practices isolation for leafcutter bee pollination is a science-based determination; it is five times greater than the standard isolation requirement for Certified seeds. Nine hundred feet is also the AOSCA Foundation Seed Class isolation standard. In unique geographies of the West where alkali bees pollinate alfalfa, NAFA Best Practices require that RRA be planted with at least 1 mile of isolation to existing conventional seed fields. For areas where honeybees are used, a 3 mile isolation distance between seed fields ensures that AP is zero or near zero (it is non-detectable). The rigorous 3 mile isolation standard is 95 times that of the standard isolation requirement for conventional Certified Seed and has been adopted by consensus of a California alfalfa industry stakeholder group convened by the University of California-Seed Biotech Center in early 2005 (UCSBC, 2005).

Based on the data collected on alfalfa gene flow, a comprehensive best management practice for RRA seed production has been established by NAFA member consensus which includes the following:

1. Isolation distances that are pollinator specific and market sensitive should be maintained. To assist conventional producers in planning their seed field locations, all RRA seed field locations will be reported to local seed certification organizations.

2. Bee management
 1. Location of hives/huts near to center of seed field
 2. Bees may not be moved from RRA to conventional seed field
3. RRA seed field stand take out reporting and monitoring. Routine management of feral alfalfa plants to reduce the risk of gene flow.
4. Proper sanitation of equipment by seed growers and seed processors
5. A coordinated industry sampling program to monitor stewardship requirements directed by the Association of Official Seed Certifying Agencies (AOSCA) and a mechanism for best management protocol modification.

In addition to best management practices for RRA seed producers, the NAFA forum developed other recommendations for seed production intended for GE sensitive markets, such as:

1. Use of increased isolation distances in seed production - including production in non-GE seed production zones. GE-sensitive seed producers can utilize AOSCA's Alfalfa Seed Stewardship Program and should consult with State seed certification programs.
2. Use of GE trait detection methods and seed certification to ensure seed stock suitability.
3. Use of field border areas along with careful selection of pollinator species used.
4. Routine elimination of feral alfalfa plants to reduce the risk of gene flow.

To determine whether the NAFA best management practices for RRA would be effective under actual field conditions, Fitzpatrick, et al. (2007) evaluated more than 122 conventional, commercial seed samples for the presence of the RRA seed that were produced in eight western states in 2006 and 2007. When the percentages of RRA seed were plotted against the isolation distance to the nearest Roundup Ready seed field they found it to be four to five times lower than predicted by the smaller-field research experiments used to develop isolation distances. AP monitoring has been continued by RRA seed-producing companies per the requirements of the NAFA best practices.

NEW TRAITS

Understanding GE sensitive markets and the potential for gene flow from GE to conventional seed or hay is the foundation for developing coexistence strategies. Successful implementation of coexistence strategies not only paves the way for the successful reintroduction of Roundup Ready alfalfa, but it also sets a standard that will enable the introduction of new biotech traits in alfalfa.

The success of Roundup Ready alfalfa has paved the way for new research supporting a second wave of biotech traits. These new biotech traits can be divided into two categories: *output traits* that enhance forage quality and *input traits* that improve efficiency of crop production.

Output traits

Reduced Lignin Alfalfa

Alfalfa is an important source of fiber in most dairy rations. Lignin is an anti-quality compound in alfalfa cell walls that increases with advanced plant maturity, and reduces the digestibility of alfalfa fiber. There are various biotech tools that can be used to turn off or “silence” native genes. Scientists at the Noble Foundation have now systematically silenced virtually each of the genes that code enzymes required for lignin synthesis in alfalfa. Based on multiple lab and field studies initiated since 2000, we have learned that transgenic plants with reduced expression of two key lignin enzymes, COMT and CCOMT have decreased lignin, increased fiber digestibility and acceptable agronomic performance. Elite alfalfa populations containing the COMT- or CCOMT- transgene have been developed. In 2007 hay was produced to that enabled sheep and dairy feeding studies confirming improved animal performance of these reduced lignin alfalfa plants. Positive results from these feeding trials has moved the project into an accelerated development mode. Reduced lignin alfalfa may provide an important new genetic tool for hay producers, providing more flexibility in harvest management and increasing forage quality and/or forage yield.

Tannin Alfalfa

Condensed tannins are a class of phenolic compounds found in many plants. Tannins bind with proteins and slow the rate of protein degradation in the rumen. Tannin containing forages (e.g. birdsfoot trefoil and sanfoin) have more bypass protein and are non-bloating when grazed by ruminants. Alfalfa produces condensed tannins, but only in the seedcoat. Various biotech strategies are being explored for production of condensed tannins in leaves and stems of alfalfa. The U.S. Dairy Forage Research Center estimates that tannin alfalfa could decrease protein feed supplement costs for dairy by 60% and significantly decrease N losses to the environment. In addition, UC Davis scientists are exploring strategies for production of hydrolysable tannins in alfalfa, unrelated compounds with positive attributes similar to condensed tannins.

Input traits

Industry, non-profit and public research institutions are investing several hundred million dollars per year in gene discovery programs aimed at improving crop performance. These genomics-based gene discovery programs are turning up hundreds of gene candidates for numerous value-added traits.

More than half of the alfalfa grown in the U.S. is produced under irrigation or under dryland conditions where moisture commonly limits productivity. Several biotech companies are currently exploring and testing transgenes that increase drought tolerance and water use efficiency when expressed in crop plants. Several of these gene candidates for drought tolerance are now being expressed in alfalfa. In collaboration with Monsanto, Forage Genetics has begun testing several new potential drought resistant transgenic alfalfa plants.

Although genes for increased biomass or delayed flowering are of little interest for grain crops, they offer exciting potential for alfalfa. Several such genes have been identified in general phenotypic assays of new gene candidates and are now being inserted into alfalfa. These new transgenes may offer our best opportunity to significantly increase forage yield in alfalfa.

SUMMARY

It's an exciting time for those involved in alfalfa improvement. Using conventional breeding techniques, alfalfa breeders continue to make incremental progress in improving yield, persistence and forage quality. Biotech traits offer a new and exciting tool for break-through improvements in crop performance and crop value, and stewardship strategies are being refined to facilitate coexistence of biotech, conventional and organic alfalfa production. Several new traits are currently in testing and development in alfalfa. We see significant potential for these to increase forage yield, improve forage quality and/or increase the role of alfalfa in animal diets.

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