

HISTORY OF ROUNDUP READY[®] ALFALFA

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In late 1997 Forage Genetics International (FGI) and Monsanto began a joint project to develop Roundup Ready alfalfa. FGI collaborated with Montana State University to produce the first transgenic Roundup Ready alfalfa plants. The same CP4 glyphosate tolerance gene that has been effectively used in developing multiple other Roundup Ready crops, was successfully inserted into an elite FGI alfalfa plant in early 1998.

EVENT SORTING

A transgenic event is the insertion of a transgene (e.g. CP4) into the plant genome. The physical location of a transgene insertion in the genome is relatively random, and location of the insertion can have a strong influence on transgene expression. For that reason, hundreds of Roundup Ready transformants, called T0 plants, were produced in the laboratory and were screened for tolerance to Roundup[®] herbicide, in order to find elite events. The T0 plants that exhibited Roundup tolerance were transplanted to field nurseries at four FGI research stations for evaluation of agronomic performance. This began a two year process called “event sorting”, where multiple transgenic events were evaluated to identify a few T0 plants that combined agronomic performance equal to the original clone plus commercial tolerance to Roundup herbicide. Two events were ultimately selected for deregulation and commercialization.

TRAIT INTEGRATION

In late 1998 about 50 Roundup tolerant T0 plants were crossed with numerous elite FGI clones representing a wide array of conventional germplasm from very winterhardy (fall dormant 3) to non-dormant (fall dormant 9) types. A modified backcrossing program (“forward breeding”) was used to integrate the Roundup Ready trait into the best FGI FD3 to FD9 germplasm. In each crossing cycle, plants that contained events that were dropped in the event selection process were eliminated and destroyed. By the time the final two commercial events were selected in 2000, the events had been backcrossed for several generations into elite dormant and non-dormant breeding populations. These populations were screened for multiple pest resistance and established in FGI breeding nurseries in Wisconsin, Indiana, Iowa, Idaho and California. Elite plants selected from these nurseries became parents for the first generation Roundup Ready cultivars.

PRODUCT DEVELOPMENT

The term “trait purity” is used to quantify the % of plants in a specific seed lot which express the Roundup Ready trait. The target threshold for minimum trait purity for Roundup Ready alfalfa was established at 90%. For crops with diploid inheritance this level of trait purity can be achieved by simply incorporating the trait in a conventional breeding program using a single transgenic event. The more complex genetics and autotetraploid inheritance of alfalfa required a different approach. FGI developed a marker-assisted breeding system using two independent transgenic events to accomplish the development of Roundup Ready alfalfa varieties with >90% trait purity. All first generation Roundup Ready cultivars will contain both of the commercially registered CP4 events.

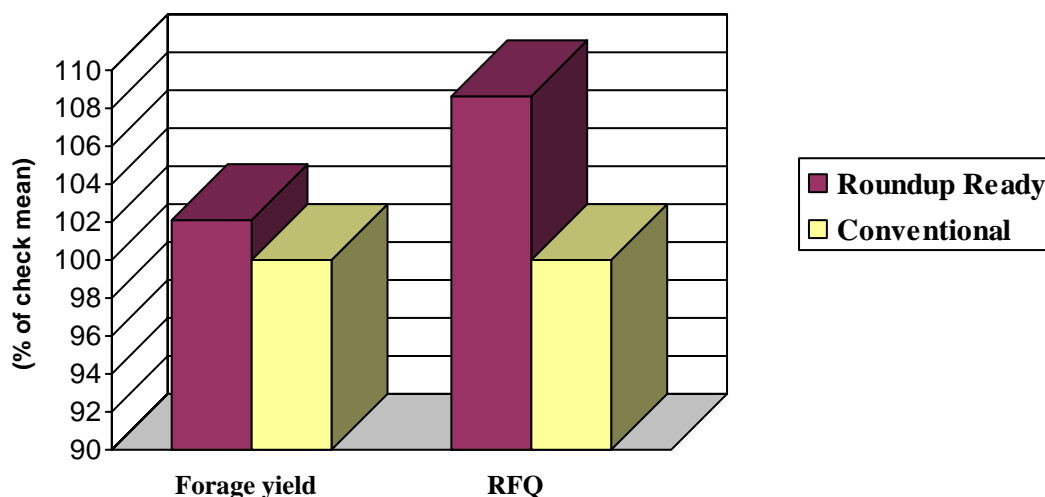
Parents for Roundup Ready cultivars were selected from FGI breeding nurseries for high forage yield, superior forage quality, persistence and tolerance to multiple applications of Roundup herbicide at the maximum labeled rate. Greenhouse crosses were made between plants containing one of each of the two commercial events, and molecular markers were used to identify progeny containing both events (i.e. dihomogenic). The dihomogenic progeny from a particular cross were shipped to Idaho for the production of Syn1 Breeder seed. Varieties produced ranged from FD3 to FD8 types with two or more varieties adapted to each major alfalfa production area in the U.S.

EXPERIMENTAL VARIETY TESTING

Various tests were initiated to evaluate the first group of Roundup Ready alfalfa experimental varieties. Multiple pest resistance, fall dormancy and winter survival were evaluated using standard tests. Forage yield trials were established at multiple locations to compare agronomic performance of the Roundup Ready varieties to a set of commercial check cultivars. Trials comparing forage yield with and without Roundup application were established to monitor crop safety. In these trials the “Roundup treatment” had maximum proposed labeled rates of Roundup herbicide applied at establishment and three times during the first harvest year. The conventional treatment used a conventional herbicide at establishment, with no herbicide treatment thereafter. All Roundup Ready varieties had equivalent performance when treated with Roundup as compared to conventional weed control treatments in these crop safety tests.

Across trials at six locations, 2004 mean performance of the Roundup Ready alfalfa varieties was competitive with the commercial check cultivar mean for forage yield and forage quality (Figure 1).

Figure 1. Performance of RR and conventional alfalfa cultivars



PRODUCT CONCEPT TRIALS

Product concept trials have been established to compare the Roundup Ready system with conventional methods of weed control in alfalfa and to develop regionally relevant “best use” recommendations for the technology. These trials, initiated in 2000, were conducted by university weed scientists and/or forage agronomists. The results of these trials have been used to formulate time and rate application recommendations for Roundup herbicide applied to Roundup Ready alfalfa, establish management options for stand take-out and volunteer management, and provide comparison data on the value of the technology compared to current weed control practices. Data from some of these trials has been published by the scientific collaborators. A summary of these data is available in a revised Roundup Ready Alfalfa Technical Update bulletin. The bulletin is available on-line at http://www.monsanto.com/monsanto/content/media/pubs/alfalfa_tech.pdf.

TRAIT STEWARDSHIP

Trait stewardship generally includes those elements required to insure that a new biotech trait is commercialized using best available practices to minimize potential disruption to current markets. New biotech traits are typically regulated by one or more agencies of the U.S. Federal government, which may include the USDA, EPA, and FDA. Commercialization of biotech traits in the U.S. is only possible after the appropriate agencies have all determined that the technology is safe and therefore the trait can be deregulated. Many other countries have a similar regulatory structure in place for biotech traits in crop plants. A cornerstone of the RR alfalfa stewardship

program has been to insure the trait has been deregulated for feed and food use in the U.S. and for key alfalfa hay export markets. Other important components of the Roundup Ready alfalfa stewardship plan have included: developing a science-based plan for managing pollen-mediated gene flow in commercial seed production (www.foragegenetics.com); availability of simple “protein-based test strips” for determining the presence of the Roundup Ready trait in seed and hay; and, the development of best practices for seed production and processing. The trait stewardship program for Roundup Ready alfalfa is based on programs that have been successfully implemented with other biotech crops and from suggestions and feedback provided from various stakeholders in the alfalfa industry.

SUMMARY

The development of Roundup Ready alfalfa has been an eight year process, which started in 1997 with transformation experiments in the laboratory and which is anticipated to culminate with a 2005 commercial release of elite Roundup Ready cultivars. Concurrent with the technical evaluation activities associated with new product development, FGI and Monsanto have implemented the necessary programs to support U.S. and international regulatory approvals of key importers (e.g., Japan, Mexico, Canada), developed and implemented trait stewardship protocols, and developed practical technical use guidelines for growers. The Roundup Ready alfalfa team at Forage Genetics includes Jose Arias, Holly Deery, Doug Elkins, Sharie Fitzpatrick, Mark McCaslin, Peter Reisen, Stephen Temple, and Joe Waldo. Tom McCoy and Pam Border at Montana State University conducted the Roundup Ready alfalfa transformation. The Roundup Ready alfalfa team at Monsanto includes Dan Foor, Tom Helscher, Bill Hiatt, Louis Meyer, Jennifer Ralston, Carlos Reyes, and Glen Rogan.