

ROUNDUP READY ALFALFA – A PROGRESS REPORT

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ABSTRACT

Roundup Ready alfalfa varieties are currently under development. Field and greenhouse tests in 1999 and 2000 show that RR alfalfa has excellent tolerance to Roundup Ultra™ at all stages of plant development. There appear to be no negative effects of the RR transgene on forage yield or other important agronomic traits and the combination of RR alfalfa/Roundup Ultra™ appear to offer some advantages over other weed control strategies.

Key words: alfalfa, genetic engineering, Roundup, Roundup Ready, weed control

INTRODUCTION

Roundup™ (glyphosate) is a widely used broad-spectrum herbicide with unique, favorable safety characteristics. Genetic engineering has been used to develop Roundup tolerant (i.e. Roundup Ready) transgenic varieties in several crop species (e.g. soybean, corn and cotton). These Roundup Ready (RR) cultivars allow the use of Roundup Ultra™ for broad-spectrum weed control. There is a significant interest in the use of RR alfalfa to improve options for effective, crop-safe weed control, both for stand establishment and for the control of tough perennial weeds in established stands.

The project to develop Roundup Ready alfalfa is collaboration between Monsanto, Montana State University and Forage Genetics International (FGI). Monsanto provided the transformation vectors and expression cassettes, Montana State University performed the transformation and molecular analysis of elite transformation events, and FGI provided the germplasm, and conducted the trait integration and product development.

TRANSFORMATION AND TRAIT INTEGRATION

An elite, proprietary FGI alfalfa clone was transformed with a commercial RR construct by Pam Border and Tom McCoy at Montana State University. Four commercial transgenic events were identified at FGI that combined the following traits: excellent tolerance to Roundup Ultra™, reproductive stability of the transgenic trait, normal seed and forage yield, and simple molecular integration. These four elite transgenic events were backcrossed into four broad genetic backgrounds to enable the development of RR alfalfa varieties adapted to the major market areas in North America. The four genetic backgrounds were as follows:

- FD3HQ – FGI fall dormancy 3 types adapted to the northern U.S. with excellent forage yield potential, exceptional winter hardiness, multiple pest resistance and enhanced forage quality.
- FD4 – FGI fall dormancy 4/5 types adapted to the northern and central U.S. with exceptional forage yield potential, winter hardiness and multiple pest resistance.

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- GH – FGI glandular-hair populations with excellent forage yield potential and high resistance to potato leafhopper.
- FD8 – non-dormant FGI breeding populations adapted to the San Joaquin Valley of California and the Desert Southwest with excellent forage yield potential, persistence and multiple pest resistance.

1999 FIELD TESTS – VEGETATIVE TOLERANCE

In 1999, elite MBC1 (modified backcross 1) RR lines (events) and appropriate control lines were established in field tests to evaluate tolerance to Roundup Ultra™ in alfalfa forage production. These tests were established at West Salem, WI, Boone, IA, West Lafayette, IN and Nampa, ID. The events were evaluated at each location, with the FD4, GH and FD8 germplasm groups represented across locations. This allowed the evaluation of all elite events over multiple environments and in three distinct germplasm backgrounds.

The MBC1 plots were established with RR and non-RR plants derived from the same population. The non-RR plants were used as negative controls. One commercial check per germplasm group was also included in the trials. Plots in this study were composed of eight plants spaced 15” apart within a row. Two spray treatments - Roundup Ultra treatment and non-treated control, were included in a replicated, split-plot design. In the sprayed treatment Roundup Ultra was applied in June, July and October of the 1999 growing season. The Roundup Ultra application rate was 4 qts/A in June and July and 2 qts/A in October. Non-sprayed plots were kept weed-free by cultivation. Sprayed plots were likewise cultivated to afford similar tilth.

The table below summarizes forage yield of 12 elite RR lines pooled over locations and genetic backgrounds.

RR Alfalfa Event Sorting Summary – 1999 Forage Tolerance Tests
(1999 total kg fresh weight/plot)

Herbicide treatment	Elite RR lines	Negative Control	Commercial checks
Roundup Ultra application	4.15	0.00	0.00
Non-sprayed control	4.25	4.14	3.35

There was no significant difference in forage yield between the RR elite MBC1 mean and the negative control in the non-sprayed tests. This suggests there is no yield drag associated with the RR transgene in the elite transgenic events. There was also no significant difference between RR elite MBC1 mean yield in Roundup treated plots vs. the non-treated control. This demonstrates the effectiveness of the transgene in providing tolerance to Roundup at high application rates (3.5 gallons cumulative/A in 1999). In the non-sprayed treatment the MBC1 populations had significantly higher yield than the commercial checks in all three germplasm backgrounds. This demonstrates the effectiveness of using a modified backcrossing method for forward breeding to insure competitive agronomic performance of transgenic alfalfa cultivars.

1999 FIELD TEST – REPRODUCTIVE TOLERANCE

A second study was conducted at Nampa, ID to assess reproductive tolerance (seed yield) of the same elite RR MBC1 lines under four Roundup application treatments.

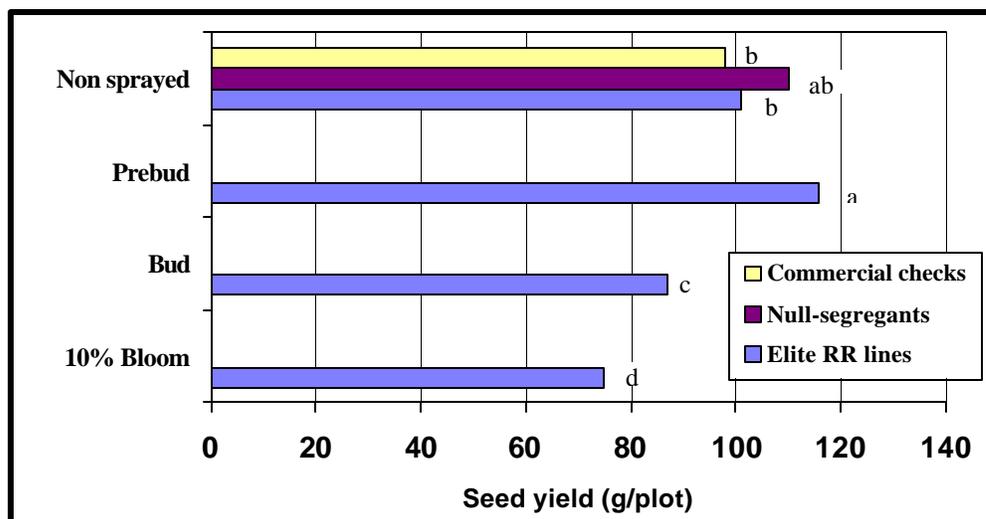
The FD4 and FD8 MBC1 germplasm backgrounds were evaluated. The following Roundup application treatments were used:

- Prebud stage Roundup application of 4 qts/A
- Bud stage Roundup application of 4 qts/A
- 1/10 bloom stage Roundup application of 4 qts/A
- Non treated control

The elite RR lines were again compared with their near isogenic negative controls (developed as described above) and commercial checks. Plots in this study were composed of five plants spaced 15” apart within a row. Non-sprayed plots were kept weed-free by cultivation. Sprayed plots were likewise cultivated to afford similar tillth.

The figure below summarizes seed yield of 12 elite RR lines pooled over genetic backgrounds. These elite lines were selected based on detailed molecular analysis of the transgene insert, trait stability in advanced generations, and performance in the 1999 field tests.

RR Alfalfa Event Sorting Summary – 1999 Reproductive Tolerance Test



In other species, RR transgenic plants have been more sensitive to Roundup Ultra application in reproductive development than in the vegetative stage. This appears to be the case in alfalfa. Roundup Ultra application after the bud stage had a negative effect on seed yield of elite RR alfalfa lines. The more advanced the reproductive development at herbicide application, the more reduction the seed yield. Seed yield was significantly enhanced by Roundup Ultra application in the pre-bud stage. There was no significant difference in seed yield between the elite RR lines, the negative controls and the commercial checks in the non-sprayed control treatment.

2000 FIELD TESTS – WEED CONTROL COMPARISONS

In May 2000, RR test plots were seeded in West Salem, WI, Lafayette, IN, Ames, IA and Nampa, ID. These tests were designed to compare the combination of Roundup UltraTM/RR alfalfa with Pursuit/Post Plus and no chemical weed control. The herbicide treatments were applied only on the first (July) harvest. The yield summaries below are a sum of three harvests at Nampa, ID. Forage quality (first and second cut mean) is expressed as a percentage of the non-sprayed check.

Variety	Treatment	DM T/A	Forage quality
RR alfalfa	Roundup Ultra	4.17	105.2
RR alfalfa	Pursuit/Post+	3.42	105.3
RR alfalfa	No weed control	4.56	100.0

In three of the five locations, significant stunting and yield loss were associated with the Pursuit/Post+ treatment. This was most evident in the first cutting, but noticeable throughout the season. This suggests crop safety risk under some environmental conditions with this commonly used herbicide treatment. At all locations the RR/Roundup Ultra treatment provided the best weed control, but both herbicide treatments showed significantly better weed control than the non-sprayed check. At Nampa, heavy weed infestation in the first cut had a significant positive effect on yield, and a significant negative effect on forage quality. Both effects decreased in the second and third harvests.

The data from these trials illustrate three advantages of RR alfalfa/Roundup Ultra:

- 1) Improved crop safety/increased flexibility of application timing – current data show that RR alfalfa can be sprayed at any stage of development and under various environmental conditions without concern for crop safety. Virtually all other chemical weed control options pose some crop safety risk when not applied under very specific conditions (e.g. crop growth stage, ambient temperature, etc.).
- 2) Improved weed control – at all locations RR alfalfa/Roundup Ultra provided virtually perfect weed control, significantly better than the other treatments.
- 3) Enhanced forage quality – significant weed biomass in the non-sprayed check plots led to higher yield than the herbicide treatments, but significantly lower forage quality. Poor weed control during establishment often results in the production of lower value hay, due to poor forage quality of weedy alfalfa.

PRODUCT DEVELOPMENT

RR alfalfa varieties are now being developed. The RR transgene has been backcrossed into a wide variety of germplasm sources. RR alfalfa varieties will be available with excellent adaptation to most major alfalfa production areas in the world (FD3 – FD9). Preliminary forage yield data suggests that the first RR alfalfa varieties will yield equal to or better than commercial check cultivars of the same fall dormancy. RR alfalfa cultivars will also have the excellent

multiple pest resistance and high forage quality that characterize many of the newer commercial varieties.

All RR alfalfa varieties will have at least 90% trait purity. This means that $\geq 90\%$ of the plants in a RR variety will show tolerance to RoundupTM. The excellent herbicide tolerance of RR alfalfa varieties will allow flexibility in application timing, making the technology a useful tool in both stand establishment and in the control of perennial weeds in established alfalfa. Product concept tests, to help identify best agronomic practices for optimizing use of the technology, will be carried out on a regional basis by agronomists and weed scientists at several major universities.

Commercial release of RR alfalfa is expected in 2004 or 2005. The date of release is primarily dependent on the duration of review prior to regulatory approval. Multiple location forage yield trials of RR alfalfa experimentals will begin in late 2001. All Roundup Ready alfalfa varieties will meet the trait purity and product performance standards established by Monsanto