ROUNDUP™ Pre-emergence Treatment to Determine the Presence of the Roundup Ready™ Gene in Soybean Seed: A Laboratory Test.

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ABSTRACT

A laboratory test for determining the presence of the Roundup Ready™ gene in soybean seeds was developed by the ISU Seed Testing Laboratory and approved by Monsanto. The procedure recommended to evaluate the percent expression of the Roundup Ready™ gene includes a seed lot of unknown tolerance and two controls, a susceptible soybean seed lot and a known Roundup Ready™ soybean seed lot. All seed lots are imbibed in a 2% solution of the ROUNDUP™ ULTRA formulation (41% active ingredient), for a concentration of 0.82% active ingredient, glyphosate, in the solution. Two replications of 100 seeds of each lot are placed overnight in paper towels treated with the ROUNDUP™ solutions. Imbibed seeds are then germinated following the prescribed procedure for soybeans (AOSA Rules for Testing Seeds) and evaluated after 7 days. A standard germination test is also planted to use as a comparison of the abnormal seedlings. Susceptible seeds present severe toxicity symptoms. Radicles of the affected seedlings are yellow to brown and stunted, with little or no secondary root growth. Seedlings of Roundup Ready™ soybeans develop normally.

EXPERIMENTAL TECHNIQUES

During preliminary developmental work, two methodologies were used: pre-emergence and post-emergence application of full strength herbicide solution. Planting and evaluation of results were similar in both methods, except for the time at which the herbicide solution was applied. The pre-emergence method allowed the seeds to imbibe overnight in paper towels treated with the ROUNDUP™ solutions prior to planting on crepe cellulose paper. The post-emergence method consisted of germinating the soybean seeds on crepe cellulose paper and spraying the 7-day-old seedlings with the ROUNDUP™ solutions. This second method was later abandoned because of the longer duration of the test (7 days to grow seedlings and 10 days after spraying for symptoms to appear).

Seeds from 10 susceptible soybean seed lots and one Roundup Ready™ soybean seed lot were imbibed in the ROUNDUP™ solutions. Two concentrations were used: 2% and 3% of the ROUNDUP™ ULTRA formulation (0.82% and 1.23% active ingredient glyphosate, respectively), following product manufacturer's recommendations. Two replications of 100 seeds were imbibed

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FIGURE 1. Herbicide pre-treated, susceptible seedlings presenting severe toxicity symptoms, with yellow to brown and stunted radicles, and little or no secondary root growth.

FIGURE 2. Herbicide pre-treated, Roundup Ready™ soybeans, normal seedlings.

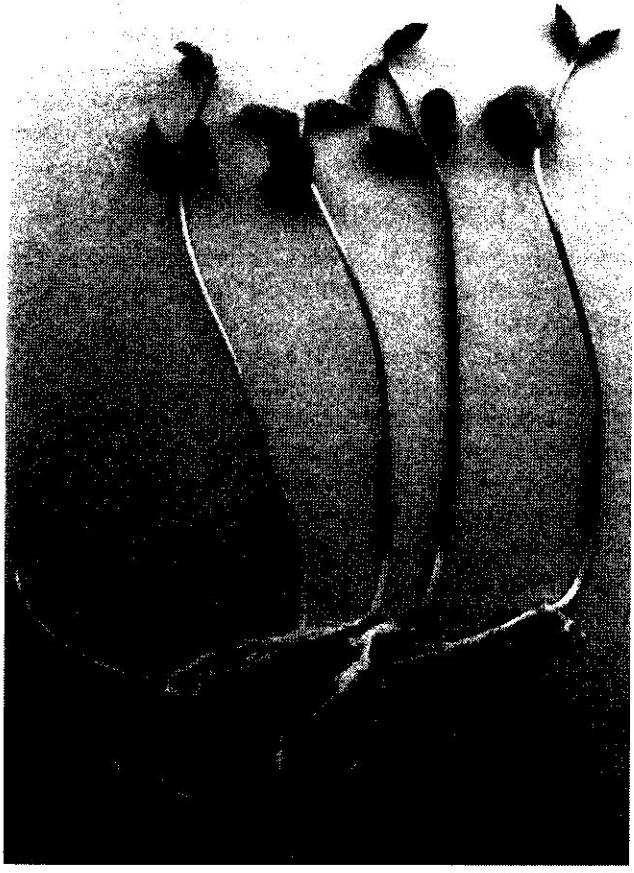
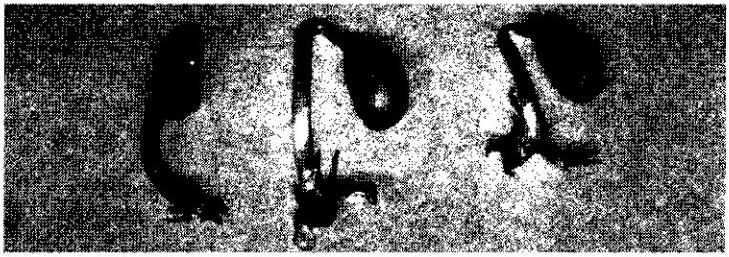


FIGURE 3. Herbicide pre-treated, abnormal seedlings for reasons other than herbicide toxicity, i.e. mechanical damage, lesions extending into the central conducting tissue and/or insufficient roots.



between paper towels treated with the ROUNDUP™ solutions for 18 hours at room temperature. A known Roundup Ready™ soybean seed lot was also tested for a control. Seeds were planted on crepe cellulose paper (Kimpak™), covered with moist sand (approximately 1 inch deep) and placed at 25 °C with 12 hours of light. After 7 days the sand was removed, and tests were evaluated for normal and abnormal seedling development following the AOSA (Association of Official Seed Analysts) Rules for Testing Seeds (1995).

RESULTS AND DISCUSSION

After 7 days, herbicide pre-treated seedlings of the susceptible seed lots presented severe toxicity symptoms (Figure 1). Most seedlings ceased radicle growth, however, elongation of the hypocotyl occurred in some seedlings. Radicles of the affected seedlings were yellow to brown and stunted, with little or no secondary root growth. These seedlings are classified as susceptible. Seedlings of Roundup Ready™ soybeans were fully developed (Figure 2) and are classified as normal. Abnormal seedlings for reasons other than herbicide toxicity (i.e. mechanical damage, lesions extending into the central conducting tissue and/or insufficient roots) (Figure 3) were removed during evaluation and not included in the calculation of the percent Roundup Ready™.

Seedlings are classified into four categories. The first three are according to the AOSA Rules for testing seeds: normal seedlings, abnormal seedlings and dead seed. Abnormal seedlings and dead seed are not included in the equation. The fourth category comprises seedlings with herbicide toxicity symptoms and are classified as susceptible seedlings. The percentage of Roundup Ready™soybeans was calculated using the formula:

$$\frac{\%\ \text{Normal}}{\%\ \text{Normal} + \text{susceptible} \times 100} = \%\ \text{Roundup Ready}^{\text{TM}}$$

The results for the 11 seed lots evaluated are presented in Table 1.

CONCLUSIONS

The pre-emergence treatment of soybean seed using either 2% or 3% solution of the commercial formulation of ROUNDUP™ (41% active ingredient)

TABLE 1. Percentage of Roundup Ready™ soybeans in eleven samples tested using the pre-emergence herbicide-application method. Lots 1 to 10 are susceptible to the herbicide ROUNDUP™ and lot 11 is resistant.

Herbicide concentration	Lot Number	Normal	Susceptible	Abnormal & Dead	Calculation	Roundup Ready™
		%				
2%	1 to 10	0	100	—	0/100	0
	11	98	0	2	98/98 + 0	100
3%	1 to 10	0	100	—	0/100	0
	11	99	0	1	99/99 + 0	100

proved effective. The presence or absence of the Roundup Ready™ gene in soybean seed and seedlings was clear. The characteristic discoloration of the roots and the lack of secondary root growth allowed for easy screening of susceptible seedlings .

Since there was no difference in the results when using 2% or 3% ROUNDUP™ solutions, we recommend the use of the more diluted, 2% solution. The total length of the test is seven days.

Some considerations when testing for herbicide resistance:

- A susceptible and resistant check sample should be planted in conjunction with the herbicide test as a comparison, to assure proper solution concentrations.
- A placebo (water) test should be conducted on the sample to aid in the correct evaluation of abnormal seedlings.
- All equipment used in herbicide-resistance testing should be clearly labeled, and used exclusively with the same herbicide. Resistance to one herbicide does not confer resistance to all herbicides.

The report to the customer should include the germination results based on a standard germination test according to the AOSA (Association of Official Seed Analysts) and the percentage of the normal seedlings that express the Roundup Ready™ gene.

REFERENCES

AOSA Rules for Testing Seeds. 1995. Ed. Association of Official Seed Analysts, Journal of Seed Technology, Vol. 16:3, 1993 (1995 update).