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ROUNDUP READY ALFALFA TEST KITS AND INFLUENCE ON THE MARKET PLACE

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A GMO (genetically modified organism) debate that has continued in the past few years on crops such as corn, cotton, canola, soybean, and wheat as well as other crops has also targeted alfalfa. With the released date of herbicide resistant alfalfa coming closer, there are concerns about the effects of a GMO on the forage export marketplace. Although export alfalfa is produced in a smaller area in the United States than wheat, the same concerns for wheat can be made for alfalfa. Many growers and exporters agree that herbicide resistant alfalfa cultivars would help ease the production of weed free hay. However, they fear that the introduction of Roundup Ready alfalfa could dramatically harm the export market. Seventy-five percent of export hay from the US goes to Japan, 16% to Korea, and 7% to Taiwan. The Pacific Rim countries import about 99% of all hay exported from the US. Therefore, they have the influence on the type of product required for their use for livestock.

Japan Product Approval for Foods and Food Additives Produced by Recombinant DNA Techniques

The Japanese Ministry of Health and Welfare has been assessing the safety of foods and food additives produced by recombinant DNA since 1991. Early on, the safety assessment of such foods and food additives was conducted on a voluntary basis.

Because foods, and food additives of this type are expected to increase, the Ministry of Health and Welfare decided to introduce mandatory requirement for safety assessment of such foods and food additives and published relevant announcements to amend existing regulations on May 1, 2000 in order to avoid the distribution of such foods and food additives that has no safety assessment. According to these announcements, any foods and food additives produced by recombinant DNA techniques that have no safety assessment shall be neither imported nor sold in Japan on and after April 1, 2001.

So far, in Japan, 59 genetic events have been evaluated based on the Guideline for safety assessment, declared by the Food Safety Investigation Council and have been confirmed individually by the Minister for Health and Welfare (MHLW). The crops involved so far are Potato, Soybean, Sugar Beet, Corn, Canola and Cotton. As of October 7, 2004, two new crops appeared on the "list of products whose safety assessments are being examined by the MHLW". They include a virus resistant Papaya and three herbicide tolerant alfalfa products. As of October 2005, the herbicide tolerant alfalfa products have been moved to the list of products that have been evaluated. The alfalfa products were Roundup Ready submitted by Monsanto Japan Ltd. and developed by Monsanto Company and Forage Genetics Inc. (USA). Because herbicide resistant crops have made up over 70% of the foods that have been evaluated and confirmed, it is likely that

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Roundup Ready alfalfa will be approved. However, the issues are more of a concern with the customer than with government approval. Most of the alfalfa hay customers have indicated a low tolerance for GMO's in hay products.

Washington Alfalfa Hay Production

According to the USDA National Agricultural Statistics Service, the value of all hay in Washington in 2004 was about $371 million while the value of alfalfa hay was about $252 million. Among agricultural commodities, hay value ranks sixth in Washington. Figure 1 shows the acreage of the four top hay producing counties in the state while Figure 2 shows the alfalfa hay production for the same counties. Grant, Adams, Benton and Franklin Counties have about 50% of Washington's alfalfa hay acreage while producing almost 70% of the alfalfa hay. Although Washington's alfalfa hay exports are estimated to be about 20%, hay processors estimate that 35-50% of the export alfalfa hay is produced in the Columbia Basin where the four primary counties exist.

Firgure 1: Four County Alfalfa Acreage

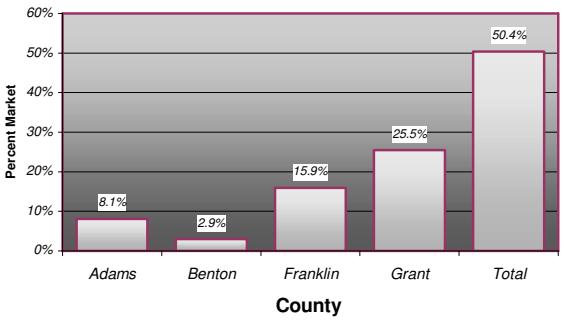
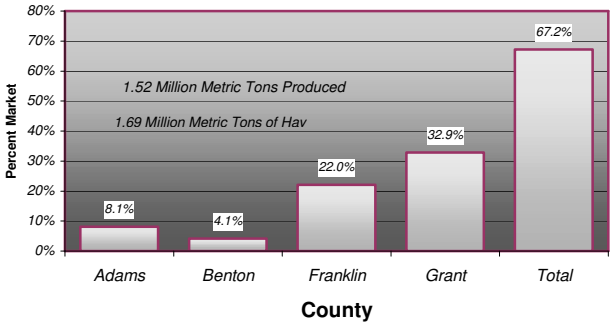


Figure 2: Four County Alfalfa Production



Forage Export Market

Exports are an important market for the PNW forage producer. The primary export products produced in Washington are double compressed bales of alfalfa and timothy as well as alfalfa cubes. The United States exported almost 3 million metric tons of forage products to the Pacific Rim in 2004. Japan's share of the market was about 74 percent with Korea's share at 19 percent. Taiwan's share was 5.7%.

While Japan's alfalfa cube imports from the US continued to decline (Figure 3), baled hay imports continue to grow, exceeding the 1.6 million metric tons mark in 2003 (Figure 4). Japan's total hay imports from the US passed the 2 million metric ton mark in 2003 (Figure 5) and will likely continue in 2004. The value of forage products as received by Japan from the US approached $480 million in 2003. With 59% of the market, the value for the PNW would be about $283 million and at 30 to 50% of the PNW's share, the value for the Columbia Basin could be estimated at $85 to $142 million. In 2003, about 70% of forage products for Japan were shipped from the United States with about 17% from Canada and 11% from Australia.

Figure 3: Total US Alfalfa Cube Imports (Japan)

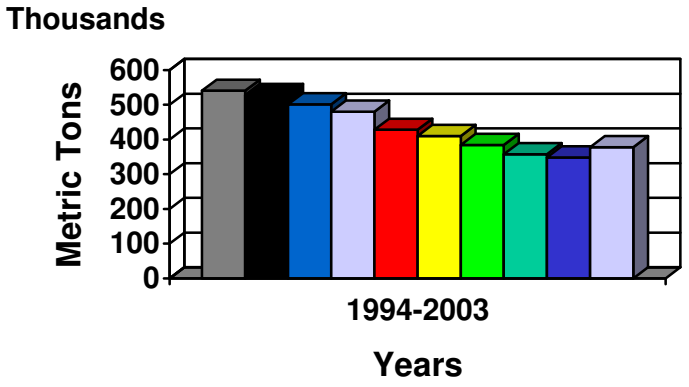


Figure 4: Total US Baled Hay Imports (Japan)

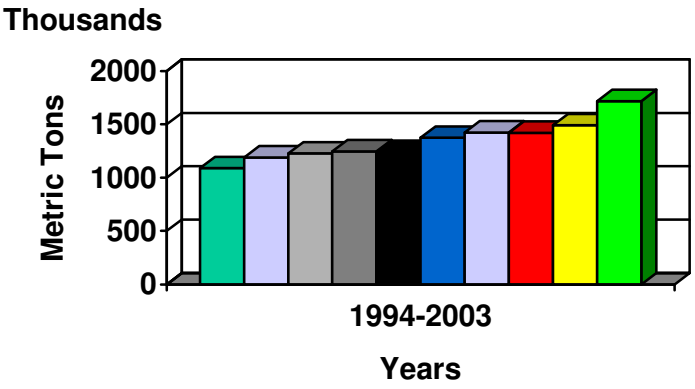
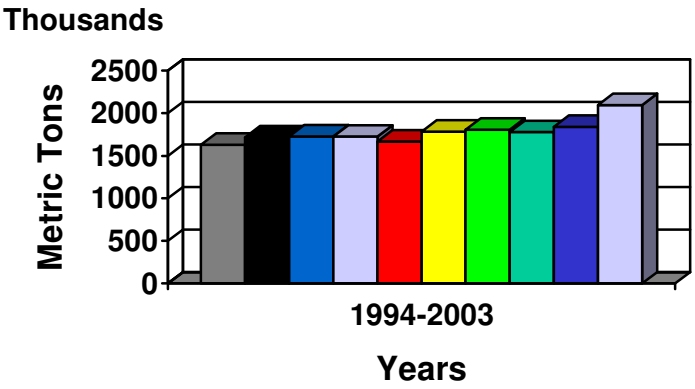


Figure 5: Total US Forage Imports (Japan)



Production Considerations

Although there could be some concerns with alfalfa used in the health and sprout market, most of the concerns involve hay headed for the export market. It is questionable if the health or sprout market knows that an alfalfa GMO will be on the market and that they might have to test for its presence.

Because 99% of hay export is from the western states, the western producers will need to know where the crop will be marketed. This will require some planning, as alfalfa is a perennial crop with stands lasting up to eight years and harvested up to five times per year in the PNW. Furthermore, the grower may export only one of those harvests in a year. It is estimated that over 80% of the growers in the Columbia Basin in Washington will export at least one of their harvests per year.

A hay processor and exporter will need to know if there is a tolerance level for the Roundup Ready gene. Although the Japanese Government might set a tolerance level of 5% for the GMO, the customer may want as little as 0.1% tolerance. Many of the alfalfa processors and exporters have indicated that their Japanese customers have asked that there be no GMO's in their forage products.

Pollen from an adjacent field can contaminate a neighboring field as the pollinator (bees) can fly several miles. The contamination could occur in both the seed field and, to a lesser extent, in a hay field. The most likely contamination could be in purchased seed because of seed production practices that may not allow adequate isolation distances. Although contamination is expected to be low, tests may unexpectedly detect the Roundup Ready gene. It is difficult to certify that a non-GMO will not be contaminated if grown in an area where GMO alfalfa cultivars are produced.

Tests and Sampling Procedures

ELISA tests need to be developed for sensitive, quick and easy use. Each haystack could be potentially sampled and tested by the producer or the processor. A sampling procedure needs to be developed that is simple and acceptable to the export markets. The procedure will need to state sample numbers and size based on the volume of hay being tested. There will need to be instructions on using the tests for judgment of the results. The same type of procedures will need to be developed for sampling containers both before and after loading.

Liability and Contracts

Segregation of hay and seed may not be simple. Non-GMO alfalfa seed may contain low levels of GMO germplasm. Because of this, growers will want to save a sample of the seed lot and a sample of the hay lot. Contamination from pollen or volunteers from a GMO previous alfalfa crop may add to the level of detection. There are risks that could occur from planters, balers, storage areas, and trucks.

Producers will need to be careful if they are asked if the crop is not GMO. It is important that the grower watches what is signed or said. There is one exporter that stated on his contract, prior to the release of Roundup Ready alfalfa products in 2003, that no GMO is to be tolerated in

purchased hay. It is expected that more hay exporters will incorporate statements such as this in their contracts. The grower will need to stick to statements such as:

- The seed company stated that the seed I planted was not GMO.
- Care was taken to avoid contamination by equipment and storage areas.

They should not make statements such as:

- The crop has no GMO germplasm.
- No contamination has occurred from equipment or storage.
- No contamination has occurred from pollen drift.

Testing for Adventitious Presence in Roundup Ready Alfalfa

Although Roundup Ready alfalfa would provide for a clean field of alfalfa, the export market should be a consideration prior to release of a cultivar. Because the western alfalfa producers are more likely to use a herbicide for weed control than mid-western and eastern US producers, western alfalfa production would be a likely target for herbicide resistant alfalfas. However, the export hay market is important to both processors and alfalfa producers in the west where 99% of the export market occurs. There is a need to be well prepared with procedures for sampling and testing prior to Roundup Ready alfalfa cultivars appearance on the market.

Considering the lack of a procedure to determine adventitious presence of Roundup Ready alfalfa in haystacks, a collaborative study was established among Washington State University (William T. W. Woodward), University of California (Dan Putnam), and Forage Genetics International (Peter Reisen).

Test Kit Companies

Two companies, Strategic Diagnostics Inc. and EnviroLogix, were selected by Forage Genetics and Monsanto to develop test kits for determining the presence of adventitious Roundup Ready (RR) alfalfa in haystacks, hay fields and seed. The test kits were developed by the end of the summer of 2005. The Lateral Flow Test Strip in a double antibody sandwich format. The protein caused by the RR gene is coupled to a color reagent and binding occurs between antibody and protein resulting in red color. When reading the test strips, the presence of one line indicates a negative sample while the presence of two red line indicate the presence of the protein, thus the presences of RR alfalfa. The seed test is to determine presence of adventitious seeds in a non-GMO alfalfa while the fresh hay test is to allow a grower to determine which of his alfalfa fields are Roundup Ready or non-GMO. The cored and ground hay test is to allow a grower, buyer, processor or exporter to determine the adventitious presence of RR alfalfa for sensitive markets such a Japan, Korea and Taiwan. Both EnviroLogix and Strategic Diagnostics Inc. have the protocol for running these tests on their websites at:
http://www.sdix.com/ProductSpecs.asp?nProductID=19,
http://www.envirologix.com/library/AS045AHF.pdf

RR Hay Strip Test

The objective for the cooperative hay test was to verify the accuracy of test strips for detecting the presence of Roundup Ready Alfalfa in non-Roundup Ready hay bales and to determine a protocol for sampling procedures to assure detection.

Four 1 acre fields were planted with alfalfa containing 0%, 1%, 5% and 10% mixtures of RR alfalfa seed with non-RR alfalfa seed. The seeding rates were 15 lbs. per acre. Each field was harvested in a fashion to ensure that no contamination was allowed on adjacent treatments. Two cuts were harvested and stacked in separate haystacks. Because it is recommended to sample 20 bales at random for running hay quality tests, five replicated random samples were taken from each haystack treatment over two cuts. Samples were both cored and ground to evaluate procedures for both cored and ground samples for both companies test kits. Tests were then run at three locations including University of California at Davis, CA, Forage Genetics in Idaho and Washington State University in Pasco, Washington.

Results

Results so far have been obtained for the second cut and the results are presented in Table1 for the core samples and Table 2 for the ground samples.

Table 1: Test Results for Cored Samples

			11-Nov-05		18-Nov-05		1-Dec-05	
			ENV	SDI	ENV	SDI	ENV	SDI
cut	trt	rep	ID	ID	WA	WA	CA	CA
cut 2		1	-	-	-	-	-	-
cut 2		2	-	-	-	-	-	-
cut 2	Zero %	3	-	-	-	-	-	-
cut 2		4	-	-	-	-	-	-
cut 2		5	-	-	-	-	-	-
cut 2		1	-	+	+***	+*	+***	+**
cut 2		2	+*	+	+**	+	+***	+**
cut 2	One %	3	-	-	+**	+**	-	+***
cut 2		4	-*	+	+*	+*	-	+**
cut 2		5	+*	+	+**	+**	-	+**
cut 2		1	+	+	+*	+	+	+
cut 2		2	+	+	+*	+	+***	+*
cut 2	Five %	3	+	+	+*	+	+***	+
cut 2		4	+	+	+*	+	+	+
cut 2		5	+	+	+	+	+*	+
cut 2		1	+	+	+	+	+	+
cut 2		2	+	+	+	+	+	+
cut 2	Ten %	3	+	+	+	+	+	+
cut 2		4	+	+	+	+	+	+
cut 2		5	+	+	+	+	+	+

Table 2: Test Results for Ground Samples

cut	trt	rep	11-Nov-05 ENV ID	11-Nov-05 SDI ID	18-Nov-05 ENV WA	18-Nov-05 SDI WA	1-Dec-05 ENV CA	1-Dec-05 SDI CA
cut 2		1	-	-	-	-	-	-
cut 2		2	-	-	-	-	-	-
cut 2	Zero %	3	-	-	-	-	-	-
cut 2		4	-	-	-	-	-	-
cut 2		5	-	-	-	-	-	-
cut 2		1	-	-	+***	+***	-	+**
cut 2		2	-*	+*	+***	+**	+***	+*
cut 2	One %	3	-	+*	+***	+**	+***	+*
cut 2		4	-*	+*	+**	+**	-	+*
cut 2		5	-*	+*	+***	+**	+***	+*
cut 2		1	+	+	+	+	+**	+
cut 2		2	+*	+*	+	+**	+***	+
cut 2	Five %	3	+	+	+	+*	+***	+
cut 2		4	+	+	+	+	+	+
cut 2		5	+	+	+	+	+*	+
cut 2		1	+	+	+	+	+*	+
cut 2		2	+	+	+	+	+	+
cut 2	Ten %	3	+	+	+	+	+	+
cut 2		4	+	+	+	+	+	+
cut 2		5	+	+	+	+	+	+

Conclusions

1. The test kits identified zero percent RRA 100% of the time.
2. Test kits identified five percent RRA 100% of the time.
3. Tests are fast and easy to use.
4. Test kits were developed to determine five % or greater RRA and would not be recommended for stating that hay has any less than 5%.