Measurement of Gene Flow in Alfalfa Seed Production using the Dominant Red Root Genetic Marker

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In anticipation of transforming alfalfa in the late 1980s, the dominant red root genetic marker in alfalfa was used to measure gene flow in alfalfa seed production. This spontaneous mutant in alfalfa was used to avoid the chance of recombinant DNA escaping into the alfalfa population.

Two rectangular fields, each about one hectare, were direct seeded in 1989, and data collected in 1989 and 1990. Fields were on Wisconsin Agricultural Experiment Stations, located about 25 miles apart at Madison and Arlington, respectively. Red root alfalfa, Wisconsin 90 -10, was seeded at a rate of about ten pounds per acre in a 15 X 15 meter plot in the center of each field, and Vernal alfalfa seeded at the same rate in the remainder of each field. The first growth of each field was allowed to flower and set seed in 1989, whereas in 1990, the second cutting was allowed to flower and set seed.

Leafcutter bee boards with about 1000 holes were placed in each red root plot, and foraging honey bees and bumble bees were observed in both fields. Each field permitted monitoring pollen flow from the red root plot to about 80 meters, and volunteer alfalfa plants in fence lines extended the measurements to 140 meters, at both locations. Results were essentially the same at both locations.

Pollen flow was measured by the number of red root progeny in seed samples collected at increasing distances from the red root plots (Fig. 1). Red root pollen flow dropped off quickly over a relatively short distance of 10 meters, but never reached zero out to the 140 meter limit of measurement. Furthermore, it must be assumed that insects transported by wind and hitchhiking preclude absolute containment of pollen flow.

A study using RAPD markers in alfalfa to study pollen movement under seed production conditions in Washington State, concluded that complete containment of transgenes within alfalfa seed and hay production fields would be highly unlikely (P.C. St. Amamd. D.Z. Skinner, and R. N. Peaden. Theor. Appl. Genet. (2000) 101:107 -114.).

The following is an abstract handout for an Environmental Protection Agency workshop held at Baltimore, Maryland in 1992. The abstract contains general comments about containment of transgenes, and a summary of the red root marker results. In view of the fact that only sterile plants will provide complete containment, a strategy was developed for alfalfa whereby a transgene can be co-segregated with a sterile trait to produce a genotype with no flowers, no

pollen, no seed, and therefore no risk of transgene flow or escape. Once such a genotype is selected, it would be cloned for production of a value-added trait for specialized purposes. The system is called Sterile Alfalfa For Experiments – SAFE. At the time the system was developed, laboratories in California and Canada were experimenting with production of a toxin for research, on the one hand, and various pharmaceuticals on the other. The stock segregating for the sterile condition in alfalfa, and the SAFE strategy, were released as germplasm from the Wisconsin Agricultural Experiment Station, and provided to the interested parties. Clonal reproduction would only be practical for high value products with limited acreage requirements, but the technology exists to do this when it is needed.

Details of the SAFE strategy are reported in Volume 2 of this web site.

The following is an abstract passed out at an Environmental Protection Agency workshop held at Baltimore, Maryland in 1992. The abstract contains general comments about containment of transgenes, and a summary of the pollen flow results using the red root marker in alfalfa.

Advance to the next page to read the abstracted from the EPA workshop

Statement for EPA Meeting

Control of Pollen Flow from Recombinant Plants via Reproduction Strategies

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Complete prevention of pollen flow requires some form of sterility. Several plants currently are utilized as vegetatively reproduced sterile forms. Such sterile forms could be developed in most other species. This could be done for special cases; however, the majority of recombinant plants for research or utilization probably will not require such precautions.

Banana is a sterile triploid that is vegetatively reproduced, garlic has not been sexual for centuries, some white and sweet potato cultivars are sterile, as are several other root crops. Then, there are seedless grapes, watermelons, cucumber, and certain citrus cultivars. Sugar cane has limited seed production, as do many fruits and berries, and sterile types could be selected. These sterile plants are immediate candidates for recombinant products that are considered risks to the environment. Some pharmaceutical and chemical products and resistances may fall into this category.

In crop plants that are reproduced and/or utilized as seed, gene flow may be controlled by genetic manipulations using existing mutants and breeding strategies. An example is development of sterile forms of recombinant alfalfa genetically engineered to produce industrial enzymes. In some cases, sterile plants may be desired to prevent gene flow to normal alfalfa fed to livestock. In special cases, it may be desirable to prevent pirating of a valuable product.

Three separate sterility mechanisms in alfalfa can be dovetailed with genetic engineering to produce value-added products in vegetative tissues that are both environmentally safe and commercially protected. Three types of sterile plants have been developed: 1) male sterile (no risk of pollen flow) but seeded; 2) sterile triploids with no pollen flow nor seed; and 3) male and female sterile barren plants. All three types can be vegetatively propagated. Straight seed reproduction is possible for number one, and seed reproduction combined with rogueing normal segregates is possible for number three.

Practical prevention of pollen flow from self or cross pollinated plants can be achieved by spatial isolation whether wind or insects move pollen. Pollen flow drops off quickly in a quantitative way as distance increases, but wind gusts and hitchhiking insects preclude absolute containment. Quantitative data on pollen flow in alfalfa were obtained using a dominant red root genetic marker. The data are being used to determine practical isolation distances for recombinant alfalfa with value-added traits.

When the value-added recombinant product is in the seed, pollen flow could be controlled by using a male sterile recombinant seed parent pollinated by a normal male parent. This is a proven hybrid seed production method in maize, sorghum, sugarbeet, onion, wheat, etc. Moreover restorer genes for male fertility could be left out of the system, so that the next generation also would be male sterile. Then, if seeds were shattered on the production field or dispersed in transit, they would give rise to male sterile plants. Finally, obligate apomictic seed formation from a vegetative cell, would be a perfect containment mechanism. Although none of the major grain crops have the mechanism, several grass and wild species have this form of apomixis and could be used to produce a risky, high-value recombinant product.

In conclusion, a variety of methods are available to control gene flow of recombinant plants producing value-added products. In some cases the methods are in the plants and waiting for a value-added recombinant product. In other cases plant materials to control pollen flow could be bred in the time it takes to do the genetic engineering -- about two years!

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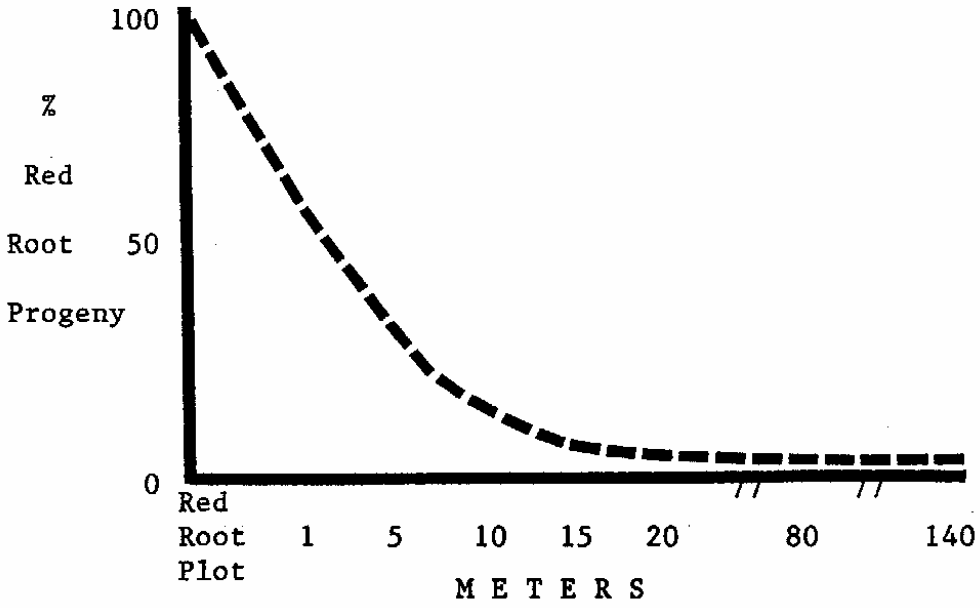
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