

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Seed Shattering - A key constraint to seed yield in perennial ryegrass seed production

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SUMMARY:

Shattering is a widespread natural phenomenon in plants and serves as a mechanism for dispersal of seed to favorable environments. However, in agriculture the loss of seeds as a result of shattering prior to and during crop harvest can be an important constraint to yield. In perennial ryegrass, shattering is estimated to cause seed yield losses of 10-20% or more. Seed yield is reduced by shattering because a lower number of seeds are harvested from the field. Seed lost during shattering is the cause of a major weed problem in perennial ryegrass seed production because of its ability to produce a volunteer crop. Removal of the volunteers increases the cost of production and can damage the seed crop, further reducing seed yield. Breeding will be the best way to increase seed retention in perennial ryegrass. Our plan is to develop mapping populations for seed retention in perennial ryegrass and use these to develop QTL maps for shatter-resistant perennial ryegrass. This work is needed to assist breeders in improving seed retention in one of Oregon's most important crops.

OBJECTIVES:

1. Conduct space-planted field trials with clonal lines of perennial ryegrass for characterization and phenotypic assessment of seed shattering resistance.
2. Create crosses among clonally propagated lines and characterize seed shattering resistance of the resulting perennial ryegrass hybrids in space-planted field trials.
3. Use seed shattering resistant and susceptible phenotypes of clonal lines and hybrids in the development of QTL mapping of seed retention traits in perennial ryegrass.
4. Share mapping results with plant breeders for use in developing shatter-resistant cultivars.

PROCEDURES:

Field and greenhouse trials will be conducted in two steps:

Step 1. Phenotypic assessment of seed shattering in field-grown perennial ryegrass clonal lines.

One each of the most shatter resistant and susceptible cultivars/breeding lines from 4 private sector breeders based on their field observations will be obtained. The most shatter resistant and susceptible PI lines from USDA Plant Introduction Center will also be obtained. The goal is to obtain 10 total lines: 5 resistant and 5 susceptible from breeders and from plant accessions.

Seeds of the shattering resistant and susceptible lines will be planted in the greenhouse and grown into plants robust enough for cloning. These plants will be used as the source for creating vegetative clones of each of the lines for field planting. These source plants will be retained for Step 2. Clonal lines will be transplanted to the field with 4 replications of the lines. Each clonal plant will be space-planted on 2 meter centers to permit access.

Shattering phenotype(s) will be characterized and losses of seed for each of the clonal plants in the trial will be quantified. The progress to maturity in each clone will be ascertained through periodic seed moisture testing and tracked by growing degree day accumulation. The seed loss and retention for each clonal line will be determined by periodically removing 3 spikes from each plant starting at late flowering and continuing until seed has reached 25% seed moisture content.

Each spike will be wrapped in pollination envelopes at the time of collection to prevent premature shattering as a result of handling. The number of spikelets per spike and length of the spike will be determined. The spikes will each be clamped into a modified wrist action shaker and subjected to a standardized shaking time (20 seconds) and rate (127 rpm) of shaking. The number of filled seeds lost as spikelets and florets from the spike will be caught in a tray and recorded. Remaining seed will be stripped from the spike and the weight and number of seeds will be determined.

The architecture of spikes and spikelets in resistant and susceptible lines will be compared and contrasted. Compactness or other morphological modifications of the spike and spikelet that contribute to shattering resistance will be noted in characterizing the degree of seed retention among the phenotypes. Microscopy will be used to illustrate these differences in seed retention resulting from spike and spikelet morphology.

Step 2. Field trials characterizing shattering phenotypes of perennial ryegrass hybrids.

Results of Step 1 will confirm or identify shattering phenotypes among the clonal lines. Hybrids (breeding population) will be created by crossing shattering resistant and susceptible phenotypes (greenhouse-grown clonal plants retained from Step 1). Crosses will be made and spikes will be covered to prevent unwanted pollination. Mature seeds will be harvested and re-planted to create clones for field trial. Procedures in Step 1 will be repeated to characterize shattering of hybrids and parental clones.

Research results and photos from the field trials will be posted periodically on OSU's Seed Production Blog site (<http://blogs.oregonstate.edu/seedproduction/>) for public viewing as they become available. Results will also be published in refereed journals and in practitioner-

oriented publications. Seed growers and other industry practitioners will have an opportunity to view and comment on the results and recommendations produced by the study at OSU Field Days, and at various OSU Extension meetings.

SIGNIFICANT ACCOMPLISHMENTS:

Requests for seed were sent out to private sector plant breeders in the USA and the USDA Plant Introduction Center. Following some delays in filling all those requests needed for the experiments, the seed was finally received. The seed were processed and then planted in the greenhouse for the establishment of shattering resistant and susceptible clonal lines. The clonal lines are growing at this time and were transplanted to the field for characterization of shattering phenotypes. Space-planted field trials are being used to ascertain the degree of seed retention in clonal lines.

Seeds of the USDA PI and plant breeder accessions were planted in the greenhouse and grown into plants robust enough for cloning. Each accession was represented by 4 plants derived from 4 different seeds to better understand the variation within accessions. These plants were used as the source for creating vegetative clones of each of the lines for field planting (Figure 1).



Figure 1. Cloned genotypic lines of perennial ryegrass grown in the greenhouse.

A total of 160 genotypic lines were established from the clonal lines and planted in the field (Figure 2). Each line was replicated 4 times for a total of 640 transplants.



Figure 2. Perennial ryegrass clonal lines ready for transplanting.

The assessment of shattering (seed loss and retention) is underway for each of the 160 genotypes. The progress to maturity in each clone is also being ascertained through periodic seed moisture testing and tracked by growing degree day accumulation. Extreme variation in spike size and architecture has been observed among the genotypes (Figure 3).



Figure 3. Variation in spike size among accessions of perennial ryegrass.

The research project is continuing and is not completed at this time. We also lost our partners in the research and had to develop a new partnership. The administration of New Zealand's AgResearch (federal research program) decided to eliminate their seed research program, ending our planned cooperation with this program in this research. A new cooperator on the QTL mapping portion of the project has been identified at the USDA-ARS National Forage Seed Production Center in Corvallis.

BENEFITS & IMPACT:

The acreage of perennial ryegrass in Oregon has averaged 104,133 acres over the past three years. The estimated farm gate value of perennial ryegrass seed produced in Oregon was \$133 million per year over this period. Since the average seed yield during these 3 years was 1501 lbs/acre, a conservative seed yield increase of 10% with effective shattering resistance or control would produce an additional 150 lbs/acre yield. This is important because perennial ryegrass seed growers only harvest 20% of the potential seed production. The gross economic effect from increased seed yield would be over \$13 million annually for the state's agricultural economy. Further economic benefits could be realized with the reduction of perennial ryegrass volunteers in seed fields.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

The architecture of spikes and spikelets in resistant and susceptible genotypic lines will be compared and contrasted in the field trials by new Ph.D. student Travis Tubbs. The research work done on the shattering project by Mr. Tubbs will be funded by the US Air Force as part of their support for his Ph.D. program at OSU. Mr. Tubbs is an Air Force expert in remote sensing technologies similar to agriculture's high-throughput phenotyping. Tubbs is charged with developing new methodologies for measuring the compactness or other morphological modifications of the spike and spikelet that contribute to shattering resistance among the shattering phenotypes.

FUTURE FUNDING POSSIBILITIES:

None at this time.