CONSERVATION OF GRASS COLLECTIONS AT THE WESTERN REGIONAL PLANT INTRODUCTION STATION

V.L. Bradley and R.C. Johnson
USDA-ARS Regional PI Station, 59 Johnson Hall, P.O. Box 646402, Washington State University, Pullman, WA 99164-6402 USA

ABSTRACT
Germplasm managers at the Western Regional Plant Introduction Station (WRPIS) have focused on improving seed regeneration in forage and turf grass species through studies of diversity maintenance, using isozyme markers in annual ryegrass (*Lolium multiflorum* Lam.) and through studies of pollen isolation, using strains of dominant pubescent and recessive glabrous smooth bromegrass (*Bromus inermis* Leyss.). Balanced samples of annual ryegrass (an equal number of seeds per plant) from field plots were best for maintaining allelic frequencies, but genetic diversity (heterozygosity and allelic richness) was maintained nearly as well in bulk samples (seeds proportionally assembled according to seeds per plant) during early regeneration cycles. In 1995, bromegrass marker plots integrated into WRPIS seed-regeneration nurseries at two locations resulted in average contamination of 4.2% at distances between 22 and 27 m. Diversity maintenance in early regeneration cycles and a relatively low level of pollen contamination appear possible in bulk samples and with modest isolation distances.

KEYWORDS
Grass, germplasm, diversity, seed, accession, isolation distance

INTRODUCTION
The USDA-ARS Western Regional Plant Introduction Station (WRPIS), Pullman, WA, USA, maintains more than 15,000 germplasm accessions of forage and turf grass species.

Seed is obtained by the WRPIS from both foreign and domestic sources including plant exploration trips, breeders, and other international plant germplasm systems. Original seed samples received are usually of low quantity or quality, making them unavailable for distribution and unacceptable for long-term storage. It is therefore necessary to regenerate these accessions. Low seed quantity due to seed distributions and loss of viability of stored seed over time are other reasons for regeneration.

Samples maintained at the WRPIS should represent the genetic composition of the original population as closely as possible. Evaluation of regeneration practices has indicated that harvest technique and isolation distance are two factors critical for maintaining the original genetic profiles of accessions.

MATERIALS AND METHODS
In 1993, original seeds from three annual ryegrass (*Lolium multiflorum* Lam.) accessions, PI 339701, PI 295600, and PI 321395, were planted in flats and grown under greenhouse conditions. The plants were transplanted into the field at the Central Ferry, WA, Research Farm, in two furrows approximately 0.25 m deep and 0.1 m wide made by a V-blade attached to a lawn tractor. Each irrigated plot consisted of 88 plants arranged in two rows of 44 plants spaced 0.3 m apart and with 0.3 m between plants within rows and isolated by a 50 m wide wheat border. As seeds ripened they were hand-harvested to develop three types of regeneration samples: 1) balanced (an equal number of seeds per plant), 2) spike (an equal number of spikes per plant), and 3) bulk (seeds proportionally assembled according to seeds per plant). Seeds from each sample type were pre-germinated at room temperature in plastic germination boxes filled with moist vermiculite. Individual plants were transplanted into cone-tainers filled with potting soil and maintained in the greenhouse. Analyses of eight isozymes: Aspartate aminotransferase, Acid phosphatase, Glucose-6-phosphate isomerase, Isocitrate dehydrogenase, Phosphogluconate dehydrogenase, Phosphoglucomutase, Shikimate dehydrogenase, and Triose-phosphate isomerase, were completed on sections of single, fully-emerged, leaves collected from each of 88 plants from each population using procedures outlined in Soltis and Soltis (1989). In 1995, populations from a second regeneration cycle of balanced, spike, and bulk samples were grown and harvested for each accession. Allelic frequency, heterozygosity, and allelic richness wASdetermined at each resolved loci, and allelic frequency of regenerated populations was compared with original populations at each loci using chi-squares.

Pubescent and glabrous smooth bromegrass (*Bromus inermis* Leyss.) strains developed by Knowles (1980) were used in isolation distance studies. Fertilization of glabrous plants by pollen from dominant pubescent strains results in seeds that produce pubescent seedlings. Tests were performed as described by Johnson, Bradley, and Knowles (1996).

The 1994-95 grass-regeneration nurseries at the WRPIS were configured as shown in Figures 1 and 2. These nurseries included regeneration plots of hundreds of accessions and numerous grass species. Accessions were arranged in strips four plots wide (Figure 1) at the Central Ferry, WA Research Farm, and five plots wide (Figure 2) at the Pullman, WA, Plant Materials Center Farm. At Central Ferry (Figure 1) bromegrass plots were replicated three times and at Pullman (Figure 2) they were replicated twice in randomized complete blocks. Ten irrigated strips 90 m long were established at Central Ferry. At Pullman, two strips, each 275 m long were established and grown under dryland conditions. Seeds of the bromegrass pubescent strain S-8753 (PI 557438) and of the glabrous strain S-9077 (PI 576975) were planted under greenhouse conditions, thinned to one plant per pot, then transplanted in the field as described above, except each plot consisted of 60 plants arranged in two rows of 30 plants. In 1995, plants in each row were hand-rubbed and the seeds were placed in paper bags, dried, and cleaned. Seeds from plants within a plot were bulked and were used to grow seedlings under greenhouse conditions for counts of pubescence. Leaves of at least 100 seedlings at the two- to three-leaf stage were examined, and percent pubescence calculated. Data were arc-sine transformed and analyses of variance conducted.

RESULTS AND DISCUSSION
Isozyme analyses on regeneration populations of annual ryegrass indicated that populations from balanced samples had allelic frequencies that were closest to that of original populations. After the first regeneration cycle, allelic frequencies differed from original populations at 8% of the polymorphic loci for balanced, 15% for spike, and 19% for bulk populations. Nevertheless, measurements of diversity, such as heterozygosity and allelic richness did not change appreciably in balanced, spike and bulk samples.

There were no significant differences among the glabrous treatment positions in pollen contamination in the isolation distance plots at Central Ferry, or at Pullman. Contamination values in glabrous plots

at Central Ferry averaged 3.7% (Figure 1), and at Pullman, 4.7% (Figure 2).

The regeneration sample study suggested that key components of diversity could be maintained through bulk or spike samples, and at far less cost than developing balanced samples. Despite this, the greater tendency for genetic drift (random change in allelic frequencies) in bulk and spike samples was likely the result of reduced effective population size associated with high variation in seeds per plant (Heywood, 1986). These effects would become more pronounced with numerous regeneration cycles. Isolation distance studies suggested that a relatively low level of genetic contamination, averaging less than 5%, appears possible through modest isolation distances.

Resource limitations and the backlog of accessions needing regeneration at many repositories, including the WRPIS, result in difficult choices. If there is zero tolerance for pollen contamination, and if it is necessary to maintain nearly exact allelic frequencies of the original populations, required regeneration and harvest procedures would necessitate greatly decreasing the number of accessions regenerated and made available for distribution. Since the goal is to maintain diversity and ensure accessibility, the WRPIS germplasm managers are using available resources in a manner that best meets both components of that goal. Current regeneration procedures appear to maintain the diversity of accessions in bulk harvest samples and limit pollen contamination to less than 5%.

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Figure 1
Percent genetic contamination by pollen in smooth bromegrass plots arranged within regeneration nurseries at Central Ferry, WA, 1995. The bold solid lines represent glabrous plots and the dotted line the pubescent plot. The number in parentheses is the percentage data subjected to an arc -sine transformation in degrees used for statistical comparisons among plots. The LSD $_{0.005}$ for comparing differences in plot position was 7.1 degrees. Plots were 9 m long, and spaced 1.5 m apart.

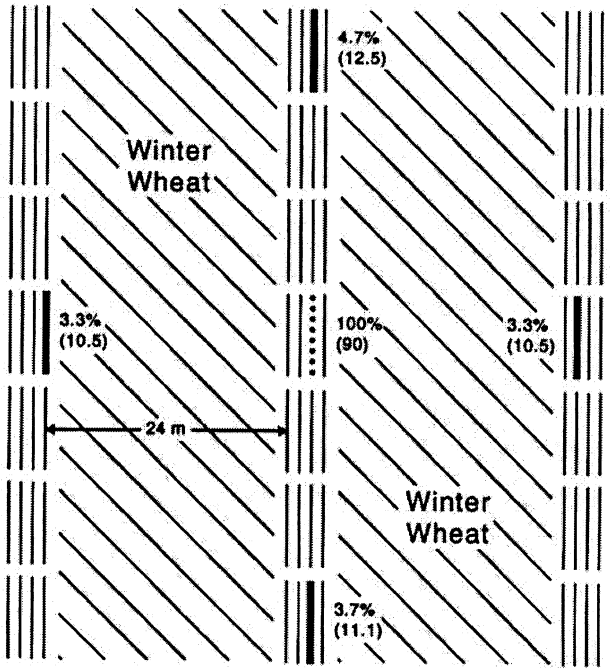

Figure 2
Percent genetic contamination by pollen in smooth bromegrass plots arranged within regeneration nurseries at Pullman, WA, 1995. The bold solid lines represent glabrous plots and the dotted line the pubescent plot. The number in parentheses is the percentage data subjected to an arc -sine transformation in degrees used for statistical comparisons among plots. The LSD $_{0.005}$ for comparing differences in plot position was 6.6 degrees. Plots were 9 m long, and spaced 1.5 m apart.