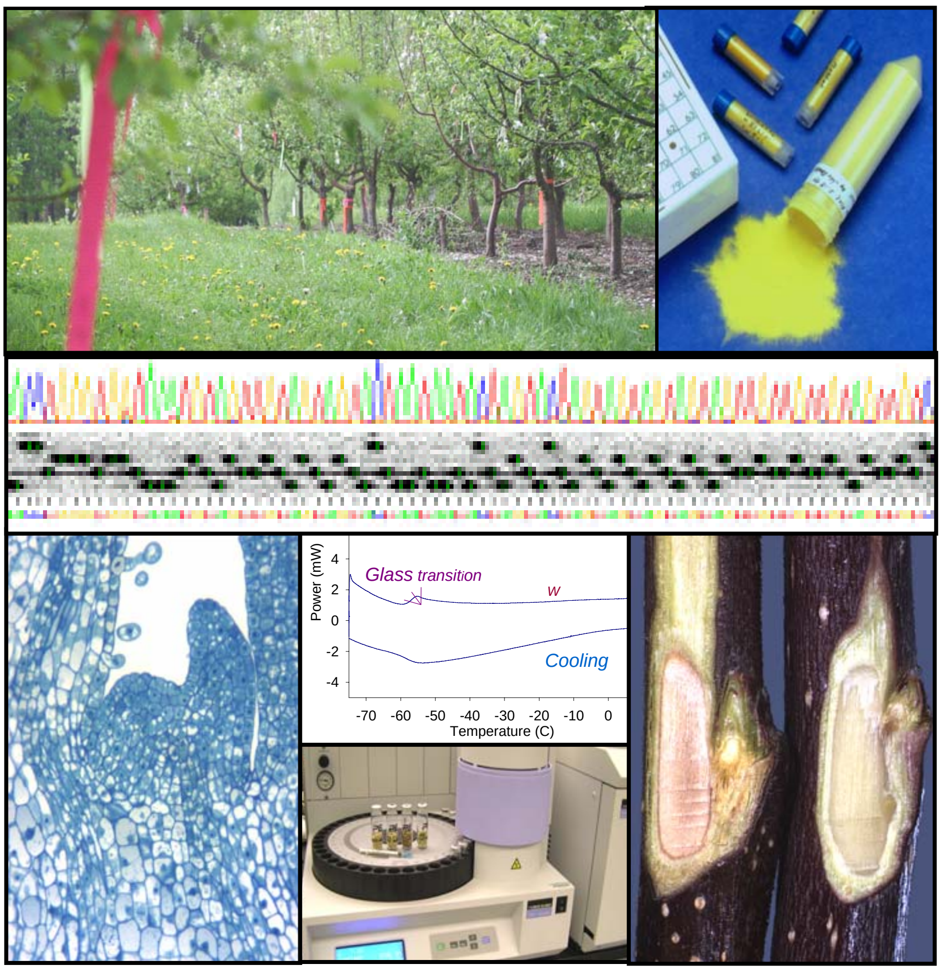
PLANT GERMPLASM PRESERVATION RESEARCH UNIT



2005 ANNUAL REPORT

CRYOPRESERVATION OF WILLOW SPECIES

Problem: The National Plant Germplasm System maintains many vegetatively-propagated species that are not backed-up at the National Center for Genetic Resources Preservation. Cryopreservation is currently the only method that provides long-term, low maintenance storage for vegetative propagules. Methods must be developed for successful *Salix* (willow) cryopreservation.

Findings: A two-step cryopreservation procedure was developed using sections from willow scions collected in mid-winter. Samples cooled at 5 degrees Celsius per day to –30 or –35 ^{o}C and then transferred to liquid nitrogen vapor gave the greatest survival, defined as shoot formation. The cooling rate to –35 ^{o}C had a major influence on survival. The cooling rate from –35 ^{o}C to –180 ^{o}C or –209 ^{o}C did not influence survival. Eight of 11 *Salix* species tested using the established protocol had significant levels of survival after cryogenic treatment.

Interpretations and Recommendations: Willow germplasm can now be successfully backed-up at NCGRP. The woody ornamentals curator of the North Central Regional Plant Introduction Station has expressed an interest in having NCGRP back-up the field collection. Ongoing research will identify other woody species that can be cryopreserved using the two-step cooling method.

- L. Towill

MECHANISM OF PROTECTIVE ACTION IN CRYOPROTECTANT SOLUTIONS

Problem: Genetic resources of several vegetatively-propagated plant species are preserved at liquid nitrogen temperatures at the National Center for Genetic Resources Preservation. Cryoprotectant solutions are routinely used to prevent lethal damage in shoot tips during the cryopreservation procedure; however, the mode of protection is unknown. We have investigated the effect of PVS2, a common cryoprotectant containing 30% glycerol, 15% ethylene glycol, 15% dimethyl sulfoxide, and 0.4 M sucrose, on shoot tips of mint and garlic. We have also performed phytotoxicity assessments of the PVS2 components.

Findings: Using differential scanning calorimetry techniques, we show that exposure of plant shoot tips to PVS2 removes water that could freeze and limits ice crystal formation during the cooling process. Ethylene glycol and dimethyl sulfoxide replace water in the shoot tips and alter the biophysical properties of the water remaining in the cells. We found that PVS2 exposures at 22 ^{o}C were more damaging than exposures at 0 ^{o}C. Glycerol is one of the toxic components of PVS2.

Interpretations and Recommendations: Our experiments suggest that PVS2 protects cells by dehydration and it may structure water molecules to inhibit ice formation but not necessarily induce glass formation. Our methods provide needed tools to study the thermodynamic effects of other solutions purported to have cryoprotectant activity. This information will allow us to develop cryoprotectant formulations that are highly protective and have lower toxicity.

- G. Volk
- C. Walters

GENETIC DIVERSITY OF WILD PEARS

Problem: NPGS collections of wild relatives of fruit crops are usually maintained vegetatively. These field collections are labor intensive and expensive. If a portion of this germplasm could be preserved as seeds, a broader genetic representation of wild-collected accessions could be included in germplasm collections. Conservation strategies for the wild relatives of *Pyrus communis* L., the European pear, can be determined when their genetic diversity has been characterized. We collected genotype, phenotype, and geographic origin data for 145 *Pyrus communis* individuals derived from seeds collected in the wild in eastern Europe, Turkey, and the Caucasus Mountain region of Russia and currently maintained in the USDA-ARS National Plant Germplasm System at the National Clonal Germplasm Repository in Corvallis, Oregon.

Findings: Data were collected for 13 pairs of microsatellite markers on 145 *Pyrus communis* individuals. A Bayesian clustering method grouped the individual pear genoytpes into 12 clusters. *Pyrus communis* ssp. *caucasica* (Fed.) Browicz, native to the Caucasus Mountains of Russia, Crimea, and Armenia, can be genetically differentiated from *P. communis* ssp. *pyraster* L., native to eastern European countries. Pears with large fruit cluster closely together and are most closely related to a group of genotypes that are intermediate to the *P. communis* ssp. *pyraster* and the *P. communis* subspecies *caucasica* groups.

Interpretation and Recommendations: Based on the high number of unique alleles and heterozygosity in each of the 12 clusters, we conclude that the genetic diversity of wild *P. communis* is not fully represented in the NPGS. Additional diversity may be present in seed accessions stored in the NPGS and more pear diversity could be captured through supplementary collection trips to Eastern Europe, the Caucasus Mountains, and the surrounding countries.

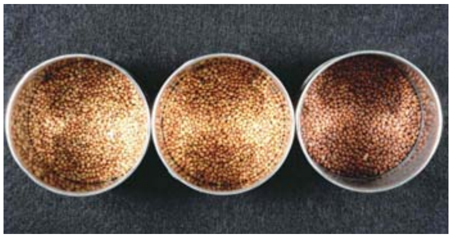
- G. Volk
- C. Richards

HISTORICAL EXPERIMENTS PROVIDE UNIQUE INFORMATION ABOUT SEED LONGEVITY

Problem: Improving storage conditions in genebanks and predicting how long seeds can survive under conventional storage conditions requires an understanding of how storage temperature and seed moisture content interact to affect seed longevity. This information is hard to collect because seeds can survive for decades and there are few experiments that can be used to document survival when water content or temperature varies. Scientists at the PGPRU are using "historical experiments," that were initiated 10 to 40 years ago, to track differences in seed viability with storage conditions.

Findings: An experiment initiated in the 1960s by Dr. Louis Bass provides longevity data for seeds of several crop species that were stored at temperatures ranging from -12 to 35^{o}C, water contents of 4, 7 and 10%, and gases such as air, nitrogen, argon and carbon dioxide. Another experiment, commissioned by the International Plant Genetic Resources Institute in 1994 to address a controversy on whether drying or cooling is more effective in preserving seed viability, is nearing completion. The experiments confirm a limit to the beneficial effect of drying and demonstrate that this moisture limit is dependent on temperature. These experiments also reveal how temperature affects aging rate.

Measuring the pressure in Dr. Bass's sealed can experiments to determine if the gaseous environment was maintained during storage.



Crimson clover seeds stored for 42 years in air at 10C and 4, 7 and 10% water content. The progressively darker color of the seeds suggests Maillard-type reactions are promoted during storage at higher water contents.

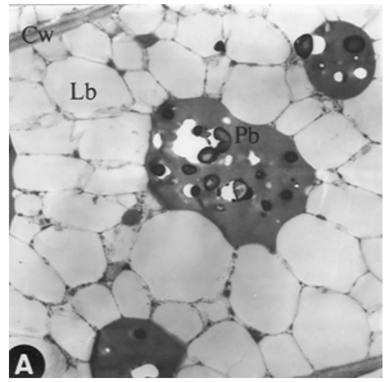
Interpretation and Recommendations: Knowledge of how temperature and water content interact provides the basis for optimizing storage environments for long and short term needs. These interactions also provide the basis for biophysical models that predict aging at extremely low temperatures. We now understand that limits to the beneficial effect of drying imply limits to the beneficial effects of low temperature storage and this has major implications for the applicability of liquid nitrogen storage of dry seeds. We will link models of seed deterioration under various storage conditions to models of genetic erosion and to estimates of the cost of seed storage under different storage environments.

- C. Walters

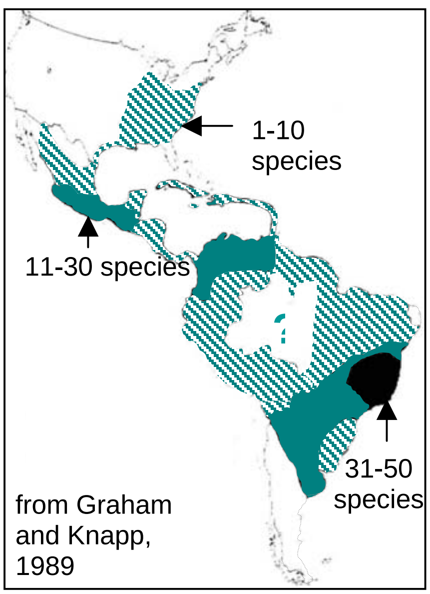
SEED OIL AND OIL BODIES REVEAL SEED QUALITY TRAITS

Problem: Seed longevity is determined by seed quality. Seed quality is controlled by genetics and conditions during plant growth and embryo maturation. Little is known about cellular constituents or structures that confer seed quality. An anecdotal correlation between high oil content and poor storage behavior has been described for decades, but never tested.

Findings: Seed longevity does not correlate with the oil content of seeds, according to a survey of ~275 species stored at NCGRP. None-the-less, there is an important association of seed quality and oil characteristics that is based on the chemical composition of the oil and its organization within the seed cell. We are elucidating these patterns by studying the tendency of oils to crystallize when seeds are exposed to low storage temperatures. Crystallization is slow in oils compared to other molecules, like water, because oil molecules are large. Presence of other molecules, such as proteins, and the size of oil bodies may influence the rate of crystallization and overall crystallinity. Changes in seed quality by priming and accelerated aging (warm, humid storage) alter the tendency of oils to crystallize. We have also discovered that water interaction with crystallized lipid is lethal. This interaction explains "intermediate" storage behavior of many seed species originating from tropical and subtropical areas.



Electron micrograph of cells of sunflower cotyledons showing that they are filled with oil (Lb) and protein bodies (Pb).



The geographic distribution of *Cuphea* species. Some *Cuphea* species exhibit intermediate storage behavior, which is linked to the chemical composition of the oils.

Interpretation and Recommendations: Crystallization behavior of lipids is currently being used as a screen for seeds that may be susceptible to damage during low temperature storage. Damage to intermediate seeds can be avoided by melting oils before seed imbibition. Changes in crystallization behavior can also be used to track the progress of deterioration during seed storage. Anomalous aging kinetics at some temperatures is likely related to the phase behavior of oils. Storage conditions that avoid these changes are recommended.

- C. Walters
- G. Volk
- C. Bailly (Univ de Pierre et Marie Curie, Paris)

SEQUENCE DIVERSITY AT FUNCTIONAL LOCI IN SUGAR BEET

Problem: There are few studies that compare diversity measured using genotypic data in genes of known function with phenotypic data traditionally used to evaluate genetic diversity of collections. Regulatory genes controlling flower timing and duration may have particular utility in monitoring diversity in *ex situ* collections. These genes may be under strong selection during seed production and, as a consequence, may be particularly informative for estimating genetic shifts in *ex situ* conditions.

Findings: Many plants require a lengthy cold treatment, namely winter, in order to flower. This process is termed "vernalization." The vernalization response in the model organism *Arabidopsis thaliana* is controlled by the interaction of two genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). Neither gene has been found outside the plant family *Brassicaceae*, to which *Arabidopsis* belongs, in spite of numerous efforts to do so. We used phylogenetic analysis of protein sequences "mined" from expressed sequence tag databases to identify FLC-like genes in tomato, poplar, and sugar beet, distant relatives of *Arabidopsis*. Using a series of complimentary genomic techniques, we were able to sequence the gene, characterize its expression during experimental cold-treatments and show that it represses flowering in FLC null mutants in *Arabidopsis.* The FLC-like gene in sugar beet, which we named BvFL1, behaves in a manner similar to Arabidopsis FLC during a vernalization treatment: it is a repressor of flowering that is down-regulated by cold.

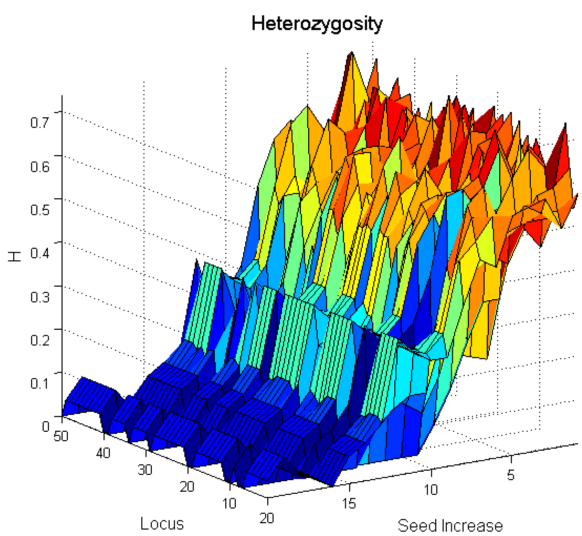
Interpretations and Recommendations We conclude that the fundamental elements of the vernalization response are conserved between Arabidopsis and sugar beet. Based on known flowering plant phylogenetic relationships, we predict that FLC-like genes are present in the majority of dicot species. These loci may provide important information on genetic changes (especially in highly variable wild accession) in *ex situ* conditions. Moreover, conservation of the basic genetic mechanism underlying the vernalization response provides an opportunity to control flowering time, a trait of critical agronomic importance, in most dicot crop species.

- C. Richards
- L. Panella (Sugar Beet Research Unit, Fort Collins, CO)
- R. Amasino (Dept of Biochemistry, University of Wisconsin-Madison)

SAMPLING STRATEGIES FOR COLLECTING AND MAINTAINING GENETIC DIVERSITY

Problem: In collaboration with CSU's Program in Mathematics, Ecology and Statistics (PRIMES), we have been examining the limitations and caveats associated with theoretical sampling models proposed to give collectors of germplasm specific guidelines on samples sizes and distributions. Many of these models have focused on efficiently collecting the most diversity with minimal collection size. In addition, one can view the process of *ex situ* conservation as a series of sampling events, starting with the initial collection and carrying through seed increases and storage in *ex situ* conditions.

Findings: Our review of the literature (Lockwood et al., 2006) described over 30 different approaches for sampling genetic diversity in the wild—each with different assumptions regarding the sampling scale and the basis for diversity. We examined two widely used quantitative models and extended their ability to accommodate rare alleles, multiple loci and multiple populations (Lockwood et al., in review). In addition, we have embarked on a simulation study of how accession inventory history impacts long-term retention of genetic diversity in the NPGS. Each inventory of a wild accession may trace its history back to the most original sample through different pathways each with different seed increase conditions, sample sizes and environmental conditions. We have constructed a computer model to examine how parameters associated with genetic drift, selection and biophysical decay of seeds interact to influence the genetic diversity of an accession kept in *ex situ* conditions. We will work with broad input from curators working in the NPGS to make realistic parameter choices and to apply this analysis to a wide spectrum of taxa.



Interpretations and Recommendations: Currently the model framework has been implemented to address a number of parameters and is flexible enough to accommodate a wide range of *ex situ* scenarios. We are developing close working relationships with curators interested in this analysis. The model will be used as a tool to can help predict the how long an accession can be maintained in *ex situ* conditions before is looses a substantial fraction of its initial diversity. Results of these simulations may provide data to help tailor *ex situ* management for diverse species that differ in their reproductive and life history traits.

- C. Richards
- G. Volk
- D. Lockwood (PRIMES post-doc)

PGPRU COLLABORATIONS

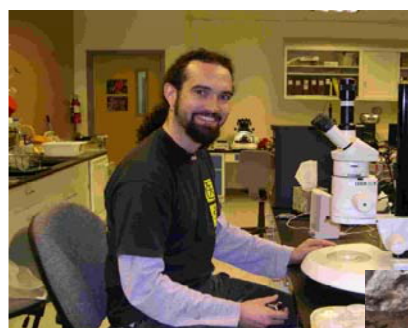
Problem: *Ex situ* preservation of genetic diversity is a global concern and requires collaboration among scientists with diverse backgrounds and interests.

Findings: The collaborative and multidisciplinary approach of the PGPRU offers an attractive environment for scientists and visiting scholars.

Interpretation and Recommendations: External collaborations contribute new talent and perspectives that enrich the research environment and encourage cultural diversity.

Dr. Pawel Chmielarz, from the Institute of Dendrology, Kórnik, Poland, develops methods to cryopreserve oak germplasm in a project funded by a competitive grant from the Kosciuszko Foundation. Pawel visited from 8/2004 to 6/2005.

Cathy Hargreaves, Forest Research, Rotorua, New Zealand, works with Monterey pine to develop cryopreservation protocols for seeds and shoot tips. Cathy visited from 3/2005 to 7/2005 as part of her sabbatical.



Daniel Ballestros, a graduate student from Jardí Botànic, Universitat de València, received a competitive grant to come to the US to study storage physiology of fern spores for species native to Spain. Dani is visiting from 10/2005 to 5/2006.

Dr. Luciano Nass, Embrapa, Brazil begins a two-year collaboration on effective genebanking approaches. Luciano was selected to study in the US as part of the Labex program.

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