

**S-9 Committee Meeting
June 3-4, 1997
Griffin, GA**

Agenda

Tuesday, 3 June

- 8:00 a.m. Call to order
 B. Rhodes, Chair, S-9 Regional Technical Advisory Committee
 Introduction of Attendees
 Official Welcome -- G. F. Arkin, Assistant Dean, CAES, Griffin
 Additions to Agenda for 1997 meeting
 Appointment of Committees
 Nominations
 Future Meeting Plans
 Resolutions
- 8:30 a.m. Activities of the U. S. National Genetic Resources Program
- 9:00 a.m. Activities of the National Germplasm Resources Laboratory
- 9:00 a.m. Activities of the National Seed Storage Laboratory
- 10:00 a.m. Coffee Break
- 10:30 a.m. CSREES Activities on Genetic Resources
- 11:00 a.m. Overview of Unit Activities/plans for Review of Efforts
- 12:00 p.m. Lunch
- 1:30 p.m. Curation activities in Griffin
 Peanut
 Cowpea
 Clovers and special-purpose legumes
 Grasses
 Vegetable Crops
- Support Groups
- 6:00 p.m. Dinner

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1997 S-9 Committe

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12:00 p.m. A

7 Meeting

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l Branch
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al Genetic Resources Program

arded a report which was prepared by Henry L. Shands and Peter K. Bretting
. The National Program Staff has been reorganized and Peter Bretting is the
lant Germplasm and Henry L. Shands is the Acting Assistant Administrator,
v. A note was made that no one from the National Program Staff attended the

ermplasm Resources Laboratory

arded a report of the National Germplasm Resources Laboratory (Appendix
sed. First, international property rights have become a major issue in the
mplasm. The U.S. Treasury Department administers and enforces sanctions on
the U.S. and several countries including: Iran, Libya, Iraq, North Korea, and
problem between Chilean officials and the U.S. regarding germplasm exchange,
ed to fill Chilean requests. A national initiative has begun on a Corn Genome

e a problem for seed distribution. Bob Jarret indicated that there is a 2-year

1. Call to order

The Region
by Chairman Bill F
Griffin, GA.

2. Official welco

Dr. G. F.
Agricultural Expe:
the Southern Regio
budgets have been
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during the review.
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An issue fa
infrastructure, peo

Next year a
Technical Advisor

A note was

turnaround for introducing sweetpotatoes. Forty to fifty entries are received each year by the USDA. Roy Pittman is cooperating with Drs. Karen Williams and David Williams to increase *Arachis hypogaea* collections from Ecuador. He wants feedback on whether setting aside \$3,000 for an Ecuadorian grow-out is a reasonable expense. Dr. Pittman plans to serve as a pass-through for seed harvested in Ecuador, while testing for viruses in Griffin. The issue of assuring that disease-free seed is introduced from Ecuador needs to be presented to the Peanut CGC in July. Gil Lovell indicated that there is strict quarantine on millet introduction to the U.S. A review is being made of the St. Croix location for clearance to plant pearl millet. The sorghum collection is being grown at Isabella and Mayaguez, Puerto Rico. About 2,500 accessions of *Sorghum bicolor* need to be regenerated, but there is a new ergot on the island and regeneration in Puerto Rico is not possible. Further, a disclaimer is being distributed along with sorghum seed indicating that "before 1995 seed was grown and regenerated in an area known to be free of ergot and therefore is free of the disease." For seed grown in 1995 and after a disclaimer is included which states, "Seed regenerated since 1995 will now be treated with Captan 400 as a preventive procedure against sorghum ergot. Though much of the seed has been grown in areas free of ergot, this procedure is being done to ensure delivery of high quality, disease-free seed."

In situ preservation of grapes is in the initial phases.

8. Activities of the National Seed Storage Laboratory

Dr. Loren Wiesner, Research Leader of the Seed Viability and Storage Unit, presented the National Seed Storage Laboratory report (Appendix 3). Corn grow-outs in St. Croix are being downsized. Eric Roos is taking a leave of absence. There are problems with some materials at the Pullman Station because materials no longer appear to be the same as at the National Seed Storage Laboratory. Experimental work in seed germination is on going.

Apples are being regenerated in Ottawa and Saskatchewan and buds from Canadian apples are being stored in liquid nitrogen. Three hundred twenty four accessions are currently in storage.

A seed Research Symposia will be held at Ft. Collins from August 13-15, 1997, with the title: "What Does Seed Research Need for the Future?"

9. Overview of unit activities

The budget of \$1.6 million per year has been flat for the past 10 years. Salaries account for about 70% of the budget. S-9 dollars are being applied to labor, seed storage, regeneration, and field activities. Salaries for graduate students and labor are taken initially and disposable funds are then used for operating activities. A summary of discretionary funds is presented in Appendix 4.

Attempts are being made to backup samples at the NSSL. Germplasm of original seed samples is being moved to -18°C storage facilities and packages are being bar coded. At the same time, backup packages are being prepared for the NSSL. Bill Branch asked if core collections are being treated differently than the individual collections as a whole, and Steve Kresovich indicated that they are not being treated differently except for regeneration, evaluation, and characterization priorities.

Qualitative data is being checked in databases. Collections need to be better organized. Further, difficulties have been encountered for obtaining information from the international community. Introductions during the next five years will need to have viability tests before seed is placed into storage.

The size of the collections has greatly increased during the past few years. For example, in 1987 there were 13,300 sorghum accessions in the germplasm bank whereas in 1997 the number has increased to 29,100. The General Accounting Office has requested information related to the unit's activities (Appendix 6).

10. Curation activities in Griffin

A general discussion of curation activities was held, with each curator presenting a brief summary of introductions and grow-outs for the past year (Appendix 7). Activities of the Applied Genetic Analysis Lab were also presented along with interactions with crop curators and other activities at Griffin (Appendix 7). Although entries of each crop in the unit's collection are being regenerated, long-term plans are needed for seed regeneration. These crop regeneration plans should consider methods to avoid materials from being lost and to limit genetic frequency changes in existing plant populations. This is especially important to be implemented before germination percentages become low. The plans should include the base collection for each crop, mutant collections, core collections, and anticipated introductions.

Plans are also needed to answer critical questions about photoperiod sensitivity, germination percentages, storage requirements, and reproductive biology of many species.

11. Support Groups

A discussion was held concerning data which is being introduced into the GRIN system. Significant progress is being made (Appendix 8).

12. Proposed relocation of the Germplasm Unit to Athens (R. Breeze):

The ARS is seeking new directions in safety, meeting the needs of small farms, etc., and is evaluating CRIS projects for fit to national priorities. Costs need to be cut while government performance has become outcome oriented. The General Accounting Office Report on the USDA and Land Grant University System concluded that there is not a national research agenda. Ten years ago the ARS had 3100 scientists whereas in 1996 the number fell to about 1900. A corresponding decrease in support staff has occurred. Thus, in the process to become more cost efficient, the USDA is investigating the feasibility of moving the germplasm unit from Griffin to Athens. A cost analysis is yet to be done, and a final decision has not been made to move the facilities and personnel.

13. Call to Order

The S-9 TAC Committee was called to order by Chairman Bill Rhodes and resumed activities at 8 a.m. on 4 June 1997.

14. Discussion/Review of Unit Activities

The question of charging for seed to persons outside the research and international communities was discussed. Every curator has his/her own standards for distributing seeds. The creation of web sites and subsequent listing of collections and their availability has resulted in a many-fold increase in seed requests. Listings on the Internet indicate that seed is freely available upon request from the USDA, so curators cannot easily deny access to the public. However, preparation of small packages of seed is expensive. Answers to issues

related to seed dispersal to persons outside the research and international communities will be needed in the near future.

Guidelines need to be set up to establish priorities for each crop to make sure germplasm is being properly maintained and evaluated. There seem to be mixed signals from National Program Staff where one group says to concentrate on collection and maintenance and another group emphasizes molecular approaches to classification and management. A synthesis is needed to make sure the germplasm unit has a unified program with the several components interacting to achieve common goals.

Gil Lovell summarized the efforts with several crops as follows:

Crop	PIs/ year	Cost/year	Cross-pollinated collections
Grasses	100	\$65,911	\$ --
Pearl Millet	125	4,733	37
Fiber crops	50	5,362	107
Oil crops	45	11,267	250

New regeneration sites are needed for several crops. Mayaguez, Puerto Rico was suggested as one possibility for additional plant germplasm activity.

A discussion was held about the information forwarded to the committee by the germplasm unit and TAC's ability to adequately review the activities being conducted by the unit as well as issues related to proper maintenance of germplasm collections. Without knowing the budget of each curator and related expenses for regeneration and evaluation, it is difficult to know whether individuals and/or programs are efficiently operating. An outline of long and short term project objectives and plans for maintenance and evaluation would be helpful to the TAC. Further, more detail about the activities of the curators would aid in discussions.

15. Committee Reports

Nominations Committee

Tom Stalker was nominated for the 1998 TAC chairmanship.
 Jorge A. Mosjidis was nominated as the 1998 TAC Secretary.

Resolutions Committee

1. The S-9 Technical Advisory Committee thanks Dr. G. F. Arkin for his guidance as Administrative Advisor, for his hospitality, and for the use of facilities at the Georgia Experiment Station.
2. The S-9 TAC thanks Dr. Stephen Kresovich and the Curators and staff of the Southern Atlantic Region Plant Genetic Resources Conservation Unit for hosting the committee and updating activities of the unit over the past year.
3. The S-9 TAC appreciates the attendance and participation at our annual meeting of Loren Wiesner from the National Seed Storage Laboratory.
4. The S-9 TAC members participating in this meeting strongly encourage greater attendance by all S-9

TAC members at future annual meetings.

5. The S-9 TAC thanks Bill Rhodes and Tom Stalker for their service as chairman and secretary, respectively.
6. Tom Stalker and Jorge Mosjidis are selected as the new chairman and secretary of the S-9 TAC, respectively.
7. The S-9 TAC commends the South Atlantic Region Plant Genetic Resources Conservation Unit for its long-term service and the committee noted improvements over the past several years in the areas of maintenance of collections.
8. Be it resolved that the S-9 TAC is concerned about the potential transfer of the Unit and earnestly requests that the USDA-ARS and the University of Georgia most carefully consider all costs, both monetary and human, before moving the Plant Genetic Resources Conservation Unit from its present location at Griffin. The S-9 TAC further resolves that these entities assess both the short and long-term impacts of such a move on the Unit's viability and effectiveness and suggests consulting the experiment station directors of individual contributing states early in the evaluation process.

Future Meeting Plans Committee Report

A motion was made and passed at last year's meeting (see p. 13 of the 1996 S-9 TAC Report) to have the 1998 S-9 meeting as a combined meeting at Ames, Iowa. Dr. Steve Kresovich recently indicated that this joint meeting will be held on July 20-24, 1998.

16. Adjournment

10:40 a.m. on 4 June, 1997

Appendix 1

USDA/ARS National Program Staff and Office of the Administrator Report

Prepared for 1997 Regional Technical Advisory Committee Meetings

June 1, 1997

Henry L. Shands, Acting Assistant Administrator, Genetic Resources and Biodiversity

Peter K. Bretting, Acting National Program Leader, Plant Germplasm

Budget. Budget continues to play a large part in reshaping the research programs of the Agricultural Research Service (ARS) as both the Administration and Congress have agreed to seek a zero budget deficit by FY 2002. Funding for agricultural research has remained relatively constant so far but ARS has redirected about 3-4 percent of its budget in recent years to pay for new projects. Germplasm management remains a high priority at ARS, with National Plant Germplasm System (NPGS) activities suffering about a 2 percent budget reduction as a result of budgetary cuts made across all of ARS's projects. The National Seed Storage Laboratory received a \$500,000 increase in the FY 1997 Fiscal Year which began on October 1, 1996.

Flooding of Grand Forks, ND by the Red River in April caused at least \$6 million of damage to the ARS Grand Forks Human Nutrition Center. As of this date, funds for repairing this facility have not been included in disaster-relief legislation before Congress. Consequently, ARS will be managing end-of-the-year funds to account for emergency needs at the Grand Forks location.

Acting Under Secretary Catherine Woteki proposed a Fund for Genetic Security and plans to renew that effort as the Department develops the new Research Title to the Farm Bill in 1997. The Department recognizes that many aspects of germplasm management, research, and genome mapping/sequencing research require enhanced support. The Congress asked the Department to develop a response to a proposal from the National Corn Growers Association for an intensive program of corn genome mapping, gene sequencing, and identification of the biological function of selected genomic segments.

USDA/ARS Germplasm Funding:

<u>Activity (STP Codes)</u>	<u>FY 1995</u>	<u>FY 1996</u>	<u>FY 1997</u>
Acquisition 2111,2112	\$4,097,200	\$3,855,100	\$3,951,700
Preservation 2113	<u>12,559,325</u>	<u>12,732,100</u>	<u>13,018,200</u>
SUB-TOTALS: 1/	16,656,500	16,587,200	16,969,900
Characterization 2114	3,377,300	2,900,100	3,088,000
 TOTALS:	 <u>20,073,800</u>	 <u>19,487,300</u>	 <u>20,057,900</u>

1/ Funding level most consistent with activities relating to National Genetic Resources Program.

Reorganization of ARS's National Program Staff. The National Program Staff is undergoing a reorganization which will place all of ARS's programs into three areas: Crop Production, Product Value, and Safety; Animal Production, Product Value, and Safety; and Natural Resources and Sustainable Agricultural Systems. The previous national programs of Genetic Resources and

proposal was included in text of the new negotiating draft for consideration by the Commission.

Plant and Animal Genome Conference V. The fifth genome conference was held January 12-16, 1997, in San Diego, California. For the first time, animal genome research was included. More than 800 scientists from 29 countries were present and represented a strong core of molecular biologists and users of genetic resources. The major genome challenge is the cross-linking of data from one crop to another.

Review of the Plant Genome Database Program. A team of external reviewers examined the Plant Genome Database Program during a review held in Beltsville on May 6-8, 1997. This was the first comprehensive review held since the program was founded. The report of this review committee will be forthcoming shortly.

Appendix 2

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1

National Germplasm Resources Laboratory (NGRL) Programs - Allan K. Stoner

The programs of the National Germplasm Resources Laboratory (NGRL) support the mission of the National Plant Germplasm System (NPGS), which is: "To effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops." The NGRL activities are performed by the following groups; the Plant Germplasm Quarantine Office (PGQO), the Plant Exchange Office (PEO), the Germplasm Resources Information Network/Database Management Unit (GRIN/DBMU), and the molecular virology investigations. In addition, the Laboratory facilitates the activities of the 40 Crop Germplasm Committees (CGC) that provide technical advice to the NPGS.

Particularly significant accomplishments during the past year included the continued progress made by the PGQO in eliminating backlogs of germplasm in quarantine and shortening the time required to process quarantined germplasm. New procedures were implemented to secure germplasm, conduct therapy of infected accessions, and more reliably and more quickly test for pathogens.

The constant evolution of the GRIN hardware and software continues to make the database more useful to the NPGS maintenance sites and to the user community. Access to the GRIN data via the Internet has greatly increased the number of users viewing and downloading data about NPGS germplasm collections.

During the past year the PEO began several new projects to identify and prioritize the germplasm acquisition needs of the NPGS, to study *in situ* conservation of plant genetic resources and to apply the Geographical Information System (GIS) and computer mapping technologies.

More specific information of the Laboratory's activities is contained in the individual reports that follow. If you have questions or comments about any of the programs, please communicate them to me or to individuals involved.

2

Plant Germplasm Quarantine Office

Progress Report on Quarantined Pome Fruits, Potatoes, Sweet Potatoes and Sugarcane - Suzanne Hurtt

Acquisitions for the period included 48 apple and 17 pears/quince accessions received as dormant budwood.. Six were subsequently discarded as unwanted by the designated recipients. One to four trees of each accession is established in the quarantine greenhouses or screenhouses.

Apples and pears are each tested in the greenhouse during the winter and spring for 3 common viruses. Three indicator species are used in graft-transmission bioassays to detect infections and accessions are tested by nucleic acid cRNA hybridization assay for apple scar skin and pear blister canker viroids. Pome fruit trees are further assayed in orchards (2 for apples, 4-5 for pear/quince) for numerous fruit and bark/trunk marking pathogens.

The 1997 winter assays/laboratory tests are still in progress at this writing. Greenhouse assays on 76 apples and 53 pears/quince are nearing completion. These tests consist of repeat- (2nd) assays on 63 pome fruit trees that tested negative for pathogens last year and initial (1st) tests on 65 trees. The latter includes new accessions (22 apples and 8 pears), older accessions that were previously too small to sample (6), and heat-treated propagations of infected accessions (23). To date, 6 of 22 apples and 6 of 8 pears imported during this reporting period were found to be infected. The majority of the heat-treated pears indexed this year were treated for virus-elimination by Joseph Postman, pathologist at the clonal repository in Corvallis, OR.

The *Solanum* quarantine includes testing of both true potato seed and clonal germplasm. During this reporting period, NGRL received 87 lots of seed and 68 clonal accessions.

NGRL continued to cooperate with the NRSP-6 to test seed lots. Seeds are germinated at Sturgeon Bay and up to 20 plants from each seed lot are grown to maturity and selfed or crossed

for seed increase. Leaf samples from the plants are shipped to Beltsville where bioassays for seed-borne viruses are performed. If the bioassays are negative for virus and spindle tuber viroid, seeds produced from the crosses are released from quarantine. 225 bioassays were performed and 115 seed lots were increased and released. Five lots were not released because of viral-like symptoms on some seedlings. An additional 25 lots of true potato seed were released from Beltsville grown seedlings.

Eighty-one vegetatively propagated *Solanum* accessions were released during 1996. When 1997 tests are completed, release of 64 additional clonal accessions is anticipated. Twenty-five additional lines entered testing this spring. Completion of these tests and release of the germplasm are anticipated in early fall, 1997.

Thirty new *Ipomoea* spp. accessions were received during the year. This increased the current inventory to 79 clones for the 1997 testing period. These include 43 accessions that have undergone one set of tests with negative results and 15 that will be tested for the first time after undergoing meristem tip culture for elimination of virus-like agents. Thirty-nine sweet potato accessions were released and distributed in the fall of 1996.

The sugarcane quarantine program supports both the international and interstate exchange of germplasm. For the interstate program, we acquired 92 clones from LA and 9 from TX and released/distributed 74 accessions to FL, 7 to LA, and 2 to NE.

Foreign accessions were received from Argentina (4), Japan (2), and Taiwan (2). Fifty-three foreign accessions were released. All went to FL. The Hawaiian Agricultural Research Center (formerly HSPA) sent 155 lines destined for the sugarcane repository at Miami. Germplasm from HI is subject to a two-year quarantine period and handled as though it were a foreign clone. The quantity of sugarcane from Hawaii was 150% of the anticipated amount. Consequently, importation of additional foreign clones will be discouraged until this germplasm is processed.

In cooperation with U. S. Sugar Cooperation, FL, we began using RT-PCR methods for detection of the luteovirus associated with yellow leaf syndrome (YLS). Approximately 250 samples were tested and YLS was detected in 51 clones from Brazil and 8 from Mauritius. Concurrent with the YLS tests, 138 clones were tested serologically for sugarcane bacilliform badnavirus (SCBV). Ninety-eight clones were found to be infected with SCBV. More than 400 immunoassays for leaf scald or ratoon stunt and > 200 inoculations to semi-selective medium for leaf scald bacterium detection were performed during the year. Approximately 300 bioassays on sorghum 'Rio' for mosaic-like viruses were conducted.

Progress Report on Quarantined Stone Fruits, Grasses, Ornamentals, Small Fruits and Tropical Crops - Howard Waterworth

Germplasm Distributions and Receipts

Tests for pathogens in germplasm of most of the plants in quarantine were completed or in progress. Among the accessions distributed to repositories and importers were 42 grasses, and 852 genotypes of stone fruits (no. items x recipients). This results in very low inventories (1 to 10 accessions) of mostly recently received accessions of the above genera except for *Ribes* (55 accessions) and stone fruits (c.300 accessions) which require long term tests. Included in the stone fruits are some 800 potted seedlings and another 150 being stratified, each of which require tests for viruses.

During the past 12 months 144 accessions of *Prunus* budwood and 66 accessions of *Prunus* seed were received; in addition to 12 accessions of small fruits and 7 of grasses.

Pathogen Detection Research

Until now the detection of phytoplasmas (=MLOs) in *Prunus* was accomplished by grafting to sensitive indicator varieties and observing trees for 3 years for development of disease symptoms. We have successfully adapted a laboratory test involving nucleic acid extraction, PCR and electrophoresis that appears to detect all known phytoplasmas that infect stone and pome fruits. We are incorporating this new technology into the program on a routine basis. But, there are many other infectious 'agents' for which rapid testing methods are not yet available, so that graft tests will continue to be needed.

Vacated Old Glenn Dale Station

We have vacated the old plant introduction and quarantine station at Glenn Dale, MD. It has served the department well during its 76 years of existence. Many are not aware that the Bradford pear was selected at that location and named after the station superintendent, and that B. Y. Morrison developed c. 450 cvs of azaleas at Glenn Dale. The New Guinea impatiens, new cvs of camellias, chestnuts and hibiscus, early agronomic work on kenaf for paper pulp, and a project on quinine during WW II are among the products of research from that Station. It continues to serve as a satellite location for the National Arboretum in Washington, D. C.

Most ARS plant germplasm/quarantine personnel and plants are now located in a state-of-the-art new facility, known as Building 580 on Powder Mill Rd in Beltsville, MD; with additional staff

and plants tentatively located in Bldg 465 on Entomology Road. We invite you to visit us anytime.

We are available to assist you regarding procedures to follow and the paper work needed in order to import germplasm that is in the prohibited quarantine category. Contact us at 301-504-6563, fax at 301-504-6737 or by email: pgqohw@ars-grin.gov.

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Progress Report on Potato, Sweetpotato, and Small Fruit Tissue Culture and Rice Quarantine - Bruce Parlman

All quarantined potato and sweetpotato accessions received as vegetative material are established and secured in tissue culture before samples are acclimated to the greenhouse for pathogen testing. Germplasm security is improved and all accessions released from quarantine are shipped as in vitro cultures. As of 5/1/97 the tissue culture potato inventory consists of 145 vegetative accessions and 43 clones of 3 seed accessions. Fourteen accessions are positive controls or for research. In 1996, for the first time, seed accessions (with very low seed counts) were germinated and propagated in vitro prior to testing. All other accessions, 131, are either in testing or ready for testing and no accessions require therapy at this time.

Of the 88 sweetpotato accessions currently in the tissue culture inventory 14 therapy-derived clones and clones of 21 new accessions are ready for testing in 1997. With the exception of one accession requiring additional therapy and 9 accessions being held as positive controls or for research, the 43 other accessions are already in testing for the second year. The indicator *Ipomoea setosa* now is being propagated in tissue culture for the second year for use in greenhouse testing procedures.

In March of 1996 the tissue culture lab began to establish, maintain, and conduct therapy on *Ribes* and *Rubus* accessions. To date 57% of the 60 quarantined *Ribes* and 66% of the 15 quarantined *Rubus* accessions have been established in tissue culture and therapy has been conducted on 33% of the *Rubus* accessions. Work continues to improve procedures for *Ribes* culture and to improve the efficiency of the therapy procedures for both genera.

In vitro cultures of other accessions, indicators, and positive controls in genera such as *Malus*, *Pyrus*, *Prunus*, and *Manihot* have been handled in the tissue culture laboratory in 1996. The volume and complexity of this work has been limited by resources and priorities.

Rice quarantine activities have been moved from the old Glenn Dale site. As a result greenhouse production space has been reduced to approximately one third of the old space. By decreasing the space between accessions and rearranging bench space, the new facility will be able to produce approximately 184 accessions per year or approximately 50% of the old production. From March 1, 1996 to March of 1997, 734 quarantine released accessions were sent to recipients. The current inventory includes 92 accessions growing in the greenhouse and 196 accessions at this facility and waiting quarantine processing. The number of quarantined rice accessions being held at NSSL is 2415. Accessions at NSSL are quarantine processed in Beltsville as space allows, but usually after other new imports.

3

Plant Exchange Office (PEO) - Ned Garvey

Exchanges

Maryann Loftus continues to forward germplasm letter requests to the appropriate maintenance sites, and has assumed responsibility for forwarding requests received through GRIN via the World Wide Web. She also provides alternate germplasm sources for requested items not maintained by NPGS and continues to place orders for foreign germplasm for U.S. scientists.

During 1996, 17,878 items in 362 shipments destined for scientists in 73 foreign countries passed through the Beltsville Quarantine Center. Additionally, 1,317 non-permit items were received from foreign cooperators for use by U.S. scientists.

Shipping Update

All the NPGS sites and many university scientists are now screening germplasm requests through the A.P.H.I.S. inspectors at the Beltsville Quarantine Center before sending materials to Beltsville for inspection and shipping. This has greatly reduced backlogs while packages wait for receipt of required documentation or permits. Turn-around time of shipments from receipt to inspection and mailing has been reduced to a few days, with priority handling given to perishable shipments.

PI Documentation

Becky Norris continues to coordinate the documentation of germplasm entering the NPGS and assignment of PI numbers. During the last year, 3,900 PI numbers were assigned and passport data verified for inclusion in the GRIN database. This includes 461 Crop Science registrations and 503 PVP accessions. Approximately 529 CSR certificates were issued and distributed to the authors.

Becky also assists curators and NPGS site personnel with adding and updating passport data and with DBMU personnel standardizing and correcting records in the GRIN database.

Plant Inventory No. 205 for 1996 has been sent to the publishers and should be distributed soon. It contains 420 pages of text and indexes.

A crop germplasm vulnerability database was created this year that will be used to assist in the planning of future plant explorations. This database will be continuously updated as CGC reports are received.

In Situ Projects

Dr. Diane Pavek joined the PEO in March, 1997 as a Postdoc. Diane is doing a two year ecogeographic survey of select grape species (*Vitis* spp.) native to North America. In collaboration with grape germplasm curator, Warren Lamboy, Diane will be evaluating the genetic diversity present in these wild populations using molecular and morphological methods and determining suitability of the populations for *in situ* conservation efforts. This project will be one step in a multi-step process to establish strategies for planning *in situ* preserves for conservation.

Diane has examined wild grape specimens from 12 herbaria to analyze geographic ranges and morphologic variability within and between taxa. This summer Diane will be locating populations of sand grape (*V. rupestris* Scheele, a rare grape threatened by habitat loss) in eight south-central and eastern states. She will record morphologic data and collect leaves for DNA extraction.

We continue to participate in the Federal Native Plant Collection Committee which meets bi-monthly to discuss conservation activities of member agencies. Extensive information about the committee is included on its website: www.aqd.nps.gov/natnet/npci/.

The PEO is supporting a project in Israel for the emergency collecting of crop relatives in areas designated for development. Both *ex situ* and *in situ* methods are being considered. Dr. Y. Anikster of Tel Aviv University is the cooperating PI for the project. Dr. Harold Bockelman, curator of the U.S. National Small Grain Collection will also be involved in the project.

Plant Exploration

The USDA Plant Exploration Program is coordinated by Karen Williams. The PEO provided support for 3 domestic and 8 foreign plant explorations in FY96. Seven explorations are being supported in FY97. Plant exploration proposals for FY98 are due in the PEO on June 15, 1997. It is no longer necessary to send them to the Regional Coordinators. The Plant Exploration Tracking System, a relatively new database application designed to track accessions collected on USDA plant explorations, shows that approximately 1500 accessions a year were collected in 1994 and 1995.

In August and September of 1996, Karen participated in a joint INIAP, USDA, IPGRI exploration to Ecuador to complete a country-wide exploration for peanut landraces. Associated with this, a project was developed with INIAP to increase and characterize accessions collected in Ecuador in 1995. Charles Simpson, peanut breeder from Texas A & M University, served as an advisor to this project and traveled to Ecuador in December, 1996 to assist Ecuadorian scientists in the characterization process. This activity helps enhance germplasm exchange, transfer of technology to a germplasm donor country, and improve professional interactions between U.S. and Ecuadorian scientists. In addition, this type of activity has potential as a cost-effective method of increasing and characterizing USDA germplasm accessions.

A collaborative project with IPGRI and CIAT is being undertaken to test the potential of Geographical Information Systems for locating cultivated plant diversity. This project will investigate the relationship between known sites of landrace peanut diversity in Ecuador and their cultural and biophysical environment. After the identification of parameters that are useful in locating peanut genetic diversity, a predictive model will be developed for use in areas of unknown peanut landrace genetic diversity. This model will be tested in Guatemala in November, 1997.

In collaboration with the PEO, the GRIN Database Management Unit is developing a computerized data form that will be used by germplasm collectors to enter their passport data. Upon completion, collectors funded through the USDA Plant Exploration Program will be asked to use this package to provide their passport data to PEO. Data entered into this application will be loaded into GRIN and the Plant Exploration Tracking System. The package can also be used by collectors to print reports or to transfer data to other databases.

Ned Garvey, Dr. Robert Krueger, USDA, ARS, NGR, Riverside, CA and Dr. Kim Bowman, USDA, ARS, HRL, Orlando, FL participated in a citrus collecting trip to India in January 1996. This trip was funded through the USDA-FAS.

Ned also participated with Dr. Fred Muehlbauer, USDA, ARS, Pullman, WA in a collecting trip to Albania in August-September 1996. This trip was partially funded through USAID.

USDA/ARS Plant Explorations Undertaken in FY 1996

Plant Exploration	Country	Principal Contacts
Woody landscape plants	People's Rep. of China	R. Lewandowski, K. Conrad
<i>Fragaria virginiana</i>	USA	J. Ballington, J. Payne, K. Williams
Oilseed Crops	USA	D. Dierig, T. Coffelt, A. Salywon
Legumes and other vegetables	Bulgaria	R. Hannan, W. Kaiser, M. Mihov
Small fruits	People's Rep. of China	M. Thompson, J. Postman, C. Finn
<i>Glycine</i> spp.	Australia	T. Hymowitz, A. Brown, J. Grace
<i>Solanum</i> spp.	Costa Rica	D. Spooner, W. Quirós
Cool-season grasses/legumes	Mongolia	D. Johnson, D. Sheehy
Small fruits	USA	K. Hummer, C. Wright
Cucurbits	South Africa	J. McCreight, T. Wehner
<i>Arachis hypogaea</i>	Ecuador	K. Williams, C. Simpson, C. Tapia

USDA/ARS Plant Explorations Planned/Undertaken in FY 1997

Plant Exploration	Country	Principal Contacts
<i>Leucaena pallida</i>	Mexico	J. Brewbaker, C. Beust
<i>Malus</i> spp.	People's Rep. of China	P. Forsline, H. Aldwinckle, L. Benson
Cool-season grasses/legumes	People's Rep. of China	D. Johnson, K. Jensen
<i>Vigna angularis</i>	People's Rep. of China	T. Lumpkin, E. Yee
<i>Solanum</i> spp.	Mexico	D. Spooner, H. Lazoya
<i>Echinacea</i> spp.	USA	K. McKeown, R. Bernatsky, M. Widrechner
<i>Gossypium</i> spp.	Australia	J. Stewart, J. Wendel, C. Brubaker

Germplasm Needs - CGC Reports

CGC reports continue to be screened for reported germplasm needs. The PEO relies upon the knowledge of the NPGS curators and CGC members in identifying germplasm needs. We welcome everyone's input. Precise germplasm needs from specific countries or regions with the reason(s) it is needed is most valuable to us. Also important is relationship of the target species to the crop species, i.e. if it crosses readily, with difficulty or not at all at this time. This information is useful to maximize the efficiency of our plant exploration and exchange programs.

4

Germplasm Resources Information Network (GRIN) - Jimmie Mowder

Continued maintenance of GRIN for the plant introduction stations and other NPGS management units is the major priority for the Database Management Unit (DBMU). The GRIN database was accessible over 97% of the time on a 7 day a week and 24 hour a day schedule.

We are now in the design phase of the GRIN Windows project that will provide a graphical user interface to the database. The GRIN Advisory Committee reviewed and commented on some early prototypes in October 1996. We will incorporate their design considerations into the new system.

The pcGRIN maintenance system has been re-written to incorporate all of the functionality of the larger GRIN system. This system will be available for use by smaller genebanks around the world that need an information system but currently lack resources to develop one. A pcGRIN workshop was held at Beltsville in May 1997 to have the software reviewed by scientists from 4 Latin American countries. The workshop was jointly prepared and supported by the International Plant Genetics Resources Institute and NGRIL.

Considerable effort was given to the preparation of reports for internal management and the GAO study of the NPGS.

The GRIN computer was moved from the National Agriculture Library to Building 003.

The GRIN disk storage space was expanded with the installation of a second disk array. This allows mirroring of all system and user data across separate devices increasing data security.

The DBMU assisted NPGS maintenance site personnel with questions concerning data entry and retrieval and provided GRIN "hands-on" training to site personnel visiting Beltsville. Data from the Desert Legume Project and the Pea Genetic Stocks Collection were incorporated into GRIN. A member of the DBMU participated in a workshop for the FAO World Information and Early Warning System (WIEWS) project.

The DBMU is working with Agriculture and Agri-Food Canada in the installation of the GRIN system at the Saskatoon Research Facility. Two Canadian computer specialists visited Beltsville in May to become more knowledgeable of GRIN's capabilities. We continue to work with India, Brazil, and the Russian Federation in the development of germplasm information systems.

The DBMU continues to lead the implementation of the Beltsville Agriculture Research Center network project that will provide high speed telecommunications to all management units at the research facility.

DBMU personnel continue to perform as webmasters for Agency Headquarters and Beltsville Area efforts. This entails daily management and maintenance for 50-60 individual management unit web pages.

5

Crop Germplasm Committee Facilitation - Mark Bohning

Forty Crop Germplasm Committees (CGC) continue to provide support to the National Plant Germplasm System (NPGS) and most have been active over the last year. The NGRL continues to assist in coordinating their activities and participated in 32 of their meetings during the year. Though not all inclusive, the CGC's are supporting the NPGS by:

- identifying gaps in U.S. collections and developing proposals to fill them through exchanges or collecting trips
- assisting crop curators in identifying duplications in collections
- prioritizing traits for evaluation and developing evaluation proposals
- assisting curators and GRIN personnel in correcting and standardizing passport and evaluation data and ensuring that complete information is entered into the GRIN database
- assisting curators with regeneration projects
- identifying germplasm in breeder working collections that should be incorporated into NPGS collections and assisting with arrangements to accomplish this
- working with quarantine officials to identify and ensure implementation of new techniques for pathogen identification
- evaluating the potential benefits and problems associated with the development and use of core subsets

A sixth biennial CGC Chairs meeting was held in Beltsville, MD, July 15-16, 1996. This meeting provided an opportunity for the 40 chairs (or their designated representative) to interact with each other, NPGS personnel and the National Program Staff. Numerous topics relating to the NPGS and genetic resources maintenance and use in general were discussed. Some of these topics included: status reports from NPGS active and base collections, the role and expectations of CGCs, impact of the Convention on Biological Diversity, the status of international genetic resource programs, intellectual property rights, plant quarantine issues, the role of core subsets in managing collections, updating and correcting GRIN data, the status of

plant breeding programs in the U.S. and the need to increase the usefulness of germplasm collections through enhancement programs. A discussion was also held on the CGC crop vulnerability reports. These reports contain information on the status and future needs of plant genetic resources regarding acquisition, preservation, evaluation and enhancement. These reports are compiled and summarized by the NGRL and priorities identified across crops. They will also be made available over the Internet on the GRIN World Wide Web page (<http://www.ars-grin.gov>).

6

Molecular Virology Investigations - A. Hadidi

The primary mission of the unit is to develop new techniques for detection of pathogens in quarantined germplasm. During the year, nucleotide sequence analysis as well as serological reactivity demonstrated that the sour cherry strain of plum pox virus from Moldova is a prototype for a new subgroup of plum pox virus termed plum pox virus-cherry. Similar molecular and serological analyses also demonstrated that the sweet cherry isolate of plum pox virus from Italy is a member of plum pox virus-cherry subgroup and that it is very similar to the sour cherry strain of the virus in many respects. Sensitive and rapid methods, based on polymerase chain reaction (PCR) technologies have been developed for detection and identification of plum pox virus-cherry subgroups; pear decline, peach liptoncrosis, aster yellows, and peach x-disease phytoplasmas. The phytoplasma associated with cherry lethal yellows disease from China was completely characterized and found to be closely related to the phytoplasma causing jujube witches broom in China. Based on 16 S RNA sequence, the two pathogens form a new subgroup within the elm yellows group of phytoplasmas. Also, the complete nucleotide sequence of a viroid from sweet cherry plants has been determined. The viroid is a strain of peach latent mosaic viroid. This is the first report of a viroid infecting cherry. The results of research will be used by plant pathologists or plant quarantine officials responsible for keeping foreign plant diseases from entering the United States.

Publications:

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- . Hooftman, R., Arts, M-J., Shamloul, A. M., Van Zaayen, A., and Hadidi, A. 1996. Detection of chrysanthemum stunt viroid by reverse transcription-polymerase chain reaction and by tissue blot hybridization. *Acta Horticulturae* 432:120-128.
- . Nemchinov, L. and Hadidi, A. 1996. Characterization of the sour cherry strain of plum pox virus. *Phytopathology* 86:575-580.

- Nemchinov, L., Hadidi, Maiss, E., Cambra, M., Candresse, T., and Ramsteegt, V. 1996. Sour cherry strain of plum pox potyvirus (PPV): Molecular and serological evidence for a new subgroup of PPV strains.
- Shamloul, A. M., Van Zaayen, A., Hooftman, R. Arts, M-J., and Hadidi, A. 1996. Detection of chrysanthemum stunt viroid by reverse transcription-polymerase chain reaction and by tissue blot hybridization. 9th International Symposium on Virus Diseases of Ornamental Plants. Herzliga, Israel. p. 15.
- Crescenzi, A., d'Aquino, L., Comes, S., Nuzzaci, M., Piazzolla, P., and Hadidi, A. 1996. Characterization of the sweet cherry isolate of plum pox potyvirus. Middle European Meeting '96 on Plum Pox, Budapest. p. 42.
- Zhu, S. F., Lee, I-M., Chiu, W., and Hadidi, A. 1996. Use of amplified restriction fragment polymorphism for generating specific cRNA probes for aster yellows and x-disease phytoplasmas. 11th International Congress of the International Organization for Mycoplasmaology, Orlando, FL. p. 107.
- Zhu, S. F., Lee, I-M., Gundersen, D. E., Zhang, C. L., and Hadidi, A. 1996. Phytoplasmas associated with cherry lethal yellows and jujube witches broom in China represent a new Candidatus subspecies level taxon. 11th International Congress of the International Organization for Mycoplasmaology, Orlando, FL. p. 218.
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- Zhu, S. F., Tian, G. Z., Peng, W., Liu, C., Lee, I-M., and Hadidi, A. 1996. PCR detection and identification of phytoplasmas associated with nine different diseases from China. 5th International Workshop on Phytoplasmas, Beijing, P.R. China. p. 22. *Phytopathology* 86:1215-1221.
- Shamloul, A. M., Hadidi, A., Zhu, S. F., Singh, R. P., and Sagredo, B. 1996. Sensitive detection of potato spindle tuber viroid using RT-PCR and identification of a viroid variant naturally infecting pepino plants. *Canadian Journal of Plant Pathology* (in press).
- Giunchedi, L., Hadidi, A., Poggi-Pollini, C., Bissani, R., Mordenti, G. L., and Lugaresi, C. 1997. Plum spotted fruit, a disease associated with peach latent mosaic viroid. *Revista di Frutticoltura e di ortofloricoltura* L1X92-94.
- Crescenzi, A., d'Aquino, L., Comes, S., Nuzzaci, M., Boscia, D., Pazzolla, P., and Hadidi, A. 1997. Characterization of the sweet cherry isolate of plum pox potyvirus. *Plant Disease* (in press).

Appendix 3



United States
Department of
Agriculture

Agricultural
Research
Service

Northern Plains Area
National Seed
Storage Laboratory

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May 5, 1997

SUBJECT: NSSL 1996 Progress Report

TO: Regional Technical Advisory
Committees on Plant Germplasm

FROM: National Seed Storage Laboratory
Steve A. Eberhart, Director
Eric E. Roos, Research Leader
Loren E. Wiesner, Research Leader

ADMINISTRATION
Steve A. Eberhart, Director

The increase in the operating budget of one half million dollars starting with FY 97 will provide funds for the increased operating and maintenance costs, for temporary staff to reduce the backlog of incoming samples, and to purchase needed equipment.

Two cooperative projects (Regeneration and Genetic shifts) with the Botanical Garden of the Polish Academy of Sciences in Warsaw, Poland have been completed. The International Conference "Crop Germplasm Conservation Problems with Special Emphasis on Rye" was held in Konstancin-Jeziorna, Poland July 2 - 6, 1996. Christina Walters and Steve Eberhart participated in the conference and presented papers.

Eric Roos and Steve Eberhart attended the 2nd International Crop Science Congress in New Delhi, India, November 17 to 22. They were invited guests at the inauguration of India's new genebank in New Delhi.

Regeneration of endangered Latin American maize landrace accessions will continue in a cooperative project with 13 countries under a new Specific Cooperative Agreement with CIMMYT. NSSL received 4,195 samples in 1996 bringing the total to 9,374 maize landrace accessions regenerated and stored under this project. Franco, Crossa, Diaz, Taba, and Villasenor, CIMMYT, have developed "A Sequential Clustering Strategy for Classifying Gene Bank Accessions" to be published in Crop Science that utilizes both quantitative and qualitative traits. These methodologies will be used with LAMP and CIMMYT data to develop a maize core subset.

The LAMP project has been completed and the Final LAMP Report is being prepared for distribution. The principal objective of LAMP was to provide and facilitate access to this information to breeders so that they can use this germplasm to create superior varieties and hybrids. In the U.S., an unprecedented public/private research effort to broaden the genetic diversity of U.S. corn hybrids using enhanced maize germplasm derived from selected LAMP accessions has been initiated as the Germplasm Enhancement Maize project (GEM). This is a unique case of collaboration in which 19 public institutions and 21 private seed companies are working together with the objective of increasing the productivity and genetic diversity of maize grown in the U.S.

SEED VIABILITY AND STORAGE RESEARCH UNIT
Loren E. Wiesner, Research Leader

A total of 38,279 samples were placed into storage: 996 into cryostorage and 37,283 into -18C storage. A total of 31,289 new samples were received during 1996; 424 from Plant Variety Protection Office, 624 apple buds which included 343 from Canada, 34 endangered species from Botanical Gardens, 175 plant quarantine, 24,766 seed samples from Regional Plant Introduction Stations, 461 Crop Science registration, 4,010 Latin American Maize regeneration, 77 Desert Legume Program, 241 Charles Rick collection and the remaining 477 samples from other individuals and organizations. The 1996 samples received plus carryover samples

from 1995 give us a backlog of 35,388 samples. A total of 2,553 accessions were distributed to 13 countries and 108 scientists. Sorghum (69) and corn (228) accessions were packaged, washed and sent to St. Croix for quarantine increase.

A new maize regeneration project was initiated and an inventory made of the maize germplasm collections preserved at CIMMYT, National Seed Storage Laboratory (NSSL), and National Genebanks in Latin America and the Caribbean. The inventory identified collections of maize which are low in viability and /or with low seed numbers and need to be regenerated. Plans for the regeneration of about 3,000 landrace collections at these locations are being prepared and will be implemented during the duration of this project.

The National Seed Storage Laboratory has received 4,195 samples of Latin American Maize landrace accessions, for storage in 1996, from the cooperative maize regeneration project with CIMMYT, USDA-ARS, and USAID. Of these samples, 3,964 were received through CIMMYT and 231 samples were received directly from Venezuela and Peru. In addition to the countries sending seed directly to NSSL, we have received seed from Brazil, Columbia, Cuba, and Mexico. In total, this project has sent 9,374 accessions for storage when duplicate samples are removed. Work is continuing on the development of a Latin American Maize core subset.

Germination tests were completed by the Colorado Seed Testing Laboratory under the terms of this Specific Cooperative Agreement on 1101 samples. The samples consisted of 323 onion samples and the remaining 778 samples were of lettuce and other species which were in need of retesting for germination. Our retest schedule is being determined based on the storability of each crop. We have determined that onion, lettuce, carrot, peppers, peanut, brassica, clover, sorghum, rye, bluestem, tomato, tobacco, and beet should be the first crops to be retested. Retest lists will be prepared of accessions not tested in the last ten years. As these samples are pulled for retesting we are obtaining random samples for moisture determination at the time of retesting.

We have been studying the effects of dormancy breaking chemicals on dry seed of sunflower. These studies are designed to find a method of overcoming sunflower seed dormancy before the seed is field planted. The effectiveness of the treatments appears to be cultivar and /or strain related. Modifications have been made to the apple bud drying room and the drying procedures. These procedures have reduced drying time and made moisture testing less time consuming. We have continued to look at ways to evaluate duplication in the pea collection at NSSL. Many primers have been evaluated for use in PCR studies.

PLANT GERMPLASM PRESERVATION RESEARCH UNIT

Eric E. Roos, Research Leader

The following report summarizes research activities by the scientists in the Plant Germplasm Preservation Research Unit at NSSL. Each project is briefly summarized and includes personnel assigned, the problem area, approach, and results for the past year. Following the reports is a list of publications appearing in print in 1996.

PERSONNEL: Ms. Jennifer Crane, research technician for Dr. Walters, left the unit to move to California. Ms. Lisa Hill has assumed a temporary biological technician position with Dr. Walters and Ms. Tammi Cooper has taken a similar position with Dr. Towill.

VISITING SCIENTISTS: Dr. Abdullah Alsadon from King Saud University completed his sabbatical year and has returned to Saudi Arabia. Dr. Ming Zhang, from China, joined us in June of 1996 to work on a post-doctoral position jointly with Drs. Roos and Walters in the area of production of seed volatiles during storage. We received the usual large number of visitors from all over the world for periods of 1-2 days.

GRADUATE STUDENTS: Mr. Jian Fang, from the Peoples Republic of China is working on his Ph.D. under the direction of Dr. Roos. Ms. Joyce Pennycooke from the Commonwealth of Dominica is working on a M.Sc. degree in the area of cryogenic storage of sweet potato under the guidance of Dr. Towill. Ms. Kim Davidson is doing her Ph.D. research under the guidance of Dr. Walters. Ms. Terri Christensen is working on a M.Sc. degree with Dr. Roos.

TECHNOLOGY TRANSFER: A complete list of publications, except for abstracts, from the National Seed Storage Laboratory dating from 1960, is available for distribution. Copies of papers can be requested from: Dr. Eric E. Roos, Research Leader, Plant Germplasm Preservation Research, National Seed Storage Laboratory, 1111 South Mason Street, Fort Collins, CO 80521-4500. We have set up a World Wide Web page on the INTERNET that can be accessed via the following:

<http://www.ars-grin.gov/ars/NoPlains/FtCollins/nsslmain.html>

The list of publications is also available through this web site.

The National Seed Storage Laboratory will host a symposium entitled "Seed Biology and Technology: Applications and Advances" on August 13-15, 1997. This symposium is sponsored by Regional Research Project W-168. Please write for further information or access the www site listed above to direct you to the www site for this symposium.

FOREIGN TRAVEL: In July of 1996, Dr. Walters attended the international conference "Crop Germplasm Conservation Problems with Special emphasis on Rye" in Poland where she presented papers on her research. In November of 1996, Dr. Roos attended the 2nd International Crop Science Congress in New Delhi, India and also was an invited guest at the inauguration of India's new genebank.

INJURY MECHANISMS DURING MAIZE SEED IMBIBITION AND DRYBACK

ERIC E ROOS (Supvry PI Physiol); Christina Walters (PI Physiol); Don Davidson (Biol Sci Tech); Jian Fang (Grad Student, China); Frank D Moore, III (Prof CO St Univ)

Problem: In previous cooperative work with Dr. Frank Moore at Colorado State University, we attempted to develop a non-destructive test for seed viability using the concept of seed leachate conductivity as a measure of seed deterioration. Theory says that membrane damage occurs during deterioration which results in a loss of cell electrolytes when seeds are imbibed. Key to developing such a test is to understand the role of various processes during early imbibition, including imbibition damage, priming effects, accelerated aging, and desiccation sensitivity as related to loss of viability during hydration/dehydration treatments.

Approach: Two experiments were conducted with maize to determine the moisture level at which we see imbibition damage, and to determine the effect of using excised embryos to determine the relationship between germination and leachate conductivity. Maize seeds were adjusted to moisture levels from 5 to 12% (dwb) and soaked in distilled water for periods of 2 to 12 hr, followed by rapid dryback to the initial seed moisture content (mc). Control seeds were rehydrated over water in a desiccator to alleviate the moisture stress upon imbibition. For the second experiment seeds were hydrated to three levels (27, 34 and 40% mc) and held for up to 10 days in a desiccator at room temperature. Each day whole seeds, and also excised embryos from these seeds, were dried to 12% prior to germination and leachate conductivity tests.

Results: Maize seed dried to 8% mc showed no imbibition injury for either germination or vigor (root lengths). However, there did appear to be some damage at 5% moisture content (reduced root lengths). This confirms our previous work which indicated that seeds having 12% mc are in no danger of imbibition damage.

In the second experiment we found that using excised embryos gave a very good correlation between conductivity readings and germination. For whole seeds the regression was significant ($r^2 = 0.41^{**}$), however for excised embryos the correlation coefficients were much higher (0.80**, 0.92** and 0.91**, for 27%, 34% and 40%mc, respectively). Thus, it would appear that the conductivity test is useful as a predictor of seed quality, if excised embryos can be used.

SEED LONGEVITY STUDIES

ERIC E. ROOS (Supvry Plant Physiol); Terri Christensen (Grad Student), Don Davidson (Biol Sci Tech)

Problem: Long-term preservation of plant genetic diversity using the most cost-effective means will usually involve storage of seeds under optimum storage conditions. Although we generally know that this is best

accomplished by cold, dry storage, we are unable to reliably predict how long seeds will remain viable under these conditions. Nor do we know how different species will store, or how seed lots within a species will behave. The problem then is to acquire longevity data on seeds stored under controlled temperatures and seed moisture contents for a wide variety of species and seed lot accessions in order to validate theoretical approaches.

Approach: Seed longevity data are acquired from several sources including experiments initiated by previous scientists at NSSL or elsewhere. Included in these are seeds from the Rincker study on forage legume and grass seed; various vegetable seed lots from earlier work by James and Bass; and seeds of several desert species from the Went longevity study. Also, seed lots in storage at NSSL since the early 1960's are available for viability assessment. Seed moisture contents are determined along with the germination level. Seedling vigor may also be assessed.

Results: We have initiated tests on the Fritz Went long-term storage experiment as part of a MSc thesis for Ms. Terri Christensen. These seeds were initially sealed in 1948 and have never been stored at low temperatures, and have not been tested since 1969. As these seeds were stored in vacuum sealed glass tubes, the first test will be to determine if the vacuum seal is still intact. Data to be taken include moisture content, germination, and headspace gases, if possible. As we have just begun this experiment it is possible to say only that some tubes did not have a vacuum seal, and we know that some seeds in the first tests showed some positive geminations. We will report more on this next year.

EVALUATION OF PLANT GERMPLASM USING DIGITAL IMAGE ANALYSIS AND CRYOPRESERVATION OF PLANT GENETIC RESOURCES

PHILLIP C. STANWOOD, (Res Agron); Lana Wheeler, (Biol Sci Tech)

Problem: Seeds are used as the primary means of preserving plant diversity for future generations. In a very practical sense, seed moisture content and storage temperature are the two primary factors that one can control to lengthen the time seeds can be preserved. The use of ultra-cold temperatures for storage, cryopreservation (-196C), has been suggested as a means of greatly extending the storage life of seeds and other biological materials. Short-term studies (< 2 years), have demonstrated the efficacy of seed cryopreservation on over 130 species. However, longer-term responses are needed to evaluate the full potential of this technology and how cryopreservation relates to current storage methodologies. Part of the problem is how one evaluates and monitors the deterioration of seeds over time. Seed germination has been and is currently used as the evaluation technique. There are certain limitations to this technique. Early detection of deterioration before loss of germination would be highly desirable, reducing the likelihood of loss of genetic diversity from reduced seed viability.

Approach: Current research is directed at: 1) development and understanding of cryopreservation of seed and pollen using liquid nitrogen (-196 C) as a storage medium; 2) development and use of computer digital analysis to measure vigor (deterioration) of seed germplasm; and 3) evaluation of the concept of image oriented databases (electronic seed herbarium).

A robotic system based on computer image analysis was built to simultaneously conduct 50 seedling root growth vigor tests. Several species have been successfully grown on the slant board robotic system including lettuce, onion, cucumber and sorghum seed. The SGRobot is temperature controlled with slant boards slewing continuously, providing the same micro-environment to each board which significantly reduces experimental variation. This reduced error allows for more reliable results, use of small number of seeds per test, reduced labor and material cost and enhanced evaluation of the seed germplasm. The output of the SGR system is a series of time course root growth curves for individual germinating seeds. From these curves, analyses can be done to determine the relative vigor (deterioration) of a sample. Reduction of vigor precedes loss of seed germination, thus identification of vigor loss provides an extremely sensitive and valuable tool in accessing the storability of a sample and thus expected longevity. This information greatly

enhances the management of genetic resources, ultimately improving the preservation of the material while reducing costs and labor inputs.

A digital image oriented database concept is being investigated to enhance the collection, storage and dissemination of information concerning our preserved plant genetic resources. Chickpea, lettuce, and sugar beet image sets are being used as test species for this part of the project. Information from these studies is consolidated and provided on photo CD-Rom. Information and images from these image databases are also placed on the USDA-ARS, Germplasm Resources Information Network (GRIN) in Beltsville, Maryland which is available through the INTERNET and world wide web (www.ars-grin.gov).

Cryopreservation of plant genetic resources using liquid nitrogen (-196 C, -320 F) offers the opportunity to enhance the longevity and quality of stored materials such as seeds, plantlets, cell suspension, pollen, and buds. This technique can also improve the reliability of the storage system, reduce costs and other resources needed per sample. Seeds from over 130 species have been successfully exposed and stored in liquid nitrogen. Long-term preservation studies are underway to determine the practical and biological feasibility of the cryopreservation technique for seed germplasm.

Highlights for 1996: Most of the 1996 effort was directed at completing image databases and image analysis of seed materials. Two trials were concluded in cooperation with Dr. Reid Palmer, Ames, IA, investigating genetic inheritance of seed color in soybean seed germplasm. The Cicer image database work was completed. A Cicer disease and Cicer insect image database were created. These with seed images, field grown plant images, literature citations and a cooperator image database were combined into a working CD-Rom distribution copy. This GARBS CD-Rom was distributed to a number of individuals for evaluation. The Garbs CD is available to interested researchers. The images from the Cicer image project (approximately 1,500 high color images) were placed on the USDA-ARS GRIN (Genetic Resources Information Network) National database and are available to the general public and researchers through the GRIN WWW (world wide web) site (<http://www.ars-grin.gov>). Approximately 500 images of mature Cicer field grown plants have also been submitted to GRIN for inclusion in the USDA-ARS GRIN database. In cooperation with the USDA in Fargo, ND, approximately 165 images of sugar beets were processed and a image database created. These images have been placed on the USDA-ARS National Plant Germplasm GRIN database. In cooperation with Pullman, WA, approximately 970 images of field grown lettuce were digitized and also placed on the GRIN database. The total number of images that have been prepared and sent to GRIN is approximately 3,500. Related to the above image database activity, a www site for the USDA-ARS National Seed Storage Laboratory was developed (<http://www.ars-grin.gov/ars/NoPlains/FtCollins/nsslmain.html>).

A device (SlantGrowthRobotics, SGR), developed in our laboratory to automatically measure seedling root growth as a measure of seed vigor, was redesigned to enhance lighting options of the seedling roots. In 1996, new programs were written to allow multiple experiments to be conducted simultaneously. Several new algorithms were developed to enhance the analysis of the resulting root images. Development of a new robotic system was begun with the objective of measuring the shoot growth of seedlings as a way of identifying seed vigor, especially in the grass and cereal species. Experiments have been started to define the relative deterioration of seed germplasm samples that have been stored for extended periods in time (up to 20 years) at -196C (liquid nitrogen), -18C, and 5C. There are approximately 200 samples, representing 45 species, that will be tested in this study.

CRYOPRESERVATION OF APPLE FROM THE CANADIAN CLONAL GENE BANK

LEIGH E. TOWILL (PI Physiol), John W. Waddell (Biol Sci Techn), Tammi Cooper (hourly employee), John Warner and Margie Luffman (Agriculture and Agrifood Canada, Canadian Clonal Genebank, Smithfield)

Problem: The Canadian Clonal Genebank has been interested in methods that have been developed for the apple cryopreservation at NSSL for application to their *Malus* collection. This need has been accentuated by the move of the genebank from Smithfield, Ontario to Harrow, Ontario, necessitating establishment of new orchards. We have agreed to help them with establishing cryopreservation methods to mitigate against germplasm loss during the move. This also promotes international cooperation in preserving genetic resources and in sharing technological advances.

Approach: In conjunction with the Canadian clonal genebank, 324 lines were identified at Smithfield for long-term conservation. To facilitate application of the cryogenic methods, cold hardy twigs were sent to NSSL and processed using the dormant vegetative bud method.

Results: The lines were processed as previously described and are being held in the vapor phase over liquid nitrogen. Where enough material was available: 1) a small sample was processed without desiccation and is also stored in the vapor phase; 2) a subsample was warmed and the viability estimated using the oxidative browning test. Results of samples stored in liquid nitrogen are similar to what was observed in 1996 with the Geneva apple lines. Viability will be tested later at the Harrow location by budding to rootstocks; an initial test of 50 lines will be done in March of 1997. The Canadian genebank is in the process of obtaining equipment to process and store lines at the Harrow location.

CRYOPRESERVATION OF WOODY, VEGETATIVELY PROPAGATED GENETIC RESOURCES

LEIGH E. TOWILL (PI Physiol), John W. Waddell (Biol. Sci. Techn), Tammi Cooper (hourly employee) and Phillip Forsline (National Clonal Germplasm Repository, Geneva, NY)

Problem: Long-term preservation of species that are vegetatively propagated is needed to avoid potential loss of germplasm and is a priority area for NSSL. Cryopreservation allows for safe, long term storage which then gives clonal repository options for minimizing costs with field or greenhouse maintenance. Apple (*Malus* spp.) was the first clonal species to be routinely placed into cryogenic storage at NSSL using dormant vegetative buds. Other cold hardy, woody species were also shown to survive such a method, but often in lower percentages. Certain aspects of the method still need examination. How can the procedure be modified to be more efficient and effective for a given crop? Since glasses are implicated in attaining the cryopreserved state, how do cooling rates from -30C to -160C and warming rates from -160C to 22C affect survival? What alternatives can be used with particularly cold-tender lines? Some of these studies are ongoing and are summations from 2-3 years of work.

Approach: In 1996, we processed 252 apple lines as part of routine cryopreservation at NSSL using the dormant vegetative bud method. In experimental materials, variables of the procedure were again examined with selected lines, including time held at -30°C, cooling rate between -30°C and -196°C, and warming rates for tender and hardy lines. Encapsulation/sucrose treatment/dehydration for 1 bud twig sections was examined as a proposed method that may enhance viability after cryotreatment. Other species were examined for possible inclusion into routine storage using techniques similar to that used for apple and with encapsulation sequence.

Results: The cryogenic survivals for the routine preservation of Geneva materials were not as high as in past years (226 lines showed some survival after -196°C exposure and 172 lines showed at least 40% survival), but the reason for this decrease is unknown. A retest (budding) is being done at Geneva NY with the lines showing low viabilities. Samples that have been held for up to 8 years at ca. -180C showed no decline of viability during storage. Retests of some previous experiments confirmed past findings: for dried and undried materials, increasing the holding time at -30C improved survival in some lines, but was not consistent or predictable enough to be generally useful. Cooling rates between -30°C and -196°C did not greatly influence survival; again some lines benefitted from faster rates. Experiments using longitudinally-sliced twigs and desiccation at other temperatures were initiated to see if some aspects of the procedure can be expedited, but results after cryogenic treatment were generally poorer than the use of whole twigs. Two species from *Pyrus*, *Ribes*, and *Vaccinium* from Corvallis, OR were examined, but in preliminary tests viability was very low, probably due to the very low extent of acclimation occurring at the Corvallis location. Nineteen lines of sour cherry, *Prunus cerasus*, from Michigan (being included into the Geneva repository's collection), were examined in greater detail for routine storage because of positive results from 1995 preliminary tests. Assessments of viability by browning generally corresponded with the more intensive test of budding. Survival was enhanced in 1995 by using sections adjusted to 25% moisture compared to the routine 30% 1996, but the low levels of survival in 1996 in some dehydrated (but uncooled) samples to 25% moisture suggest that

overdrying occurred. Growth conditions during the summer of 1996 (drought) may have contributed to the quality of the initial material.

Some tests of the encapsulation/sucrose exposure/dehydration method were performed. In observations done with oxidative browning test, levels of survival were about comparable to those found with the routine test. Sucrose exposure and desiccation alone were sufficient in some tests. Encapsulation, sucrose exposure and desiccation were beneficial in other tests. Some materials showed low levels of viability after treatments (including controls). Gas chromatographic tests of the vapors from some encapsulated tips held in plastic bags showed acetaldehyde and ethanol -- suggesting that fermentative pathways were occurring, probably during the encapsulation/soaking period. This may account for the erratic and often low viabilities observed.

Many of the observations made are probably related to extent of cold acclimation and the potential to attain this within the genotype. Presently most lines are not characterized with regard to extent of acclimation in the buds and bark in a given year or what maximum hardiness potential is. It is not feasible to do the many tests each year to ascertain this information, but storage systems would benefit from a simple gauge to assess cold acclimation. We will be initiating tests to see if sugar concentration and composition may be a useful gauge.

CRYOPRESERVATION OF SHOOT TIPS USING VITRIFICATION METHODS

LEIGH E. TOWILL (PI Physiol), Tammi Cooper and Michelle Wagner (student hourly employees), Joyce Pennycooke (graduate student, Commonwealth of Dominica)

Problem: Vitrification, a method to cryopreserve diverse cells, tissues and organs, has been shown by us and others to be effective for a range of species. This is a relatively new method and is, as yet, not used as a routine method for cryopreservation. Vitrification is a process containing a series of steps which must be optimized. Modifications of the vitrification process usually need to be explored to develop an efficient, effective procedure. When incorporated, cryopreservation will allow clonal repositories options for cost savings in managing their field, greenhouse or in vitro collections. We continue to investigate aspects of vitrification for several species.

Approach: Most of these studies were with axillary buds excised from plants maintained in vitro. Such systems are axenic and minimize contamination when the treated axillary buds are cultured to produce the shoots. Both in vitro stock plants and the buds isolated from them may be treated prior to cryopreservation. Both liquid-based and encapsulation-based vitrification procedures are being examined.

Results: 1. Experiments this past year have used a single line of sweet potato (*Ipomoea batatas*) and were intended to examine how certain pre-cryogenic treatments of the plant and isolated shoot tip, and post-treatments of the growth medium may influence viability after low temperature exposure. A vitrification procedure previously devised by us was modified by exposing shoot tips to sucrose and a brief glycerol-sucrose treatment. Survival improved by immersion into LN at faster rates (use of foil strips as contrasted to either semen straws or cryovials), and by immersion into a cooled LN solution (ca. -208C) to prevent a vapor effect. Harvesting of the shoot tips immediately after a dark treatment results in higher survivals and suggests that endogenous sugar levels are important.

2. 'Encapsulation and dehydration' methods for cryopreservation also are also a form of the vitrification procedure. Preliminarily, we determined kinetics of drying and survival for sweet potato shoot tips within alginate capsules. Shoot tips survive desiccation to about 16-20%, but have not routinely survived subsequent exposure to -196°C. Results on moisture content with drying time have been erratic. Preculture treatments are being studied to determine their effect on survival with this method.

IN VITRO BACKUP OF CLONAL GERMPLASM ACCESSIONS

LEIGH E. TOWILL (PI Physiol), Tammie Cooper and Michelle Wagner (student hourly employees), Robert Jarret (Geneticist, Plant Genetic Resources Unit, Griffin, GA)

Problem: Germplasm collections that are maintained in vitro are often at risk unless a second site is available for storage of a duplicate. Development of a suitable cryopreserved backup for some of these in vitro-maintained crops is still a research phase project.

Approach: The National Seed Storage Laboratory has the capability to store some in vitro lines under slow growth conditions, and under minimal labor requirements. The sweet potato collection at the Plant Genetic Resources Unit at Griffin, GA is held entirely in vitro and is a representative crops for establishing protocols for such medium term storage between units.

Results: A medium term project for storage of in vitro-maintained sweet potato lines from Griffin, GA was initiated at NSSL in 1995 and observations on this stored material were continued during 1996. The objective was to provide a second site backup at NSSL since materials at GA are all held in one growth room and are at risk. Sweet potato is cold sensitive so lines cannot be stored below about +14C (preliminary tests). We selected +18C since cultures grew slower, but did not develop some injury-like symptoms as observed at +14C. Most lines survived at least 9 months at this temperature, but some lines have survived almost 20 months of storage. Microbial contamination has been a problem, probably because of the existence of some spores between the lip of the tube and the cap. The parafilm barrier generally has not deteriorated. It is obvious that a different type of vessel is desirable and sealable bags are envisioned as a more desirable system. This project drew awareness to the need for a backup site and currently materials are to be held at two physically different sites at the Griffin station.

OPTIMIZING SEED WATER CONTENT TO IMPROVE LONGEVITY IN EX SITU GENE BANKS

CHRISTINA T. WALTERS (PI Physiol), Jennifer Crane (Biol Sci Tech), Lisa Hill (Biol Sci Tech), Eric Roos (Supvry PI Physiol), N. Kameswara Rao (ICRISAT, India), Chen Shuping, (ICGR, CAAS, China), Hu Xiaorong (ICGR, CAAS, China), Jan Engels (IPGRI, Rome)

Problem: Seed genebanks must store seeds under optimum conditions in order to maximize longevity. However, there is disagreement regarding what the optimum conditions are. Some research has suggested that the drier the seeds, the longer they will survive in storage. In contrast, work at the National Seed Storage Laboratory has shown that seeds dried below a critical water content age faster and that the benefits of refrigeration or liquid nitrogen storage can be lost if seeds are overly dried. This research, funded by the International Plant Genetic Resources Institute, addresses the controversy in an experiment which tests the effect of very low water contents on seed aging rates for lettuce seed stored at different temperatures. The experiment is being conducted simultaneously in several laboratories around the world.

Approach: We used lettuce seeds because seeds from this species tend to age rapidly and because there is conflicting data for this species regarding the existence of an optimum water content for storage. The experiment was started in 1995. The water content of seeds was adjusted at the NSSL to 12 levels ranging from 0 to 9%, then the seeds were sealed in air-tight foil packets. Sets of seeds were kept at the NSSL or shipped to India and China where they were placed in storage at 20, 35, or 50C. Seed germination and seedling growth were monitored.

Results: Data from China, India and the US are comparable indicating that the experiment is valid. As expected, seeds stored at lower temperatures aged less rapidly. Deterioration was negligible for seeds stored at RH < 20, 50 and 75% and temperatures of 50, 35 and 20C, respectively. Until the seeds deteriorate further, we will not be able to ascertain whether drying to extremely low RHs increases, decreases, or has no effect on seed longevity. Conclusive information is not expected for at least another year.

A COST-BENEFIT ANALYSIS OF SEED STORAGE PRACTICES

CHRISTINA T. WALTERS (PI Physiol)

Problem: Breeders and genebank operators require seed storage systems which maintain highly vigorous seeds for a few years to several decades to perhaps centuries. The procedures used to preserve valuable germplasm may vary with the longevity required and the resources available. Unfortunately, there are few predictive tools to determine the longevity of seeds stored under different storage regimes and the cost of maintaining those storage conditions. This research is designed to provide guidelines for genebank operators so that they can design genebanks that will provide the required longevity at the minimum cost.

Approach: The research involves developing a cost-benefit model where the energy costs for drying and storing seeds are compared with the predicted longevity. Energy costs for drying and storing seeds are calculated from variables such as building specifications, drying protocols, packaging procedures, storage temperatures, and outside climate. Longevity of seeds are predicted from models of the effect of temperature and water content on seed aging rates. The volume of seeds that can be processed and the viability of the seeds following drying are evaluated from the drying temperature, RH, and packing density.

Results: The model shows a number of interesting features that should help genebank operators in making decisions on how to process and store seeds. Questions addressed by the model include where genebanks should be located, if seeds should be dried under ambient or refrigerated conditions and what RH should be used, should seeds be sealed in air-tight containers, whether cryopreservation is needed, and are separate active and base collections recommended.

THE ACQUISITION AND LOSS OF DESICCATION TOLERANCE DURING SEED DEVELOPMENT AND GERMINATION

CHRISTINA T. WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), Kim Davidson (graduate student), P. Berjak (U of Natal, So. Africa), N.W. Pammenter (U of Natal, So. Africa) J. Farrant (U of Cape Town, So. Africa), O. Leprince (Agricultural U, Netherlands), M. Eira (EMBRAPA, Brazil)

Problem: Seeds are divided into two categories based on their storage behavior. Orthodox seeds survive desiccation and so can be stored under cold, dry conditions. Recalcitrant seeds, on the other hand, do not survive drying and so cannot be stored for more than a year. Many economically-important species produce recalcitrant seeds. The mechanism(s) by which recalcitrant seeds are damaged by drying and orthodox seeds are protected are unknown, and yet this information is critical for successful germplasm preservation.

Approach: Orthodox seeds acquire desiccation tolerance during maturation and lose tolerance during germination. We are comparing the developmental patterns of orthodox seeds and recalcitrant seeds in order to quantify the level of desiccation tolerances achieved in the two seed categories and to gain insights into the mechanisms of damage and protection. We are considering ultrastructural, biochemical and biophysical changes in embryonic cells in the context of the water potential required to mediate these changes.

Results: We have shown that orthodox and recalcitrant seeds behave very similarly during the early stages of embryogenesis. As maturation proceeds, recalcitrant seeds initiate germination processes and maintain high levels of metabolism. In contrast, orthodox seeds tend to switch off their metabolism and enter into a state of quiescence. In spite of differences in desiccation tolerances, we do not see differences in the levels of putative protectants (sugars, LEA proteins) among different seed types. We have noticed changes in the physical behavior of water in orthodox seeds as they enter into the desiccation tolerant state. The cause of this change and its importance for the tolerant condition is conjectural.

PROPERTIES OF LEA-LIKE PROTEINS

CHRISTINA T. WALTERS (PI Physiol), M.K. Walker-Simmons (ARS, Pullman, WA)

Problem: During the final stages of maturation, seeds produce an abundance of proteins for which there is no known function. These proteins, called late-embryogenic-abundant (LEA) proteins, constitute as much as 40% of the total soluble protein in seeds and have the unusual property of remaining soluble when boiled. Because these proteins are produced in organisms with high tolerances to drying or freezing, it is believed that they may play a role in conferring tolerance. In an attempt to learn more about the role of these proteins under dry and cold conditions, we are studying their hydrophilic properties.

Approach: Heat-soluble proteins were extracted from wheat embryos and the water absorbing capacity was characterized by the rate and total amount of water absorbed at different relative humidities. In addition, the freezing and glass transition behavior of protein fractions were analyzed using differential scanning calorimetry.

Results: Heat soluble proteins from wheat had a high affinity for sugars, and in combination with sugars, had unusual hydrophilic properties. Some protein fractions absorbed more than 2x their weight in water and resisted drying. Based on information from this work, we have developed a working hypothesis which suggests that LEA proteins serve as a hydration buffer in desiccating tissues.

THE KINETICS AND MECHANISM OF DETERIORATION IN DRIED ORGANISMS

CHRISTINA T. WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), M. Zhang (post-doctoral research associate, China), J. Butink (Agricultural U, Netherlands), M. Eira (EMBRAPA, Brazil)

Problem: Organisms that survive drying can be placed in "suspended animation" and remain viable for a long time. However, all organisms eventually die. The inevitable loss of viability presents a problem for genebanks since it necessitates monitoring germplasm and periodically regenerating it. Genebank operators need to predict which samples are more susceptible to deterioration and to know how to prevent deterioration. To address these needs, we must elucidate the mechanism(s) of deterioration during storage and the precise relationships among the kinetics of aging, the temperature and relative humidity of storage, and intrinsic properties of cells.

Approach: Our approach to the study of the mechanisms and kinetics of deterioration can be divided into three separate studies: 1) determination of optimum RH for storage, 2) determination of the interaction between RH and temperature on aging rates, and 3) determination of genetic and environmental factors in seeds which contribute to propensity to age rapidly. The last project was recently initiated and we have nothing to report as yet.

In previous work we established that an RH of about 22% gave maximum longevity for seeds from cultivated crops. More recent findings suggest the optimum ranges from between 14 and 28% RH. We would like to know whether this optimum humidity range is universal among desiccation tolerant organisms and whether the mechanism of deterioration is different at sub- and supra-optimal hydration levels. To address the first question, we are monitoring viability of phylogenetically diverse organisms stored at relative humidities between 0 and 75%. To address the second question, we are studying the physical property of water in tissues and the nature of reactions associated with water behavior.

The interaction of water and temperature in controlling aging rates is a relatively new concept in the seed aging literature. We would like to know if dynamic models used in food deterioration (aqueous glasses) or traditional thermodynamic models (Arrhenius behavior) provide a better description of aging kinetics. We would also like to know whether the interaction of temperature and relative humidity is similar among different seed species and tissue types. The research involves storing pea, soybean, peanut, sunflower, and pollen at relative humidities between 0 and 75% and temperatures between -18 and 45C. Because aging is very

slow at low RH and temperatures, the experiment is necessarily long-term. We are in our 7th year of data collection.

Results: We have developed phase diagrams for seeds based on calibrating the measurements of water. Our results indicate that the nature of deteriorative reactions for all desiccation tolerant organisms are dependent on the phase properties of water. Temperature effects can be described by Arrhenius behavior with apparent activation energy similar among tissue types. This information gives us powerful tools to predict aging rates for different seed species and lots.

DETERMINING STORAGE PROTOCOLS FOR SEEDS FROM ENDANGERED HAWAIIAN FLORA

CHRISTINA T. WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), Ken Wood (NTBG, Hawaii)

Problem: Numerous species endemic to Hawaii are in danger of extinction. Storage in ex situ genebanks is one method of preserving the remaining genetic diversity until habitats can be restored. Information about the physiology of seeds from these species will ensure that seeds are stored under optimal conditions and monitored appropriately. This project is funded by the US Fish and Wildlife, Pacific Islands Division.

Approach: We are assaying small quantities of seed for basic information such as seed size, water content at harvest, level of desiccation tolerance, tolerance to freezing temperature, optimum water content for storage and projected longevity. Experimental protocols are similar to those used with cultivated species, except we are trying to use non-destructive assays in order to limit the number of seeds required.

Results: We have tested over 50 species of seeds. Some endangered species produce seeds prolifically, while others have poor seed set. In spite of our non-destructive assays, the small number of seeds in each sample confounds our efforts to obtain complete data sets. While experiments on optimum water contents and longevity are inconclusive as yet, preliminary results indicate that most of the species exhibit orthodox behavior (are desiccation tolerant) and should be stored at an RH between 15 and 25%.

EVOLUTION OF VOLATILES DURING SEED AGING

MING ZHANG (Post-doc Fellow, People's Republic of China), E.E. Roos (Supvry PI Physiol), C. Walters (PI Physiol)

Problem: Most seed aging experiments utilize rapid aging techniques in order to produce aged seeds for studies within a reasonable time frame. However, different mechanisms of deterioration may operate depending on the aging conditions used. Seeds emit volatile compounds during storage and deterioration. In this experiment we wanted to investigate the production of volatile compounds, such as methanol, ethanol, pentanal, and hexanal as a function of seed deterioration method.

Approach: Lettuce seeds were adjusted to various moisture contents (mc) ranging from 1.0% to 5.6% and sealed in glass bottles for storage at 35C. Headspace gas samples were sampled periodically and analyzed using gas chromatography. In another experiment, lettuce seeds were imbibed for 9 hours and dried back to the original seed mc (4%) before being adjusted to various mc (0.8 to 5.7%) and stored at 25 and 35C. Seeds were analyzed for germination and vigor (3 day root lengths).

Results: After 5 months storage at 35C lettuce seeds with 5.6% mc were all dead. Seeds with lower mc lost germination and/or vigor. Methanol and ethanol production were detected from seeds with mc of 3.3% and above, and increased with storage time. Hexanal and pentanal were detected from seeds with 1.1 and 2.3% mc.

Initial vigor of imbibed/dried seeds was higher than control seeds, however, vigor declined more rapidly during storage for imbibed/dried seeds. Soaking seeds in this manner greatly promoted the production of

hexanal and pentanal. Production of methanol and ethanol were only slightly promoted by this treatment, but does not sufficiently explain the more rapid aging of soaked seeds during storage. These experiments are continuing.

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Note: Names in caps are present or former ARS employees, or were supported on NSSL funds.

Appendix 4

CURATORS' ALLOTMENT FOR FY 97 (Tentatively)

DISCRETIONARY FUNDS

09/24/96

	FINANCIAL PLAN -----	GILLASPIE CODE GG -----	JARRET CODE JJ -----	LOVELL CODE LL -----	MORRIS CODE MM -----	PITTMAN CODE PP -----	KRESOVICH CODE KK -----
ACCOUNT: 601-6607-050:							
2100 - Travel	16,000.00	1,712.00	3,000.00	1,000.00	1,000.00	3,000.00	6,288.00
2600 - Supplies & Materials	36,209.00	3,874.36	10,000.00	3,000.00	3,000.00	10,000.00	6,334.64
TOTAL 050	52,209.00	5,586.36	13,000.00	4,000.00	4,000.00	13,000.00	12,622.64
ACCOUNT: 601-6607-910:							
2600 - Supplies & Materials	61,777.00	2,563.75	12,000.00	6,000.00	6,000.00	12,000.00	23,213.25
TOTAL 910 AND 050:	113,986.00	8,150.11	25,000.00	10,000.00	10,000.00	25,000.00	35,835.89

* FY 97 - Allocation 00 - \$1,427,795 - Kresovich

* FY 97 - Allocation 00 - \$151,709 - Gillaspie

* As of this date, I do not have a subobject class breakdown per CRIS. I only have the overall breakdown for Account 050. ARMPS shows that Gillaspie has 10.70 percent of the total of Account 050. The figures for Gillaspie is based on that percentage per subobject class.

* Gillaspie's has a 4.15 percentage for Account 910.

* These figures are tentatively until I receive a CRIS breakdown of subobject class.

* I did not focus on Subobject Class 2500, it involves: SCA, transfers, maintenance agreements, task orders, repairs and maintenance, etc. At the end of the first quarter, it would be safer to analyze the situation closer. (January 1997).

* I RECOMMEND each curator is allotted their funds quarterly. This allows for better analysis and planning. These amounts could decrease or increase; it depends on whether the actual salaries and other fixed cost are the same as the FY 97 ARMPS.

* Please note the attached sheet, from The ARS Resources Management System (ARMS), as amended June 1992, "Discretionary Funds".

* Kresovich's fund includes allotments for Hopkins, Strickland, Spinks, L. Chalkley, Administration, and DNA Labs.

Appendix 5

PLANT GENETIC RESOURCES CONSERVATION UNIT
GRIFFIN, GEORGIA

MAY, 1996 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED UP	CRITICAL BACKUP	CORE	NSSL ONLY ¹
Grasses & others	Lovell, Gil	Bamboo	70	70	0	50			
		Castorbean	357	272	85	325			672
		Hibiscus	361	225	134	46			380
		Miscellaneous	264	198	66	98			156
		Pearl millet	527	357	170	29			683
		Sesame	1,084	1,065	19	949			146
		Warm Season Grasses	7,171	6,154	1,017	1,243			2,429
		Sorghum	27,280	25,152	2,123	10,525			12,112
Legumes	Morris, Brad	Guar	350	347	3	259	84		951
		Legumes	2,906	2,148	757	515			94
		Trifolium	1,957	1,303	654	416		95 ²	46
		Winged bean	165	18	147	1	17		
Peanuts (8,804)	Pittman, Roy	Cultivated	8,224	7,379	845	6,064		798	60
		Wild	580	477	103	5			

¹Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.

²Core includes three clover species: *T. alexandrinum*, *T. resupinatum*, *T. subterraneum*

PLANT GENETIC RESOURCES CONSERVATION UNIT
 GRIFFIN, GEORGIA

MAY, 1996 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED UP	CRITICAL BACKUP	CORE	NSSL ONLY ¹
Vegetable Crops	Jarret, Bob	Citrullus	1,611	1,335	276	720			317
		Cucurbits	1,276	496	774	195			47
		Eggplant	864	841	22	501	242		40
		Gourds	458	259	199	121	103		12
		Ipomoea	1,053	725	328	4			34
		Luffa	159	129	29	52	60		1
		Okra	1,927	1,534	393	368	1,079		35
		Pepper	3,802	3,615	187	1,018			174
Vigna (12,391)	Gillaspie, Graves	Cowpea	7,603	4,625	2,978	2,657		700	247
		Mung bean	4,186	2,417	1,768	670			29
		Other Vigna spp.	602	137	465	56			1
Grand Total for Griffin crops			74,837	61,278	13,542	26,887	1,585	1,593	18,666
Percent (%)				81.9%	18.1%	35.9%	2.1%		

¹Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.

PLANT GENETIC RESOURCES CONSERVATION UNIT
GRIFFIN, GEORGIA

MAY 1997 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED ¹ UP	CRITICAL ² BACKUP	CORE	NSSL ³ ONLY
Grasses & others	Lovell, Gil	Bamboo	98	98	0	50			
		Castorbean	358	255	103	348	19		672
		Hibiscus	366	239	127	86			382
		Miscellaneous	263	207	56	239	36		140
		Pearl millet	448	373	75	398	63		688
		Sesame	1,084	1,069	15	1083	9		146
		Warm Season Grasses	7,137	6,112	1,025	1,349			2,430
		Sorghum	29,170	27,040	2,130	15,223			11,139
Legumes	Morris, Brad	Guar	413	404	9	406	84		888
		Legumes	2,902	2,236	666	675			85
		Trifolium	1,939	1,304	635	436		95 ⁴	37
		Winged bean	165	17	148	18	17		
Peanuts (9,115)	Pittman, Roy	Cultivated	8,476	7,683	793	6,318		798	63
		Wild	639	523	116	7			

¹Total accessions backed up for the site crop.

²Number of accessions in "BACKED UP" column where quantity on hand was too low for a normal backup.

³Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.

⁴Core includes three clover species: *T. alexandrinum*, *T. resupinatum*, *T. subterraneum*.

PLANT GENETIC RESOURCES CONSERVATION UNIT
GRIFFIN, GEORGIA

MAY 1997 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED ¹ UP	CRITICAL ² BACKUP	CORE	NSSL ³ ONLY
Vegetable Crops	Jarret, Bob	Citrullus	1,640	1,387	253	1,388	391		315
		Cucurbits	1,315	505	810	200			46
		Eggplant	897	851	46	873	160		51
		Gourds	464	257	207	257	120		12
		Ipomoea	1,092	767	325	151			34
		Luffa	159	127	32	128	60		10
		Okra	3,046	1,534	1,512	1,725	1,079	165	35
		Pepper	3,809	3,705	104	1,607			173
Vigna (12,600)	Gillaspie, Graves	Cowpea	7,820	4,640	3,180	3,508		699	250
		Mung bean	4,188	3,890	298	1925	384	410	28
		Other Vigna spp.	592	270	322	187	56		1
Grand Total for Griffin crops			78,480	65,493	12,987	38,585	2,478	2,167	17,625
Percent (%)				83.5%	16.5%	49.2%	6.4%		

¹Total accessions backed up for the site crop.

²Number of accessions in "BACKED UP" column where quantity on hand was too low for a normal backup.

³Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.

S9 Distributions May 1996 - May 1997 to Southern Region

State	Category	Cooperator	Totals	
Alabama	STA	Chapman, L., Alabama Cooperative Extension Service	1	
		Mosjidis, J., Auburn University	7	
		Prakash, C., Tuskegee University	3	
		Robertson, G., Auburn University	5	
	UCOM	Gorgus, B., Plantwell Inc.	6	
	UIND	Brook, D.	21	

			43	
Arkansas	STA	Anderson, E., University of Arkansas	497	

			497	
Florida	STA	Ali-Ahmad, M., Florida A&M University	2	
		Carle, R., University of Florida	4	
		Dunavin, L., University of Florida	6	
		Hopkins, D., University of Florida	669	
		Kretschmer, A., Ft. Pierce Agriculture Research	1	
		Kucharek, T., University of Florida	4	
		McCuiston, F., University of Florida	4	
		Nuessly, G., University of Florida	2	
		Quesenberry, K., University of Florida	30	
		de Sa Guimaraes, P., University of Florida	5	
		UARS	Schnell, R., USDA, ARS	6
		UCOM	Baldwin, T., Deepwater Enterprises	31
			Grant, R., Exoticus II Inc.	9
	Ikeda, S., Sakata Seed America, Inc.		9	
	Williams, T., Rogers Seed Co.		4	
	UIND	Baldwin, E.	9	
		Clark, D.	5	
		Eigsti, N.	4	
		Fertitta, D.	10	
		Koehl, C.	3	
		Kulp, E.	12	
		Morehead, L.	12	
Shannan, S.	11			
Strickland, T.	18			
Wilson, R.	4			
UIND	Worth, S.	3		
UPRU	Hemenway, D., Elfin Permaculture	6		

			883	

S9 Distributions May 1996 - May 1997 to Southern Region

State	Category	Cooperator	Totals
Georgia	STA	Dhir, S., The Fort Valley State College	4
		Dyer, M., University of Georgia	1
		Hall, M., University of Georgia	2
		Ingram, K., University of Georgia	8
		Kays, S., University of Georgia	94
		Libombo, M., The University of Georgia	64
		Oetting, R., University of Georgia	11
		Pappert, R., University of Georgia	2
		Todd, J., University of Georgia	654
		Wetzstein, H., University of Georgia	4
	UARS	Gillaspie, G., USDA, ARS	11
		Hopkins, M., USDA, ARS	2
		Jarret, R., USDA, ARS	64
		Moncrief, K., USDA-ARS	2
		Pinnow, D., USDA, ARS	70
		Pittman, R., USDA, ARS	2
	UCOM	Jahromi, S., International Paper	1
		Sutton, J., Mycogen Plant Sciences	13
		Tolla, G., Asgrow Seed Co.	4
	UIND	Bekemeyer, G.	3
		Brantley, T.	12
		Greene, E.	3
		Haden, K.	3
		LaBorde, T., B.I.G.	6
		McKenzie, H.	5
		Mock, S.	14
		Mora, P.	5
		Roberts, H.	3
		Russell, R.	11
Shortman, S.		27	
Thomaston, W.		3	
Tyson, J.	1		

			1,109
Hawaii	UIND	Cook, W.	35
		Lawless, A.	3

			38
Kentucky	STA	Phillips, T., University of Kentucky	1
		Roberts, L., University of Kentucky	2
		Taylor, N., University of Kentucky	13

			16
Louisiana	STA	Clark, C., Louisiana State University	78
		La Bonte, D., Louisiana State University	3

S9 Distributions May 1996 - May 1997 to Southern Region

State	Category	Cooperator	Totals
Louisiana	STA	LaBonte, D., Louisiana State University	8
		Venuto, B., Louisiana State University	6

			95
Mississippi	STA	Baldwin, B., Mississippi State University	19
		Hovermale, C., Mississippi State University	59
		Ivy, R., Mississippi State University	51
		Maddox, V., Mississippi State University	35
	UARS	Fairbrother, T., USDA/ARS	18
		Pederson, G., USDA, ARS	49
	UFED	Lockley, T., USDA-APHIS	11

			242
North Carolina	STA	Counell, J., North Carolina State Univ. Arboretum	19
		Eubanks, M., Duke University	8
		Lommel, S., North Carolina State University	12
		Wehner, T., North Carolina State University	1,280
	UCOM	Kurgen-Chen, Z., Shelton Herb Farm	85
	UIND	Arthur, J.	21
		Cutter, J.	2

			1,427
Oklahoma	STA	Kirby, J., Oklahoma State University	1
		Kochneower, R., Oklahoma State University	3
	UARS	Johnson, M., USDA-ARS	2
		Rao, S., Grazinglands Research Laboratory	10
	UIND	Reed, E.	1
	UPRU	Baker, J., The Samuel Roberts Noble Foundation, Inc.	28

			45
South Carolina	UARS	Dukes, P., USDA, ARS	12
		Fery, R., USDA, ARS	1
		Harrison, H., USDA/ARS	61

S9 Distributions May 1996 - May 1997 to Southern Region

State	Category	Cooperator	Totals
South Carolina	UCOM	Stanton, D., Stanton Pedigreed Pepper Seed Co.	43
	UIND	Bundurant, M.	10
			----- 127
Tennessee	STA	Duck, B., University of Tennessee	7
	UCOM	Turtle, A., Our Nursery	6
	UIND	Reynolds, D.	9
		Wood, W.	2
		----- 24	
Texas	STA	Auld, D., Texas Tech University	262
		Hart, G., Texas A & M University	443
		Hussey, M., Texas A&M University	31
		Mullet, J., Texas A & M University	445
		Peterson, G., Texas A&M University	3
		Rosenow, D., Texas Agriculture Exp. Stn.	77
		Scholthof, K., Texas A&M University	6
		Scott, C., Texas A&M University	23
	UARS	Cook, C., USDA, ARS	2
		Grusak, M., USDA-ARS	2
		Rooney, B., USDA, ARS	1
	UCOM	Cline, A., Pioneer Hi-Bred International, Inc.	255
		Dobbs, D., Wilhite Seed Co.	4
		Rodriguez, O., G. E. Pogue Seed Co., Inc.	362
		Rodriguez, O., G.E. Pogue Seed Co.	10
	UIND	Barham, J., Mexia High School	1
		Longley, M.	5
		Morse, M.	87
		Parker, T.	7
		Steed, C.	12
		----- 2,208	
Virginia	STA	Bhardwaj, H., Virginia State University	3
		Hilu, K., Virginia Tech	14
		Mozingo, R., Tidewater Agricultural Research and Extension Center	12
		Riopel, J., University of Virginia	12
		Zhang, W., Virginia Polytechnic & State University	20
	UCOM	Walker, K., Claude Moore Colonial Farm	6
			----- 67 ===== 6,821

Appendix 6

United States General Accounting OfficeTo: *Dr Kresovich*Fax: *770-229-3323*From: **Beverly Peterson, Sr. Evaluator**

Ph. 202-512-7438
Fax 202-512-9936
Email petersonb.rced@gao.gov
Address GAO RCED
Room 2474
441 G Street, NW
Washington D.C. 20548-0001

*Hi. hope all is
well... Thank for
your help.
-Beverly*

Subject:

REQUEST FOR DATA ON ACTIVE SITE COLLECTIONS

As part of GAO's review of the NPGS, GAO is requesting data on NPGS active site collections. Attached is a data request sheet which GAO is requesting each site fill out for each major crop genera (representing in total at least 80% of the site's collection). Ames pilot tested our data request form and has developed a program that is useful for retrieving some of the needed GRIN data (for those whose data is in GRIN). Please contact Mark Widrechner for the program Ames used. If the data is not available, please indicate NA.

Please call me if you have any questions. Otherwise, please fax or email your responses by May 16. Thank you for your time and effort.

3 pages, including this cover sheet, are being transmitted.

REGENERATION

Of the number of Acc. unavallable in 1997,
how many cannot be regenerated because
too few viable seeds remain? _____

OF ACC. WHICH REQUIRE REGENERATION DUE TO:

- Original sample (received on or after 1/95) _____
- Original sample (received before 1/95) _____
- Low seed quantity _____
- Low Germination _____
- Low population size of unavallable acc. _____
- Other factors _____

Available accessions regenerated
with low population size or other insufficiency: _____

Years required to regenerate entire backlog at
current resource levels and success rates based
on current technology: _____

of ACC. (est.) at risk of losing genetic integrity= _____ OR
If there is little or no basis to judge # of Acc. at risk then check here _____

IN THE LAST 5 YEARS, THIS BACKLOG HAS (circle one):

- Greatly Increased (>20%) _____
- Increased (6 to 20%) _____
- Remained Static (\pm 5%) _____
- Decreased (-6 to -20%) _____
- Greatly decreased (< -20%) _____

IN THE NEXT 5 YEARS, THIS BACKLOG IS EXPECTED TO: (circle one)

- Greatly increase (>20%) _____
- Increase (6 to 20%) _____
- Remain Static (\pm 5%) _____
- Decrease (-6 to -20%) _____
- Greatly decrease (< -20%) _____

Appendix 7

Arachis Curation Project

1997

Roy N. Pittman

I. Objectives

The *Arachis* collection which is currently made up of nine sections and 70 described species. Duties include the acquisition, maintenance, evaluation, and distribution of germplasm. In addition, germplasm is screened for the presence of pathogens and therapeutic procedures used to eliminate pathogens prior to release to the user public.

II. Status

A. Accomplishments

1. Regeneration of 1250 cultivated peanuts at Griffin in the field in 1996.
2. Regeneration of cultivated peanuts through quarantine in the greenhouse.
3. China which coordinates the peanut program. In addition, contacts were made with the regional peanut programs in the three regions of productions. Received xx accessions from China
4. Received 6 accessions from Korea.
5. Received 11 accessions from Bolivia and exchanged 20 accessions from breeding programs in Florida, Georgia, and Texas.
6. Regenerated 115 cultivated accessions in the greenhouse.
7. Regenerated 68 cultivated accessions in the greenhouse which were rescued from old seed.
8. Developed a artificial soil mixture suitable for growing *A. marginata*, *A. tuberosa*, and *A. quarantica*.
9. Species names being assigned based on Krapovickas and Gregory's Monograph.

B. Work in progress

1. 725 accessions of cultivated peanuts have been planted this year for increase. In addition, 1200 accessions are being grown by cooperators in Georgia, Florida, and Oklahoma.
2. Material from the '96 Williams collection has been started through in house quarantine to evaluated possible difference in testa color prior to assigning PI numbers.
5. An agreement in cooperation with the Plant Introduction Office continues with Ecuadorian cooperators to increase material from the '96 Williams and Williams collection.
6. Virus elimination from wild peanut germplasm continues.
7. the '96 cultivated increase is being germination tested.
8. Wild peanut sieve has been made to sieve peanuts with.

III. Needs

1. Need to start increasing wild peanut germplasm for seed as no increases have been since Simpson's contract was not renewed.
2. Cages for wild peanut increases to eliminate out crossing if grown in a field.
3. Land for wild peanut increases at Ft. Pierce, Fl. needs to be developed for use.
4. Additional good land with irrigation for cultivated increases needs to be found near by.
5. A drying room to store field increases prior to shelling needs to be build. This needs to be dehumidified with heating kept to a minium.

PLANT PATHOLOGY AND VIGNA CURATION PROJECTS

A. Graves Gillaspie

I. Objectives

Develop and apply new technologies for detection, characterization, and elimination of pathogens on introduced plant germplasm. Curate the National Plant Germplasm System *Vigna* germplasm collection including acquisition, maintenance, evaluation, and distribution. Test introduced germplasm for the presence of pathogens and conduct therapeutic procedures to eliminate such pathogens prior to the release of the germplasm.

II. Status

A. Accomplishments

1. Greenhouse grow out of 22 cowpea lines (due to limited space) previously put through greenhouse virus elimination, but failing to produce seed in Georgia field regeneration tests.
2. Greenhouse testing of 73 Botswana lines from 1991 University of California Riverside increase for presence of seedborne viruses and regeneration of 10 lines found to be infected.
3. Regeneration of 153 virus-free lines of Botswana cowpeas in the field at Griffin and 35 lines at St. Croix.
4. Inventorying of *Vigna* collection has been completed with the exception of those items only recently received. Samples of cowpea collection have been pulled for regular NSSL backup, but still need to do critical backup on the cowpeas. Samples have been taken for over half of the mung bean collection for backup, rebagging, and barcoding. All mung bean accessions not in the core collection are being placed at -20C with the core remaining at 5C 25 RH.
5. Characterization completed on a new strain of cucumber mosaic virus prevalent on cowpeas in Georgia and on a bean yellow mosaic subgroup virus isolated from Sesbania. Work almost completed on a strain of bean common mosaic virus found on guar and on peanut stunt virus isolated on Desmodium. Other viruses/mixtures thus far have not been characterized. Good progress has been made on a PCR-based detection method for cowpea mottle virus and work almost ready to begin on testing a similar type test for detection of the white-fly transmitted virus important in sweet potatoes.

B. Work in progress

1. Regeneration of 107 lines of cowpeas in the field at Griffin.
2. Tests of regeneration capacities and virus reinfection of photoperiod sensitive cowpea lines at Griffin, St. Croix, and Puerto Rico to determine regeneration potentials of locations for such lines. Greenhouse growout (based on space available) of photoperiod sensitive cowpea lines and possibly some additional growout of such lines at St. Croix and/or Puerto Rico in spring/summer 1998 based upon the results of the 1997 tests discussed above.
3. Continuing tests to characterize viruses found on germplasm during regeneration attempts at Griffin and elsewhere. Continuing work to develop PCR-based methods for detection of viruses in peanuts and sweet potatoes.
4. Work continuing on sampling for backup, rebagging, and barcoding of the *Vigna* collection. This should be completed during the coming year.

CLOVER AND SPECIAL-PURPOSE LEGUME CURATORIAL ACTIVITIES

Brad Morris

I. Objectives

Curate the National Plant Germplasm System's clover and special-purpose legume germplasm collection which consists of 5378 accessions of approximately 424 horticultural, agronomic, ornamental, medicinal, and industrial species. The mission and objectives include acquisition, maintenance, characterization, evaluation, documentation, and distribution of germplasm. In addition, collaborative work is ongoing for the utilization of breakdown products from soil amendments of legume species for nematicidal properties.

II. Status

A. Accomplishments

1. Approximately ninety percent of the clover and legume collections seed database are complete.
2. A total of 50 pollination cages were designed, built and utilized for regenerating cross-pollinated clover species.
3. Established a contract with a beekeeper to provide and maintain honeybees used in regenerating cross-pollinated species.
4. Regeneration (conventional and/or embryo rescue) of 73 PI's from several important legume species including *Trifolium* spp., *Canavalia* spp., *Desmodium* spp., *Indigofera* spp., *Senna* spp., and *Sesbania* spp.

B. Work in progress

1. The seed database work continues.
2. One hundred clover PI's (50 cross-pollinated and 50 self-pollinated) were transplanted to the field. Bee hives will be placed in each cage at approximately 50% flowering for 2-3 weeks.
3. A test regeneration for forty Ethiopian clovers in the greenhouse has also been initiated. Several African clover species are being tested and grown in Beeville, Texas to determine their photoperiod requirements for regeneration.
4. Two *Desmanthus* spp. will be regenerated in the field this year.
5. A collaborative strategy is being developed to eliminate redundancies in the largest clover collections.

C. Future

1. Complete the seed database work this year.
2. Regenerate 100-200 cross and self-pollinated clovers next year.
3. Establish collaborative efforts for screening legume species and identifying useful phytochemicals for forage use, phytomedicinals, and disease/nematode resistance plus inheritance studies for these traits.

Robert L. Jarret, Research Horticulturist/Vegetable Crops Curator

Incumbent is responsible for the management (maintenance/characterization/acquisition) of vegetable crops genetic resources in the S-9 Plant Germplasm Collection. Primary crop mandates include; sweetpotato (*Ipomoea* spp.), pepper (*Capsicum* spp.), watermelon (*Citrullus* spp.), okra (*Abelmoschus* spp.), eggplant (*Solanum* spp.), squash (*Cucurbita* spp.), and gourds (misc. genera). At the present time, total holdings of vegetable crop plant introductions (PIs) in the S-9 collection number about 22,000.

Seed increase activities take place predominately in Byron, GA and also Griffin, GA. Mr. J. Leaptrot (ARS) is coordinating watermelon and okra germplasm seed regeneration in Byron. Mr. Kevin Mataxas (ARS) coordinates germplasm increase in Griffin and assists with seed regeneration in Byron, GA.

Individual Crop Activities (1997)

An okra core collection was identified in 1997 and 55 accessions from this core collection are being regenerated/increased in Byron, GA using controlled pollination techniques. Characterization data will be taken on core collection accessions and entered into the GRIN data base.

Additional sweetpotato clones were received for entry into the clonal (in vitro) germplasm collection. A total of twenty-four sweetpotato PIs were virus indexed in 1997. Plans are being formulated to increase seed of *Ipomoea* spp. in Puerto Rico, pending the availability of funds.

One-hundred and five watermelon PIs are being increased in Byron, GA (caged with honey bees). Characterization data will be recorded for entry into the GRIN data base. Additional pollination cages are in the process of being procured. It is anticipated that 150 PIs will be increased in 1998.

Three-hundred pepper (*Capsicum* spp.) PIs are being increased via the continuation of a cooperative agreement with New Mexico state University. Characterization data will be acquired and entered into the GRIN data base.

Twenty *Solanum* (eggplant-related) PIs are being increased in the screenhouse. Characterization data and herbarium specimens will be collected. Requests for *Solanum* spp. have increased since a gene governing nematode resistance in *Solanum* was reported.

Fifty *Cucurbita moschata* PIs are being increased in Byron and Griffin, GA. These plants are very large and seed production is being accomplished by growing plants in isolation plots. The GA Station has assisted in providing land ground preparation support for this activity. Characterization data will be collected and entered into the GRIN data base.

Research: Current research activities involve the isolation and characterization of microsatellites in vegetable crop germplasm, their use in genetic and paternity analysis studies, and the evaluation of SSR-library enrichment procedures.

Annual Report to the Sweetpotato Crop Germplasm Committee
Submitted February 1997
Robert L. Jarret and Kevin G. Mataxa

Personnel

R.L. Jarret (Research Horticulturalist/Curator)

K.G. Mataxas (Biological Science Laboratory Technician/Assistant Vegetable Crops Curator). Kevin was responsible for overseeing and assisting with the periodic reculture of accessions in the collection, receiving and shipping orders for germplasm and coordinating field evaluation of newly released accessions. As of 1/97 Kevin will assume responsibility for increase of vegetable crops germplasm and will occasionally supervise Ms. Stephanie Dunn.

Stephanie L. Dunn (Biological Science Aid). Stephanie has assumed the responsibility of direct maintenance of the Sweetpotato In-Vitro Collection, including periodic reculture of accessions and filling germplasm orders.

Current Inventory

Current holdings in the collection include:

Ipomoea batatas (in vitro): 668
Related Ipomoea spp. (seed): 219

New additions to the sweetpotato in vitro collection include 40 clones released from Quarantine in late 1996 (see Appendix 1). Forty clones were released in 10/96. Two of these clones were in poor health and did not survive subculture. A request for replacement cultures has been made for Q35655 and Q24534, and cultures will be sent as soon as they are ready. All of the remaining material in the shipment received 10/96 has been established in vitro plantlets, and PI Numbers have been assigned to them. These materials are available for distribution. All newly received clones will be grown in the field in Griffin during the summer of 97, and descriptor data taken for entry into GRIN.

No related sweetpotato-species were increased in 1996 due to a lack of greenhouse space. However, many Ipomoea spp. await regeneration. The curator is in the process of establishing arrangements for regeneration of Ipomoea spp. in Puerto Rico, but implementation will require additional financial support.

Germplasm Distribution

In calendar year 1996, there were a total of 14 requests for sweetpotato germplasm as follows:

13 domestic requests (93% of Total)
1 international request (7% of Total)

Included in this distribution was a large number of clones; 500+, that were sent to Dr. Stanley Kays, at the University of Georgia, as part of a project to evaluate the collection for various storage root characteristics. To date, the project is ongoing. These were initially sent as acclimated plants, approximately 8 plants per PI. This was found to be very time consuming and subsequently in vitro cultures were sent and acclimated in greenhouses at UGA.

Among the remaining 13 requests, 69 clones were distributed (10 propagules each) for a total to 552 node propagated plantlets shipped in 1996.

Requests for germplasm continue to average 1-3 per month. The total number of requests for 1996 was down 7 from 21, in 1995, with domestic requests outnumbering international requests.

Germplasm Maintenance (clonal)

All clonal materials continue to be maintained in vitro. 12 tubes are maintained for each clone, 10 in the working collection used for distribution and 2 held in a separate incubator in a location separate from the main collection. The back up of cultures at NSSL has been discontinued due to the labor required to maintain the materials and the damage to the cultures incurred during shipping to Colorado.

The collection is on a 8-9 month, as needed, reculture schedule. Material is available upon request, and orders for germplasm are filled and shipped in 6-8 days, depending on growth rate of subcultures.

Exploration

No proposals for plant exploration were received in 1996 for 1997 funding. Copies of the guidelines for preparation and submissions of plant exploration proposals are available from the Germplasm Services Laboratory, USDA/ARS/BA, Bldg. 003, Room 224, BARC-West, Beltsville, MD 20705.



United States
Department of
Agriculture

Agricultural
Research
Service

Beltsville Area
Beltsville Agricultural
Research Center

Beltsville, Maryland
20705
Plant Quarantine
Bldg 580, BARC-East

October 10, 1996

SUBJECT: Release of *Ipomoea* germplasm from quarantine

TO: Dr. Joseph A. Foster
USDA, APHIS, PPQ
Bldg 580, BARC-East
Beltsville, MD 20705

THROUGH: Suzanne Hurtt *S. Hurtt*
Plant Pathologist

FROM: Renee De Vries *R. De Vries*
Research Technician

I hereby recommend unconditional release from quarantine of *Ipomoea batatas* var. *batatas* cv. *Rusenya-BDI*, accession Q35651-A, from CIP-Peru.

This accession was graft inoculated to the indicators *I. setosa* and the sweet potato clone TIB8, preinfected with the aphid-borne component (feathery mottle virus). Bioassays were each performed twice on triplicate sets of indicators about one year apart. Tests in both years were negative. No symptoms of viral-like infection have been observed on the sweet potato plant, grown in the greenhouse.

On the basis of the procedures and results submitted, I unconditionally release Q35651-A, *Ipomoea batatas* var. *batatas* cv. *Rusenya-BDI*.

Joseph A. Foster

Joseph A. Foster
Plant Pathologist
USDA, APHIS, PPQ

10/17/96
Date



United States
Department of
Agriculture

Agricultural
Research
Service

Beltsville Area
Beltsville Agricultural
Research Center

Beltsville, Maryland
20705
Plant Quarantine
Bldg 580, BARC-East

September 18, 1996

SUBJECT: Release of *Ipomoea* germplasm from quarantine

TO: Dr. Joseph A. Foster
USDA, APHIS, PPQ
Bldg 580, BARC-East
Beltsville, MD 20705

THROUGH: Suzanne Hurtt *S. Hurtt*
Plant Pathologist

FROM: Renee De Vries *R. De Vries*
Research Technician

I hereby recommend unconditional release from quarantine of the 38 *Ipomoea* sp. accessions on the attached list. Seventeen accessions, with a "T" prefix to the quarantine tracking number (Qnumber), are derived from meristem tip cultures of sweet potatoes that previously tested positive for viral-like agents.

All 38 were graft inoculated to the indicators *I. setosa* and the sweet potato clone TIB8, preinfected with the aphid-borne component (feathery mottle virus). The bioassays were each performed twice on triplicate sets of indicators about one year apart. Tests in both years were negative. No symptoms of viral-like infection have been observed on the sweet potatoes, grown in the greenhouse.

On the basis of the procedures and results submitted, I unconditionally release the 38 *Ipomoea* sp. introductions on the attached list.

Joseph A. Foster

Joseph A. Foster
Plant Pathologist
USDA, APHIS, PPQ

9/24/96

Date

09/18/96

SWEET POTATO RELEASES

QNUMBER	SUBCLONE	CLONAL IDENTIFICATION	DATE RECEIVED	ORIGIN/DONOR
T24514 ✓	01	Camote Morado	03/27/84	Guatemala
T24520 ✓	16	Camote Remolacha	03/27/84	Guatemala
T24521 ✓	02	Camote Amarillo	03/27/84	Guatemala
T24534 ✓	04	Camote Blanco	03/27/84	Guatemala
T25346 ✓	02	#406	01/07/85	Guatemala
T25347 ✓	01	#407	01/07/85	Guatemala
T25348 ✓	01	#512	01/07/85	Guatemala
T26770 ✓	07	SPV-73	04/27/87	Puerto Rico
T26992 ✓	A27	Cuba 6	10/26/87	Cuba/Mexico
T26996 ✓	02	Coleccion Bajo Papaloapan	10/26/87	Cuba/Mexico
T26997 ✓	08	Cuba 9	10/26/87	Cuba/Mexico
T27222 ✓	A16	SPV-52	08/08/88	Puerto Rico
T27227 ✓	01	SPV-95	08/08/88	Puerto Rico
T27837 ✓	01	Benihayato	11/15/89	Japan
T28457 ✓	03	Honiara	06/13/91	Fiji
Q28745 ✓	E	G113-2B	10/10/91	Philippines/Australia
T29652 ✓	07	IPS 153	02/09/93	Bangladesh/Australia
T29666 ✓	04	87049-3	02/09/93	Tonga/Australia
Q35192 ✓	A	Xushu 18	08/10/94	China/CIP
Q35646 ✓	A	Huarmeyano	03/20/95	Peru/CIP
Q35649 ✓	A	Zapallo	03/20/95	Peru/CIP
Q35650 ✓	B	IITA-TIB 10	03/20/95	Nigeria/CIP
Q35652 ✓	A	Mogamba	03/20/95	Burundi/CIP
Q35653 ✓	A	Luby 3074	03/20/95	Burundi/CIP
Q35654 ✓	B	Imby 3102	03/20/95	Burundi/CIP
Q35655 ✓	A	IITA-TIS 1487	03/20/95	Nigeria/CIP
Q35656 ✓	B	IITA-TIS 2544	03/20/95	Nigeria/CIP
Q35657 ✓	B	IITA-TIS 3290	03/20/95	Nigeria/CIP
Q35658 ✓	B	IITA-TIS 9162	03/20/95	Nigeria/CIP
Q35659 ✓	B	IITA-TIS 9232	03/20/95	Nigeria/CIP
Q35660 ✓	A	K 51/3251	03/20/95	Rwanda/CIP
Q35665 ✓	A	Tanzania	03/20/95	Uganda/CIP
Q35666 ✓	A	Wagabolige	03/20/95	Uganda/CIP
Q35667 ✓	B	No. 29	03/20/95	Uganda/CIP
Q35668 ✓	A	KEMB 10	03/20/95	Kenya/CIP
Q35669 ✓	A	KEMB 37	03/20/95	Kenya/CIP
Q35670 ✓	A	Rusanya-RWA	03/20/95	Rwanda/CIP
Q35671 ✓	B	CN 1869-13	03/20/95	Taiwan/CIP

Date shipped: 10/24/96

Invoice 96106
102/68

Plant Material Shipping Invoice from USDA-ARS-WGRU-Plant Germplasm Quarantine Office

Destination:

Dr. Robert Jarret
USDA-ARS S9 Plant Gen. Res. Cons. Unit
1109-Experiment St. U of Ga.-Redding Bldg.
Griffin, Georgia 30223-1797= denotes that material
OK to destroy in quarantine.

Q-number	Genus/species/subtaxa	Plant name	Origin	Form shipped
5855 T 24514	Ipomoea sp.	Plant Name: CANOTE MORADO ✓	GUATEMALA	3 in vitro
	Comment: Actual material sent was T 24514 01, post-therapy material			
5856 T 24520	Ipomoea sp.	Plant Name: CANOTE REMOLACHA ✓	GUATEMALA	2 3 in vitro
	Comment: Actual material sent was T 24520 16, post-therapy material			
5857 T 24521	Ipomoea sp.	Plant Name: CANOTE AMARILLO ✓	GUATEMALA	3 in vitro
	Comment: Actual material sent was T 24521 02, post-therapy material			
5858 T 24534	Ipomoea sp.	Plant Name: CANOTE BLANCO ✓	GUATEMALA	3 in vitro
	Comment: Actual material sent was T 24534 04, post-therapy material			
5859 T 25346	Ipomoea batatas var. ba	Plant Name: #0406 ✓	GUATEMALA	4 in vitro
	Comment: Actual material sent was T 25346 02, post-therapy material			
360 T 25347	Ipomoea batatas var. ba	Plant Name: #0407 ✓	GUATEMALA	4 in vitro
	Comment: Actual material sent was T 25347 01, post-therapy material			
361 T 25348	Ipomoea batatas var. ba	Plant Name: #0512 ✓	GUATEMALA	4 in vitro
	Comment: Actual material sent was T 25348 01, post-therapy material			
362 T 26770	Ipomoea batatas var. ba	Plant Name: DONE ✓	PUERTO RICO	4 in vitro
	Comment: Actual material sent was T 26770 07, post-therapy material			
363 T 26992	Ipomoea batatas var. ba	Plant Name: CUBA 6	CUBA	10 cuttings
	Comment: Actual material sent was T 26992 127, post-therapy material			
364 T 26996	Ipomoea batatas var. ba	Plant Name: COLECCION BAJO PAPALOAPAN ✓	CUBA	4 in vitro
	Comment: Actual material sent was T 26996 02, post-therapy material			
365 T 26997	Ipomoea batatas var. ba	Plant Name: CUBA 9 ✓	CUBA	4 in vitro
	Comment: Actual material sent was T 26997 08, post-therapy material			
366 T 27222	Ipomoea batatas var. ba	Plant Name: NINETY-NINE ✓	PUERTO RICO	4 in vitro
	Comment: Actual material sent was T 27222 116, post-therapy material			
367 T 27227	Ipomoea batatas var. ba	Plant Name: STONECORE	PUERTO RICO	4 in vitro
	Comment: Actual material sent was T 27227 01, post-therapy material			

Date shipped: 10/23/96

Invoice 96106
102168

Plant Material Shipping Invoice from USDA-ARS-NGRL-Plant Germplasm Quarantine Office

Destination:

Dr. Robert Jarret
USDA-ARS S9 Plant Gen. Res. Cons. Unit
1109 Experiment St. U of Ga.-Redding Bldg.
Griffin, Georgia 30223-1797

Q-number	Genus/species/subtaxa	Plant name	Origin	Form shipped
868 T 27837	Ipomoea batatas var. ba	Plant Name: BENIHAYATO	JAPAN	2 in vitro
	Comment: Actual material sent was T 27837 01, post-therapy material			
69 T 28457	Ipomoea batatas var. ba	Plant Name: HONIARA	FIJI	3 in vitro
	Comment: Actual material sent was T 28457 03, post-therapy material ✓			
370 Q 28745	Ipomoea batatas var. ba	Plant Name: G113-2B ✓	PHILIPPINES	4 in vitro
	Comment: Actual material sent was Q 28745 E		1 contaminated	
871 T 29652	Ipomoea batatas var. ba	Plant Name: SP-117	AUSTRALIA	4 in vitro
	Comment: Actual material sent was T 29652 07, post-therapy material ✓			
372 T 29666	Ipomoea batatas var. ba	Plant Name: 87049-3	AUSTRALIA	10 cuttings
	Comment: Actual material sent was T 29666 04, post-therapy material ✓			
373 Q 35192	Ipomoea batatas var. ba	Plant Name: KOSHU 18 ✓	PERU	3 in vitro
	Comment: Actual material sent was Q 35192 A			
374 Q 35646	Ipomoea batatas var. ba	Plant Name: HUARNAYANO ✓	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35646 A			
375 Q 35649	Ipomoea batatas var. ba	Plant Name: ZAPALLO ✓	CIP-PERU	2 in vitro
	Comment: Actual material sent was Q 35649 A			
376 Q 35650	Ipomoea batatas var. ba	Plant Name: IITA-TIB 10 ✓	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35650 B			
377 Q 35651	Ipomoea batatas var. ba	Plant Name: ROSENYA-BDI ✓	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35651 A			
378 Q 35652	Ipomoea batatas var. ba	Plant Name: MOGANBA ✓	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35652 A			
379 Q 35653	Ipomoea batatas var. ba	Plant Name: LOBY 3074	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35653 A			
80 Q 35654	Ipomoea batatas var. ba	Plant Name: INBY 3102 ✓	CIP-PERU	4 in vitro
	Comment: Actual material sent was Q 35654 B			

Date shipped: 10/23/96

Invoice 96106

Plant Material Shipping Invoice from USDA-ARS-NGRL-Plant Germplasm Quarantine Office

Destination:

Dr. Robert Jarret
USDA-ARS S9 Plant Gen. Res. Cons. Unit
1109 Experiment St. U of Ga.-Redding Bldg.
Griffin, Georgia 30223-1797

Q-number	Genus/species/subtaxa	Plant name	Origin	Form shipped
✓ 35655 5881	Ipomoea batatas var. ba Comment: Actual material sent was Q 35655 A	Plant Name: IITA-TIS 1487 <i>Poor health</i>	CIP-PERU	4 in vitro
✓ 35656 5882	Ipomoea batatas var. ba Comment: Actual material sent was Q 35656 B	Plant Name: IITA-TIS 2544	CIP-PERU	4 in vitro
✓ 35657 5883	Ipomoea batatas var. ba Comment: Actual material sent was Q 35657 B	Plant Name: IITA-TIS 3290 ✓	CIP-PERU	4 in vitro
✓ 35659 5884	Ipomoea batatas var. ba Comment: Actual material sent was Q 35659 B	Plant Name: IITA-TIS 9162 ✓	CIP-PERU	4 in vitro
✓ 35660 5885	Ipomoea batatas var. ba Comment: Actual material sent was Q 35660 A	Plant Name: IITA-TIS 9232 ✓	CIP-PERU	4 in vitro
✓ 35663 5886	Ipomoea batatas var. ba Comment: Actual material sent was Q 35663 A	Plant Name: K 51/3251 ✓	CIP-PERU	4 in vitro
✓ 35665 5887	Ipomoea batatas var. ba Comment: Actual material sent was Q 35665 A	Plant Name: TANZANIA	CIP-PERU	4 in vitro
✓ 35666 5888	Ipomoea batatas var. ba Comment: Actual material sent was Q 35666 A	Plant Name: HAGABOLIGE	CIP-PERU	4 in vitro
✓ 35667 5889	Ipomoea batatas var. ba Comment: Actual material sent was Q 35667 B	Plant Name: NO 29	CIP-PERU	4 in vitro
✓ 35668 5890	Ipomoea batatas var. ba Comment: Actual material sent was Q 35668 A	Plant Name: KEMB 10 ✓	CIP-PERU	4 in vitro
✓ 35669 5891	Ipomoea batatas var. ba Comment: Actual material sent was Q 35669 A	Plant Name: KEMB 37 ✓	CIP-PERU	4 in vitro
✓ 35670 5892	Ipomoea batatas var. ba Comment: Actual material sent was Q 35670 A	Plant Name: RUSWEYA-RWA ✓	CIP-PERU	4 in vitro
✓ 35671 5893	Ipomoea batatas var. ba Comment: Actual material sent was Q 35671 B	Plant Name: CM 1869-13 ✓	CIP-PERU	4 in vitro 4

Vegetable Crops Germplasm
Maintenance Program

Budget 1997*

<u>REPAIR & MAINTENANCE:</u>	\$6016.00**
<u>RESEARCH/LAB SUPPLIES:</u>	\$4568.64
<u>FIELD & GREENHOUSE:</u> INCLUDING GASOLINE	\$6077.25
<u>OFFICE SUPPLIES:</u> INCLUDING PHONE	\$153.08
<u>TRAVEL EXPENSES:</u>	\$136.00
<u>TISSUE CULTURE:</u>	\$1211.85
<u>MISCELLANEOUS EXPENSES:</u> INCLUDES COMPUTER PURCHASE PHYSICAL EXAMS GREENHOUSE WATERER'S SALARY	\$2349.00
<u>FEDERAL EXPRESS EXPENSES:</u>	\$223.65
<u>Total Funds Obligated to Date:</u>	<u>\$20,735.47</u>
<u>Funds Remaining:</u>	\$4204.53

*All figures approximate

**Includes replacement of supplies lost during -70C freezer malfunction.

Activity Report to the Technical Advisory Committee
S-9 Regional Project
June 3-4, 1997

Gil Lovell
Grass Curator

FORAGE AND TURF GRASS COLLECTION

A complex group of 7,137 PI's composed of 106 genera and 537 species. There are 6,112 available for distribution. However, only 1,349 are backed up at the National Seed Storage Lab (NSSL). There are 2,430 accessions held at NSSL that are not in our S-9 working collections, and a review of the passport data is required before consideration can be given to acquiring seed lots for inclusion in our working collections.

Regeneration 1996 - 253 PI's 1997 - 34 PI's

Priority has been placed on maximizing the time needed to carry out a detailed inventory of the grass seed collections. This should be completed by the end of 1997. This detailed inventory will allow us to carry out accurate, prioritized regeneration projections.

CASTOR (*Ricinus communis*) COLLECTION

There are 358 PI's in this collection; 255 are available for distribution; and 348 are backed up at NSSL.

Regeneration 1996 - 10 PI's 1997 - 30 PI's

SESAME (*Sesame indicum* & *S. radiatum*) COLLECTION

There are 1,084 PI's in this collection; 1,069 are available for distribution; and 1,083 are backed up at NSSL.

Regeneration 1996 - 13 1997 - 31 PI's

Of the 31 accessions being regenerated, 10 are for NSSL to replace seed lots classified as "at risk" because of low viability or seed quantity.

MISCELLANEOUS COLLECTION

This collection totals 264 PI's; 207 are available for distribution; and 239 are backed up at NSSL.

Regeneration 1996 - 17 PI's 1997 - 16 PI's

These regenerations (1996 & 97) are all Stokes' aster (*Stokesia laevis*). these PI's are native collections from southeast coastlines of South Carolina to Louisiana and Florida.

KENAF (*Hibiscus cannabinus*) & ROSELLE (*H. sabdariffa*) COLLECTION

There are 164 PI's of kenaf and 49 PI's of roselle in this fiber crop collection. Consideration had been given to transferring the collection to the ARS Cotton Germplasm unit at College Station, Texas but this has been dropped for an indefinite period. Through a cooperative effort with the Cotton germplasm unit regeneration will be done at the Tecoman (Mexico) Cotton Winter Nursery. Regeneration 1996-97 - 50 PI's

**Plant Genetic Resources Conservation Unit
Griffin, Georgia**

Research Scope

**Application of Molecular and Population Genetics
to Agricultural Resources Conservation and Use**

February 1997

Marker and/or Technique Development:

- * Identification and development of polymorphic, conserved, easily assayed DNA markers in cultivated crops for applications in agriculture and conservation
- * Automated genetic analysis:
DNA extraction, synthesis, amplification, sequencing, and fragment detection
 - Genomic library construction and enrichment
 - Identification of simple sequence repeats (SSRs) conserved within plant families
 - Multiplex PCR of SSRs
- * Using simple sequence repeat and AFLP analyses to characterize genetic uniqueness and variation in cultivated crop species and other agriculturally important organisms
- * Strategies for the identification of informative and useful sequences in plant genomes

Application:

- * Assessment of genetic diversity and redundancy of *ex situ* collections of crops (sorghum, maize, peanut, and forage/turf grasses)
- * Conservation of *Arabidopsis thaliana* (L.) Heynh. and *Brassica napus* L. SSRs in cultivated species of *Brassica*
- * Genetic variation and useful traits of cultivated and weedy *Brassica nigra* (L.) Koch
- * DNA typing and gene characterization of geographic isolates of *Escherichia coli* serotype 0157:H7
- * Determination of genetic variation of *Reticulitermes* spp. via sequence analysis and development of diagnostic assays
- * Determination of genetic variation of isolates of *Rhizoctonia solani* via sequence analysis of ITS regions
- * Sequence characterization of coat protein genes of peanut bud necrosis virus
- * Plant disease (virus) detection via DNA sequence-based assays

Theory and Hypothesis Testing:

- * Relationships between genomic, genetic, and phenotypic variation in cultivated crops
- * Genotype and gene identification in cultivated crops
- * Conservation of DNA sequence variation (coding and noncoding regions) in *Poacea*, *Fabaceae*, and *Cruciferae*

Appendix 8

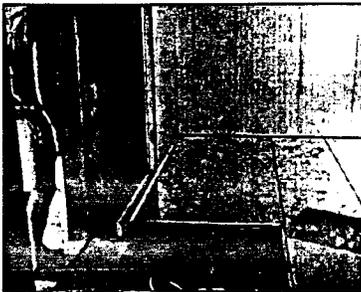
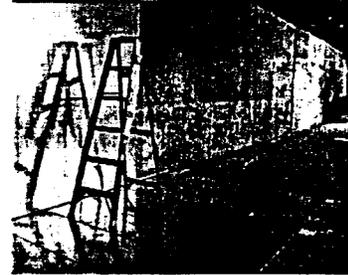
Seed Storage/Database Maintenance

May 1996 - May 1997



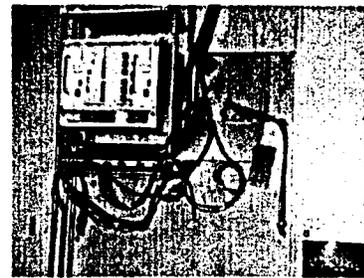
Seed Storage Renovation

Initial demolition (left) began in August 1996. Half of walls and floor installed (right). Installation of ramp and wall panel (below left) in front compartment. Completed in January 1997. Front compartment dual temperature capable 4°C or -18°C, 25% RH. Rear compartment -18°C.



Local Area Network

Completed installation of local area network in February 1997. Seven buildings connected via fiber optic cabling (right).



- Backup - 5,916 accessions in 53 orders have been sent to NSSL for backup since May 1996. Since May 1996, 11,698 additional accessions have been flagged as backed up on the GRIN for the S-9 site.
- Hundred seed weights and counts - An additional 19,965 inventory samples have been converted to a count inventory. All but four crops (legumes, peanuts, grasses, sorghum) have complete count inventory data.
- Move to -18°C - A total of 12,399 inventory samples have been sealed in foil bags, bar coded and moved into freezer storage.
- Bar codes - In addition to the accessions moved into freezer storage, 12,122 inventory samples have been re-bagged, bar coded, and put into 4°C storage.
- Orders - Processed 734 orders containing 24,849 items (459 distribution orders/12,309 items; 135 information only orders; 24 observation orders/1,648 items; 59 replenishment orders/4,958 items).
- Passport data - All passport data (41, 770 records) for *Sorghum* and *Vigna* has been verified and updated from the Plant Introduction books and old card file records.
- Database maintenance - Since May 1996, 114,578 records have been created on the GRIN and 119,946 records have been modified for the S-9 site.