

MINUTES OF THE MEETING OF  
THE S-9 TECHNICAL ADVISORY COMMITTEE  
FOR  
PLANT GENETIC RESOURCES CONSERVATION AND UTILIZATION  
S-9 REGIONAL PROJECT

Cooperative among:

THE STATE AGRICULTURAL EXPERIMENT STATIONS  
OF THE SOUTHERN REGION

the  
AGRICULTURAL RESEARCH SERVICE

the  
COOPERATIVE STATE RESEARCH, EDUCATION, AND  
EXTENSION SERVICE

and the  
NATURAL RESOURCES CONSERVATION SERVICE

of the  
UNITED STATES DEPARTMENT OF AGRICULTURE

JULY 20, 1998

IOWA STATE UNIVERSITY  
AMES, IOWA

SUBMITTED BY

H. THOMAS STALKER, CHAIRMAN

**S-9 TECHNICAL ADVISORY COMMITTEE MEETING**

**July 20, 1998**

**Ames, Iowa**

**AGENDA**

1:00 Call to order

Thomas Stalker, Chair, S-9 Regional Technical Advisory Committee

Introduction of attendees

Official Welcome - G.F. Arkin, Assistant Dean, Administrative Advisor

Minutes from 1997 Meeting

Additions to the Agenda

1:15 Curation activities at Griffin

Inventory and GRIN for S-9 crops

Research Leader vacancy

S-9 Review Committee Report

3:00 Break

3:15 Role of the S-9 Technical Advisory Committee

National Plant Germplasm System Report

Nominations

Future meeting plans

Resolutions

5:00 Adjourn

## 1. Call To Order

The regional S-9 Technical Advisory Committee (TAC) was called to order at 1:00 P.M. on July 20, 1998 by Chairman Thomas Stalker in the Conference Building on the campus of Iowa State University, Ames, Iowa. This year the meeting was held in conjunction with the national Plant Genetic Resource Management Meeting.

## 2. Introduction of Attendees

State TAC Members:

Dr. Gerald Arkin  
Administrative Advisor  
Director of Georgia Experiment Station  
Griffin, Georgia

Dr. Bryan Brunner  
University of Puerto Rico  
Lajas Substation  
Lajas, Puerto Rico

Dr. Don LaBonte  
Department of Horticulture  
Louisiana State University  
Baton Rouge, Louisiana

Dr. Billy Rhodes  
Department of Horticulture  
Clemson University  
Clemson, South Carolina

Dr. H. Thomas Stalker  
Department of Crop Science  
North Carolina State University  
Raleigh, North Carolina

Dr. David L. Coffey  
Department of Plant Sciences  
University of Tennessee  
Knoxville, Tennessee

Dr. Bill Kirby  
Department of Plant sciences  
Oklahoma State University  
Stillwater, Oklahoma

Ex-officio Members:

Dr. Peter K. Bretting  
National Program Leader, Plant Germplasm  
USDA-ARS  
Beltsville, Maryland

Dr. Thomas W. Zimmerman  
Univ. of the Virgin Islands  
Agricultural Experiment Station  
Kingshill, St. Croix, VI

Dr. Marsha Stanton  
National Program Leader, Plant  
Breeding and Genetics  
USDA, CSREES  
Washington, DC

Staff Members - S-9 Plant Introduction Station, Griffin, Georgia:

Dr. Graves Gillaspie  
Research Virologist and Vigna Curator

Dr. Robert Jarret  
Research Horticulturist  
Sweet Potato Curator

Gilbert R. Lovell  
Agronomist and Grass Curator

Dr. J. Brad Morris  
Agronomist and Curator of  
Clover and Special-purpose Legumes

Ms Lee Ann Chalkley  
Manager, Seed Storage  
and Order Processing

Ms Merrelyn Spinks  
Data Base Manager and  
GRIN Coordinator

James Strickland  
Agronomist and  
Farm Manager

### **3. OFFICIAL WELCOME**

Dr. Gerald Arkin, Administrative Advisor to the S-9 Project and representative of the Southern Agricultural Experiment Stations, welcomed the TAC committee. He discussed major points from the S-9 Program Review of August 1997. This review was conducted on behalf of the Southern Association of Agricultural Experiment Station Directors (SAAESD). Goals of the review were to evaluate (1) progress since the 1992 review in the areas of budget, infrastructure, personnel and operations; (2) strengths of the S-9 Project; (3) areas for improvement; and (4) planning and oversight for Unit personnel. The overall impression of the review team is that the Southern Region Plant Genetics Resource Conservation Unit has made important advances in all areas since the last review. A summary documenting the review is found in Appendix I of these Minutes.

Dr. Arkin pointed out that the S-9 TAC is an advisory board which should have access to budget information, so it can participate in setting priorities for expenditures for the operation of the Plant Genetics Resource Conservation Unit (PGRCU). He indicated that USDA formula funding has been declining. However, the Southern Directors had indicated that they would consider providing matching funds (up to \$150, 000) if ARS would provide funds for an additional Curator position.

Dr. Peter Bretting, USDA-ARS, National Program Leader for Plant Germplasm also welcomed the TAC. He reaffirmed that the S-9 Committee is an advisory group that is to review activities at the Griffin station and to make recommendations for funding, research, and curation activities.

#### **4. Minutes of the 1997 Meeting**

The minutes of the 1997 meeting were approved as circulated.

#### **5. Approval of Agenda**

The 1998 Agenda was approved as circulated.

#### **6. Curation Activities of the PGRCU**

Germplasm activities at the Plant Genetic Resources Conservation Unit were reported by the various staff members. Copies of their written reports are in the Appendix of the Minutes. The following are summaries of the reports:

Merrelyn Spinks distributed a data sheet which details by crop groups the status of the various germplasm collections. The grand total is now 79,427 accessions with 83.5% available for distribution and 68.8% are backed up in long-term storage at the National Seed Storage Laboratory (NSSL). Over the last five years there has been an annual increase in the numbers available for distribution and the numbers placed in backup at NSSL.

Dr. Graves Gillaspie, Vigna Curator, reported that photoperiod sensitive Vigna accessions do not regenerate adequately in greenhouses and he needs to have access to the ARS field propagation in Puerto Rico and St. Croix. However, a new policy denies access to the Puerto Rico field station (TARS), and small plantings underway at St. Croix are adversely affected by the high soil pH factors.

Gil Lovell, Grass Curator, reported that a Post-Entry Quarantine Permit was obtained from APHIS to grow Pearl Millet at the ARS unit at St. Croix, V.I. Imported accessions (700) have been in quarantine storage at NSSL for a number of years. These accessions will be regenerated over the next two years and will be available for distribution with no plant health restrictions. The warm season grass collection (7,010 accessions) cannot be regenerated adequately because of budget restrictions that adversely limits the hiring of seasonal and temporary labor. Of the total collection 3,314 are over 20 years old and only 1,351 are backed up at NSSL.

Dr. Brad Morris, Clover and Special Purpose Legumes Curator, reported that 50 pollination cages provides an efficient regeneration for cross-pollinated clovers. Beehives with each cage provide for the necessary cross-pollination. This results in adequate seed harvests with high seed quality (viability levels).Thirty self-pollinated clover accessions were direct seeded and transplanted to the greenhouse to identify an optimum method for regeneration. The methods proved equal for optimum plant and seed production. There is a need to investigate value-added traits and additional uses for the crops in his collections.

Dr. Robert L. Jarret, Vegetable Curator, reported a severe pathogen problem with Fusarium in the watermelons being regenerated in pollination cages. This is a soil-borne pathogen that is intensified by the higher temperatures and moisture levels within the cages. Additional funding is needed to fumigate the field plots and thereby control the Fusarium blight. Dr. Jarret's program has been severely limited by reduced staffing and funding. At the present staffing includes 1 PFT Curator/Research Horticulturist (RLJ). There is no technical support in-place for maintenance of the vegetable crops germplasm. A vacant position will eventually be filled. The individual in this position will ultimately have the responsibility for maintaining the in vitro sweetpotato collection in addition to assisting with the maintenance of other vegetable crops germplasm.

Dr. Roy Pittman, Peanut Curator, was unable to attend the meeting but provided a written report for distribution which is included in the Appendix to the Minutes of the meeting. The PGRCU staff is regenerating 990 peanut accessions in field plots at Byron, Georgia. Another 789 are being grown through the cooperation of four peanut breeders.

## **7. New Business**

A discussion about the duties of the Technical Advisory Committee (TAC) was held. The committee is to be an advisor to the PGRCU. The committee needs to know information about (1)crop vulnerability, data acquisition and collection needs; (2)strengths and weaknesses of programs on an annual basis; and (3) itemized budgets and allocations of funds.

A subcommittee was formed to make recommendations about poor attendance and to consider possible industry representation on the committee. Also, Dr. LaBonte is leading a subcommittee to develop a survey form for the S-9 participants to fill out before the TAC annual meeting in the summer of 1999.

The nominations of new officers for 1999 resulted in Dr. LaBonte being elected Chairperson and Dr. David Coffey elected Secretary for 1999.

The 1999 Annual Meeting will be held at the Griffin station. Dr. LaBonte will query th e TAC to determine the best date for the meeting.

Chairman Stalker adjourned the meeting at 5:00 P.M.

## APPENDIX I

## SUMMARY

### SOUTHERN ASSOCIATION OF AGRICULTURAL EXPERIMENT STATION DIRECTORS

#### Southern Region Plant Genetic Resources Conservation Unit

#### S-9 Program Review, 1997

On August 18-20 we conducted a review of the S-9 Project/Southern Region Plant Genetic Resources Conservation Unit, Griffin, Georgia. This program is jointly sponsored by the United States Department of Agriculture, Agricultural Research Service (ARS); the Southern Association of Agricultural Experiment Station Directors (SAAESD); and the University of Georgia and Georgia Agricultural Experiment Station. The present review was conducted on behalf of the Southern Association of Agricultural Experiment Station Directors. Goals of the review were to evaluate (1) progress since the last review regarding budget, infrastructure, personnel and operations; (2) strengths of the S-9 project; (3) areas for improvement; and (4) planning and oversight for Unit personnel. We found many specifics that merit a positive report. The overall impression of the review team is that the Unit has made important advances in all areas since the last review. Because of major technological advances in molecular sciences, this report focuses on achieving a contemporary plant genetic resources conservation and utilization program. This report emphasizes areas that we believe warrant immediate attention. The following are our specific findings and recommendations.

1. Human Resources. A priority of the Unit is to become current in regeneration, collection maintenance, and collection entry documentation. This task is an important goal, but cannot be achieved with current human and fiscal resources. In his leadership role for the Unit, Dr. Kresovich has appropriately charged his curator staff with responsibility for establishing a system of priorities for respective crop groups within the collection. However, Curators must move more aggressively and assume this charge as a personal commitment. Judging from our interviews, they clearly are not providing significant or sufficient leadership neither for their collections nor to their respective Crop Germplasm Committees (CGCs). We recommend that Curators accelerate their level of leadership in their respective crop areas and increase their level of interaction With CGCs regarding collection curation decisions..

Numbers of permanent scientific and technical personnel supporting the Unit seem generally adequate for meeting the program mission. The technical staff communicates well and often with staff at other germplasm centers in the system, and recognizes and values the benefits from these connections. The staff also shows commendable evidence of innovation in developing software tools and they also demonstrate conscientious dedication to germplasm maintenance and using and maintaining equipment for quantifying and cleaning seeds for storage.

We believe that additional administrative support is essential for continued development and improvement of the Unit. Dr. Kresovich does a commendable job of providing the Unit with energy, enthusiasm, vision, and especially with intellectual and programmatic leadership. In our view, he is doing everything possible to merge the classical functions of the Unit with new technology functions and efficiencies. We agree that the Unit should take the lead and serve as a model for preserving and providing genetic and genomic resources. In our view, Dr. Kresovich is gradually being overwhelmed by the daily processes of dealing with personnel, budgets and day-to-day details of Unit management. Curators must increase their support and cooperation in these areas for the Unit to achieve its potential.

We recommend that a new curator position be immediately added to the Unit. This person is needed to promote the genetics and molecular/biotechnology skills and understanding, to improve support of the Unit's genomics capacity. This recommendation is a top priority for facilitating moving the Southern Region Plant Genetic Resources Conservation Unit achieving its potential for leadership in genomics capabilities.

During our review, all support personnel were well prepared to discuss their functions, demonstrated clear understanding of their respective assignments and their respective roles in meeting the mission of the Unit. Especially noteworthy is their intellectual commitments to the Unit, and their innovative approaches to accomplishing their responsibilities.

2. Physical Facilities. The ARS has continued to invest funds to improve the physical facilities of the Unit, and with the exceptions noted below, these facilities now seem generally adequate for meeting the program mission. Moreover, the University of Georgia is a very generous host to this Unit, routinely supporting laboratory needs such as access to the worldwide-web and field space to regenerate seeds.

Equipment needs of the Unit include a power supply backup that can assure the environmental conditions needed for seed storage and additional pollination cages that are similar to those in use now.

3. Financial Resources. Financial resources in 1997 consisted of about \$ 1.5M from ARS of which about 70% is allocated for personal services, and about \$250K from the SAAESD (i.e., S-9 funds that are 'off-the-top'), of which about 70% is allocated to personal services. Three sister regional units of the National Germplasm System receive about \$450K in CSREES-based funds (Ames-North Central), \$150K (Geneva-Northeastern) and \$350K (Pullman-Western). Given the critical importance of preserving crop germplasm, it seems reasonable and appropriate that the SAAESD should increase its portion of funding. We recommend an SAAESD increase of \$200K to this Unit. This is especially important considering the major time and personnel necessary to pursue the mission of the Unit, the fact that the Unit is the largest of the four regional units, and the major use and importance of these germplasm resources to crops that are important to all the State Agricultural Experiment Stations in the Southern Region.

4. The Collection. Curators must provide stronger leadership in working with their respective Crop Germplasm Committees. In our view, they seemed inclined to only respond to desires or expectations of their respective Crop Germplasm Committees (CGCs); they are not providing significant leadership (e.g., setting the agenda for CGCs, establishing priorities for CGCs or at least negotiating priorities, providing intellectual leadership for CGCs etc.). A critical next step for curators is they must take a clear position in their respective crops for a core collection. These positions should be developed in a collective process that includes participation from CGCs, S-9, other curators, and ARS National Program Staff.

5. Leadership and Management. The general leadership and management of Unit programs clearly depend on Dr. Kresovich and his energy and vision. However, the crop curators could help this process by providing additional clear, strong and aggressive leadership for their respective crops. Also, it was unclear to us what role is played by the NSSL, NCGR, CGC, etc., in promoting leadership from curators. We believe it will be essential for the crop curators to assume far more leadership in managing the genomics of their respective crops.

Dr. Kresovich has implemented regular meetings (i.e., usually each week) of curators and administrative staff as part of the Unit management process. These meetings were noted by each curator, and is a strong indication of Dr. Kresovich's total commitment to a collective process for managing Unit resources. However, we did not sense that the curators viewed their role as being responsible for decisions and priorities for their respective crop needs and activities.

6. Moving the Griffin Unit to Athens. In our view, the proposed move of the Southern Region Plant Genetic Resources Conservation and Utilization Unit to Athens will be counterproductive. This committee in the strongest of terms recommends that the SAAESD take a strong position against the move. No worthy rationale could be found during the Review to support a move. Regardless of a decision on moving, the rationale supporting a move to Athens should be made public and discussed on its merits. A final decision should then be made within 7-10 days (see partnership below). Prolonging the discussion and delaying the decision will significantly weaken the Unit's capacity to meet its mission and will be destructive to Unit morale.

7. Partnership. A continuing partnership between the University of Georgia, the Georgia Agricultural Experiment Station, the SAAESD, and the ARS is essential to continuing the success of this Unit. We recommend that the SAAESD increase its level of priority on this Unit, and take a much stronger lead in providing needed resources. To improve this partnership, individuals should be identified as an Advisory Board, and should represent each element of the partnership. The Advisory Board should include one or more individuals from the private sector, and the Board should meet regularly with the Unit administrative leadership to facilitate recognizing unit needs and promote unit accomplishments.

We complement Dr. Kresovich in his leadership to the Griffin Unit. He is a key to the Unit's continued success and progress toward using new technologies. The new curator position described above should be designated for a scientist in molecular genetics and genomics. At the

earliest possible date, we request that the SAAESD address the need for additional unit resources. Unless the Association acts soon, the Unit's investment in the future could be compromised, and it could lose its current capacity and level of accomplishment.

## APPENDIX II

PLANT GENETIC RESOURCES CONSERVATION UNIT  
GRIFFIN, GEORGIA

JUNE 1998 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED <sup>1</sup> UP	CORE	NSSL <sup>2</sup> ONLY	OBS DATA LOADED
Grasses & others	Lovell, Gil	Bamboo	98	98	0	47			
		Castorbean	361	278	83	355	670	2,398	
		Hibiscus	357	268	89	289	382	53	
		Miscellaneous	268	210	58	242	152	985	
		Pearl millet	448	400	48	425	691	959	
		Sesame	1,203	1,190	13	1,203	27	8,698	
		Warm Season Grasses	7,010	5,996	1,014	1,351	2,425	53,128	
		Sorghum	29,881	27,922	1,959	22,423	11,249	83,590	
Legumes	Morris, Brad	Guar	413	401	12	406	888	1,890	
		Legumes	2,904	2,228	676	2,290	86	5,600	
		Trifolium	1,946	1,329	617	1,411	95 <sup>3</sup>	34	4,833
		Winged bean	166	14	152	18		50	
Peanuts (9,115)	Pittman, Roy	Cultivated	8,604	7,520	1084	6,348	798	67	56,605
		Wild	643	533	110	5			
Vegetable Crops	Jarret, Bob	Citrullus	1,642	1,370	272	1,479	315	11,137	
		Cucurbits	1,332	495	837	829	46	1,425	
		Eggplant	897	851	46	870	115	41	8,854
		Gourds	464	246	218	306	12	782	

<sup>1</sup>Total accessions backed up for the site crop.

<sup>2</sup>Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.

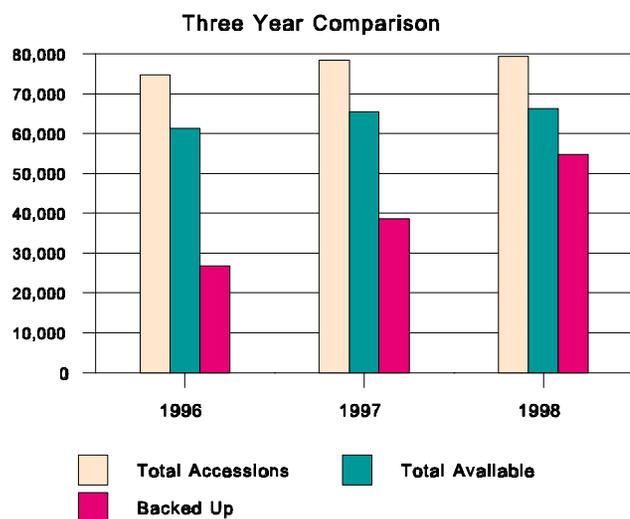
<sup>3</sup>Core includes three clover species: *T. alexandrinum*, *T. resupinatum*, *T. subterraneum*.

JUNE 1998 SUMMARY

GROUP	CURATOR	SITE CROP	TOTAL ACCESSIONS	NUMBER AVAILABLE	NOT AVAILABLE	BACKED <sup>1</sup> UP	CORE	NSSL <sup>2</sup> ONLY	OBS DATA LOADED
Vegetable Crops	Jarret, Bob	Ipomoea	1,071	752	319	307		34	9,470
		Luffa	159	128	31	129		1	193
		Okra	3,047	1,516	1,531	1,906	165	35	5,534
		Pepper	3,876	3,731	145	2,235		176	86,573
Vigna (12,600)	Gillaspie, Graves	Cowpea	7,855	4,739	3,116	5,425	699	249	33,734
		Mung bean	4,188	3,836	352	4,088	410	28	15,879
		Other Vigna spp.	594	272	322	294		1	
Grand Total for Griffin crops			79,427	66,323	13,104	54,681	2,282	17,609	392,320
Percent (%)				83.5%	16.5%	68.8%			
Change 1997 to 1998			947	830	-117	16,096	115	-16	66,464

<sup>1</sup>Total accessions backed up for the site crop.

<sup>2</sup>Figures in this column represent accessions held only at NSSL for which Griffin is the first priority site in the NPGS.



## 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 50 cowpea lines previously put through greenhouse virus elimination, but failing to produce seed in Georgia field regeneration tests.
2. Regeneration of 107 lines of cowpeas in the field at Griffin.
3. Tests of regeneration capacities and virus reinfection of six photoperiod sensitive cowpea lines at Griffin, St. Croix, and Puerto Rico to determine regeneration potentials of locations for such lines. Tests made throughout growing season for virus reinfection and random 100-seed-samples of each replication tested for seedborne viruses after the field test complete. A manuscript is in preparation.
4. Work completed and published on the characterization of a new severe strain of cucumber mosaic virus prevalent on cowpeas in Georgia, on a bean yellow mosaic subgroup virus isolated from *Sesbania*, and on a latent, highly seedborne strain of bean common mosaic virus found on guar.
5. A manuscript is in preparation on a PCR-based detection method for cowpea mottle virus in cowpeas.
6. Backup of the *Vigna* collection at NSSL is completed.

##### B. Work in progress

1. Field regeneration is under way on 150 cowpea lines that were in short supply and not available for distribution. In addition, 7 lines that had only a few seeds germinate are being grown out in the greenhouse.
2. Greenhouse growout (based on space available) of photoperiod sensitive cowpea lines is planned for the late fall-winter and 30 additional lines are being regenerated at St. Croix now. The soil pH and water problems at St. Croix continue to be a problem, but attempts

are being made to cope with these things.

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.

Activity Report to the Technical Advisory Committee  
S-9 Regional Project  
July 20, 1998

Gil Lovell  
Germplasm Curator

#### FORAGE AND TURF GRASS COLLECTION

Since June, 1997 we have placed maximum time available into detailed inventory of the collection. We have completed 97% of the PI's. This activity consists of extra seed cleaning with newer type equipment, followed by seed counts and weight for each of the 7,137 PI's in the collection. This detailed inventory will allow us to set practical priorities for regeneration based on the age of the seed or the critical volume on hand. The viability level (per cent germination), when known, is the primary criteria for regeneration.

Regeneration 1996 - 253 PI's                      1997 - 34 PI's                      1998 - 316 PI's

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

There are 1,203 PI's in this collection and all are backed up at NSSL.

Regeneration 1996 - 13    1997 - 31 PI's                      1998 - 9 PI's

The nine accessions being grown this season are for NSSL to replace seed lots classified as "at risk" because of low viability or seed quantity.

#### PEARL MILLET (*Pennisetum glaucum*)

There have been 603 PI's in quarantine since the mid-1980's. After two years of negotiating with APHIS we have finally gotten a Departmental Permit to grow these accessions at St. Croix for seed increase. This September and October, 420 will be planted with hope that we will complete the collection in two growing seasons.

Regeneration 1997 - 210    1998 - 420

#### KENAF (*Hibiscus cannabinus*) & ROSELLE (*H. sabdariffa*) Collection

In addition to an annual planting of 50 PI's of kenaf at the Tecoman (Mexico) Cotton Winter Nursery we will have five roselle PI's grown out in large plots at a special request from the University of Georgia. The University needs several pounds of each to carry out research with replicated plots. They have transferred funds to us to cover the cost of production and shipment. We will also get seed samples from this production to be included in our working collections and add to the backup collection at NSSL.

Regeneration 1996 - 50    1997 - 50    1998 - 55

**Clover and Special-Purpose Legume Curation/Research Activities**  
**July 9, 1998**  
**Brad Morris**

**I. Objectives**

Curate the National Plant Germplasm System's clover and special-purpose legume germplasm collection which consists of 5,378 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 nematicidal potential from various legume species as soil amendments and collaborating with various public and private organizations for identification, isolation, purification and enhancement of various phytoestrogens and lectins found in various legume species.

**II Status**

**A. Accomplishments**

1. Back-up samples of both annual clovers (1,419 accessions) and legumes (2,288 accessions) have been sent to NSSL. Accessions with inadequate seed numbers will not be sent until satisfactory seed numbers from regenerations are accomplished.
2. 50 pollination cages provided an efficient regeneration for cross-pollinated clover species.
3. 75 Ethiopian clovers were compared for regeneration capability in the greenhouse at Griffin, GA and Beeville, TX. Most tested can be grown under greenhouse conditions at Griffin to obtain optimum regenerations.
4. To facilitate technology transfer, various publicly viewed news articles were provided to news agencies regarding phytochemicals found in the legume collections.
5. Through collaborative efforts with UGA, *Desmodium*, *Leucaena*, *Senna*, and *Sesbania* species when added as soil amendments reduced nematode galls by up to 50%.
6. Through collaborative efforts with USDA and UGA colleagues, characterized a latent potyvirus seedborne in guar and guar green sterile virus.
7. Potential redundant accessions have been identified in clover species.

**B. Work in Progress**

1. Thirty self-pollinated clover accessions were direct seeded and transplanted to the screenhouse to identify the optimum method. Both methods were equal for optimum plant and seed production.
2. 50 cross-pollinated clover accessions were transplanted the first week of April. Pollination cages with beehives are utilized for cross-pollinations. Efficient regenerations with high seed quality are being harvested.
3. 70 self-pollinated legume species with novel value added phytochemical traits are regenerating in the field.
4. Ongoing collaborative strategy development with Texas A&M breeder for redundancy elimination in large clover collections.
5. Ongoing collaboration with UGA plant pathologist for identification of additional legume species with potential as soil amendments for nematicidal properties.

6. Collaborating with Washington State University curator for conservation strategies in clover.
7. Conducting crossing experiments for various legumes including *Crotalaria spp.*, *Canavalia spp.*, *Indigofera spp.*, and *Desmodium spp.* for establishing cross compatibilities.

### **C. Future**

1. Regenerate 100 cross and self-pollinated clovers next year.
2. Establish collaborative efforts for identification, isolation, purification, inheritance and enhancement for various phytochemicals (including phytoestrogens and lectins) from the clover and legume collections.

## **Robert L. Jarret, Research Horticulturist/Vegetable Crops Curator**

### **Introduction**

Incumbent is responsible for management (maintenance/characterization/ acquisition) of vegetable crops genetic resources at the Southern Regional Plant Introduction Station (SRPIS) in Griffin, GA. Primary crop mandates include; pepper (*Capsicum* spp.), sweetpotato (*Ipomoea* spp.), watermelon (*Citrullus* spp.), okra (*Abelmoschus* spp.), eggplant (*Solanum* spp.), squash (*Cucurbita* spp.) and gourds (various genera). The sweetpotato collection is maintained as in vitro cultures. All other germplasm is maintained as botanical seed. At the present time, germplasm of these crops and their related species account for approximately 23,000 accessions.

### **Germplasm Maintenance/Seed Regeneration**

Seed increase activities take place predominantly in Byron, GA. Mr. Jim Leaptrot coordinates vegetable seed increase activities in Byron. At the present time we are regenerating watermelon (*Citrullus lanatus* and *C. colocynthis*), squash (*Cucurbita moschata*) and a portion of the okra (*Abelmoschus* spp.) core collection. In addition, we are characterizing and photo- documenting the *Solanum melongena* (eggplant) core collection. Materials in the sweetpotato collection are recultured periodically, as required. *Ipomoea*, *Capsicum* and *Citrullus* germplasm is regenerated in the greenhouse, as space permits. Approximately 1,000 accessions of *Capsicum* are being grown in Las Cruces New Mexico in order to acquire characterization data and for photodocumentation. Representative examples of several *Capsicum* spp. are being grown in the greenhouse in order to confirm their correct botanical identification.

### **Staffing**

At the present time staffing includes I PFT curator/Research Horticulturist (RLJ). There is no technical support in-place for maintenance of vegetable crops germplasm. A vacant position (previously held by Mr. Kevin Mataxas) is in the process of being filled. The individual in this support position will ultimately have the responsibility for maintaining the in vitro sweetpotato collection in addition to assisting with maintenance of other vegetable crops germplasm. At present, vegetable crop germplasm maintenance activities utilize about 1/3 of the time of the Byron crew. The program has been employing Ms. Melanie Newman (TPT- 8 hrs/wk) since February to help the curator with the reculture the in vitro collection.

### **Acquisition**

Progress was made in identifying collaborators for plant germplasm exchange (*Citrullus* in South Africa and *Capsicum* in South America). A plant exploration proposal to Argentina to collect germplasm of *Capsicum* spp., was recently submitted. There has been an increasing interest in *Solanum* spp. by private sector plant breeders. Opportunity exists to acquire germplasm of edible-fruited *Solanum* spp.

## Research

Research relative to germplasm management is conducted, as funds permit.

### 1998 Vegetable Crops Germplasm Program Operating Budget/Expenditures\*

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Total:	\$23,000
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Field supplies (herbicides, fertilizer, etc.)	1,500
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Greenhouse supplies (soil, pots, etc.)	2,200
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Apiary services for controlled pollination of <i>Citrullus</i>	2,400
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Supplies for in vitro collection maintenance (media, culture tubes, etc.)	5,500
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Labor (M. Newman as described previously)	2,500
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R&M (lab equipment and greenhouse)	2,400
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Travel	500
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Vehicle R&M, gasoline	1,500
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Research	2,000
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Total	\$20,500
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Balance (7/98)	\$2,500
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# 1998 *Arachis* S-9 Report

Roy N. Pittman

## Plant Genetic Resources Conservation Unit

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### I. Objectives

The *Arachis* collection is currently made up of nine sections and 70 described species. Duties include the acquisition, maintenance, documentation, 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. 725 accessions of cultivated peanuts were planted last year for increase. An additional 1200 accessions were grown by cooperators in Georgia (Corley Holbrook and Kim Moore), Florida (Dan Gorbet), and Oklahoma (Jim Kirby). Many thanks to these cooperators.
2. Plant, pod, and seed descriptors for the 1997 increase will be taken at sites in Georgia and Florida on the cultivated increase material. Pod and seed descriptors were collected only from Oklahoma.
3. Increases from the '95 (77 accessions) and '96 (78 accessions) Williams collections were received from Ecuador.
4. Virus elimination from wild and cultivated peanut germplasm continues.
5. A paper titled 'Survey of the fatty-acid composition of peanut (*Arachis hypogaea*) germplasm and characterization of their epoxy and eicosenoic acids' was published in the Journal of The American Oil Chemists Society 74(10):1235-9.1997.
6. Information from my collection and Paula Bramel-Cox (ICRISAT) about the status of wild peanuts was given to Tom Stalker to combine with information from other sites maintaining material in the U.S. and elsewhere.
7. Rhizomatous peanuts have been divided and some of the material shipped to Miami. Other material will follow later in July or August.
8. Graves Gillaspie developed an immunocapture-reverse transcription-polymerase chain reaction procedure to detect PSTV which is 10X+ more sensitive than current technique. The current technique can find 1 in 10 while the new finds 1 in 100 particles tested.

#### B. Work in progress

1. 1998 increases are being increased with Tim Moore 292, Dan Gorbet 142, Tom Isleib 155, and Jim Kirby 200. In addition, I am increasing 990 at Byron. With help of many people the peanut collection is about 90%+ increased and backed up. Next year only Byron will be used for increase. This will be material from increases which did not produce much seed and recent introductions.
2. Testing using IC-RT-PCR has started on the Ecuadorian material. This should be by August and material available for distribution at that time if free of PSTV.
3. Holbrook and I are collecting descriptors for 'New' accessions (840) for selection into the, 'core.' Approximately 37 accessions are from increase material from recent Ecuador collections.

### III. Needs

1. A drying room to store field increases prior to shelling needs to be build at Byron. This needs to be dehumidified with heating kept to a minimum.
2. Evaluation funds for pests/diseases which are important to peanuts which other CGC's have access to.

### IV. Other News

1. Stephanie Dunn was selected for the USDA greenhouse/laboratory position. She will be helping with increasing material through quarantine, maintaining wilds in the greenhouse, data base records of increase and germplasm and several other tasks including general increase of material.
2. Steve Kresovich will be moving to Cornell University in Ithaca, New York. There he will be a professor in the Department of Plant Breeding, Director of the Crop Diversity Program in the Center for Germplasm Studies and Molecular Breeding and member of the University-wide Genomics Initiative.

## PRELIMINARY COMPARISONS OF GENETIC SIMILARITIES OF SELECTED BOLIVIAN CULTIVATED PEANUT ACCESSIONS

M.S. Hopkins, S.E. Mitchell, R.N. Pittman, and S. Kresovich

June 1998

Peanut (*Arachis hypogaea* L.) is an important international crop for direct human consumption and as an oilseed crop. It has been hypothesized that peanut was domesticated in South America, probably in Southern Bolivia or Northern Argentina (Gregory *et al.*, 1980; Kochert *et al.*, 1996). In an effort to obtain more genetic diversity for the U. S. National Plant Germplasm System (NPGS) collection of cultivated peanut and for subsequent breeding activities, peanut seed samples were collected in Bolivia in 1997. Two samples of seed, collected at markets from different cities, appeared morphologically similar. These were designated 'Bayo Grande Guartro Qjiatas' (BGGO) and 'Bayo Grande Guaryos' (BGG). **We were asked to determine: (1) if these two samples were genetically similar to each other, and also, (2) if they were genetically similar to other accessions already maintained in the U.S. NPGS cultivated peanut collection at Griffin.** When compared to other crop species, cultivated peanut classically has demonstrated low amounts of molecular genetic variation. However, we have developed a small set of six polymorphic simple sequence repeat (SSR) markers that have detected variation in cultivated peanut (Hopkins *et al.*, in review).

### RESULTS

**Fragment data indicated that all plants representing accessions PI 339967, BGGO, BGG, and three of the five plants from PI 497412 were identical for the six SSRs (putatively 10 loci) (Table 1).** In complement with previous seed morphology and agronomic trait evaluations, the molecular data support the hypothesis that these accessions tested are genetically similar. However, one must recognize the limited sample size of accessions, plants per accession, and markers assayed. Four of the five entries tested were genetically homogeneous. However, it is interesting that within-accession variation was detected in PI 497412. Two individuals representing this accession varied from the other three at four of the six SSRs assayed. These results suggest that there very well may be a mixture of seed within PI 497412. In addition, based on the different genotypes detected it is likely that one plant is a result of mixed seed while the other a result of an outcrossing event. As more polymorphic SSR markers become available for ready use, a more extensive examination of genetic similarities among entries in the collection may be warranted.

### Materials and Methods

Three NPGS cultivated peanut accessions were considered to be similar to Bayo Grande 'type' based on seed morphology and agronomic traits. Five seed from representing accessions PI 339967, PI 497358, PI 497412, and Bolivian market type BGGO, and four seed from a second Bolivian market type BGG were germinated and grown in the dark at 35C for 21 days. After removing the cotyledons, the etiolated seedlings were stored at -70C. DNA was purified via the CTAB method and quantified by fluorometry. Fifty ng of DNA from each seedling was used as a template in two multiplex PCR reactions incorporating the six SSRs (Table 2). In addition to the DNA each reaction contained 1 x Promega PCR buffer, 1.5 mM MgCl<sub>2</sub>, 0.20mM dNTP, 0.5 units Taq polymerase (Promega), and 10 pmoles of each primer set in a total volume of 20ul. The forward primer in each set was labeled with either 6-carboxyfluorescein (6-FAM), tetrachloro-6carboxyfluorescein (TET), or hexachloro-carboxyfluorescein (HEX) to the 5' end of the oligonucleotide during DNA synthesis. PCR cycling conditions were an initial denaturation of 95C/4 min, followed by 25 cycles of 95C/1 min, 55C/2 min, and 72C/2 min. In the final PCR cycle, the extension time at 72C was increased to 10 min. Samples containing 1ul PCR product, 0.5ul GeneScan 500 internal lane standard labeled with N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA) (Perkin- Elmer/Applied Biosystems), and 50% formamide were heated at 92C for 5 min, placed on ice, then loaded on 6% denaturing (8.3M urea) acrylamide:bisacrylamide (19: 1) gels (24cm well-toread). DNA samples were electrophoresed in 1X TBE buffer (89mM Tris, 89mM borate, 2mM EDTA pH 8.3) at constant power (3 1W) for two hours on an automatic DNA sequencer (Perkin Elmer/Applied Biosystems, model 373A) equipped with GeneScan 672 software version 1.2.1 ((Perkin Elmer/Applied Biosystems). Fragment sizes were automatically calculated using the "local Southern" algorithm.



<b>Table 1. SSR fragment sizes (bp) for selected Bayo Grande 'type' cultivated peanut.</b>									
<b>Sample</b>	<b>Ah4-004</b>	<b>Lec-1</b>	<b>Ah4-026 i</b>	<b>Ah4-026 ii</b>	<b>Ah4-026 iii</b>	<b>Ah4-024</b>	<b>Ah6-125</b>	<b>Ah4-020 i</b>	<b>Ah4-020 ii</b>
<b>339967-1</b>	82	228	157	184	211	421	190	203	211
<b>339967-2</b>	82	228	157	184	211	421	190	203	211
<b>339967-3</b>	82	228	157	184	211	421	190	203	211
<b>339967-4</b>	82	228	157	184	211	421	190	203	211
<b>339967-5</b>	82	228	157	184	211	421	190	203	211
<b>BGGO-1</b>	82	228	157	184	211	421	190	203	211
<b>BGGO-2</b>	82	228	157	184	211	421	190	203	211
<b>BGGO-3</b>	82	228	157	184	211	421	190	203	211
<b>BGGO-4</b>	82	228	157	184	211	421	190	203	211
<b>BGGO-5</b>	82	228	157	184	211	421	190	203	211
<b>BGG-1</b>	82	228	157	184	211	421	190	203	211
<b>BGG-2</b>	82	228	157	184	211	421	190	203	211
<b>BGG-3</b>	82	228	157	184	211	421	190	203	211
<b>BGG-4</b>	82	228	157	184	211	421	190	203	211
<b>497412-1</b>	82	228	157	184	211	421	190	203	211
<b>497412-2</b>	104	230	157	192	211	411	190	203	211
<b>497412-3</b>	82	228	157	184	211	421	190	203	211
<b>497412-4</b>	92 / 104	230 / 238	157	192	213	411	190	203	
<b>497412-5</b>	82	228	157	184	211	421	190	203	211
<b>497358-1</b>	98	228	157	190	211	421	190	203	211
<b>497358-2</b>	98	228	157	190	211	421	190	203	211
<b>497358-3</b>	98	228	157	190	211	421	190	203	211
<b>497358-4</b>	98	228	157	190	211	421	190	203	211
<b>497358-5</b>	98	228	157	190	211	421	190	203	211

**Table 2.** Primer pairs used (sequence, motif, length, and number of fragments) for analysis of *A. hypogaea* and peanut species.

Identification	Motif	Primer sequence	Size range
Ah4-4	(GA) <sub>19</sub>	5'- <b>TET</b> -CGATTTCTTTACTGAGTGAG-3'(F) 5'-ATTTTTTGCTCCACACA-3'(R)	82-100 1
Ah4-20	(GA) <sub>19</sub>	5'- <b>HEX</b> -ACCAAATAGGAGAGAGGGTTCT-3'(F) 5'-CTCTCTTGCTGGTTCTTTATTA ACTC-3'(R)	201-215 2
Ah4-24	(ATA) <sub>17</sub>	5'-TET-TTCTGATTTTAGTAGTCTTCTTTCACT-3'(F) 5'-CTCCTTAGCCACGGTTCT-3'(R)	403-418 2
Ah4-26	(CT) <sub>25</sub> <b>imperfect</b>	5'- <b>FAM</b> -TGGAATCTATTGCTCATCGGCTCTG-3'(F) 5'-CTCACCCATCATCATCGTCACATT-3'	156-213 1
Ah6-125	(TTC) <sub>13</sub> <b>imperfect</b>	5'- <b>TET</b> -TCGTGTTCCCGATTGCC-3'(F) 5'-GCTTTGAACATGAACATGCC-3'(R)	190-192 1
Lec- I	(AT) <sub>18</sub>	5'- <b>TET</b> -CAAGCATCAACAACAACGA-3'(F) 5'-GTCCGACCACATACAAGAGTT-3'(R)	218-281 1

Report of the

# National Germplasm Resources Laboratory

to the

**Regional Technical  
Committees on Plant  
Germplasm**

July 1998

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## **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 Plant Exchange Office (PEO), and the Germplasm Resources Information Network/Database Management Unit (GRIN/DBMU). In addition, the Laboratory has responsibility for facilitating the activities of the 40 Crop Germplasm Committees that provide technical advice to the NPGS.

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.

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.

## **Plant Exchange Office (PEO) - Ned Garvey**

### **Exchanges**

Maryann Loftus continues to forward to the appropriate NPGS site requests for germplasm received through GRIN's World Wide Web connection. As more researchers find our Web site the number of requests has increased to nearly 200 during calendar year 1997, while letter requests have dropped to only 35 for the year.

During 1997, 17,509 items in 392 shipments were forwarded to 65 different countries. Some 972 non-permit items were received from foreign cooperators for use by U.S. scientists.

Maryann continues to provide quarantine and shipping information to traveling scientists, plant collectors, and researchers, and assists returning collectors when clearing APHIS inspections at international airports.

### **Shipping Update**

Walter Denny, APHIS-PPQ Inspector at the Beltsville Quarantine Center, retired on 30 April 1998. The remaining Inspector, Pamela Waterworth, is now handling all exports, imports, and post-entry duties. Pam's phone number is 301-504-7142 and FAX is 301-504-8539. Pam is currently keeping up with the workload, but should the sites have any perishable shipments which they need to send out, it would be advisable to contact either Pam or Maryann BEFORE sending the material to Beltsville as there is no one to replace Pam when she is on leave or attending training. At this time there are no plans to fill the other inspector's vacancy.

### **PI Documentation**

Becky Norris continues to coordinate the documentation and assignment of PI numbers. 6,205 new PI numbers were assigned and the passport data reviewed. This includes 356 new Crop Science registrations and 1,480 PVP accessions. Approx. 731 CSR certificates were printed and distributed to the authors.

Becky continues to assist curators and site personnel with adding and updating passport data and work closely with DBMU personnel to standardize and correct records in the GRIN database.

Plant Inventory No. 206 for 1997 has been published. It is 2 parts and contains a total of 707

pages of text and indexes.

Becky is part of two DBMU committees, Quality Control and User Interface, providing feedback necessary for developing GRIN systems.

### **In Situ Conservation**

Dr. Diane Pavek, a postdoc with PEO, is in the second year of a two-year ecogeographic survey of select grape species (*Vitis* spp.), native to North America. During 1997, she examined a total of 84 waterways and found rock grape (*Vitis rupestris* Scheele) on only 24 waterways in nine states. In collaboration with grape germplasm curator, Warren Lamboy, and horticulturist, Ned Garvey, molecular and morphological population data were evaluated, and seven populations were proposed as in situ preserves based on the differences in genetic variation. This summer Diane will collect 50 grapes from each established in situ preserve for midterm seed storage and distribution to researchers and breeders. Currently, in cooperation with State and Federal agencies, two of the seven proposed in situ preserves have been established.

This summer, Diane located five populations of sweet mountain grape (*Vitis monticola* Buckl.), a Texas endemic found only on calcareous substrate, and four populations of Calloosa grape (*Vitis shuttleworthii* House), a Florida endemic. For each population, morphological data will be analyzed, and DNA extracted from leaf samples. These data are necessary to determine which populations are included as in situ preserves.

### **Plant Exploration**

The USDA Plant Exploration Program is coordinated by Karen Williams and supported by Judi duCellier. One domestic and five foreign plant explorations were supported in FY 1997. An exploration for *Vigna angularis* in China originally scheduled for 1997 was postponed until the fall of 1998. In FY 1998, eight explorations and one exchange trip are being supported. An exploration for forage species and vegetables in Albania has been postponed until 1999 because of political unrest in the country. Eleven proposals have been received for FY 1999.

Requests for guidelines for exploration and exchange proposals should be sent to Karen. These guidelines are updated yearly. Scientists intending to submit proposals should begin planning several months before the proposal submission date because agreements with host countries are often much more complex than in the past. It is not possible for USDA to obtain permission for plant exploration in some countries. In some cases, it is advisable to associate additional benefit sharing with plant explorations. In 1998, benefit sharing agreements were associated with explorations for wild potatoes in Peru, wild and domesticated peppers in Paraguay and peanut landraces in Guatemala. The PEO should be consulted for advice on benefit sharing associated with plant explorations.

As part of an ongoing USDA/IPGRI/CIAT collaborative project, the use of Geographical Information Systems (GIS) for targeting cultivated plant (landrace) diversity was tested in Guatemala in November, 1997. The GIS uses cultural and physical environmental parameters correlated with known sites of cultivated plant diversity to create a model that will predict the occurrence of diversity in unexplored areas. Additional testing of the model for locating cultivated peanut diversity will be done in Guatemala and Venezuela in the coming year.

During the past year, Karen Williams participated in plant explorations for peanut landraces in Guatemala and wild and landrace *Capsicum* in Paraguay.

Ned Garvey will be participating in a plant exploration to southeastern PRC, Sept. 15 - October 18, 1998 for woody landscape plants. Hemlocks will be the primary collection target.

### **Assessment of Germplasm Needs**

The PEO is currently utilizing several methods to identify and tabulate NPGS germplasm needs. One method extracts the identified needs from the CGC Vulnerability Reports. This information is loaded into an Excel database. Precise germplasm needs from specific countries or regions with the reason(s) it is needed is most valuable to us. Also important is the relationship of the target species to the crop species.

A second method is to define germplasm needs for wild crop relatives based on the climatic and geophysical variation within geographic ranges of all species within a crop. Dr. Robert Webster joined the PEO as a Botanist in November, 1997 to support this project. Robert has established a procedure for the analysis and interpretation of a broad range of taxonomic, ecological and geographic information to define the germplasm needs within the native distribution of taxa. Specifically, he has incorporated global GIS data on vegetation ecoregions, elevation, soils, temperature, moisture, and climatic regimes as a means of defining and isolating ecogeographically significant zones. Data from this approach are recorded in a format for the production of germplasm reports, information retrieval, and data interrogation. Incorporated within the system is a mechanism for mapping the defined need and assessment of priority of the needs with an individual crop.

Initially, Robert will be working with the small grains. He has successfully applied this approach to a treatment of barley (*Hordeum*) and has made significant progress in a soon to be completed treatment of wheat (*Triticum* and close relatives).

### **Cooperative Programs**

Emergency Seed Collecting in Israel. Cooperator: Dr. Y. Anikster, Tel Aviv University. This is the second year of a two year project. Germplasm collected through this project is available to the NPGS sites. Plans are to expand the project to include Palestine and Jordan if appropriate funding can be obtained.

Development of the North American Plant Collections Consortium. Cooperator: American Association of Botanic Gardens and Arboreta. The consortium at this time includes 14 collections, 20 additional gardens have been solicited. The PEO has provided the Consortium with a list of 26 additional collections from 18 AABGA member institutions that would support the NPGS collections. With assistance from the DBMU, linkages have been established between GRIN and AABGA Institutions.

Regeneration of Economically Useful Plants of South India. Cooperator: Indian Agriculture Research Institute, New Delhi, India. PL 480.

In-vitro propagation/conservation of clonally propagated crops of south India. Cooperator: Indian Agriculture Research Institute, New Delhi, India. PL 480.

#### USDA/ARS Plant Explorations Undertaken in FY 1997

Plant Exploration	Country	Principal Contacts
<i>Leucaena pallida</i>	Mexico	J. Brewbaker, C. Beust
<i>Malus spp.</i>	People's Rep. of China	P. Forsline, H. Aldwinckle, L. Benson, L. Yunong
Grasses & legumes	People's Rep. of China	D. Johnson, T.A. Campbell, Y. Zhuomeng, A. Shazhou
<i>Solanum spp.</i>	Mexico	D. Spooner, H. Lazoya
<i>Echinacea spp.</i>	USA	K. McKeown, R. Bernatskey, M. Widrechner
<i>Gossypium spp.</i>	Australia	J.McD. Stewart, J. Wendel, C. Brubaker

#### USDA/ARS Plant Explorations/Exchanges Planned/Undertaken in FY 1998

Plant Exploration/ Exchange	Country	Principal Contacts
Forage and Turf Grasses	Falkland Islands	S. Wright, R. Reid
<i>Zea mays</i>	Paraguay	W. Salhuana, S. Paniagua, G. Altamirano
<i>Capsicum spp.</i>	Paraguay	K. Williams, D. Williams, F. Mereles, P.J. Caballero
Sour cherry, cherry, apple	Russia	P. Forsline, A. Iezzoni, R. Karle, M. Plekhanova
Grasses and legumes	Mongolia	D. Johnson, D. Sheehy, B. Minzhigdorj
<i>Solanum spp.</i>	Peru	D. Spooner, Z.Huaman
<i>Glycine max</i>	Vietnam	R. Nelson, T. Van Lai
<i>Vigna angularis</i>	People's Rep. Of China	T. Lumpkin, E. Yee
<i>Arachis hypogaea</i>	Guatemala	K. Williams, D. Williams, C. Azurdia

## **Germplasm Resources Information Network (GRIN) - Jimmie Mowder**

Continued maintenance of GRIN for the plant introduction stations and other NPGS management units keeping the production database operational on a 7 day per week and 24 hour per day schedule.

The graphical user interface for GRIN is under development with many technical issues being resolved on how to manipulate data with the new Oracle tools.

A new database server was installed permitting all databases to be installed on an isolated computer without competition from other functions and increases security for the databases. The time required to backup the database has been reduced to 1½ hours a week.

pcGRIN was totally rewritten over the past year in collaboration with IPGRI including a new user manual. A training session for the trainers was given to three people in April. A formal training session will be held in Cali, Colombia, this coming August for South American countries interested in using pcGRIN. African and Asian countries are also interested in using pcGRIN as management tools for their plant genebanks.

The DBMU are developing a database for beneficial insect data and will use experience from this effort in completing the development of the GRIN Windows access.

Continue to provide assistance to site personnel in the preparation and retrieval of data.

There is considerable interest by the animal germplasm community in getting their data into GRIN. They may provide additional funds to permit the DBMU to acquire one additional computer specialist and one animal geneticist.

We have increased the security for the NPGS computers and networks. Personnel constantly monitor the many computer advisory groups that alert users to vulnerabilities in operating systems and software packages.

The GRIN Web access displays a collection locality map. We have requested funds to upgrade the quality of the maps.

DBMU participated in a review of the FAO World Information Early Warning System (WIEWS) which is to provide a single point of contact for information on all plant genebanks of the world and summarize their holdings:

## 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 37 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 seventh biennial CGC Chairs meeting will be held in Ames, IA, July 22, 1998. In conjunction with the joint Regional Technical Advisory Committee meetings. This venue provides 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 management and use in general will be discussed. Some of these topics include: 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, 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 a summary of the GAO report regarding the status of the NPGS, revising 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. When possible, these reports will be made available over the Internet on the GRIN World Wide Web page (<http://www.ars-grin.gov>).



United States Department of Agriculture

Research, Education, and Economics  
Agricultural Research Service

July 8, 1998

SUBJECT: NSSL 1997 Progress Report

TO: Regional Technical Advisory  
Committees on Plant Germplasm

FROM: National Seed Storage Laboratory  
Steve A. Eberhart, Director  
Phillip C. Stanwood, Acting Research Leader  
Loren E. Wiesner, Research Leader

ADMINISTRATION

Steve A. Eberhart, Director

The additional increase in the operating budget of \$225,000 starting with FY 98 will provide funds for a plant physiology/molecular biologist in the Plant Germplasm Preservation Research Unit to conduct research in the area of long-term preservation of vegetatively propagated genetic resources.

Regeneration of endangered Latin American maize landrace accessions will continue in a cooperative project with 13 countries under a Specific Cooperative Agreement with CIMMYT. NSSL received 2,262 samples in 1997 bringing the total to 11,636 maize landrace accessions regenerated and stored under this project. CIMMYT staff have used a sequential clustering strategy for classifying gene bank accessions that utilizes both quantitative and qualitative traits with LAMP and CIMMYT data to develop a maize core subset for Latin American landrace accessions. The LAMP project has been completed and the Final LAMP Report was prepared and distributed.

SEED VIABILITY AND STORAGE RESEARCH UNIT

Loren E. Wiesner, Research Leader

In 1997, 25,596 samples were placed into storage: 4,612 into cryostorage and 20,984 into conventional storage. The 30,038 new samples that were received during 1997 included 426 from the Plant Variety Protection Office, 385 apple buds, 33 other clonal materials, 19 endangered species from Botanical Gardens, 52 for plant quarantine, 23,854 seed samples from Regional Plant Introduction Stations, 472 Crop Science registration, 2,262 Latin American Maize regeneration, 639 Desert Legume Program, 208 Charles Rick tomato collection, 1,373 maize genetic stocks, and the remaining 315 samples from other individuals and organizations.

A total of 2,752 accessions were distributed to 115 Scientists in 15 countries. Included in these distributions were 184 corn accessions which were washed, packaged, and sent to St. Croix for quarantine increase.

Education of the public concerning the mission of NSSL is an important responsibility. This year the laboratory staff conducted 75 individual tours for a total of 823 people. Through these tours, we are able to educate students, teachers, seedsmen, scientists, and the general public. This year we had international visitors from Japan, Brazil, China, Turkey, Russia, India, and Albania. However, 61% of our visitors are students, ranging from preschool to college age.

Northern Plains Area \* National Seed Storage Laboratory

1111 South Mason Street \* Ft. Collins, CO 80521-4500

Voice: 970 495-3200 - Fax: 970 221-1427

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Studies are being conducted to evaluate the long-term storage of *Rhizoctonia* in liquid nitrogen, the breaking of seed dormancy for field emergence, methods of germination and storage of strawberry seed, duplicate pea accessions, and to complete the quality evaluation of sugar beet seed. An extensive study to reduce seed dormancy in sunflower was conducted in the field and laboratory. Field applications of dormancy reducing chemicals were applied to developing sunflower seed. These chemicals did not prevent seed dormancy. Cold storage (-5 to -12°C of seed for approximately 20 days at a moisture content of 7-9% was the most effective method of breaking sunflower seed dormancy in large seed lots. Several duplicate pea accessions were evaluated using PCR methods and 20 different primers.

Germination tests were completed by the Colorado Seed Testing Laboratory under the terms of a Specific Cooperative Agreement on 2,697 samples. Tests were conducted on 40 different species; however, the majority of the tests were conducted on lettuce, carrots, and tomato samples. Our retest schedule is being 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. Of the species found not to store as well as other species, we have completed retests on onions, lettuce, carrots, peppers, rye, and tomatoes. A database has been setup to record when various species were retested.

In the cooperative maize regeneration project with CIMMYT, USDA-ARS, and USAID, NSSL has received 2,262 samples of Latin American Maize landrace accessions for storage in 1997. In 1997, NSSL checked in 7,731 maize accessions for CIMMYT and the Latin American countries involved in this project. To date we have stored 14,873 maize accessions increased by this project and a previous maize seed increase project. Work is continuing on the development of a Latin American maize core subset. In addition to the maize seed increase project, seed increases of potato in Mexico and onion in Poland and collection trips to the Ukraine and to the Altay mountains near the border of Mongolia have been funded.

PLANT GERMLASM PRESERVATION RESEARCH UNIT  
Phillip C. Stanwood, Acting Research Leader

The following report summarizes research activities by the scientists in the Plant Germplasm Preservation Research Unit (PGPRU) 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 1997.

**PERSONNEL:** Dr. Eric Roos has been serving as Acting Assistant Area Director since June 1997. Dr. Phil Stanwood has served as Acting RL of the PGPRU. Ms. Lisa Hill filled the vacant biological technician position in Dr. Walters' laboratory in October 1997. Dr. Darren Touchell, from Australia, joined the PGPRU in April 1998 as a postdoctoral research fellow.

**VISITING SCIENTISTS:** Dr. Ming Zhang, from China, moved to a new postdoctoral position at the University of Guelph in March 1998. Ms. Mirian Eira, EMBRAPA Brazil, has been at the NSSL since April 1997 conducting research on *Coffea* germplasm. Ms. Paula Power, South West Texas State University, will visit for two weeks in July 1998 to study embryogenesis and desiccation tolerance of *Zizania texana*. We received the usual large number of visitors from all over the world for periods of one to two days.

**GRADUATE STUDENTS:** Ms. Joyce Pennycooke from the Commonwealth of Dominica received her Master's Degree from CSU in August 1997 (under the guidance of Dr. Towill). Mr. Jian Fang, from the Peoples Republic of China passed the qualifying examination for his Ph.D. program (under the guidance of Dr. Roos). Ms. Kim Davidson continues research for her Ph.D. under the guidance of Dr. Walters. Ms. Terri Christensen is writing her thesis for a M.Sc. degree (with Dr. Roos). Mr. Robert Cooke is writing his thesis for a M. Sc. degree (with Dr. Walters).

**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 805214500. 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 hosted a symposium entitled "Seed Biology and Technology: Applications and Advances" on August 13-15, 1997. This symposium was sponsored by Regional Research Project W-168. The conference was attended by 250 delegates, many of whom represented seed companies.

FOREIGN TRAVEL: In Jan 1997, Dr. Walters was invited to participate in the 2<sup>nd</sup> International Workshop on Desiccation Tolerance in Franshoek, So. Africa and to continue collaborations with colleagues at the University of Cape Town and University of Natal. In May 1997, Drs. Roos, Walters, and Zhang and Mr. Fang visited Guangzhou, China, to attend the 2<sup>nd</sup> International Conference on Seed Technology and the IPGRI-SPONSORED Satellite Symposium on ultradry technology. In January 1998, Dr. Walters met with colleagues at the University of Reading and IPGRI in Reading, UK to develop recommendations for seed storage in gene banks in developing countries. Dr. Towill was invited to spend six weeks at the New Zealand Institute for Crop and Food Research, Christchurch, New Zealand in February/March 1998. Dr. Walters spent six weeks in France in May/June 1998: five weeks at the Université de Pierre et Marie Curie, Paris, France as an invited professor to collaborate on calorimetric properties of seeds and one week visiting the germplasm repositories in Montpellier, France.

#### INJURY MECHANISMS WHEN SEEDS ARE OVER DRIED

ERIC E. ROOS (Suprvy PI Physiol); Jian Fang (Graduate Student, China); Don Davidson (Biol Sci Tech); Frank D. Moore, III (Prof CO St Univ)

Problem: It is commonly accepted that drying seeds increases their longevity, but that there are limits to this beneficial effect. Detrimental effects of over drying seeds may result from membrane changes, reducing enzyme activity, and peroxidation reactions in very dry conditions. The purpose of this research is to investigate the nature of deteriorative reactions that occur at very low moisture content and the effect of those reactions on physiological and morphological indices of seed vigor.

Approach: In this study, soybean and lettuce seeds were adjusted to 1%, 5%, and 15% moisture content (dw) at 1%, 25%, and 75% RH and then seeds were stored at 35°C. Seed were removed from storage for measuring leachate conductivity to detect any membrane damage in seeds under extremely dry conditions during storage. Seeds were also tested for viability, root length, and dehydrogenase activity to test for deterioration during storage. To measure possible peroxidation reactions in the dry state, seeds were stored in reaction flasks (with or without N<sub>2</sub>) at 35°C, and volatile production was measured using GC each month.

Results: Increases in seed leachate conductivity and decreases in dehydrogenase activity were found in 1 % and 15% moisture content seeds compared with 5% moisture seeds after seven months in storage. At this time, hexanal production was found in lettuce seeds, but not in soybean seeds. N<sub>2</sub> inhibited hexanal production during storage. These results indicated that deterioration in soybean and lettuce seeds was more rapid at the extreme seed moistures. The results of the completed experiments will provide a better understanding of some of the mechanisms of damage when seeds are over dried.

#### SEED LONGEVITY STUDIES

ERIC E. ROOS (Suprvy Plant Physiol); Teri 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 M.Sc. thesis for Ms. Teri 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 was to determine if the vacuum seal was still intact. Data taken included moisture content, germination, and head-space gases, if possible.

After further tests, it was determined that the range in moisture content for these seeds was between 0.2 and 9.5%. It was also determined that any tube not containing a vacuum showed zero percent germination and viability. Out of a total of 95 tubes, six did not have a vacuum in part due to small cracks observed and possibly microholes in the tubes. Out of the rest of the tubes, 55% of the selected California species still showed a minimum germination percent of 20% or higher after 50 years of vacuum storage at room temperature. Some species even had as high as 100% germination after 50 years in storage. On average, seeds from the families Onagraceae, Lamiaceae, and Polemoniaceae retained high germination rates. Seeds from the Asteraceae, Papaveraceae, and Chenopodiaceae had relatively lower rates. Generally, fewer seeds of most species germinated in 1997 compared to the last sample tested. However, some species had higher rates in 1997. This is most likely best explained by improved germination tests. Continuation of this study will help determine cost-effective, long-term storage practices.

#### 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 (-196°C), 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 cryo preservation 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 100 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 microenvironment 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, desert legumes, 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 USDAARS, Germplasm Resources Information Network (GRIN) in Beltsville, MD, which is available through the INTERNET and World Wide Web ([www.ars-grin.gov](http://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 1997: Most of the 1997 effort was directed at completing image databases and image analysis of seed materials. The Cicer image database work was completed and the images were linked to GRIN. These with seed images, field grown plant images, literature citations, and a cooperater 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 CID 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 (worldwide 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 an image database was 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 4,200. Digital images of 724 accessions of desert legume seed were imaged, characterized, and placed into an image database. These images have been added to the GRIN database. The images are available on CD-ROM. The project was in cooperation with the SVSRU (Cheryl Johnson) at the NSSL and Matthew Johnson, Boyce Thompson Southwestern Arboretum and the University of Arizona, Tucson. 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 (SlantGrowth Robotics, 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 1997, 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 continued 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 -196°C (liquid nitrogen), -18°C, and 5°C. There are approximately 200 samples, representing 45 species, that will be tested in this study.

## CRYOPRESERVATION OF WOODY, VEGETATIVELY PROPAGATED GENETIC RESOURCES

LEIGH E. TOWILL (PI Physiol), John W. Waddell (Biol. Sci. Techn), Tammi Cooper (Biol. Sci. Techn), L.J. Grauke (National Clonal Germplasm Repository, Brownwood, TX) Philip 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  $-30^{\circ}\text{C}$  to  $-160^{\circ}\text{C}$  and warming rates from  $-160^{\circ}\text{C}$  to  $22^{\circ}\text{C}$  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:** We examined the dormant vegetative bud cryopreservation method with pecan. 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. Cooling rates from  $-30^{\circ}\text{C}$  to  $-160^{\circ}\text{C}$  or liquid nitrogen were examined with non-desiccated and desiccated sections from several lines. Viability of desiccated and non-desiccated materials were examined during storage at  $-5^{\circ}\text{C}$ .

**Results:** Cooling rates between  $-30^{\circ}\text{C}$  and  $-196^{\circ}\text{C}$  did not greatly influence survival (oxidative browning viability test); again some lines showed some benefit from faster rates. Warming rates also did not have a dramatic effect. There also was some suggestion that an initial rapid rate may be beneficial if the subsequent rate (say from  $-15$  to  $0^{\circ}\text{C}$ ) is slower. In contrast to several other species where the cambial region is usually equal to or more cold hardy than the bud, the cambial region of pecan was injured at a warmer temperature than the vegetative bud. The dormant vegetative bud method used did not give complete survival with materials subsequently cooled to liquid nitrogen and preliminary attempts at varying moisture content, second stage cooling rate and time of holding at  $-30^{\circ}\text{C}$  prior to transfer to LN did not improve viability. Tests of the encapsulation/sucrose exposure/dehydration method were performed on apple and pecan. In observations with the oxidative browning test, levels of survival were about comparable to those found with the routine test. Sucrose exposure and desiccation alone (without encapsulation) 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). Again, gas chromatography of the vapors from some encapsulated twigs held in plastic bags showed acetaldehyde and ethanol -- suggesting that fermentative pathways were occurring, probably during the encapsulation/soaking period (ca  $+1^{\circ}\text{C}$ ). This may account for the erratic and often low viabilities observed. It is very difficult to control the moisture content of the twigs with the alginate encapsulation procedure-considerable variation occurred. Our results using this method have been disappointing and several factors may be responsible for survival variations, including variable moisture content and sucrose permeation/ metabolism.

In tests with 2 apple lines, desiccated twigs were still able to be cryopreserved 2-3 months after desiccation, but survival decreased thereafter. Non-dried apple twigs with holding of 2-4 months at  $-5^{\circ}\text{C}$  also gave similar levels of survival after cryopreservation. Although not tested directly, it is supposed that holding time at  $-5^{\circ}\text{C}$  increases the cold acclimation of woody species, and as such may be beneficial to the cryopreservation protocol. A rapid assessment of the extent of cold hardiness still is needed but it is not feasible to do the many destructive tests each year to ascertain this information (materials are limiting). We initiated tests to measure sugar type and concentrations and have devised an extraction and separation method for the Dionex system. This information may be useful to assess the hardiness condition. Sugar analyses also may be useful in determining changes in quality of materials held at  $-5^{\circ}\text{C}$ , in either the desiccated or usual moisture condition.

## CRYOPRESERVATION OF SHOOT TIPS USING VITRIFICATION METHODS

LEIGH E. TOWILL (PI Physiol), Tammi Cooper (Biol. Sci. Techn), Brennick Langston 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 still 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. Most studies have been with sweet potato, given our previous work and observations of considerable variation in survival after cryopreservation.

**Results:** Experiments 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 cryoampoules, and by immersion into a cooled LN solution (about.  $-208^{\circ}\text{C}$ )] to prevent a vapor effect; this also gave more consistent survival amongst experiments. Harvesting of the shoot tips immediately after a dark treatment resulted in higher survivals and suggested that endogenous sugar levels are important. Addition of ABA prior to cryopreservation did not influence survival. A critical factor for improved viability was the omission of ammonium within the recovery medium for a few days after cryopreservation. Other modifications of the recovery medium, such as reduced iron content and application of Pluronic F68, a surfactant, (both in the presence of ammonium) enhanced viability. These observations strongly suggest that the cryogenic treatment used is not invariably lethal, but does create a damaged condition from which recovery is influenced by post-treatments. The combination of observations made with a single line of sweet potato also seemed applicable to 4 other in vitro lines. The method also gave good survival using shoot tips from growth chamber grown plants. Thus, the findings have given a more consistent method for cryopreservation of sweet potato and are worthy of examination across a greater extent of diversity.

'Encapsulation and dehydration' methods for cryopreservation also are also a form of the vitrification procedure. We determined kinetics of drying and survival for sweet potato shoot tips within alginate capsules. Shoot tips survived desiccation to about 16-20%, but did not routinely survive subsequent exposure to  $-196^{\circ}\text{C}$ . In later experiments, the use of the no-ammonium recovery medium gave survival after LN exposure, especially between the bead moisture contents of ca. 14-20%

Another problem encountered previously with sweet potato was a more extensive callus formation from the shoot tips after treatments. In limited studies we identified a growth regulator combination that reduced callus formation and promoted shoot growth. Reduced callus formation and enhanced shoot growth also occurred with the other 4 lines tested, but the extent of reduction varied. The medium, however, does seem sufficient to be used with a greater range of diversity in a pilot project.

Because of a thrip infestation in our mint collection, we had to reinitiate our stocks for examination of routine cryopreservation. Stocks are now clean and in 1997 we reexamined our cryo preservation methods to determine what is a practicable and useful procedure. Studies are continuing, but modifications of vitrification procedures using very rapid cooling seem advantageous.

## IN VITRO BACKUP OF CLONAL GERMLASM 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 and early 1997. 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 +14°C (preliminary tests). We selected +18°C since cultures grew slower, but did not develop some injury-like symptoms as observed at +14°C. Survival ranged from about nine months to two years. 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. The remaining viable cultures were returned to Griffin for subculturing and observations on growth characteristics. In conjunction with the cryogenic phase of the project, selected lines are being sent to NSSL from GA to initiate cryopreservation and in vitro cultures are being established at +18°C as a backup.

## THE OPTIMUM WATER CONTENT FOR SURVIVAL OF DRIED GERMLASM

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

**Problem:** Germplasm must be stored at precise water contents to maximize longevity. These water contents vary among species and with storage temperature. Obviously there are insufficient resources to determine the optimum water content for all species represented in the NPGS. We are using thermodynamic principles as a tool to predict optimum water contents. This research is funded in part by the International Plant Genetic Resources Institute, Rome.

**Approach:** We have studied phylogenetically diverse organisms, pollen, and more than 30 species of seeds to determine the interaction of water content, temperature, relative humidity, and deterioration rates. Experiments are long-term, some with more than eight years of storage data.

**Results:** Regardless of the temperature, species, or tissue, optimum water contents for storage of desiccation tolerant organisms correspond to about 18-22% RH. Above and below this humidity, organisms deteriorated faster. This means that drying protocols can be easily established to obtain optimum storage conditions for any storage temperature used. Dr. Walters is guest-editor of a special issue of SEED SCIENCE RESEARCH to be published in September 1998 which summarizes the current knowledge of seed storage practices.

## THE KINETICS AND MECHANISM OF DETERIORATION IN DRIED ORGANISMS

CHRISTINA WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), M. Zhang (postdoctoral research associate, China), J. Buitink (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:** Initial studies document how water activity affects the nature and kinetics of deterioration reactions. Once this is understood, we can impose storage temperature as a variable and determine if deterioration follows Arrhenius, Avrami, or WLF kinetics. This allows us to develop predictive models of deterioration rates under numerous storage conditions. The next step is to determine how seed quality factors affect various coefficients and then to find the underlying chemical or physical properties of seeds that give rise to the quality factors.

**Results:** We have developed phase diagrams for seeds based on calorimetric measurements of water. We have established that the optimum moisture content for seed storage corresponds to a specific change in water properties. At water contents below the optimum, lipid peroxidation reactions dominate and the kinetics of these are presently being described. At water contents above the optimum, reactions involved in glycolysis and unregulated respiration. The effect of temperature on these reactions can be described by predominantly 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.

## CONSERVATION STRATEGIES FOR SEEDS FROM ENDANGERED FLORA FROM USA

CHRISTINA WALTERS (PI Physiol), DARREN TOUCHELL (post doc), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), K. Wood (NTBG, Hawaii), P. Power (U SWT), M. Antolin (CSU)

**Problem:** Numerous plant species 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 and plant parts allows us to develop preservation protocols and information on the genetics of wild populations which allow us to target populations to conserve. This project is partially funded by the US Fish and Wildlife, Pacific Islands Division and ARS Postdoctoral Research Fellowship Program.

**Approach:** The study is conducted in two parts. First we evaluate the feasibility of storing various plant parts and estimate the % survival with time of storage. Some seeds have recalcitrant characteristics and require more fine-tuning of preservation protocols. Once preservation protocols are established, strategies that conserve a representation of the genetic diversity of wild populations are developed. This requires baseline data of the genetic heterogeneity and then an evaluation of the genetic heterogeneity of cryopreserved samples.

**Results:** Of the 200 species examined, most produce orthodox seeds, and plans are underway to get these species in storage immediately. Our test study of genetic representation of wild and cryopreserved samples uses *Zizania texana*. This federally-listed endangered species has agronomic importance (relative of cultivated wild rice). We expect the baseline data for evaluating the genetics of wild populations to be completed in the fall of 1998.

## THE ACQUISITION AND LOSS OF DESICCATION TOLERANCE AND STORAGE LONGEVITY DURING SEED DEVELOPMENT AND GERMINATION

CHRISTINA WALTERS (PI Physiol), J. Crane (Biol Sci Tech), L. Hill (Biol Sci Tech), K. 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), F. Corbineau (U P.M. Curie, Paris), D. Come (U P.M. Curie, Paris), C. Bailley (U P.M. Curie, Paris)

**Problem:** Embryos acquire, to varying degrees, the ability to survive the immediate (desiccation damage) and long-term (aging damage) effects of desiccation during development. They lose this ability when they germinate. Many economically important species from tropical areas produce seeds that have limited abilities to survive drying and storage. Our task is to understand the basis of the limitations, first at a physiological level and then at a genetic level. We believe that this information will allow us to successfully cryopreserve all embryos -- somatic or zygotic-- and perhaps to artificially enhance the desiccation tolerance of other plant propagules that we wish to cryopreserve.

**Approach:** We have started to "map" out the developmental changes in seeds of diverse phylogenetic backgrounds on a biophysical, chemical, and ultrastructural basis and to determine which changes lead to greater tolerance of desiccation.

**Results:** We have characterized critical water contents at which damage occurs to seeds during development and germination and have hypothesized the underlying basis of the damage. Using principles of limiting factors, we are testing the effectiveness of various putative protectants. One extremely interesting result is the rapidity with which orthodox seeds acquire desiccation tolerance (three days after harvest, regardless of maturity status).

## PROPERTIES OF LEA-LIKE PROTEINS

CHRISTINA 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. We are also pursuing the rheological properties of these proteins in aqueous solutions.

**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.

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

### STATUS OF NPGS CROPS, July 1998

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64% %	Seed no. > 549	Core %
<i>Aegilops</i>	Aberdeen		2098	1824	87	97	1			
<i>Avena</i> / oat	Aberdeen	627	21543	20604	96	94	58	98	22	
<i>Hordeum</i> / barley	Aberdeen	731	28025	27270	97	97	51	99	82	8
<i>Oryza</i> / rice	Aberdeen	867	18146	16655	92	88	81	90	98	
<i>Secale</i> / rye	Aberdeen	5	1910	1881	98	98	86	99	100	
<i>Triticum</i> / wheat	Aberdeen	1030	47033	46741	99	99	83	98	24	10
<b>Aberdeen backup</b>			<b>118735</b>	<b>114975</b>	<b>97</b>	<b>96</b>				
<i>Amaranthus</i>	Ames		3361	2503	74	71	84	98	100	
<i>Brassica</i> / oilseed, vegetable	Ames	142	3305	3163	96	97	44	96	100	
<i>Cucumis</i> / cucumber, cantaloupe	Ames	463	5227	2972	57	48	75	94	99	
<i>Cucurbita</i> /pumpkin, squash	Ames	102	1105	702	64	52	86	97	91	
<i>Daucus</i> / carrot **	Ames	169	890	763	86	87	59	73	100	
<i>Helianthus</i> / sunflower	Ames	182	3898	2580	66	59	63	90	89	
<i>Linum</i> / flax	Ames	2	2956	2810	95	95	99	98	100	
<i>Melilotus</i> / sweetclover	Ames		919	724	79	78	75	89	100	9
<i>Zea</i> / corn	Ames	1647	16672	11861	71	74	76	90	87	
<b>Ames backup</b>			<b>38333</b>	<b>28078</b>	<b>73</b>	<b>70</b>				
<i>Gossypium</i> / cotton	College Station	882	7068	3962	56	56	98	92	90	
<i>Allium</i> / onion **	Geneva	27	1315	620	47	28	55	53	97	
<i>Brassica</i> / oilseed, vegetable	Geneva	334	2572	1084	42	29	100	88	100	
<i>Cucurbita</i> /pumpkin, squash	Geneva	105	947	543	57	57	96	96	93	
<i>Lycopersicon</i> /tomato	Geneva	1558	7349	6633	90	70	74	86	99	
<b>Geneva backup</b>			<b>12183</b>	<b>8880</b>	<b>73</b>	<b>56</b>				
<i>Abelmoschus</i> /okra	Griffin	34	3081	1940	63	57	23	85	100	5
<i>Andropogon</i> **	Griffin	1063	1099	1074	98	97	99	32	88	
<i>Arachis</i> /peanut	Griffin	66	9619	6416	61	67	84	82		8

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64% %	Seed no. > 549	Core %
<i>Capsicum</i> / pepper	Griffin	175	4050	2238	55	45	55	85	99	
<i>Citrullus</i>	Griffin	315	1923	1766	92	87	67	89	71	
<i>Cucurbita</i> /pumpkin, squash	Griffin	46	1286	783	61	19	58	90	50	
<i>Cyamopsis</i> / guar	Griffin	888	1301	1294	99	99	93	90	91	
<i>Eleusine</i> /finger millet	Griffin	701	1474	1006	68	57	96	96	100	
<i>Ipomoea</i> / sweet potato seed	Griffin	35	455	201	44	11				
<i>Pennisetum</i> / pearl millet	Griffin	106	674	523	78	76	76	78	52	
<i>Sesamum</i> / sesame	Griffin	27	1229	1229	100	100	100	92	100	
<i>Solanum</i> / eggplant	Griffin	38	967	905	94	96	58	43	62	12
<i>Sorghum</i> / sorghum	Griffin	10823	40934	32404	79	65	99	79	97	
<i>Vigna</i> / cowpea, mungbean	Griffin	278	12802	9017	70	46	59	95	92	9/11
<i>Trifolium</i> / clover	Griffin	34	1979	1445	73	24	96	93	95	
<b>Griffin backup</b>			<b>82873</b>	<b>62241</b>	<b>75</b>	<b>61</b>				
<i>Trifolium</i> / clover	Lexington		<b>253</b>	<b>175</b>	<b>69</b>	<b>70</b>	16	9	10	
<i>Nicotiana</i> / tobacco	Oxford	72	<b>2153</b>	<b>1411</b>	<b>66</b>	<b>65</b>	100	65	99	
<i>Allium</i> / onion	Pullman	9	829	168	20	21	99	84	100	
<i>Beta</i> / beet	Pullman	175	2320	1793	77	77	97	70	98	
<i>Bromus</i>	Pullman	4	1095	979	89	86	96	82	100	
<i>Carthamus</i> / safflower	Pullman	27	2451	1967	80	80	90	82	100	9
<i>Cicer</i> / Chickpea	Pullman	8	4612	4021	87	85	98	100	99	11
<i>Cucurbita</i> /pumpkin, squash	Pullman	3	30	13	43	43	90	10	90	
<i>Dactylis</i> / orchardgrass	Pullman	10	1532	1331	87	90	90	91	100	
<i>Elymus</i> / wildrye, wheatgrass	Pullman	1	1727	1146	66	65	62	90	100	
<i>Eragrostis</i>	Pullman	1	1297	1273	98	98	99	90	100	
<i>Festuca</i> / fescue	Pullman	164	2241	1922	86	81	89	86	100	
<i>Lactuca</i> / lettuce	Pullman	231	1548	1232	80	80	93	66	100	
<i>Lens</i> / lentil	Pullman			2437	84	84	93	100	100	10
<i>Lolium</i> / ryegrass	Pullman	146	1408	1089	77	79	91	92	100	5

Species/Crop	Active site location	NSSL only no.	NPGS no.	NSSL no.	Backup at NSSL % 1998	Backup at NSSL % 1997	Tested for germ %	Germ >64% %	Seed no. > 549	Core %
<i>Lotus</i> / trefoil	Pullman	26	940	471	50	48	100	92	100	8
<i>Medicago</i> / alfalfa	Pullman	441	4044	3397	84	67	86	97	100	5
<i>Medicago</i> / except alfalfa	Pullman	7	3594	2987	83	87	78	95	100	5
<i>Phaseolus</i> / bean	Pullman	267	14318	8655	60	63	92	96	72	11/7
<i>Pisum</i> /pea	Pullman	520	4008	2963	74	74	92	99	97	14
<i>Poa</i> / bluegrass	Pullman	77	901	808	90	89	96	91	100	
<i>Trifolium</i> / clover	Pullman	41	3912	1949	50	52	58	50	58	6/11
<b>Pullman backup</b>			<b>55692</b>	<b>40601</b>	<b>73</b>	<b>72</b>				
<i>Glycine</i> / soybean	Urbana	1028	<b>20133</b>	<b>16230</b>	<b>81</b>	<b>81</b>	85	92	73	
<b>Over all seed backup</b>			<b>337423</b>	<b>276553</b>	<b>82</b>	<b>77</b>				
<b>GENETIC STOCKS</b>										
<i>Hordeum</i> genetic stocks	Aberdeen GSHO		2531	1139	45	45				
<i>Lycopersicon</i> genetic stocks	Davis GSLY		2985	2985	100	100	39	38	42	
<i>Pisum</i> genetic stocks	Pullman GSPI		485		0					
<i>Zea</i> genetic stocks	Urbana GSZE		2503	2125	85	56	4	3	3	
<b>SEEDS OF VEGETATIVE</b>										
<i>Fragaria</i> / strawberry seed	Corvallis	5	5	5	100	100				
<i>Malus</i> / apple seeds	Geneva		841		0					
<i>Solanum</i> / potato seed	Sturgeon Bay	7	4451	3900	88	90	79	92	93	52
<b>VEGETATIVE</b>										
<i>Corylus</i> /filbert, hazelnut	Corvallis		574	3	1					27
<i>Cydonia</i> / quince	Corvallis		90	1	1					58
<i>Fragaria</i> / strawberry	Corvallis		1518	74	5					34
<i>Humulus</i> / hop	Corvallis		979		0					8
<i>Juglans</i> /walnut	Corvallis		14		0					
<i>Mentha</i> /mint	Corvallis		507	12	2					10
<i>Pycnanthemum</i> / mountain mint	Corvallis		109		0					28
<i>Pyrus</i> / pear	Corvallis		1851	64	3					13

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<i>Ribes</i> / currant, gooseberry.	Corvallis		1034	3	0					31
<i>Rubus</i> / raspberry, blackberry	Corvallis	1	1724	3	0					31
<i>Vaccinium</i> / blueberry, cranberry	Corvallis		1205		0					31
<i>Juglans</i> / walnut	Davis		444		0					
<i>Prunus</i>	Davis	8	1869	8	0					
<i>Vitis</i> / grape	Davis		2537		0					
<i>Malus</i> / apple	Geneva		2,989	1508	50	49				4
<i>Prunus</i>	Geneva		72	19	0					
<i>Vitis</i> / grape	Geneva		1504		0					8
<i>Ipomoea</i> / sweet potato clonal	Griffin		623	38	6					
<i>Prunus</i>	National Arboretum		254		0					
	Arboretum									
<i>Citrus</i>	Riverside		879		0					
<i>Solanum</i> /potato clonal	Sturgeon Bay		978		0					8
<b>Vegetative Backup</b>			<b>23865</b>	<b>1836</b>	<b>8</b>	<b>6</b>				

\*\*Species with limited longevity at 5°C

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