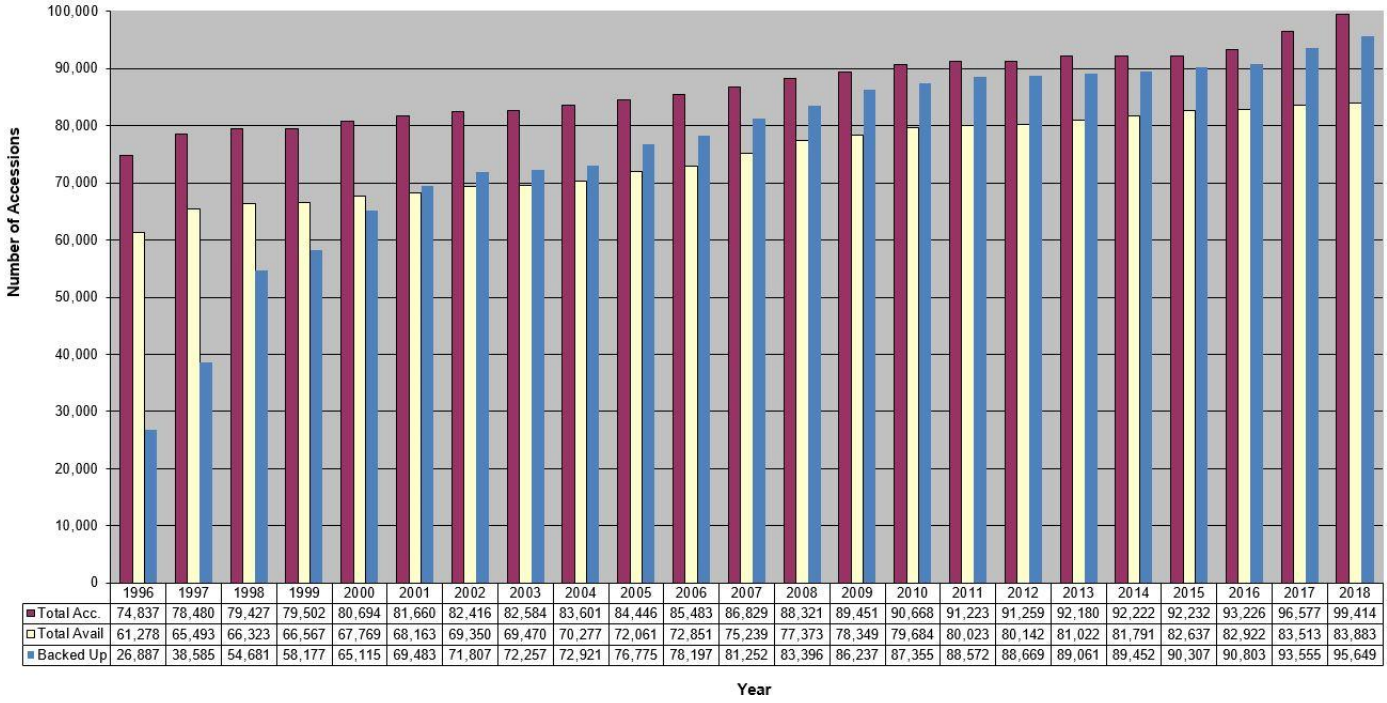
Appendix 1.

Table 1. Total number of accessions, number of available accessions, and number of accessions backed up at Ft. Collins, CO.

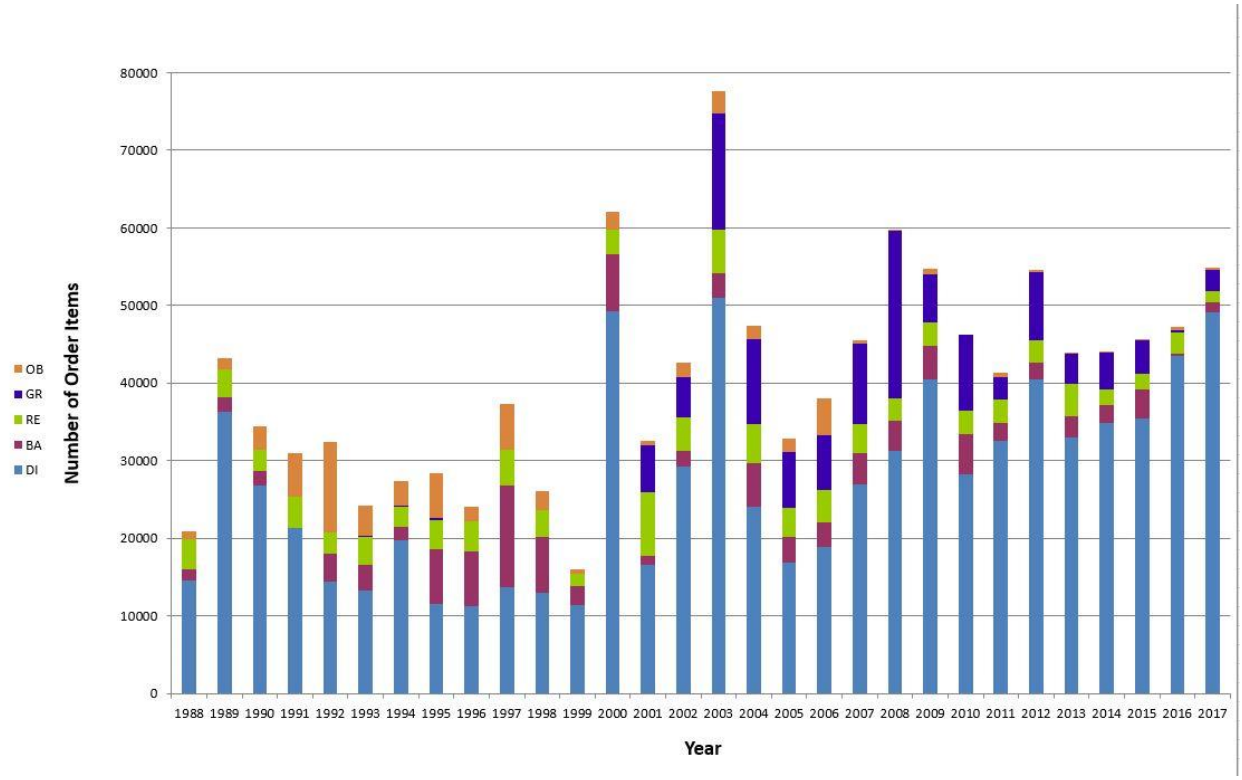


	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Total Acc.	74,837	78,480	79,427	79,502	80,694	81,660	82,416	82,584	83,601	84,446	85,483	86,829	88,321	89,451	90,668	91,223	91,259	92,180	92,222	92,232	93,226	96,577	99,414
Total Avail	61,278	65,493	66,323	66,567	67,769	68,163	69,350	69,470	70,277	72,061	72,851	75,239	77,373	78,349	79,684	80,023	80,142	81,022	81,791	82,637	82,922	83,513	83,883
Backed Up	26,887	38,585	54,681	58,177	65,115	69,483	71,807	72,257	72,921	76,775	78,197	81,252	83,396	86,237	87,355	88,572	88,669	89,061	89,452	90,307	90,803	93,555	95,649

Table 2. Number of accessions maintained in -18ºC freezer storage and number of accessions tested for germination, 2009-2018

Year	No. of accessions in -18 C	Percentage of seeded accessions in -18 C	No. of accessions tested for germination	Percentage of seeded accessions tested
2009	60,269	67.4	54,054	60.4
2010	62,524	70.1	60,207	67.5
2011	66,995	74.8	69,556	77.7
2012	67,241	74.8	73,274	81.5
2013	67,567	74.6	73,886	81.6
2014	72,478	79.8	79,4396	87.4
2015	73,212	80.6	81,778	90.0
2016	76,989	84.7	83,964	92.4
2017	79,511	85.1	84,945	90.9
2018	83,234	84.9	86,103	87.8

Table 3. Number of samples distributed to scientists and educators in S-009 region, all other U.S. states, and foreign countries and U.S. territories, 1988-2018. OB=Observation, GR=Germination, RE=Regeneration, BA=Back Up, and DI=Distribution.



Appendix 2. Additional methods for Objectives 1, 2, and 4.

Objective 1. Methods continued:

Seed viability testing is a priority at the Griffin location. Efforts will continue to conduct standard germination tests on the entire range of crop and CWR accessions in the germplasm collection with emphasis on testing new material and retesting select inventories at ten year intervals. Germination testing will follow standard procedures developed by the Association of Official Seed Analysts (AOSA, 2015) for major crop species and/or the Handbook of Seed Technology for Genebanks Volumes I, II. When standardized germination protocols do not exist for a particular species, the crop curator, cooperators at NLGRP, and curators or crop experts for other minor crops and CWR's will be consulted for alternative germination techniques. Germination testing priority will be 1) recently regenerated seeds (1,200 accessions per year); 2) recently acquired crop accessions; 3) CWR accessions with no previous germination data; and 4) additional inventories with no previous germination data. Germination testing will be conducted on a sample of accessions with a range of viability noted from previous tests. Germination retesting intervals of accessions will be adjusted depending on the species, known seed longevity of the species, and results of sample tests. The germination tests (Ellis et al., 1985) provide seed viability information to curators for establishing regeneration priorities. Germination tests will be conducted using 100 seeds for accessions with adequate seed numbers and follow a sliding scale decreasing to 10 seeds on accessions with minimal seed numbers. The germination technician

will receive an electronic file based on a GRIN-Global order from the seed storage manager. The PGRCU Digital Germination Card program, which uses bar codes to reduce errors, will be used to record all test results, make calculations, and prepare the data to drag-and-drop into GRIN-Global Curator Tool Viability Dataviews.

Accessions are evaluated for trueness to type at various stages in the conservation and maintenance process. Regenerated seeds are compared visually to original samples during seed processing. Seed mixtures, incorrect taxonomic identifications, or other problems are noted during seed cleaning, germination testing, characterizations, and regenerations. Recipients of germplasm may report that a particular accession is incorrectly identified during their research. Accessions with trueness to type problems are identified during these processes and referred to the crop curator for growouts or other evaluations to determine the correct taxon or designation for the sample. Curators will consult with outside taxonomic or crop experts as needed.

Sweetpotato, perennial wild peanuts which produce few seeds, bamboo, Chinese water chestnuts, and several warm-season grass accessions are maintained as clones instead of seeds. Many of these clones are reproductively sterile and produce few seeds in the southeastern U.S. Researchers prefer conservation of these accessions as clones to maintain the unique genetic characteristics of each individual accession. Clonal accessions of 446 warm-season grass, 144 wild peanut, and six water chestnut accessions are currently maintained as live plants in the greenhouse or field. Two pots of each warm-season grass are maintained in the greenhouse and 2-3 plants of wild peanut accessions in the *Erectoides* section will be grown in pots for maintenance in the greenhouse. Clonal bamboo accessions are maintained in separate 5 x 6 m plots at the USDA, ARS, Byron, GA, location. Below ground concrete barriers and 10 m grass spacing between each plot are used to reduce contamination. Chinese water chestnut accessions are maintained in the greenhouse as live plants growing in a re-circulating hydroponic system (Morris, 2016).

The sweetpotato clonal collection consists of over 750 accessions maintained in tissue culture. Sweetpotato accessions will be maintained *in vitro* on semi-solid Murashige and Skoog (MS) media in test tubes on a 10 hr photoperiod at ~20^{o}C. *In vitro* accessions will be examined for bacterial and fungal contamination at one month intervals, and recultured every 6 to 12 months to ensure viability and availability. New sweetpotato clones introduced into the U.S., at the request of the curator or by individual sweetpotato researchers, are processed through Plant Protection and Quarantine (PPQ). New pathogen-tested materials will be added to the *in vitro* collection when received from PPQ. Eight cultures of each accession will be maintained in Griffin, and two to five cultures sent for backup to NLGRP, Ft. Collins, CO.

Objective 2 Methods continued:
Oil and protein content measurements will be performed on a Mini-spec mq-one NMR analyzer (Bruker Optics Inc., Houston, TX, USA) and Rapid N Exceed [nitrogen/protein] analyzer (RNEA) (Elementar, New Jersey, USA), respectively. Seed oil content will be measured using an NMR consisting of a 50-ml glass tube filled with 10-20 g of seeds. Crude protein content measurements will follow the procedure described by Wang et al. (2016). Five medium-size peanut seeds will be crushed in a small plastic bag with a hammer. After crushing, a small amount of crushed seed powder (~200 mg) will be wrapped in tin foil (50 x 50 mm). The wrapped seed powder will be transferred into a small column and pressed into a small pellet (10

mm diameter x 5 mm high). Sample pellets will be combusted on RNEA at ~950ºC in a CO_2 injection, resulting in a mixture of oxidized products and ash. Water, SO_2, and HX will be removed by various absorbers while nitrogen oxides will be reduced to molecular nitrogen and detected by a thermal conductivity detector (TCD) (Winkler, 2008). Total nitrogen content will be expressed as a percentage of the sample mass, and a protein factor of 5.46 will be used to estimate crude protein content in peanut seeds (Jones, 1931). Protein content measured from other species may require a different protein factor.

Fatty acid and amino acid composition measurements will be performed on an Agilent 7890A gas chromatograph (GC) and Agilent 1100 series high performance liquid chromatographer (HPLC), respectively (Wang et al., 2011; Wang et al., 2017). Fatty acid methyl esters (FAMEs) will be prepared from seeds by alkaline transmethylation (Liu, 1994). Seeds will be ground to a fine powder by various means. Oil from a small amount (50-75 mg) of meal will be extracted in 5 ml of heptane and converted to FAMEs with 500 µl of 0.5 N sodium methoxide in methanol. Water will be added to separate the organic layer containing fatty acids from the meal. A portion of this layer will be transferred to a vial for injection to GC with a flame ionization detector (FID). Peak separation will be performed on a DB-23 capillary column (15 m x 0.25 mm i.d. with a 0.25 µm film). A fatty acid methyl ester (FAME) standard mix RM-3 (Sigma) will be used to establish peak retention time. Fatty acid composition will be determined by identifying and calculating relative peak areas. For amino acid analysis, the samples will be prepared by two different methods (acid hydrolysis and base hydrolysis). Protein prepared by acid hydrolysis yields 17 measurable amino acids, while three amino acids (tryptophan, cysteine, and methionine) are destroyed. Protein prepared by base hydrolysis yields one amino acid (tryptophan) for analysis. Approximately 120 mg of plant meal will be measured into a 5-ml reaction vial, and 4 ml of 6N HCl will be added to each vial plus 4 µl of phenol (0.1% final concentration) for acid hydrolysis. Nitrogen will be blown over the top, and vials immediately sealed with a teflon-lined cap. Vials will be incubated in a 110ºC heating block for 20 hours. Samples will then be cooled, and a 1 ml aliquot will be neutralized and diluted to 25 ml with 4.2N NaOH and water, respectively. Dilutes will be filtered, and an aliquot derivatized using AccQ-Fluor™ reagent (Waters Corp., Milford, MA). Amino acids will be separated and quantified using HPLC with a 1260 fluorescent detector (Wang et al., 2017). A standard mix containing 100 pmol/µl each of 15 amino acids will be diluted accordingly and derivatized to generate a 9-point calibration curve from 1.25 to 30 pmol/µl. Amino acid totals will be converted to mg/g by multiplying the molecular mass of each and the dilution factor, then dividing by the sample mass: mg/g = (molar concentration x molecular mass x dilution factor)/ sample mass. All samples will be prepared and analyzed twice.

Jute physical-mechanical properties including bulk density, bast to core fiber ratio for different stem diameters, moisture sorption, fiber morphology and aspect ratio will be evaluated using the active standard test method for tensile strength and Young's modulus of fibers (ASTM C1557-14, 2014) and the fiber analysis of paper and paperboard (TAPPI T401 OM-15) standards. Mineral element (P, K, Ca, Mg, Fe, Mn, Zn, B, Al, Na, S, and Cu) concentrations will be determined using recommended digestion procedures (Perkin-Elmer, 1994). Samples will be heated at 70ºC in an oven for 24 hours and placed in desiccators. Three replicates of 1 g tissue from each sample will be weighed in porcelain crucibles and incinerated at 500ºC for 4 hours and cooled overnight. The incinerated samples will be digested with 20 ml of 33% HCl until 10 ml of solution remains in the crucible. After digestion, each sample will be filtered through

Whatman filter paper (No. 541) into a 100-ml volumetric flask using hot distilled water. After cooling, the sample will be adjusted to 100 ml by adding water and then employed for nutrient analysis using ICP-OES. The mission signal of samples will be obtained by developing calibration curves. Standard reference material (SRM 1547, National Institute of Standard and Technology, Gaithersburg, MD, USA) with certified concentrations of elements indicated a recovery within the range of certified values. The results for replicate samples will be averaged, and standard deviations for each element will be calculated.

Flavonoids (quercetin, kaempferol, myricetin, genistein, cyanidin, delphinidin) and resveratrol content will be quantified on an Agilent HPLC (Wang and Pittman, 2008). Peanut flavor-related compounds will be evaluated on GC/MS (Wang, 2016). Approximately 0.5 g of seeds/accession will be ground into a fine and uniform powder using a mixer mill for flavonoid analysis. The seed powder will be mixed with 80% methanol and 1.2 M hydrochloric acid followed by incubation at 80ºC for two hours in a water bath. The sample will then be centrifuged and the supernatant removed and filtered before injection into the HPLC. About 8 g of air-dried seeds will be ground to a powder by various means for resveratrol analysis. Ground seed tissues (3 g) will be transferred into 50 Falcon tubes and homogenized with 9 ml of 80% ethanol and centrifuged. Two milliliters of supernatant will be cleaned by solid-phase extraction, and effluent collected. The collected solvent will be evaporated at 50ºC until dry with a nitrogen stream. The extracted compounds will be dissolved in 1 ml of 20% acetonitrile and filtered (0.45 µM filter) prior to injection for HPLC analysis. Flavonoids and resveratrol will be quantified using an Agilent 1100 series RP-HPLC system with a C_{18} column and a diode-array detector (DAD) at various absorption wavelengths (Wang and Pittman, 2008). For flavor, a headspace-solid phase micro-extraction (HS-SPME) technique will be employed for extraction of volatiles. About 1.5 g of ground samples will be transferred in duplicate to 20 ml screw-capped vials containing a polytetrafluoroethylene/silicone septum. Then 2 ml of distilled water will be added with 20 µl of 0.045 mg/ml 1,3-dichlorobezene solution to the vial. The vials will be equilibrated for 15 min at 50ºC in the autosampler and agitated at 250 rpm. After equilibration, a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber will be exposed to the sample headspace for 40 min at 50ºC. The analytes will be desorbed to the injection port at 250ºC for 5 min in a splitless mode. The GC-MS system of Agilent Technologies 7890A/5977A equipped with an HP-5MS column (30 m x 250 µm x 0.25 µm) will be used for separating analytes. Identification of compounds will be based on the mass spectra database (NIST/EPA/NIH mass spectra library, Version 2.2, 2014) and Kovats indices (KI).

Sugars, organic acids and fruit color will be analyzed as described by Jarret et al. (2007) and Missio et al. (2015). Fruit will likewise be evaluated by the Phenospex PlantEye F500 multispectral imager for a range of characteristics including internal and external fruit color. Twenty mature fruit per genotype will be blended with water and homogenized. Aliquots of the homogenate will be centrifuged and the supernatant filtered. HPLC analysis of sugars will be conducted as described by Baldwin et al. (1991). Individual sugars will be analyzed by injecting 20 µL extract filtrate on a Sugar Pak column at 90°C (Waters, Millipore Corp., Milford, MA) with a mobile phase of 0.0001 N methylenediamine tetraacetic acid disodium–calcium salt (CaEDTA), a flow rate of 0.5 mL·min−1, and a refractive index detector (Agilent 1100 series; Agilent Technologies, Palo Alto, CA). HPLC analysis of acids will be conducted by injecting 20 µL extract filtrate on a ThermoFinnigan SpectraSytem UV6000UL (Thermo Electron Corp., San Jose, CA) with a Prevail Organic Acid column at 35°C (Alltech, Deerfield, IL), a mobile phase

of 0.1N H2SO4, a flow rate of 0.2 mL·min−1, and a photo diode array (Thermo Electron Corp.) spectrophotometric detector at 210 nm.

Unidentified compounds of potential interest will also be isolated, separated by chromatography, and collected for further characterization by LC-MS, GC-MS or NMR. The oil and protein content, fatty acid composition, amino acid composition, fiber, mineral element concentration, flavonoid, resveratrol, and flavor-related compounds will be examined by analysis of variance and mean separations using SAS software (SAS Institute, 2002-2005). All data will be entered into GRIN-Global. Descriptors or crop traits will be developed and added to GRIN-Global as appropriate using the Curator Tool and Crop Group of Dataviews. A Method will be added to describe the environment and/or procedures. Docments summarizing various statistical data may be added using the Accession Attachment Wizard and referenced in the Method. Characterization/evaluation data will be loaded primarily from spreadsheets to GRIN-Global Dataviews.

Objective 4 Methods continued:
One to three *in vitro* cultures of sweetpotato are usually sent per request depending on the needs of the user. Cultures (semi-solid media) are packaged in boxed cardboard sleeves (25 x 150 mm culture tubes) or styrofoam packing material (liquid media). Vegetative material of other *Ipomoea* spp., perennial wild peanuts, and warm-season grass accessions which are non-seed producers will be shipped as bare-rooted cuttings; wrapped in moist paper towels, foam root cubes, or moss; placed in plastic bags; and shipped in packing material within a cardboard box. Communications with the user ensures that if damage occurs in transit, replacement of damaged plant materials can be arranged. Nursery certificates issued by the Georgia Department of Agriculture will be kept current to allow for legal domestic distribution of live plant material. Routine inspections of greenhouse facilities and bamboo field plots will be conducted twice a year by the Georgia Department of Agriculture to remain in compliance with the Imported Fire Ant Quarantine, Nematode Quarantine, Federal Japanese Beetle Quarantine, and North Carolina Tropical Spiderwort Quarantine. In addition, seed storage personnel will view Department of Agriculture websites for the destination state before the plant material is shipped to determine if specific state import requirements limit the shipment of live plant material. Where plant material cannot be distributed due to state-imposed import restrictions, the requestor will be notified by the curator. Bamboo rhizomes will be distributed once a year in February to all requesters from the bamboo plots at the USDA, ARS, Byron, GA, location. The appropriate documentation (e.g. official fire ant stamp) showing legal compliance will be sent with each live plant shipment. Chinese water chestnut corms will be distributed upon request.

Appendix 3. Response to reviewers' comments

Appendix 4. S-009 Multistate Project personnel

ADMINISTRATIVE ADVISOR
Dr. Bob Stougaard
University of Georgia
Assistant Dean for Research
107 Conner Hall
147 Cedar Street

Athens, GA 30602
(706) 542-0932
bob.stougaard@uga.edu

ALABAMA

Dr. Charles Chen
Auburn University
258 Funchess Hall
Auburn, AL 36849
(334) 844-4100
cyc0002@auburn.edu

ARKANSAS

Dr. James Correll
University of Arkansas
Department of Plant Pathology
PTSC 217
Fayetteville, AR 72701
(479) 575-2710
jcorrell@uark.edu

FLORIDA

Dr. Kevin E. Kenworthy
University of Florida
Institute of Food and Agricultural
Sciences, Agronomy Department
304 Newell Hall
P.O. Box 110965
Gainesville, FL 32611-0965
(352) 262-8719
FAX: 352-392-6110
kenworth@ufl.edu

GEORGIA

Dr. Paul L. Raymer
University of Georgia Griffin Campus
Crop & Soil Sciences
Weed Science Building
1109 Experiment Street
Griffin, GA 30223-1797
(770) 228-7324
FAX: 770-412-4734
praymer@uga.edu

GUAM

Dr. Mari Marutani

University of Guam
College of Agriculture and Life Sciences
CALS Building, Room 318
UOG Station, Mangilao, GU 96923
(671) 735-2131
FAX: 671-734-4600
marutani@uguam.uog.edu

KENTUCKY

Dr. Timothy D. Phillips
University of Kentucky
Department of Agronomy
325 Plant Science
Building 0312
Lexington, KY 40506
(859) 218-0769
FAX: 859-257-7125
tphillip@uky.edu

LOUISIANA

Dr. Don LaBonte
Louisiana State University
Department of Horticulture
137 Julian C. Miller Hall
Baton Rouge, LA 70803
(225) 578-1024
FAX: 225-578-1068
dlabonte@agcenter.lsu.edu

MISSISSIPPI

Dr. Brian S. Baldwin
Mississippi State University
Plant and Soil Sciences
Box 9555
Mississippi State, MS 39762
(662) 325-8862
FAX: 662-325-8742
bbaldwin@pss.msstate.edu

NORTH CAROLINA

Dr. Tom Stalker
North Carolina State University
Department of Crop Science
Box 7629
Raleigh, NC 27695-7629
(919) 513-2929

FAX: 919-515-5657
tom_stalker@ncsu.edu

OKLAHOMA

Dr. Yanqi Wu
Oklahoma State University
Department of Plant & Soil Sciences
368 Agricultural Hall
Stillwater, OK 74078-6028
(405) 744-9627
FAX: 405-744-5269
yanqi.wu@okstate.edu

PUERTO RICO

Dr. Bryan Brunner
University of Puerto Rico
Agricultural Experiment Station
HC-02 Box 11656
Lajas, PR 00667
(787) 899-1530
FAX: 787-899-1265
brbrunner@yahoo.com
bryan.brunner1@upr.edu

SOUTH CAROLINA

Dr. Stephen Kresovich
Clemson University
Department of Genetics and Biochemistry
318 Biosystems Research Complex
Clemson, SC 29634
(864) 656-1194
skresov@clemson.edu

TENNESSEE

Dr. Virginia R. Sykes
University of Tennessee
Department of Plant Sciences
2431 Joe Johnson Drive
Knoxville, TN 37996
(865) 974-7285
FAX: 865-974-7997
vsykes@utk.edu

TEXAS

Dr. G. Ray Smith
Texas A&M University

Texas Agricultural Experiment Station
P.O. Box 200
1710 North Highway 3053
Overton, TX 75684
(903) 834-6191
FAX: 903-834-7140
g-smith@tamu.edu

VIRGIN ISLANDS (U.S.)

Dr. Thomas W. Zimmerman
University of the Virgin Islands
Agricultural Experiment Station - Biotechnology
RR 2, Box 10,000
Kingshill, St. Croix, VI 00850
(340) 692-4074
FAX: 340-692-4035
tzimmer@uvi.edu

VIRGINIA

Dr. Richard E. Veilleux
Virginia Polytechnic Institute and State University
Department of Horticulture
Blacksburg, VA 24061-0327
(540) 231-5584
FAX: 540-231-3083
potato@vt.edu

Ex-Officio

Dr. Peter K. Bretting
National Program Leader, Plant Germplasm
USDA, ARS
Room 4-2211, Mailstop 5139
5601 Sunnyside Avenue
Beltsville, MD 20705-5139
(301) 504-5541
FAX: 301-504-6191
peter.bretting@ars.usda.gov

Mr. Archie Tucker
Area Director
USDA, ARS, Southeast Area
141 Experiment Station Road
JWDSRC
Stoneville, MS 38776
(662) 686-5266

FAX: (662) 686-5309
archie.tucker@ars.usda.gov

Dr. James Frelichowski
USDA, ARS, SPARC
Crop Germplasm Research Unit - Cotton
2881 F&B Road
College Station, TX 77845
(979) 260-9329
FAX: 979-260-9333
james.frelichowski@ars.usda.gov

Dr. Ricardo J. Goenaga
USDA, ARS
Nat'l Germplasm Repository
Tropical Agric. Res. Station
P.O. Box 70
Mayaguez, PR 00681-0070
(787) 831-3435 x238
FAX: 787-831-3386
ricardo.goenaga@ars.usda.gov

Dr. L.J. Grauke
USDA, ARS
National Germplasm Repository
Pecan Breeding & Genetics
10200 FM50
Somerville, TX 77879
(979) 272-1402
FAX: 979-272-1401
lj.grauke@ars.usda.gov

Dr. Melanie Harrison
Research Leader/Coordinator
USDA, ARS
Plant Genetic Resources Conservation Unit
1109 Experiment Street
Griffin, GA 30223-1797
(770) 228-7254
FAX: 770-229-3323
melanie.harrison@ars.usda.gov

Dr. Quinn P. Sinnott
USDA, ARS
National Germplasm Resources Laboratory
Room 400, Building 003, BARC-West

10300 Baltimore Avenue
Beltsville, MD 20705
(301) 504-6072
FAX: 301-504-5536
quinn.sinnott@ars.usda.gov

Dr. Ann Marie Thro
National Program Leader, Plant Breeding and Genetics
USDA, CSREES
Mail Stop 2220
1400 Independence Ave., SW
Washington, DC 20250-2220
(202) 401-6702
FAX: 202-401-4888
athro@csrees.usda.gov