S-009 Regional Technical Advisory Committee
Minutes of Annual Meeting July 22-23, 2014

Research and Education Garden Building
University of Georgia Griffin Campus
Griffin, GA

Members present:

Florida (University of Florida) ..... Ken Quesenberry clover@ufl.edu
(for Kevin Kenworthy)
Georgia (University of Georgia) ..... Paul Raymer praymer@uga.edu
Guam (University of Guam) ..... Mari Marutani marutani@uguam.uog.edu
Kentucky (University of Kentucky) ..... Tim Phillips tphillip@uky.edu
Louisiana (Louisiana State University) ..... Don LaBonte dlabonte@agcenter.lsu.edu
Mississippi (Mississippi State University) ..... Brian Baldwin bbaldwin@pss.msstate.edu
North Carolina (North Carolina State University) ..... Tom Stalker tom_stalker@ncsu.edu
South Carolina (Clemson University) ..... Shyam Tallury stallur@clemson.edu
(for Stephen Kresovich)
Tennessee (University of Tennessee) ..... Fred Allen allenf@utk.edu
Virgin Islands (University of the Virgin Islands) ..... Tom Zimmerman tzimmer@live.uvi.edu

Members absent:

Alabama (Auburn University) ..... Edzard van Sariten vanedza@auburn.edu
Arkansas (University of Arkansas) ..... James Correll jcorrell@uark.edu
Hawaii (University of Hawaii) ..... [vacant]
Oklahoma (Oklahoma State University) ..... Yanqi Wu yanqi.wu@okstate.edu
Puerto Rico (University of Puerto Rico) ..... Bryan Brunner brbrunner@yahoo.com
Texas (Texas A&M University) ..... Gerald Smith g-smith@tamu.edu
Virginia (Virginia Tech) ..... Richard Veilleux potato@vt.edu

USDA, ARS, PGRCU representatives present:

PGRCU, Griffin, GA ..... Gary Pederson gary.pederson@ars.usda.gov
PGRCU, Griffin, GA ..... Bob Jarret bob.jarret@ars.usda.gov
PGRCU, Griffin, GA ..... John B. Morris brad.morris@ars.usda.edu
PGRCU, Griffin, GA ..... Ming Li Wang mingli.wang@ars.usda.gov
PGRCU, Griffin, GA ..... Merrelyn Spinks merrelyn.spinks@ars.usda.gov
PGRCU, Griffin, GA ..... Melanie Harrison melanie.harrison@ars.usda.gov
PGRCU, Griffin, GA ..... Jacquie McDonald Jacquie.mcdonald@ars.usda.gov
S-009, University of Georgia, Griffin, GA ..... Donna Kent dmkent@uga.edu
S-009, University of Georgia, Griffin, GA ..... Jeremy Smith zaydoc@uga.edu
S-009, University of Georgia, Griffin, GA ..... Jill Cunningham jcunnin@uga.edu
S-009, University of Georgia, Griffin, GA ..... Angie Lewis alew71@uga.edu
PGRCU, Griffin, GA ..... David Pinnow david.pinnow@ars.usda.gov
PGRCU, Griffin, GA ..... Brandon Tonnis Brandon.tonnis@ars.usda.gov
PGRCU, Griffin, GA ..... Libbie Hudson libbie.hudson@ars.usda.gov
PGRCU, Griffin, GA ..... Kami Lindberg kami.lindberg@ars.usda.gov
PGRCU, Griffin, GA ..... Phiffie Vankus phiffie.vankus@ars.usda.gov

Others present:
Administrative Advisor.....Gerald Arkin garkin@uga.edu
University of Georgia, Tifton, GA .....Albert Culbreath spotwilt@uga.edu
Clemson University .....Brad Rauh brauh@clemson.edu

The meeting was called to order at the UGA-Griffin Research and Education Garden building at 1:00 p.m., July 22, by Chair Mari Marutani. The meeting began with self-introductions of all those present.

1) Minutes of 2013 S-009 meeting were approved as distributed.
2) Tim Phillips and Fred Allen were appointed to a committee to identify candidate(s) for 2015 S-009 Secretary to become S-009 Chair in 2016 and to propose a location for the 2015 Annual S-009 Meeting.
3) Report of National Programs: Dr. Gary Pederson reported for Dr. Peter Bretting who could not be at the meeting but provided a slide presentation (Appendix 1). The presentation illustrated locations, accession numbers, federal budget requests for 2000-2013, effects of the 2013 Federal furlough on entity operations, FAO International Treaty for germplasm sharing, partnerships of gene banks, and ARS Area changes. ARS will be changing from eight Areas to five Areas with Area Directors located in the new Southeast, Northeast, Midwest, Plains, and Pacific West Areas.
4) National Genetics Research Advisory Council: Dr. Gary Pederson is an ex-officio member of the Council. He reported that because of the impacts of the Federal Government furlough in 2013 and the delay in approving a new Farm Bill which authorized this council, not as much activity or accomplishments had been made during the past year as had been hoped. New members are being appointed to the council and NGRAC will be meeting in Ames, IA in September.
5) Dr. Jerry Arkin, S-009 Administrative Advisor, gave a welcome and made overview comments to the group. He reiterated the liaison with the Southern Experiment Station Directors with their positive view of S-009 and activities as well as their continued monetary support for germplasm conservation and utilization. He also mentioned the FAO International Treaty for germplasm sharing and the need for SAES Directors to support the legislative ratification of the Treaty. Dr. Arkin gave a brief report on the "NIFA Road Map" and transgenes practices and procedures. Lastly he announced that he plans to retire in ~2 months. The S-009 Committee expressed sincere gratitude to Dr. Arkin for his strong support, contributions and advocacy for the conservation and utilization of plant germplasm, especially the collection maintained at Griffin, GA.
6) Dr. Gary Pederson gave a summary of PGRCU activities for the past year (Appendix 2). His report included collection size (92,222 accessions), backed up accessions, germination testing of individual crops, availabilities, and distributions. S-009 staff distributed 32,958 accessions in 2013 with 8,728 accessions distributed to foreign users and 13,685 accessions distributed to S-009 users. He also reported on new improved facilities/equipment; funding and financial status; and staffing.
7) Discussion of Griffin Program: Dr. Melanie Harrison gave a report on the collection of grasses and reviewed some of the low germinations for some species/accessions. She reported that different species had different issues. Old seed (35-45 years old) has a higher priority for regeneration. She is using some seed treatments and cleaning techniques to reduce seed damage during threshing and cleaning. Other concerns include GE traits in new material that may enter the collection in the future or through inadvertent contamination. There is concern that donors may not disclose presence of GE traits in accessions added to the collection.
8) Dr. Ming Li Wang presented a report on "Characterization and Utilization of the USDA Sesame Collection by Biochemical and Genetic Analysis" (Appendix 3). His report included information

on production, utilization, genetics, and cross pollination of sesame. He reported that the maximum oil content was 65.5% and as low as 25.7%. Three accessions with high oleic acid were PI 278164, PI 599442 and PI 599435. He also reported that 1414 mutant lines had been produced via EMS seed treatment.

Brandon Tonnis (Appendix 4) presented a report on "Analysis of primary and secondary plant compounds: An overview of the PGRCU chemistry lab". The Chemistry Laboratory at Griffin evaluates primary and secondary plant compounds including polyphenol, tocopherol (Vit E), phytosterols, and capsaicenoids using Time Domain Nuclear Magnetic Resonance (TD-NMR) and Gas Chromotography.

The meeting was adjourned and reconvened on Wednesday, July 23 at 8:30am.

1) The first item on the agenda for Wednesday was a tour of facilities. University of Georgia utilization of turf germplasm was shown by Paul Raymer's graduate student, Clint Steketee, and Melanie Harrison showed the development of little bluestem for use as an ornamental grass.
2) Dr. Albert Culbreath made a presentation (Appendix 5) on "Rust Resistance in Peanut Genetic Resources." He discussed germplasm screening, incorporating resistance from peanut accessions into GA and FL breeding lines with some lines having good rust resistance. Cordillera has both leaf spot and rust tolerance. He also reported on one PI that has served as the major source of resistance to Tomato Spot Wilt Virus (TSWV) that helped save the peanut industry in the Southeastern U.S.
3) State reports were given by representatives from FL (Ken Quesenberry); GA (Paul Raymer written); Guam (Mari Marutani); KY (Tim Phillips); LA (Don LaBonte written); MS (Brian Baldwin); NC (Tom Stalker); OK (written); SC (Shyam Talbury); and TN (Fred Allen).
4) The Nominating/2015 Meeting Location Committee (Phillips and Allen) provided the name of Dr. Brian Baldwin (Mississippi State) as the nominee for Secretary in 2015 and Chair in 2016. Dr. Baldwin was elected by acclamation. The 2015 meeting will be held in Knoxville, TN, hosted by the University of Tennessee, Institute of Agriculture. The group approved the date of July 21-22, 2015 for the next meeting.

The meeting was adjourned.

Fred Allen
S-009 Secretary

Appendix 1

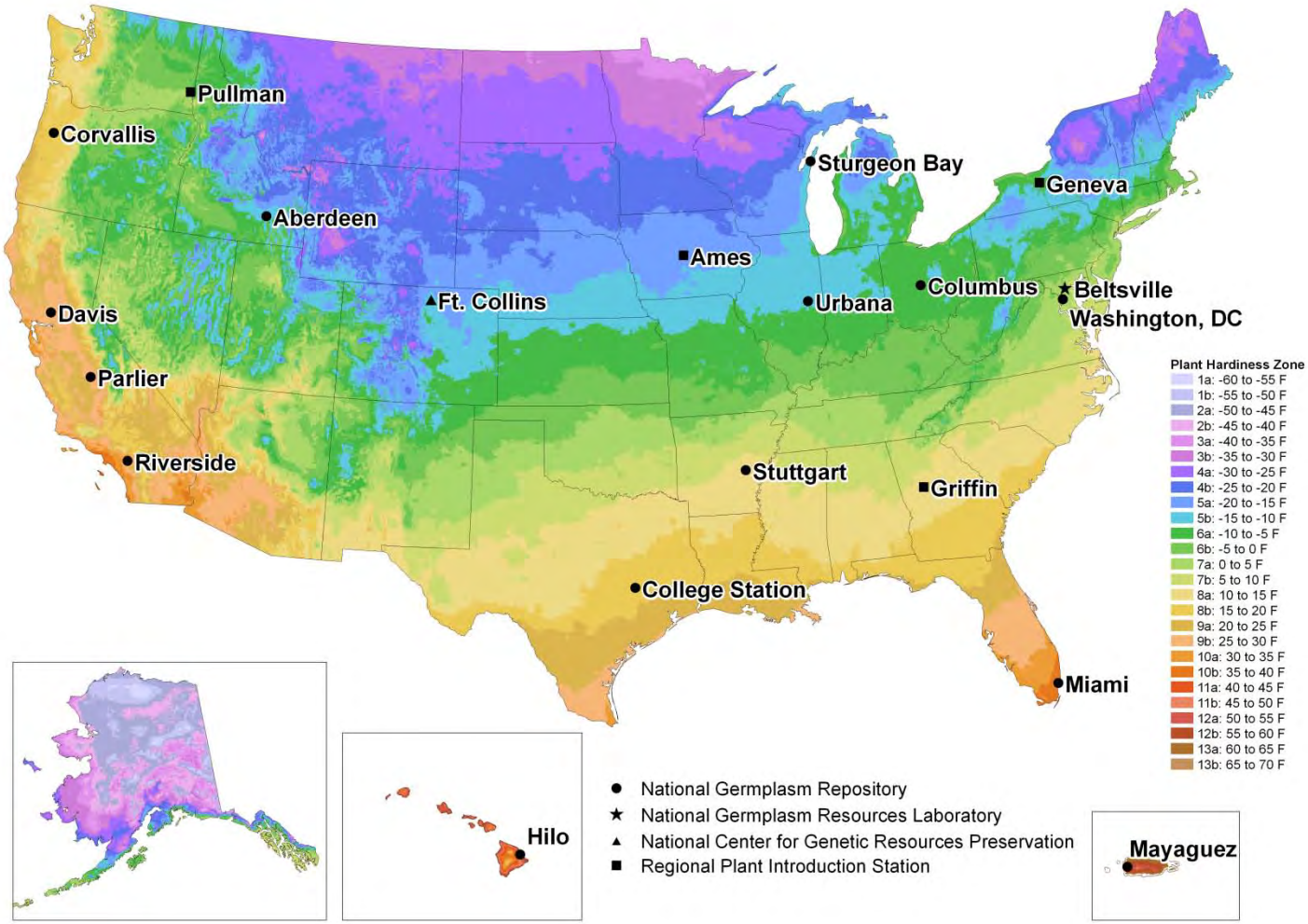
DR. PETER BRETTING

2014 OFFICE OF NATIONAL PROGRAMS REPORT

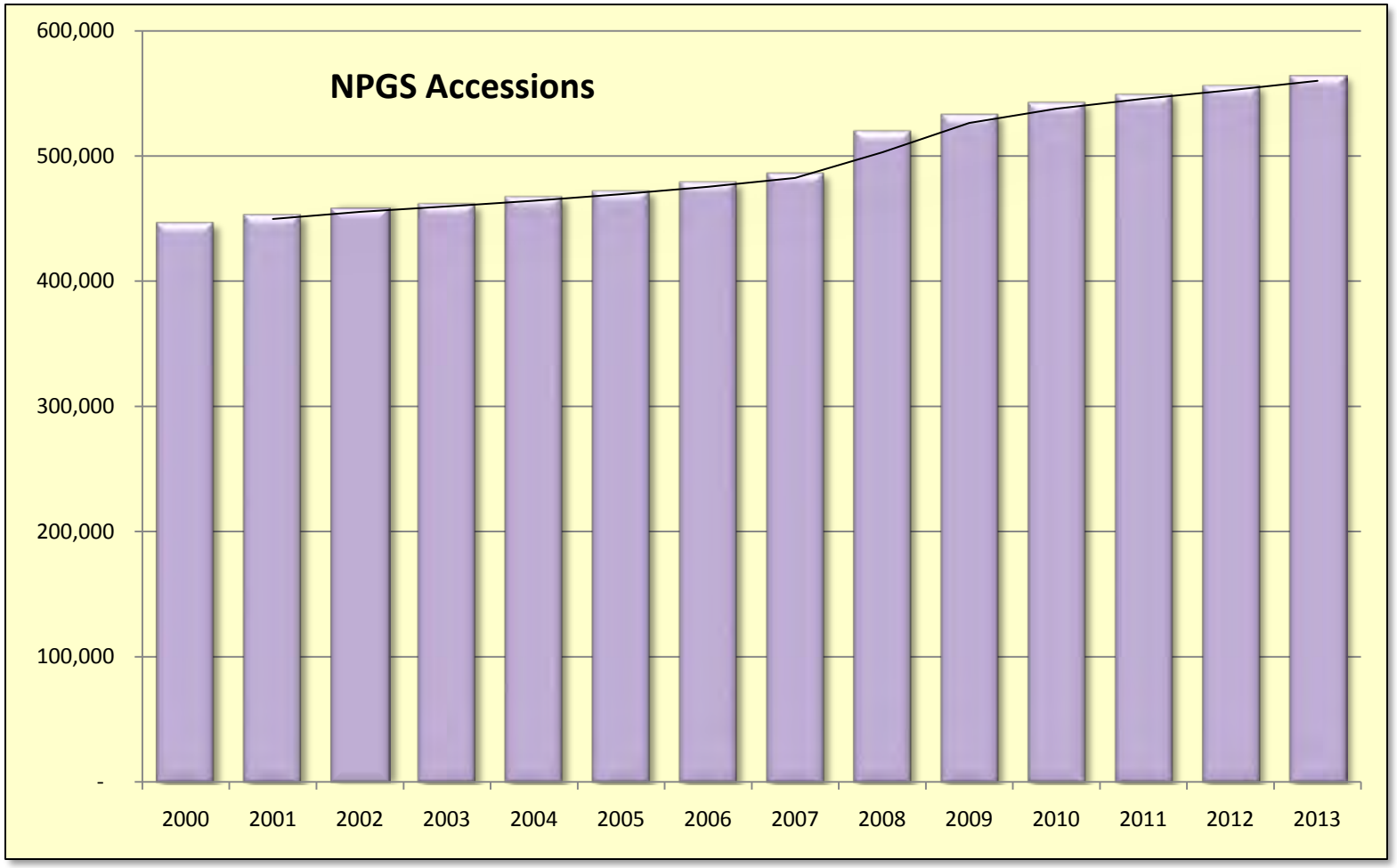
The National Plant Germplasm System: 2014 Status and Prospects

Peter Bretting
USDA/ARS Office of National Programs

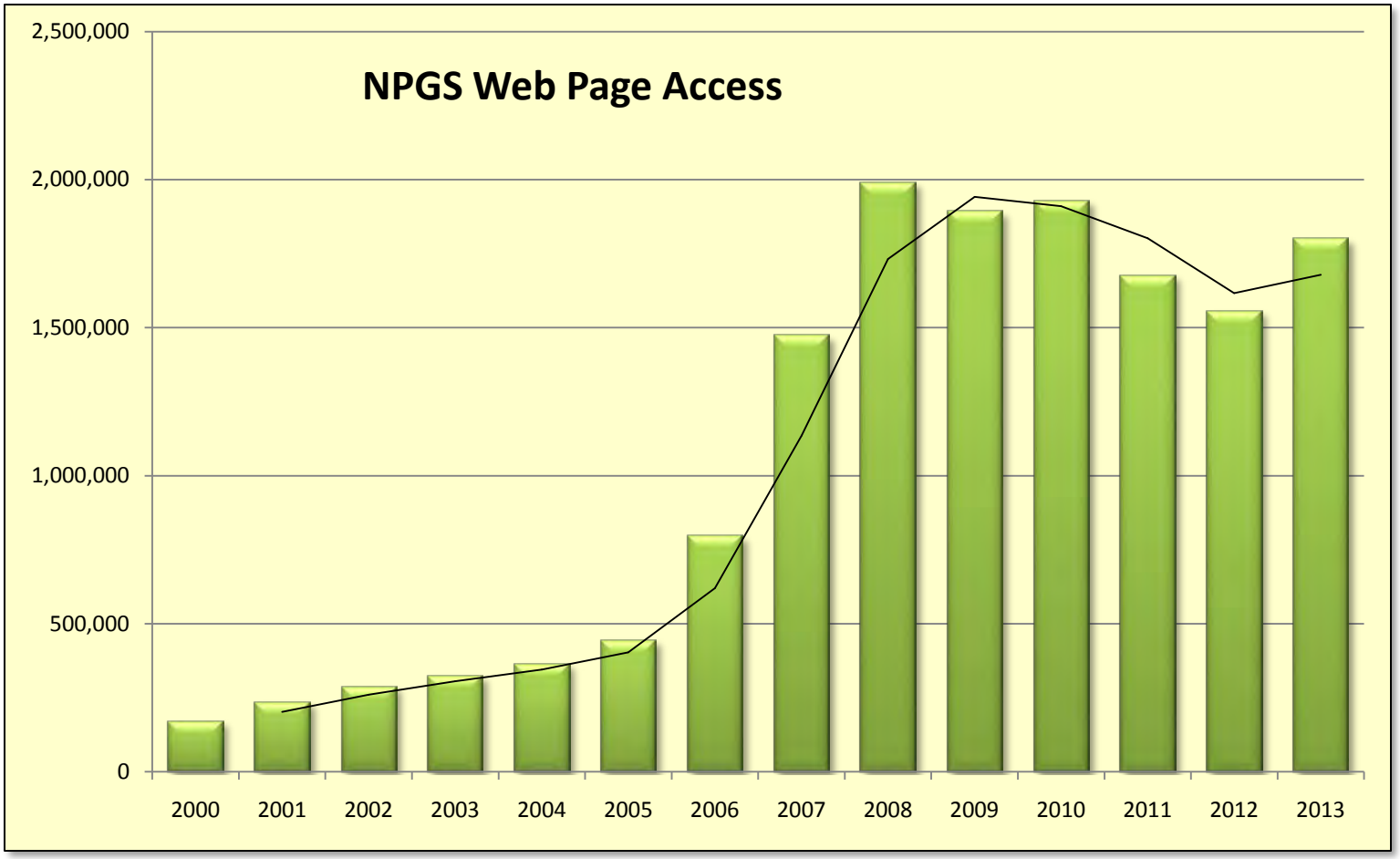
USDA National Plant Germplasm System (NPGS)



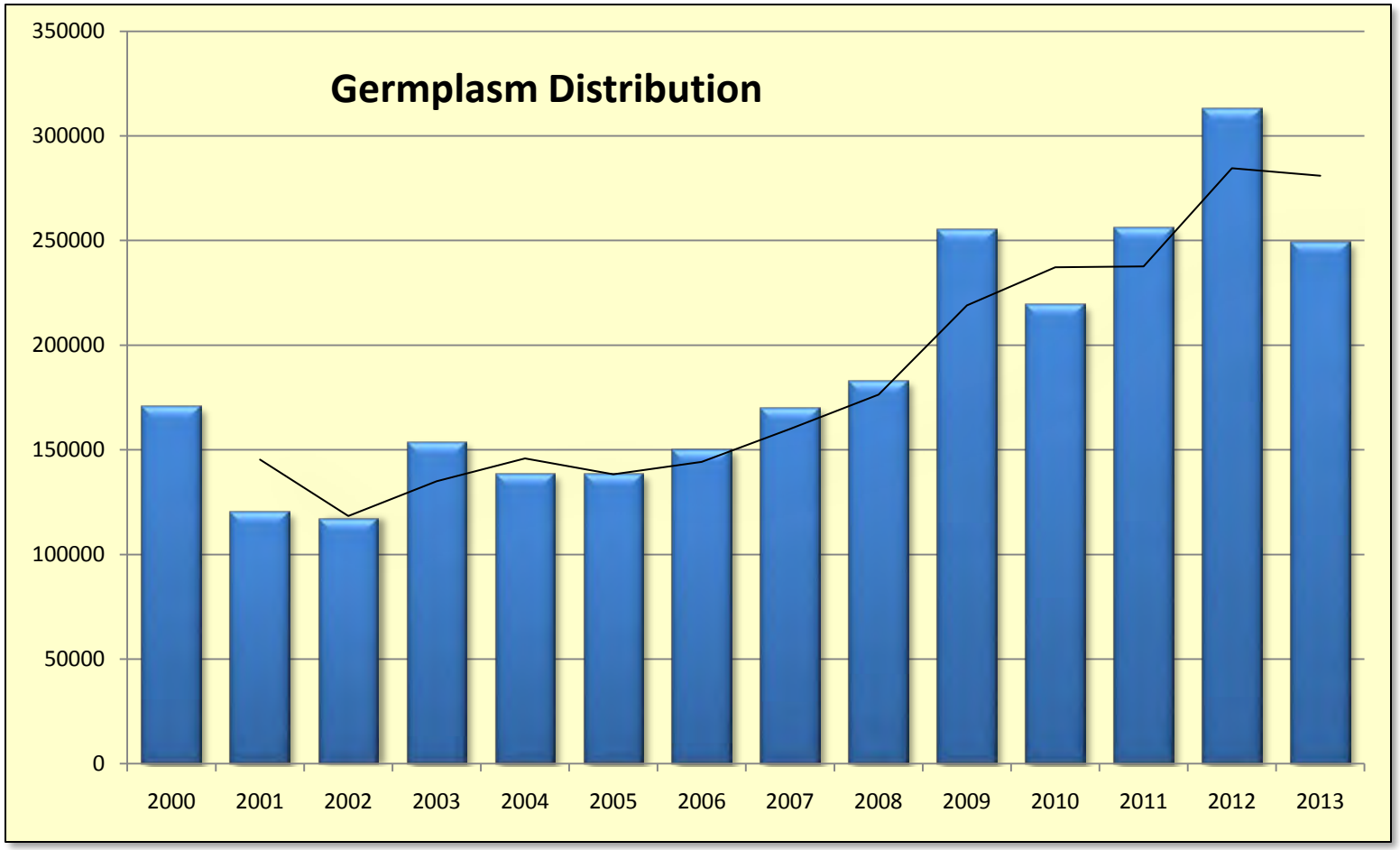
Number of NPGS Accessions 2000-2013



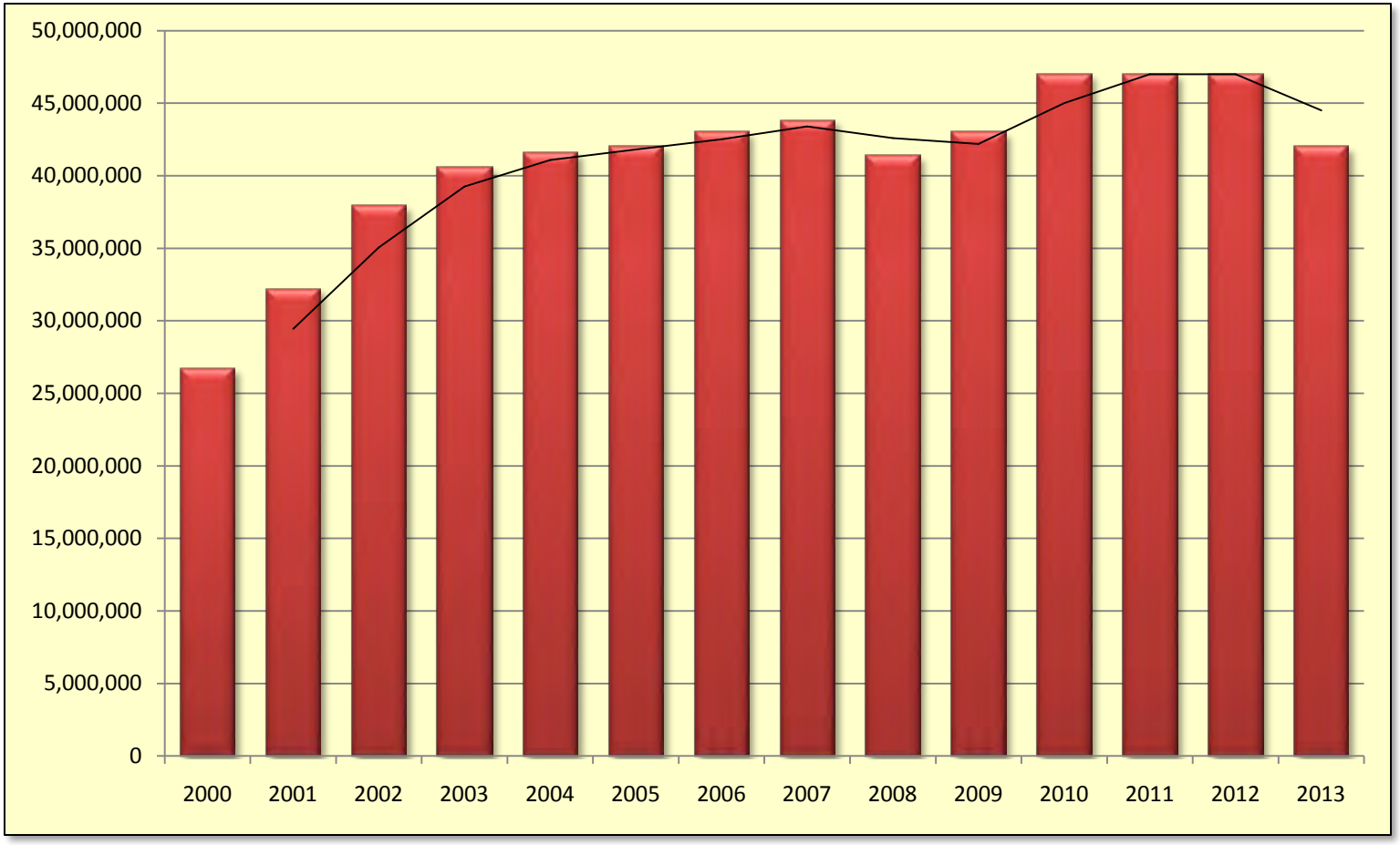
Demand for NPGS information 2000-2013



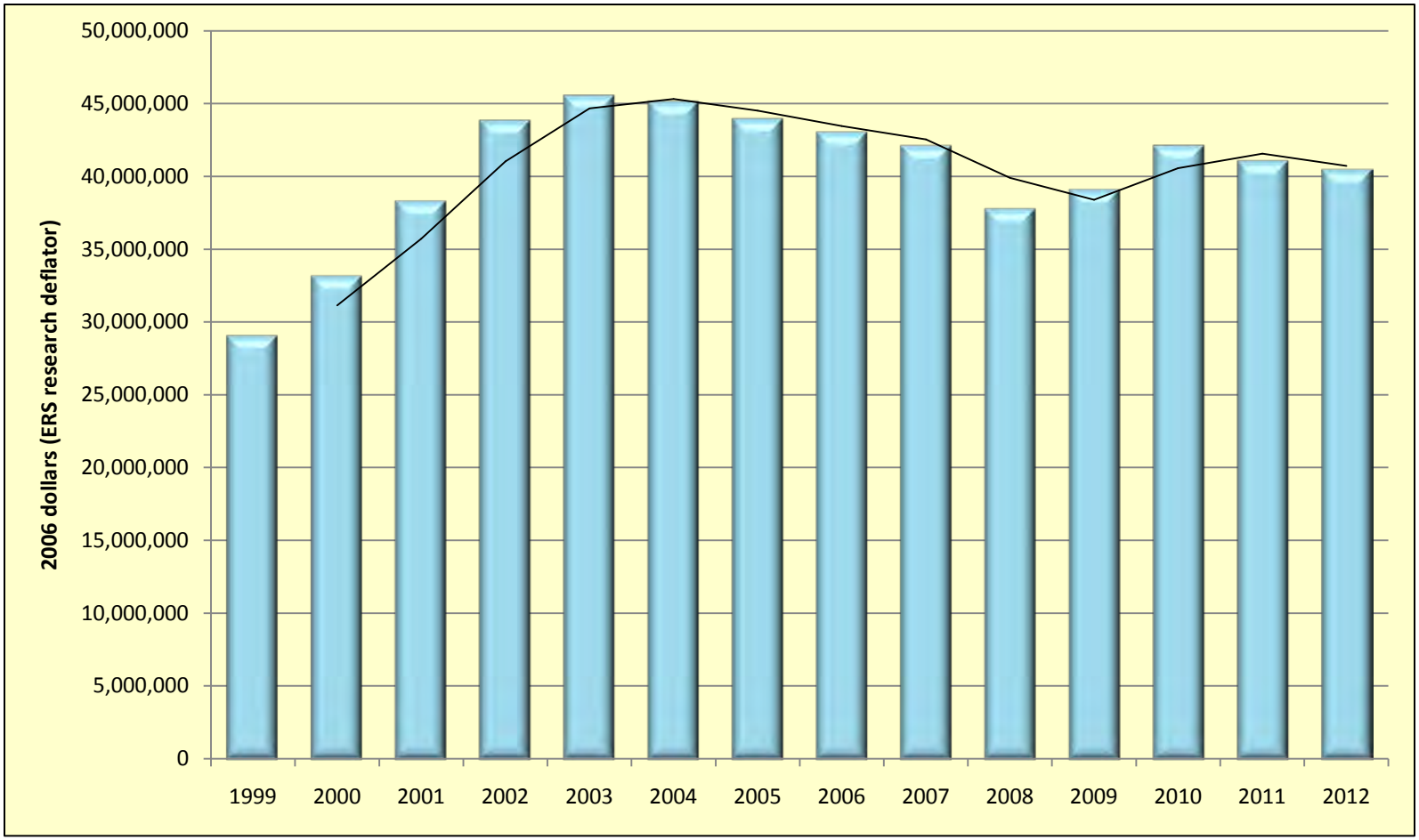
Demand for NPGS Germplasm 2000-2013



ARS National Plant Germplasm System budget 2000-2013



ARS National Plant Germplasm System Budget, Real, 1999-2012



Notable NPGS Developments

- **1-17 October 2013: Furlough**
  - **Only designated key personnel permitted to work.**
  - **No germplasm lost.**
  - **No germplasm distributed; GRIN off-line.**
  - **Delayed harvests, delayed shipments to winter nurseries.**
- **FAO International Treaty on Plant Genetic Resources for Food and Agriculture**
  - **Seed industry advocates US ratification.**

Notable NPGS Developments

- **Stronger and more extensive international partnerships**
  - **Hosted CGIAR Genebanks Annual General Meeting at NCRPIS, Ames.**
  - **Global Crop Diversity Trust: Developing international project for increasing the use of PGRFA (especially crop wild relatives)**
  - **PRC, S. Korea, Canada, Mexico, Colombia national genebanks: training at NPGS**

Pacific West Area

North East Area

Midwest Area

Plains Area

South East Area

WA
OR
ID
CA
NV
UT
AZ
MT
WY
CO
NM
ND
SD
NE
KS
OK
TX
MN
IA
MO
AR
LA
WI
IL
MI
IN
OH
KY
TN
MS
AL
GA
FL
SC
NC
VA
WV
PA
NY
VT
NH
ME
MA
CT
RI
NJ
DE
MD

The bold lines reflect the boundaries of the ARS geographic Areas.

Chavonda Jacobs-Young (ARS Administrator)

Deborah Brennan – Southeast Area Director, Stoneville, MS

Dan Upchurch – Northeast Area Director, Beltsville, MD

Larry Chandler – Plains Area Director, Ft. Collins, CO

Bob Matteri – Midwest Area Director, Peoria, IL

Andy Hammond – Pacific Northwest Area Director, Albany, CA

Appendix 2

DR. GARY PEDERSON

PLANT GENETIC RESOURCES: CURRENT STATUS

Plant Genetic Resources: Current Status

Gary A. Pederson

USDA, ARS, Plant Genetic Resources Conservation Unit

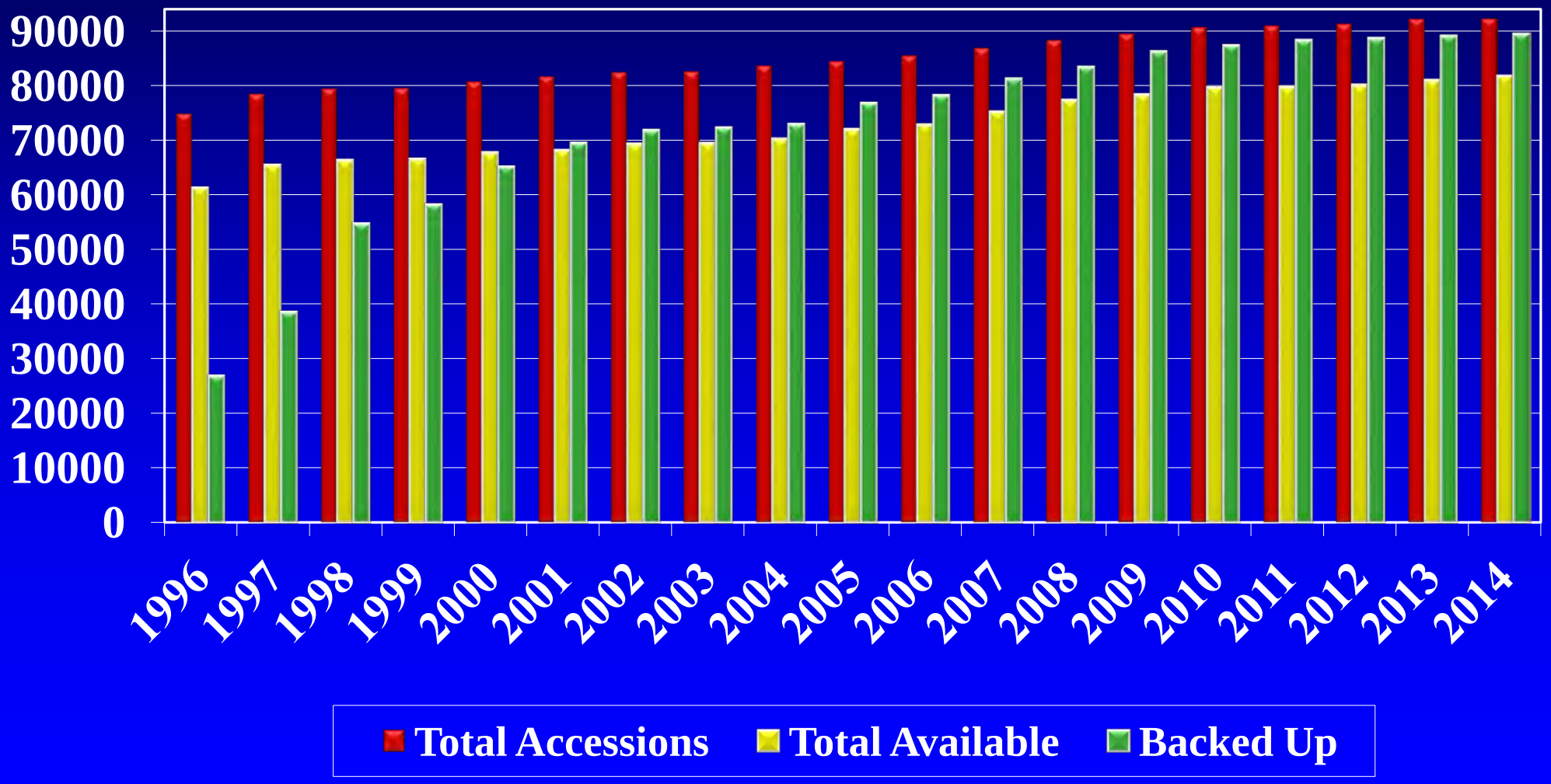
Griffin, GA

PGRCU Collection - June 2014

- Total Accessions
  - 92,222
- Total Available
  - 81,791 (88.7%)
- Backed Up
  - 89,452 (97.0%)

Acknowledgement: Merrelyn Spinks and Lee Ann Chalkley, PGRCU, compiled and summarized all numbers shown in this presentation.

PGRCU Collection 1996 - 2014



Germination Testing

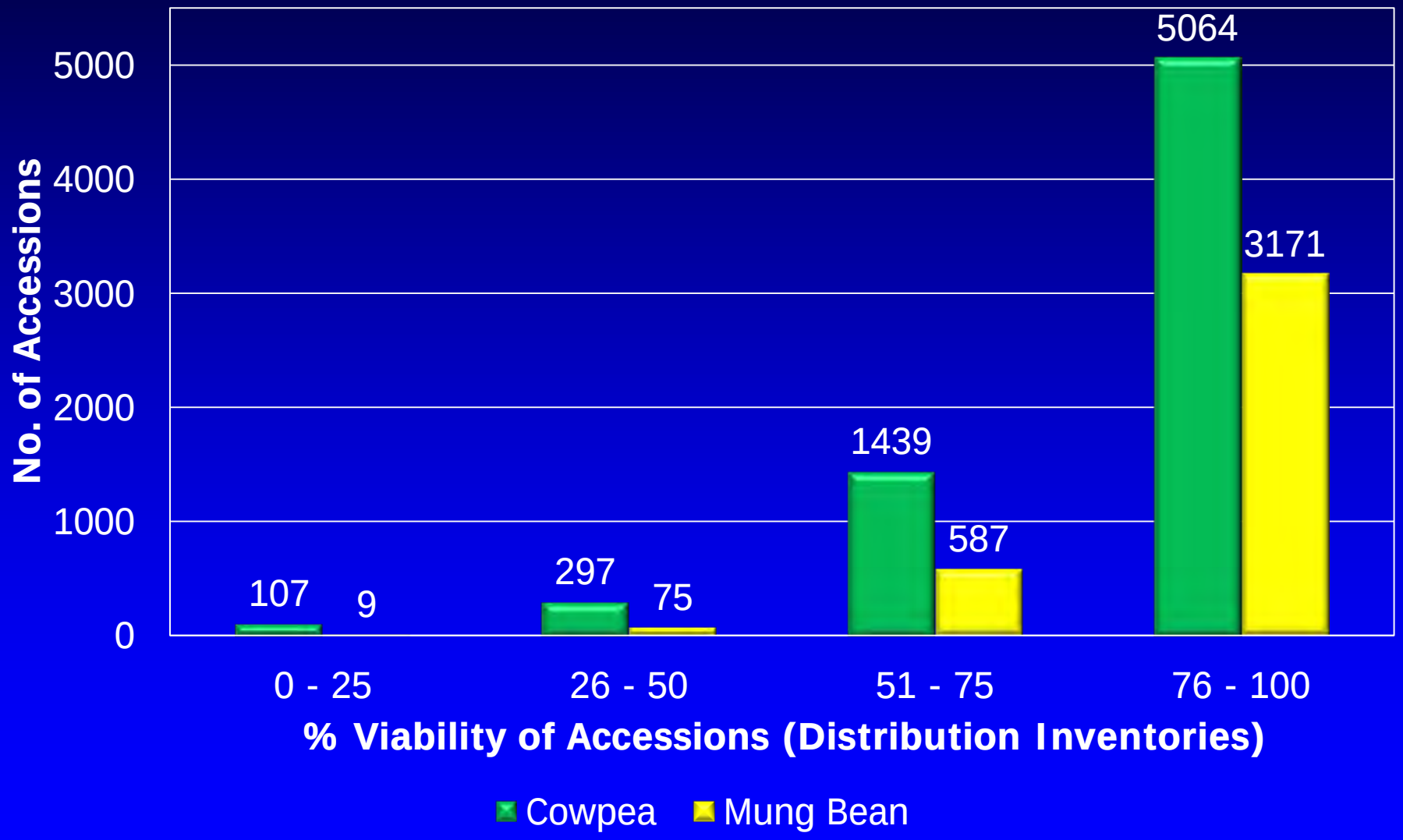
(Accessions with seed only, tested since 2002)

Crop	Accessions	%	Crop	Accessions	%
Sorghum	35,542	93.2	Clover	2,003	89.2
Peanut	8,421	90.3	Okra	1,756	59.1
Cowpea	7,241	87.7	Watermelon	1,758	92.1
Grass	6,577	87.8	Pearl millet	1,294	99.8
Pepper	4,895	99.2	Sesame	1,211	99.6
Mung bean	3,928	92.8	Eggplant	946	92.6
Legume	2,423	78.6	Cucurbit	701	49.4
			TOTAL	**81,157**	**89.3**

Vigna

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Brad Morris	Cowpea	8,254	6,913	8,207	**2,186**
	Mung bean	4,232	3,843	4,232	177
	Other Vigna spp.	496	301	353	70

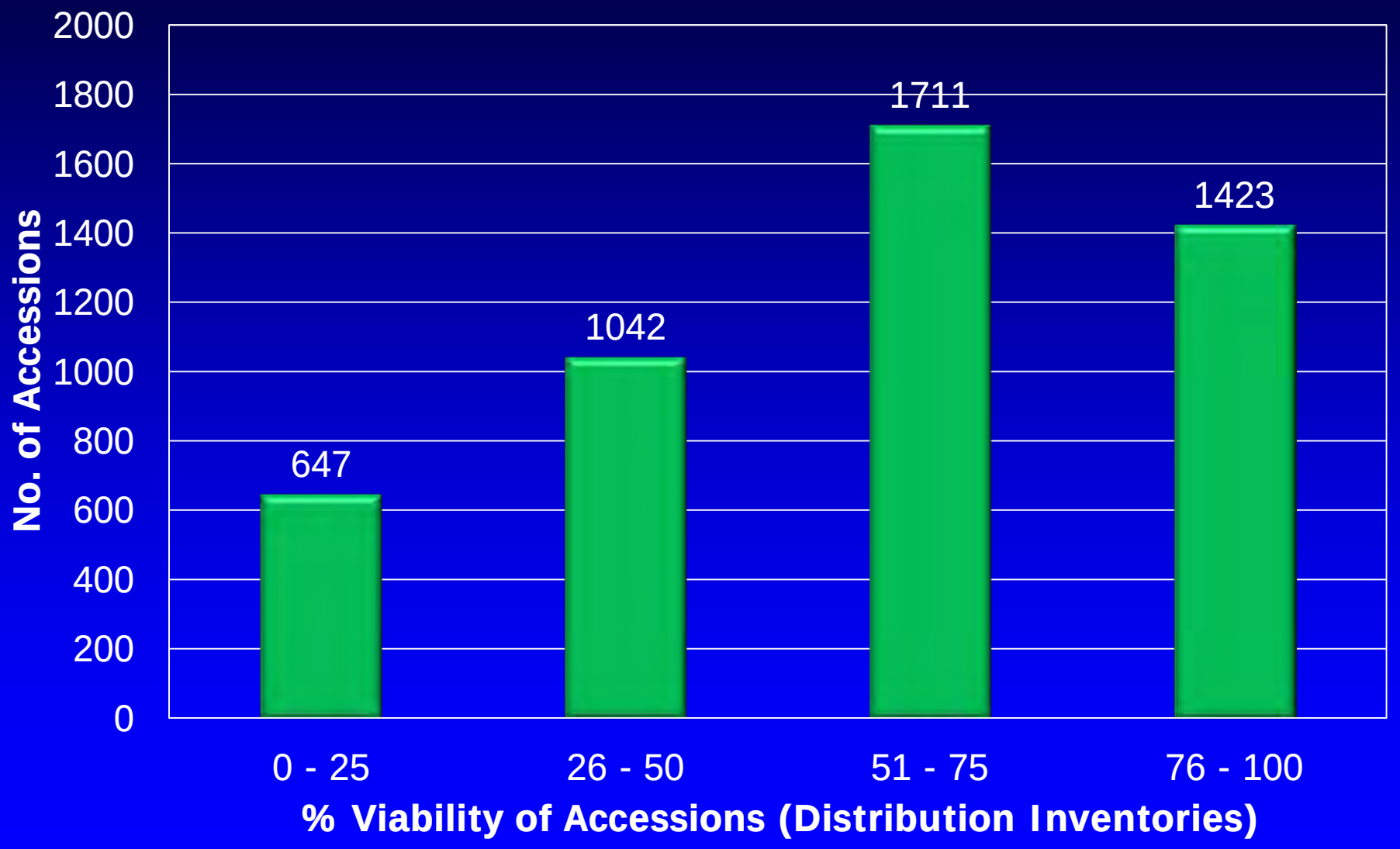
Cowpea & Mung Bean Accession Viability



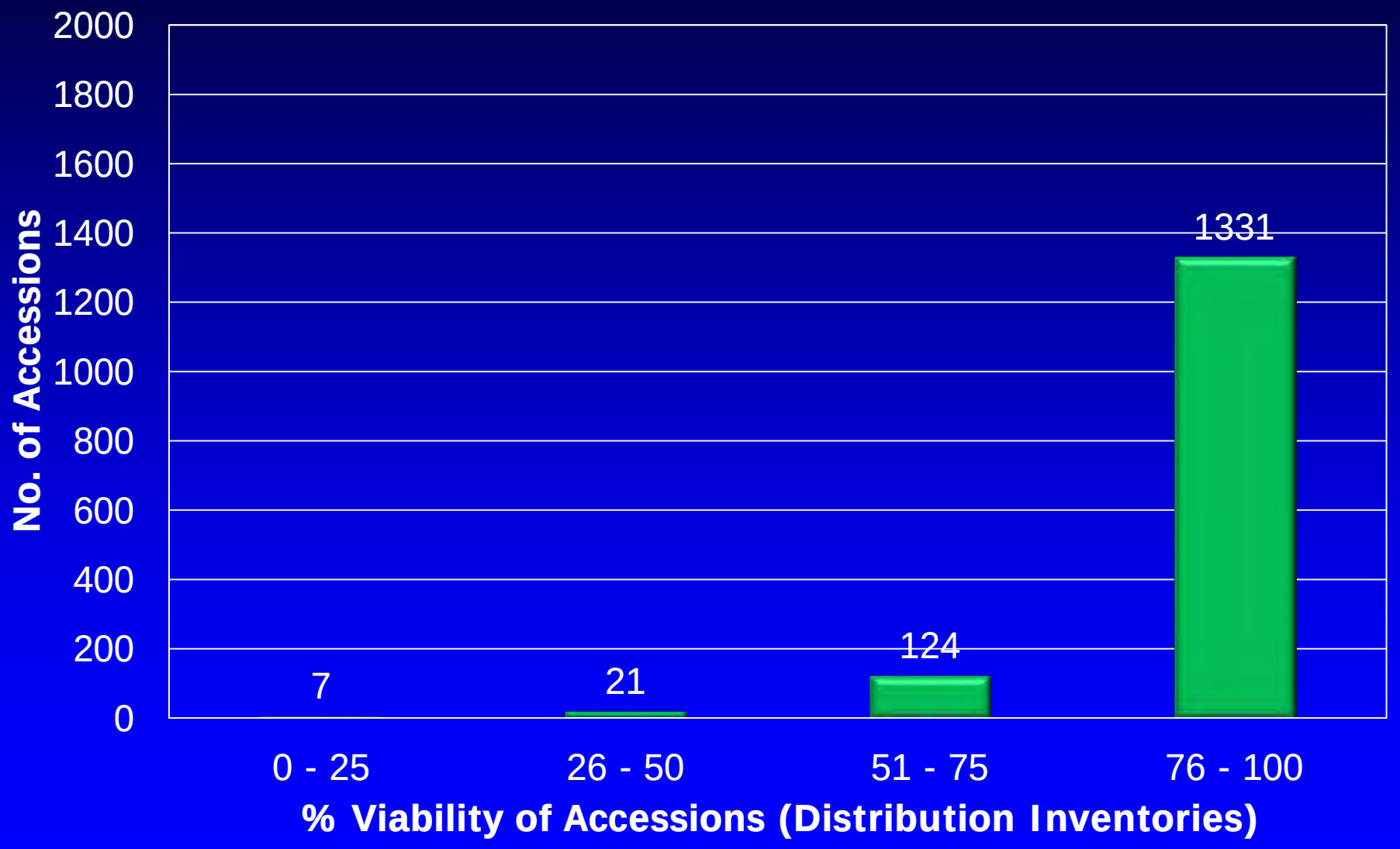
Vegetable Crops & Sweetpotato

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Bob Jarret	Cucurbits	1,420	512	1,315	1,044
	Eggplant	1,022	920	1,000	741
	Okra	2,971	1,514	2,935	203
	Peppers	4,934	4,859	4,932	**2,859**
	Sweetpotato - tissue culture	768	736	681	**640**
	Other Ipomoea spp.	461	208	406	52
	Watermelon	1,908	1,480	1,875	**3,923**

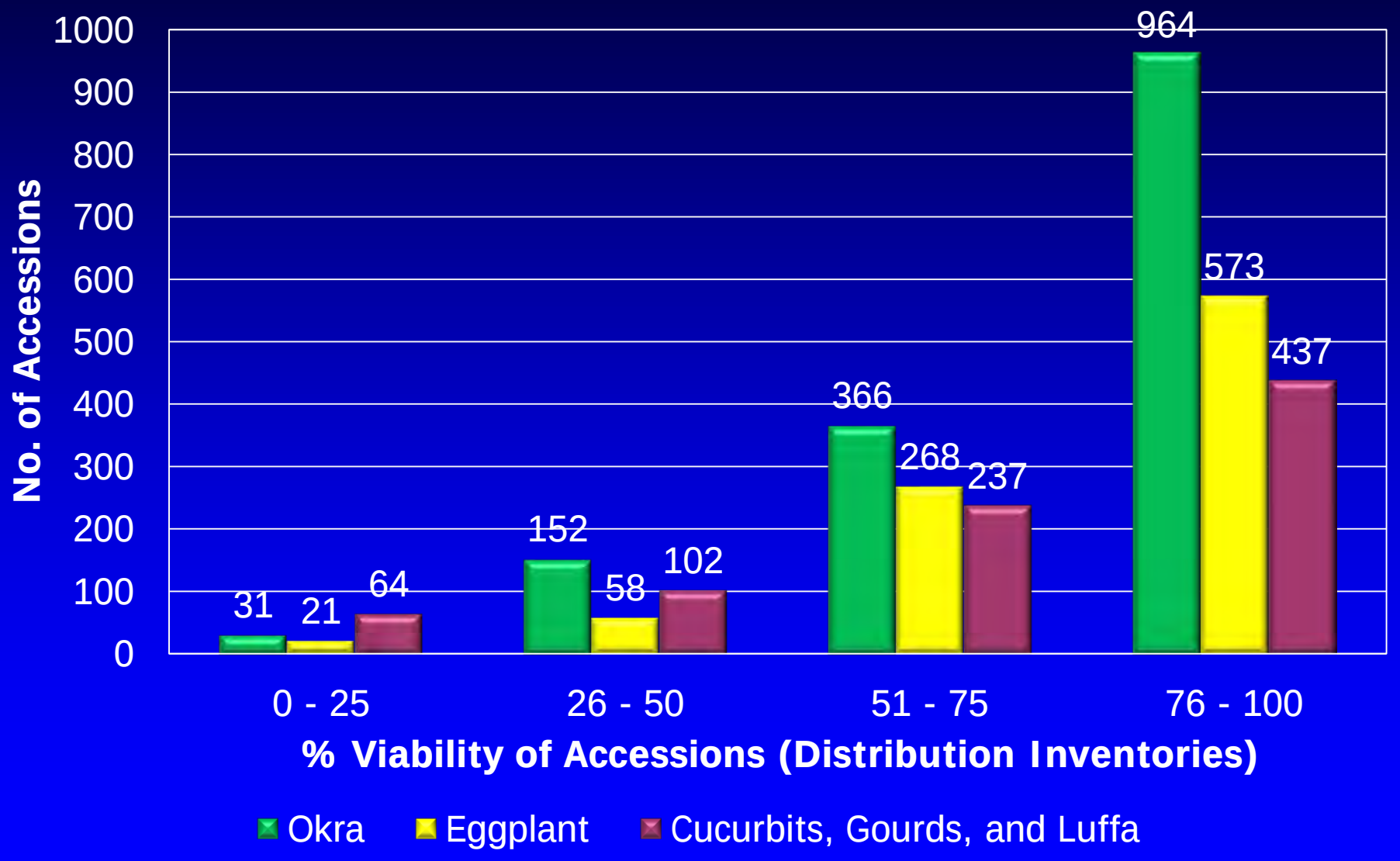
Pepper Accession Viability



Watermelon Accession Viability



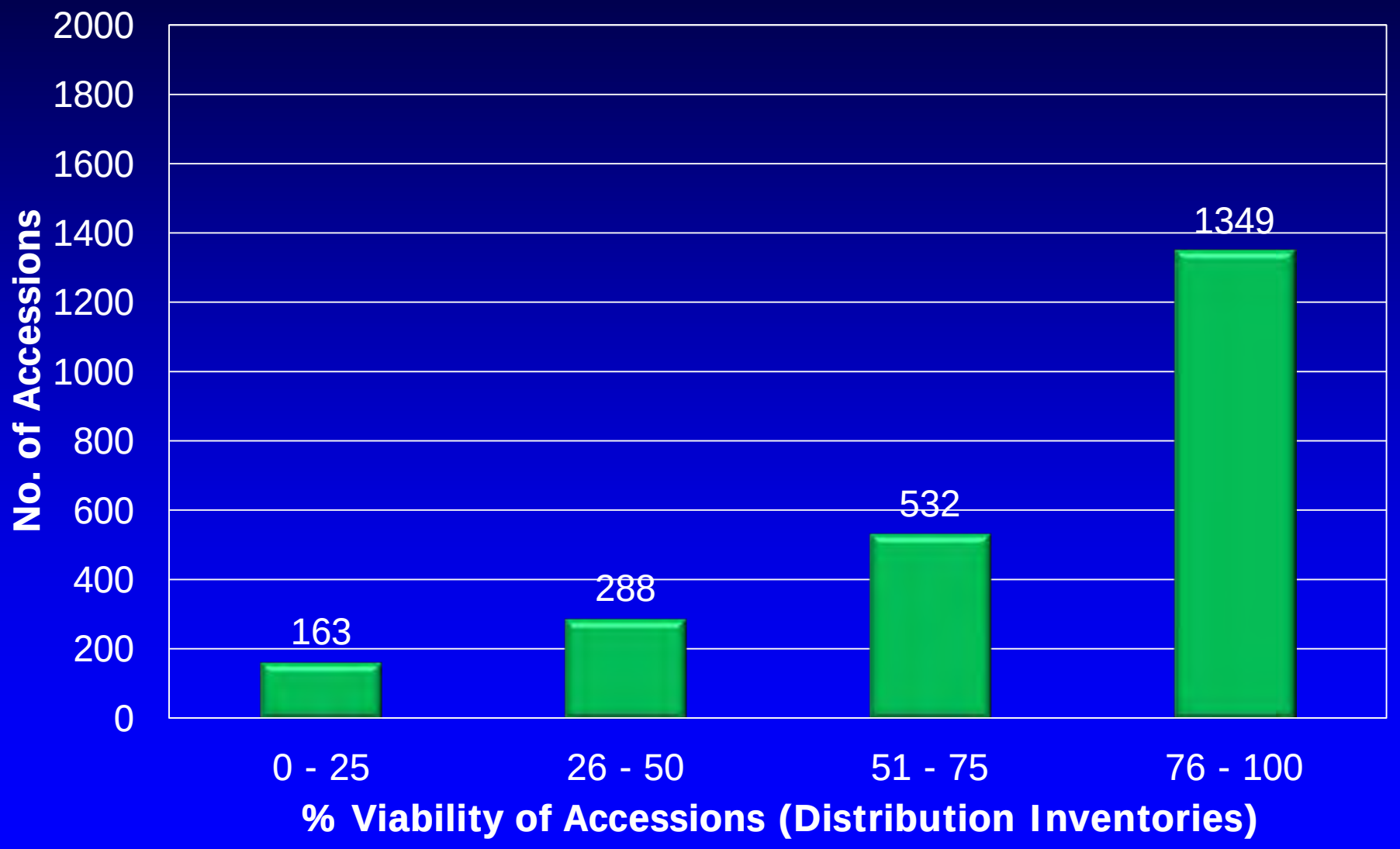
Okra, Eggplant, & Cucurbits Accession Viability



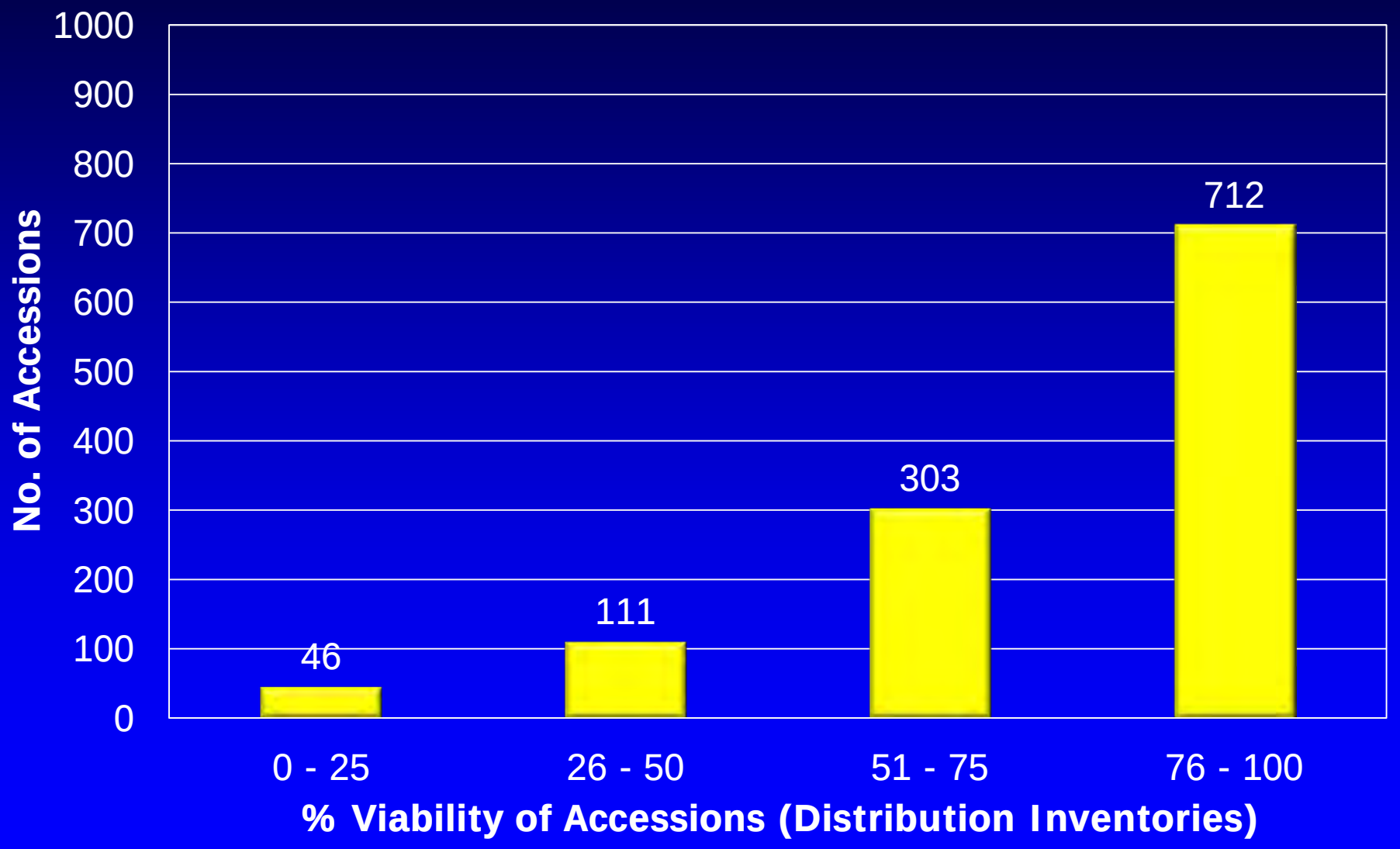
Legumes and Misc. Crops

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Brad Morris	Castor bean	378	164	377	250
	Hibiscus	346	280	344	86
	Legumes	3,083	2,342	2,997	**1,046**
	Guar, Winged Bean, Misc	721	557	633	213
	Sesame	1,216	1,172	1,216	**981**

Legume Accession Viability



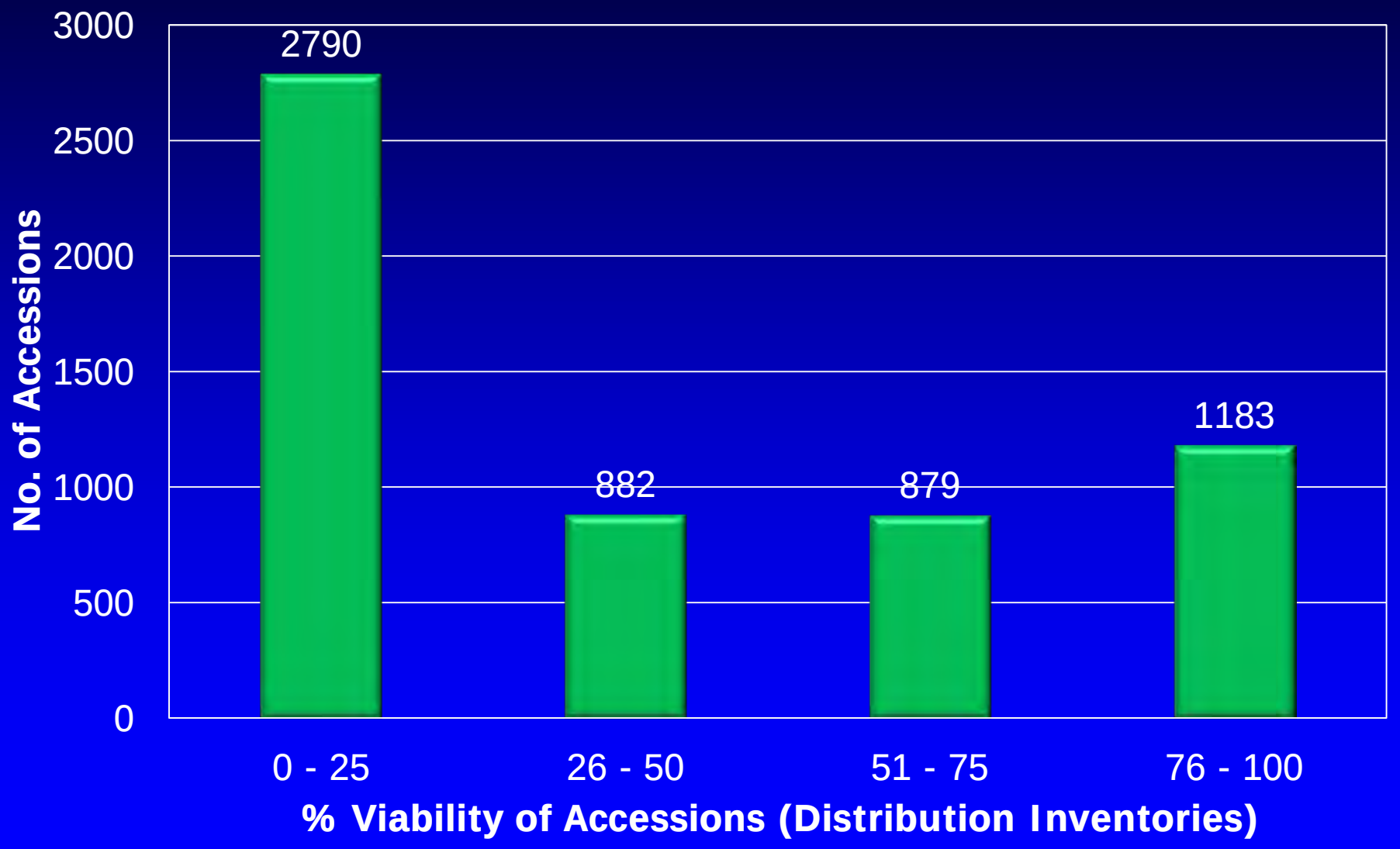
Sesame Accession Viability



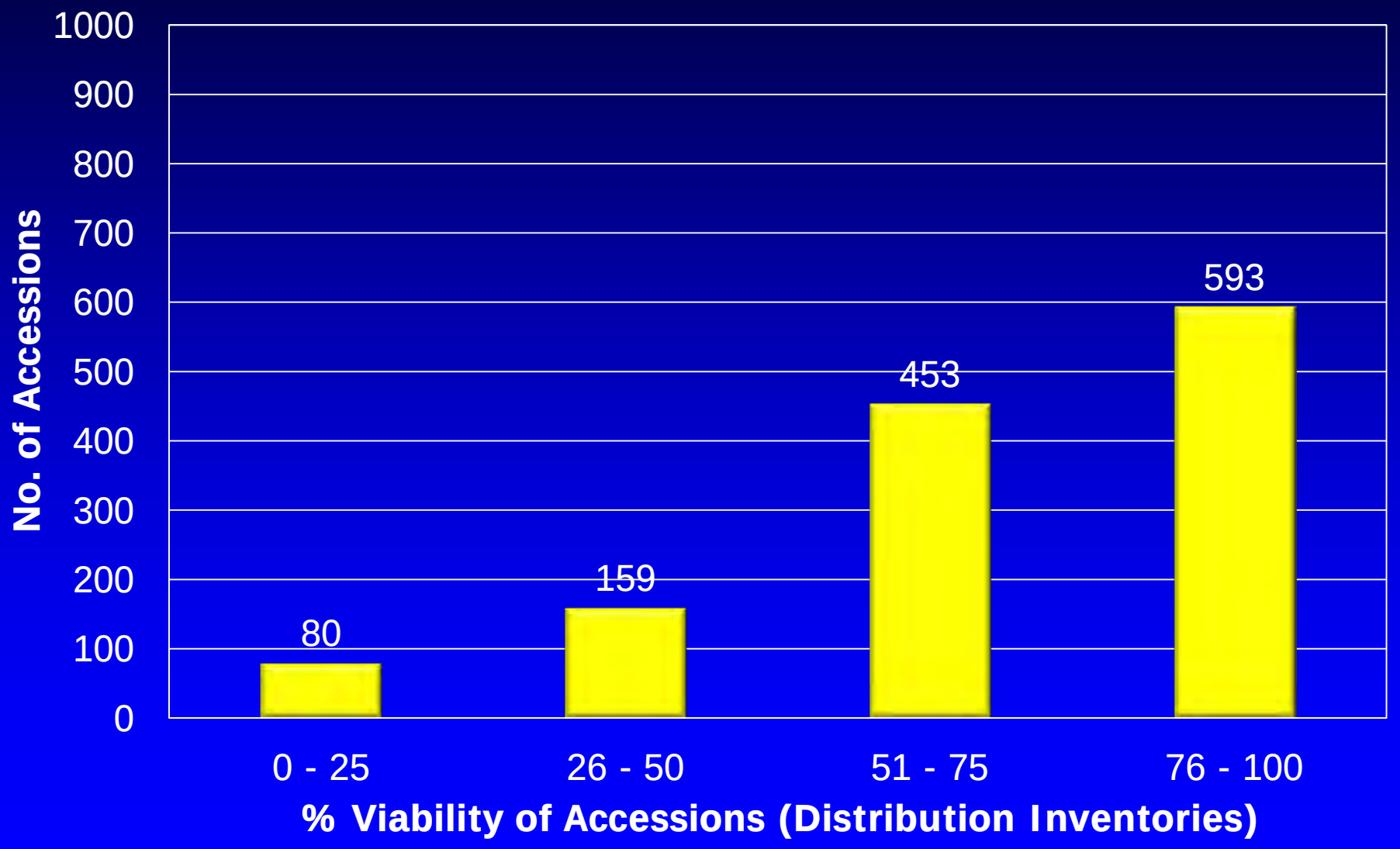
Warm-Season Grasses

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Melanie Harrison	Bamboo	96	96	2	16
	Grasses	7,488	6,114	6,866	**1,380**
	Pearl millet	1,297	1,283	1,291	361

Warm-Season Grass Accession Viability



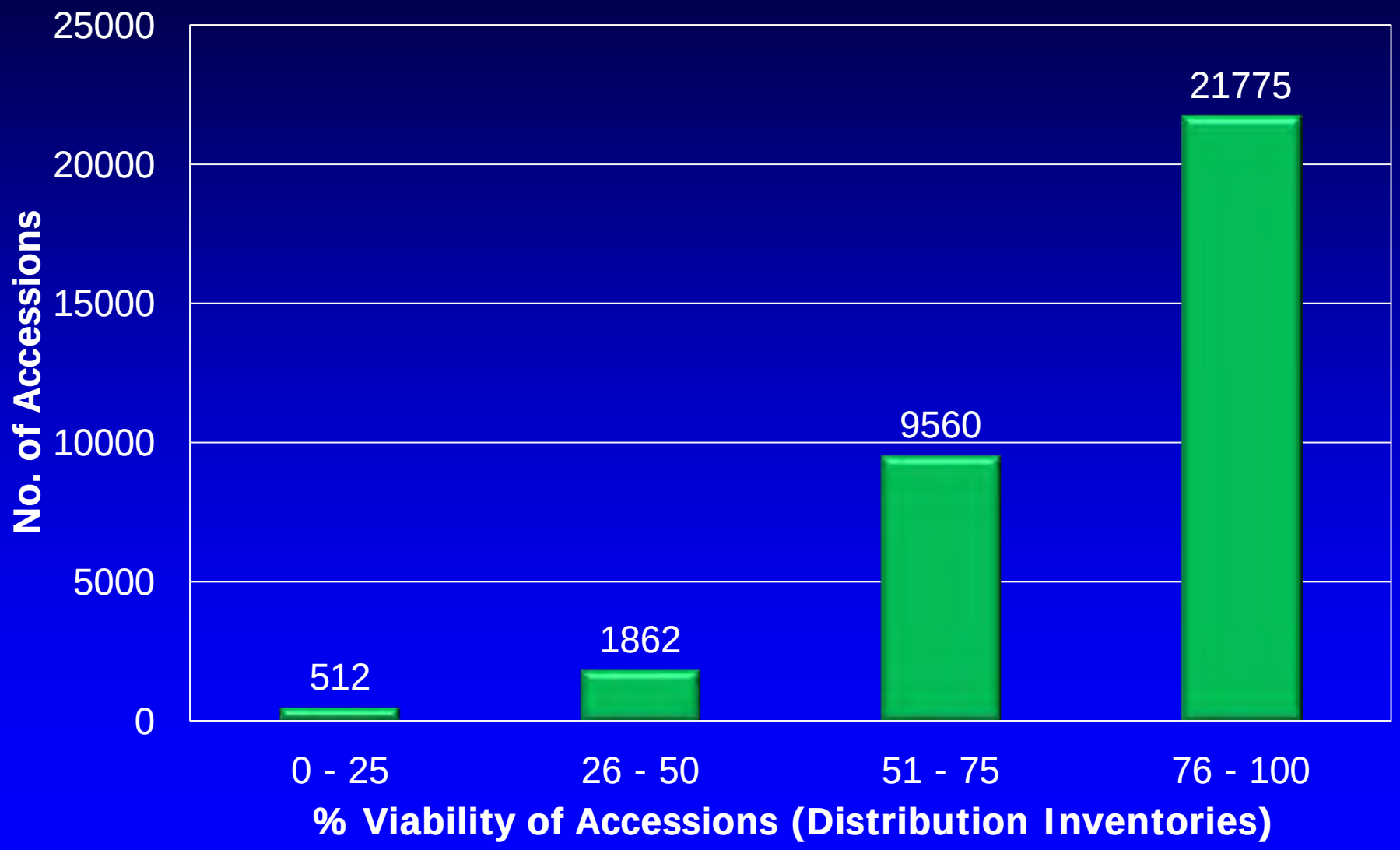
Pearl Millet Accession Viability



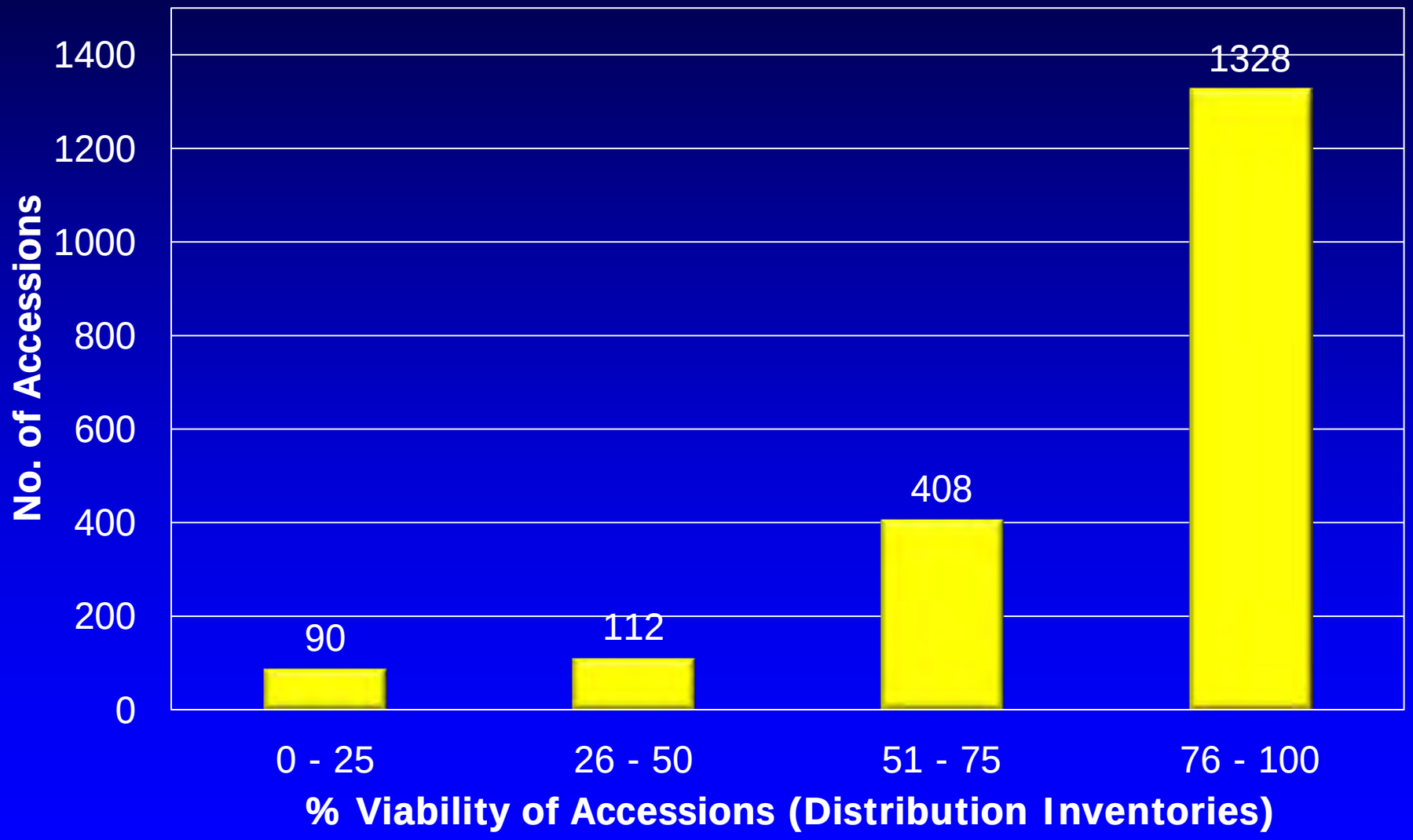
Clover and Sorghum

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Gary Pederson	Annual Clover	2,245	2,004	2,228	487
	Sorghum	38,149	37,314	37,367	**13,878**

Sorghum Accession Viability



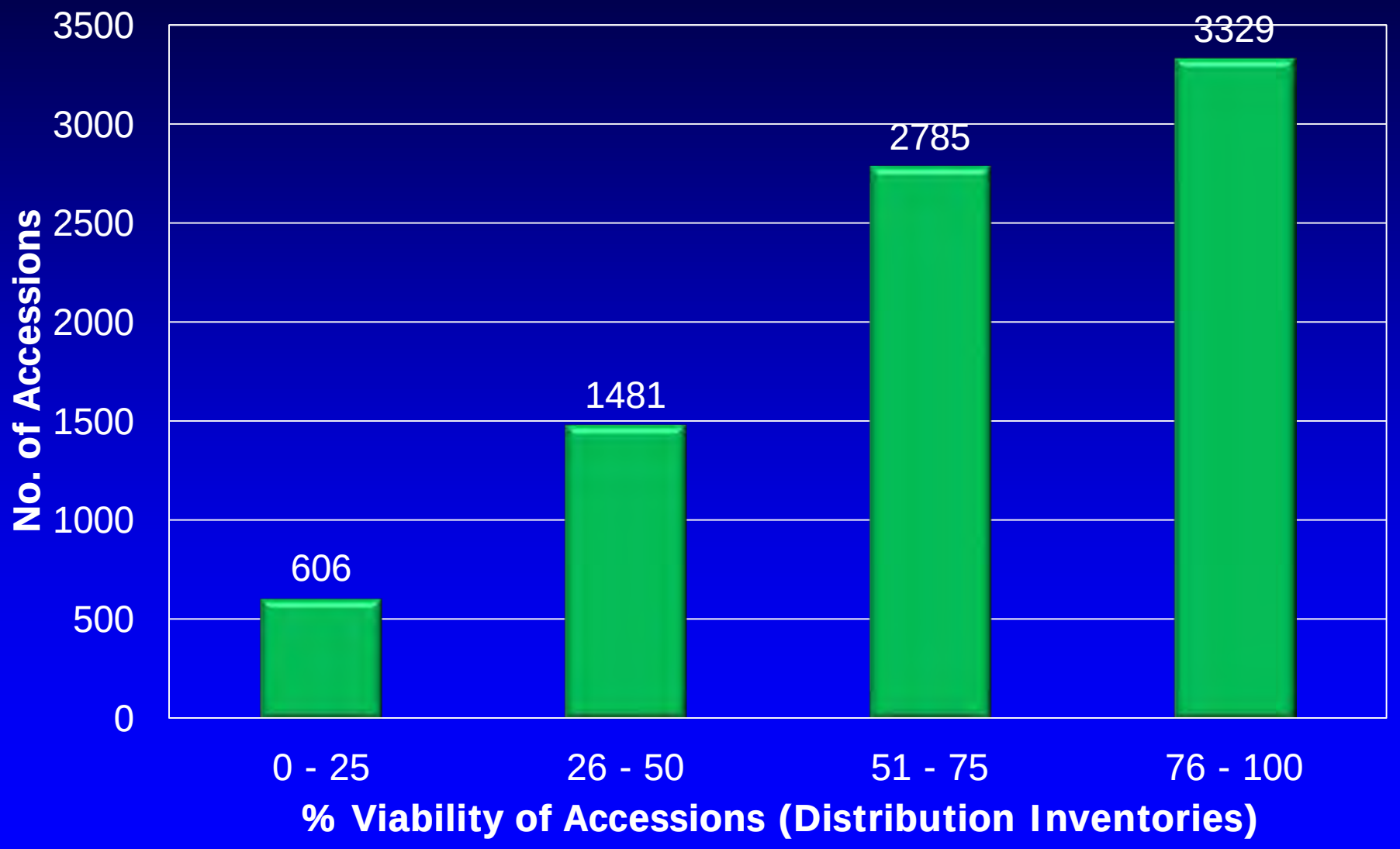
Annual Clover Accession Viability



Peanuts

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2013
Noelle Barkley	Cultivated Peanuts	9,321	8,299	9,067	**1,633**
	Wild Peanuts	655	499	438	75

Peanut Accession Viability



Number (and %) of accessions that are unavailable

Crop	2013	2014	Crop	2013	2014
Cowpea	1,428 (17%)	1,341 (16%)	Hibiscus	62 (18%)	66 (19%)
Mung bean	387 (9%)	389 (9%)	Legumes	760 (25%)	741 (24%)
Cucurbits	883 (62%)	908 (64%)	Sesame	43 (4%)	44 (4%)
Eggplant	106 (10%)	102 (10%)	Grasses	1,338 (18%)	1,374 (18%)
Okra	1,456 (49%)	1,457 (49%)	Pearl millet	51 (4%)	14 (1%)
Peppers	80 (2%)	75 (2%)	Annual clover	252 (11%)	241 (11%)
Sweetpotato (TC)	32 (4%)	32 (4%)	Sorghum	1,348 (4%)	835 (2%)
Watermelon	390 (20%)	428 (22%)	Cultivated peanuts	1,156 (12%)	1,022 (11%)
Castor bean	209 (55%)	214 (57%)	Wild peanuts	193 (31%)	156 (24%)
			TOTAL	**11,158 (12%)**	**10,431 (11%)**

Digital photos

Crop	Images
Sorghum	8,957
Cowpea	2,208
Watermelon	1,825
Pepper	1,789
Grass	1,086
Cucurbit	826
Peanut	662
Pearl millet	451
Other crops	447
Total	18,611 (20.2%)

Accessions in -18 C storage

(Accessions with seeds only, seed splitting initiated in 2001)

Crop	Accessions	%	Crop	Accessions	%
Sorghum	29,703	**77.9**	Watermelon	1,904	99.8
Peanuts	9,256	**99.3**	Cucurbits	1,405	98.9
Grasses	6,814	95.3	Sesame	1,216	100.0
Peppers	4,928	99.9	Eggplant	1,020	99.8
Mung bean	4,074	96.3	Cowpea	838	10.2
Legumes	3,078	99.9	Gourds	478	98.0
Okra	2,965	99.8	Ipomoea spp.	454	98.5
Clover	2,228	99.2	**TOTAL**	**72,761**	**80.2**

Requested for regeneration in CY2014

Crop	# accessions	Crop	# accessions
Cowpea	96	Grasses	145
Sorghum	1,268	Legumes	169
Cucurbit	26	Wingbean	32
Clovers	7	Cult peanut	178
Peppers	19	Wild peanut	48
Sesame	8	Mungbean	6
Ipomoea sp.	8	Castor bean	5
Watermelon	36	Misc crops	32
Eggplant	2		

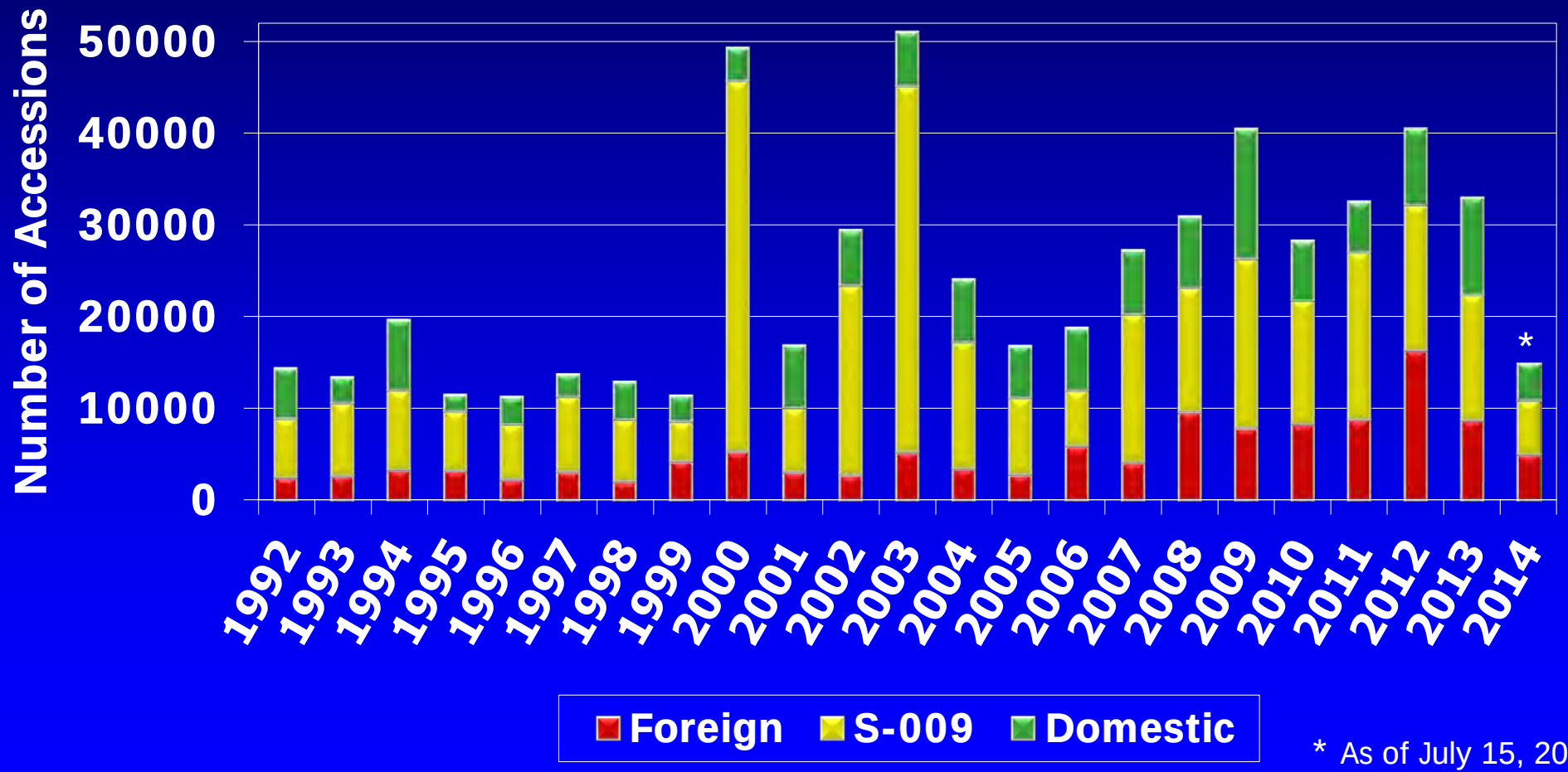
Regenerations by others (since last S-009 meeting)

- 51 cowpea and 1 cucurbit accessions
  - R. Goenaga, USDA, ARS, Puerto Rico
- 1,243 sorghum, 15 legume, and 6 wingbean accessions
  - A. Quiles, USDA, ARS, St. Croix
- 22 watermelon and 5 cucurbit accessions
  - X. Zhang, Syngenta, Woodland, CA
- 21 watermelon accessions
  - J. Green, Monsanto, Woodland, CA
- 533 peanut accessions
  - 358 accessions, J. McKinney, Univ. Florida, Citra, FL
  - 75 accessions, K. Chamberlin, USDA, ARS, Stillwater, OK
  - 100 accessions, C. Holbrook, USDA
- 189 wild peanut accessions
  - T. Stalker, North Carolina State Univ., Raleigh, NC

Distributions in CY2013

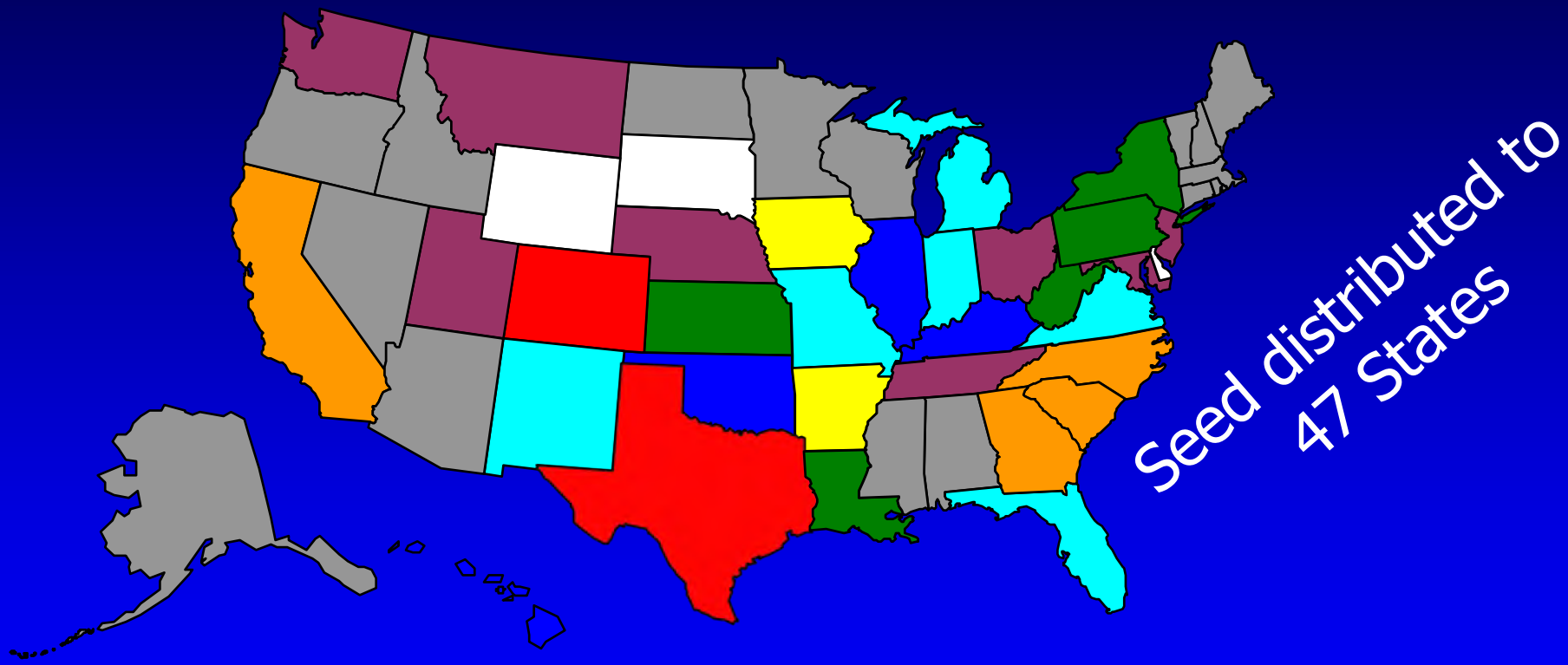
- Domestic = 24,230 items in 889 orders
  - S-9 region = 13,685 items
- Foreign = 8,728 items in 201 orders
- Total CY2012 distributions = 32,958 items

Distributions



* As of July 15, 2014

Domestic Distributions in CY2013



Total number of accessions by state

2,500 to 7,500	500 to 749	50 to 99
1,000 to 2,499	250 to 499	1 to 49
750 to 999	100 to 249	0

Distributions outside of 50 U.S. states in CY2013

Argentina	Dominican Republic	Iraq	Netherlands	Spain
Australia	Egypt	Israel	Nigeria	Taiwan
Austria	France	Italy	Pakistan	Thailand
Belgium	Germany	Japan	Peru	Turkey
Bolivia	Hong Kong	Lithuania	Poland	Ukraine
Canada	Hungary	Malaysia	Puerto Rico	United Arab Emirates
China	India	Mexico	South Africa	United Kingdom
Czech Republic	Indonesia	Morocco	South Korea	U.S. Virgin Islands

Equipment purchased

- 1,558 injection-mold, seed storage trays (SAA funded)
- Two Seedburo blowers for seed cleaning
- Double-size Percival incubator for maintaining sweetpotato tissue cultures
- One 150 HP tractor for pulling larger 3-point-hitch soil preparation equipment (SAA partially funded)

Building and Cold Room Construction

- ARS Seed Processing Building Addition
  - Contains new 4 C cold room (20 x 30')
  - Existing 4 C cold room in State Seed Storage building converted to -18 C
  - Total -18 C seed storage space = 1,897 sq ft (increased from 1,061 sq ft) to house bulk seed of entire Griffin collection in -18 C

Facility Repair and Maintenance

Items completed

- Repaired ceiling in Seed Cleaning Room due to waterline that froze and burst. Repairs included new copper waterline, insulation, ceiling drywall, painting, and light fixtures.
- Upgraded LAN facilities in three buildings.

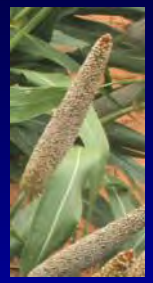
Items underway

- Convert T-12 fluorescent lighting fixtures to more efficient T-8 lighting fixtures (SAA funded).
- Replace existing racks and pinions for roof vents and replace old shade cloth system for all 10 bays in the main greenhouse complex.

PGRCU Field Day – August 21, 2013

PLANT GENETIC RESOURCES CONSERVATION UNIT
S-009 MULTISTATE PROJECT
GRIFFIN, GEORGIA
AUG. 21, 2013

Welcome to Field day 2013

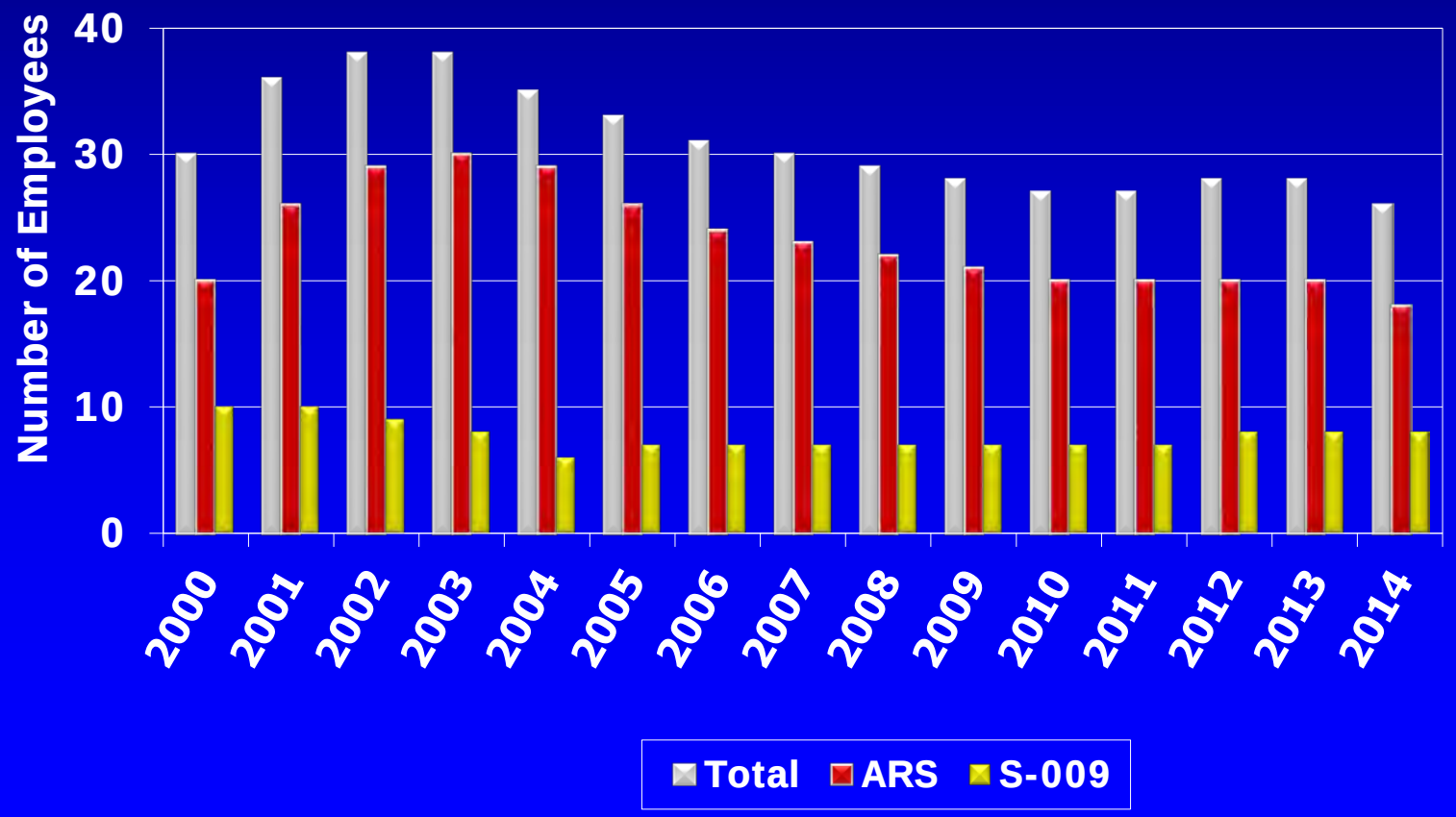


Total PGRCU Funding

- ARS base funding
  - FY2012 = $2,318,940
  - FY2013 = $2,140,251 ($178,689 decrease)
  - FY2014 = $2,410,251 ($270,000 increase)
- S-009 base funding
  - FY2013 = $417,723
  - FY2014 = $431,723 ($14,000 increase)
  - FY2015 = $450,625 ($18,902 increase)

Staffing summary

- Current staff is 26 employees (18 ARS and 8 S-009)



Funding – Last year

- All ARS salaries frozen FY2011-FY2013
- All State salaries frozen FY2010-FY2014
- ARS travel capped at 70% of FY2010 travel amounts
  - Included work travel to plant and harvest regenerations at remote locations
  - Included all soft funded travel from outside sources

Impact of FY2013 budget reduction

- After budget reduction of $178,689, federal salaries comprised 89.0% of ARS FY2013 budget.
- Travel was reduced to 31% of FY2010 actual travel.
- Office of National Programs provided $90,000 to help fund essential PGRCU ARS operations through FY2013.
- All S-009 temporary positions (other than weekend watering) were terminated on Jan. 1, 2013.
- S-009 funds provided the majority of supplies to maintain PGRCU operations.

Current Financial Situation

- Vigna curator (Roy Pittman) and technician (James Chalkley) positions were terminated upon retirement in Jan. 2014 to free up discretionary funds.
- Office of National Programs provided $90,000 in Feb. 2014 to help fund essential PGRCU ARS operations.
- ARS budget for Griffin location increased $300,000 ($270,000 net) in permanent funds in April 2014.
- ARS salaries increased 1% (FY2014) and State salaries increased average of 4% (FY2015).
- Travel cap (70% of FY2010) is still in place, however soft funded travel no longer counts against the cap.

Current Grants

Funded

- $100,000 over 2 yrs – Sorghum Checkoff Program (sorghum)
- $ 40,000 over 4 yrs – USAID (peanuts)
- $ 20,000 – Mars (bamboo)
- $ 7,000 – Peanut Foundation (peanuts)

Pending

- $ 20,750 – Peanut Foundation (peanuts)
- $ 11,250 – Georgia Peanut Commission (peanuts)
- $ 6,000 – Southeast Reg Research Initiative (peanuts)

Financial Summary

- With ARS discretionary funds available, curators and scientists again have funds to support operations.
- Five S-009 temporary employees are being hired for 2-6 months to support laboratory, grass curation, legume curation, peanut curation, and pepper curation programs.
- Six ARS temporary employees have been hired from sorghum grant funds to support sorghum seed cleaning, processing, and distribution. These employees will help free up time for permanent seed storage staff to work on other crops.
- No permanent ARS employees were hired from increased funds, so limited personnel continues to be a concern.

Plant Genetic Resources Conservation Unit

Appendix 3

DR. MING LI WANG

CHARACTERIZATION AND UTILIZATION OF THE USDA SESAME GERMPLASM COLLECTION BY BIOCHEMICAL AND GENETIC ANALYSIS

Characterization and Utilization of the USDA Sesame Germplasm Collection by Biochemical and Genetic Analysis

Ming Li Wang
Molecular and Biochemical Genetics Laboratory
PGRCU

S9 Meeting, July 22, 2014

Outline of My Talk

I. Introduction of Sesame

II. Evaluation of the Entire Collection by Chemical Analysis

III. Confirmation of the Unique Accessions by Replanting

IV. Identification of the Genetic Difference by Sequencing

V. Development of New Germplasms/cultivars by Mutagenesis

Introduction of Sesame

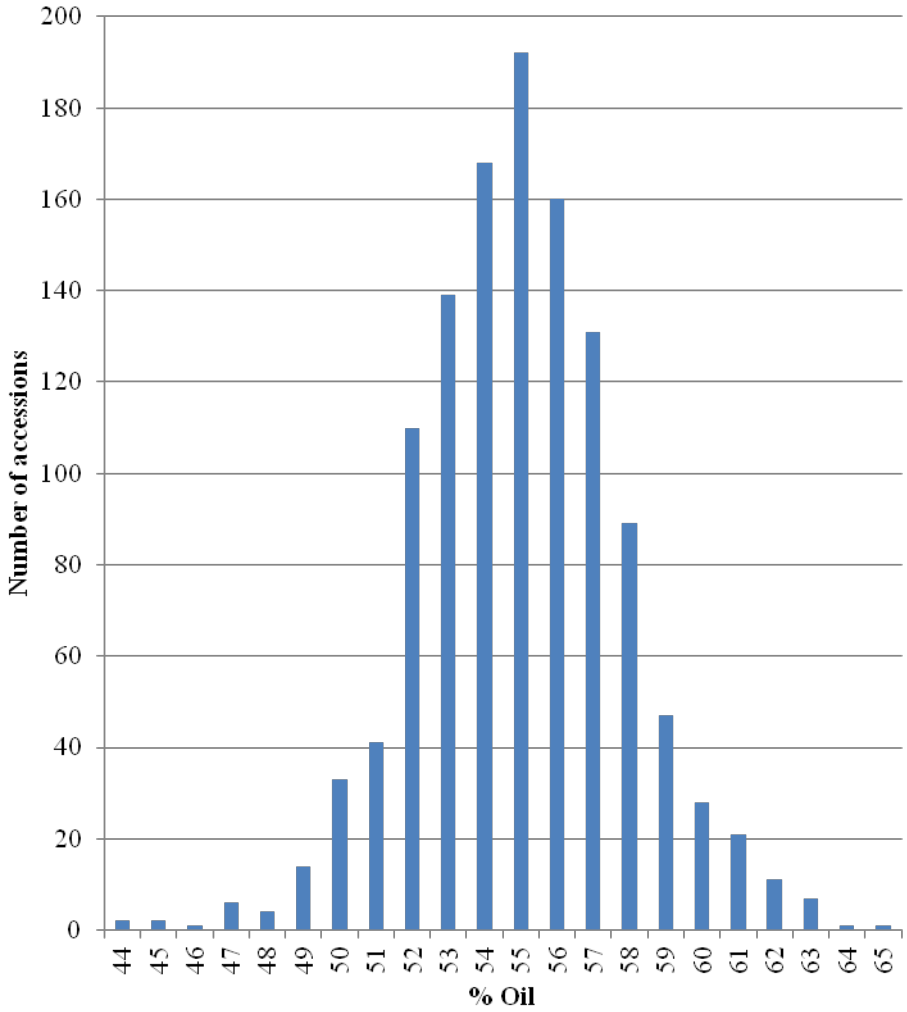
World Production and Utilization

- Production
- Utilization
- Major Cultivation Problems
  Indeterminate growth and seed shattering

Sesame Genetics and Cross Pollination

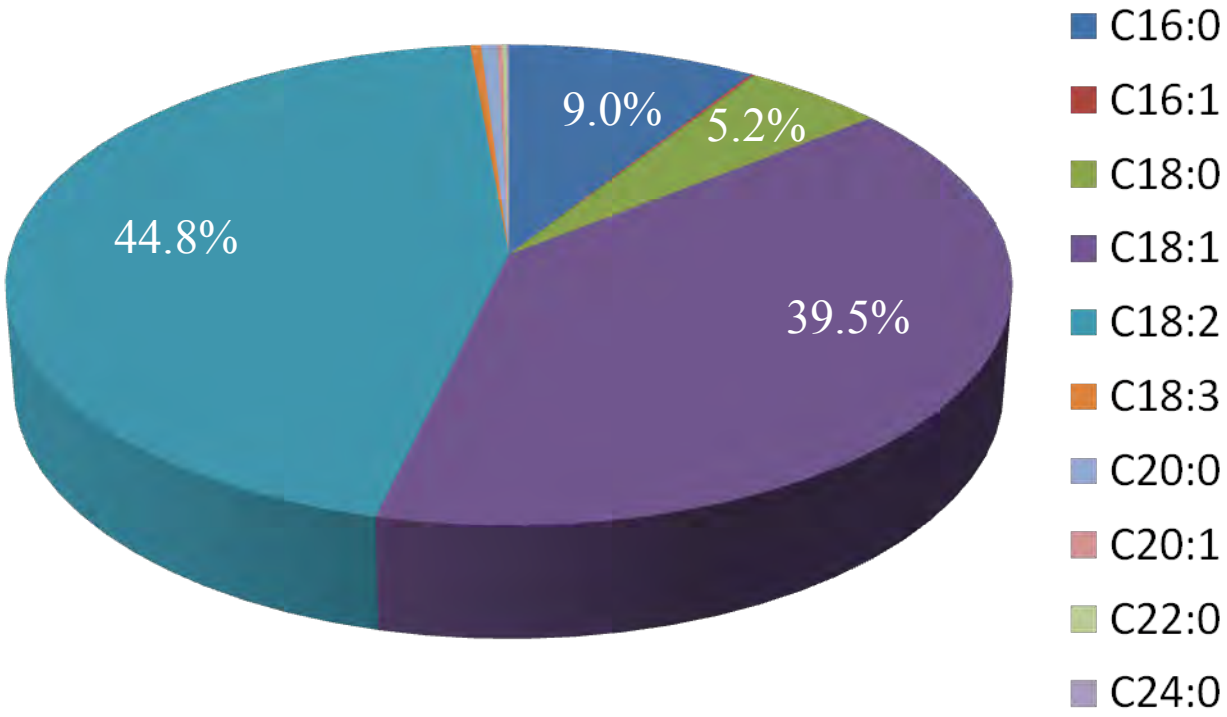
- Low path genome sequence available
- Cross pollination by bee visitation
- Variable basic chromosome number (x)
  ($2n = 26$, $2n = 32$, and $2n = 64$)

Range and Sample Distribution of Oil Content in the Entire USDA Sesame Germplasm Collection

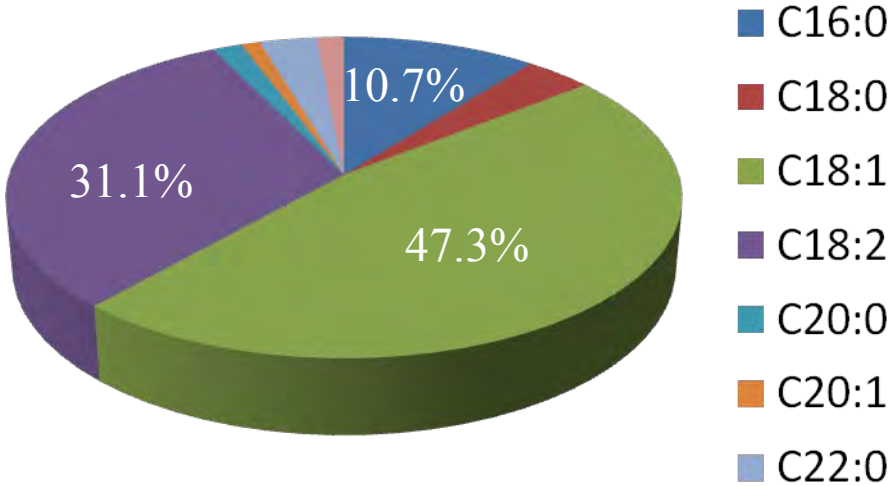


N = 1,232
Mean = 54.9%
Min = 25.7%
Max = 65.5%
6 accessions
< 35.0%

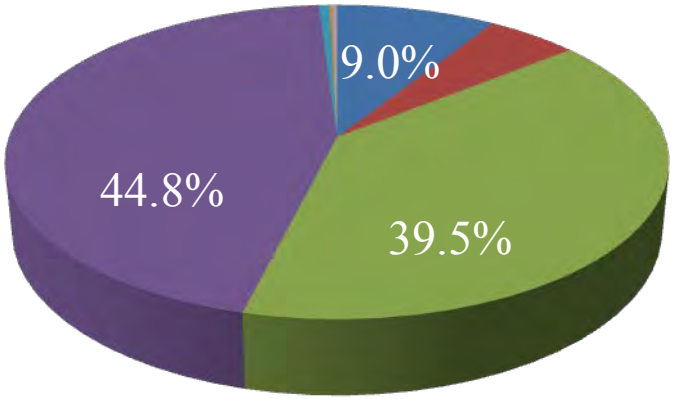
Fatty Acid Composition of Sesame Seeds



Comparison of Fatty Acid Composition and Oil Content between Peanut and Sesame Seeds

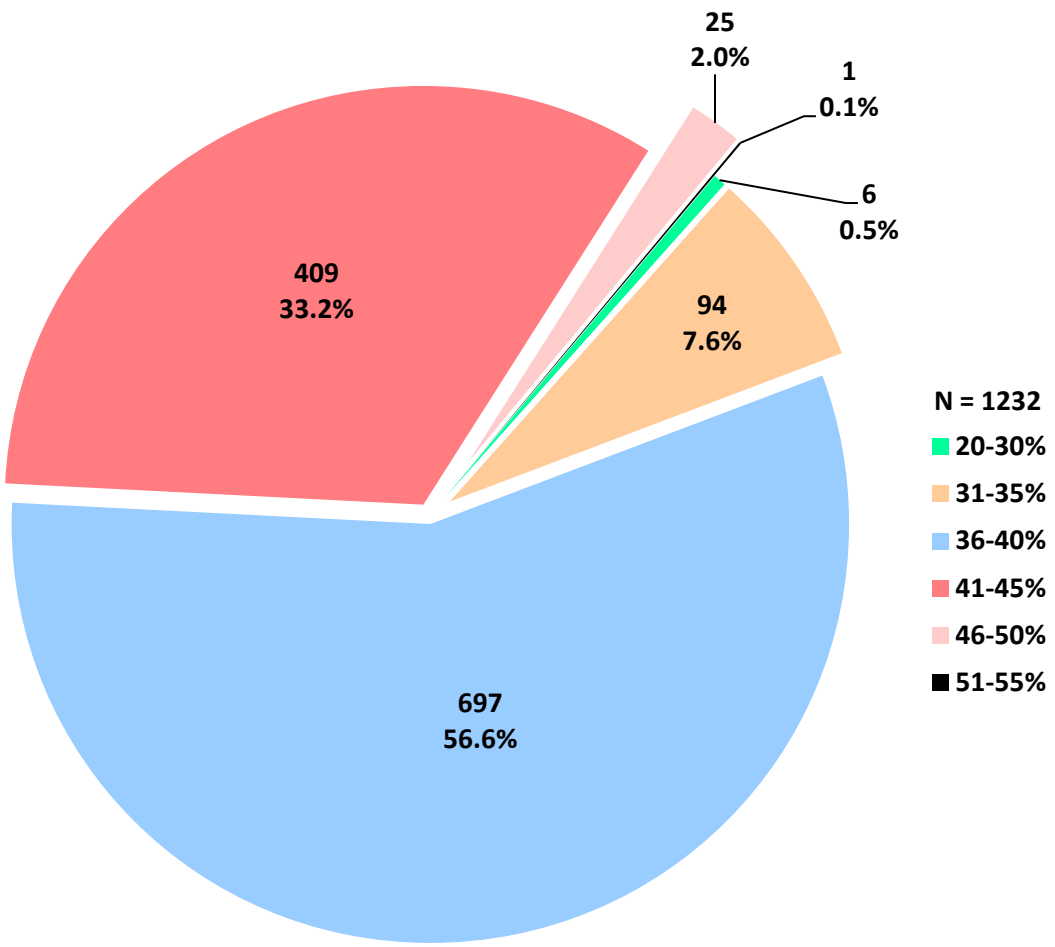


Peanut
50% oil



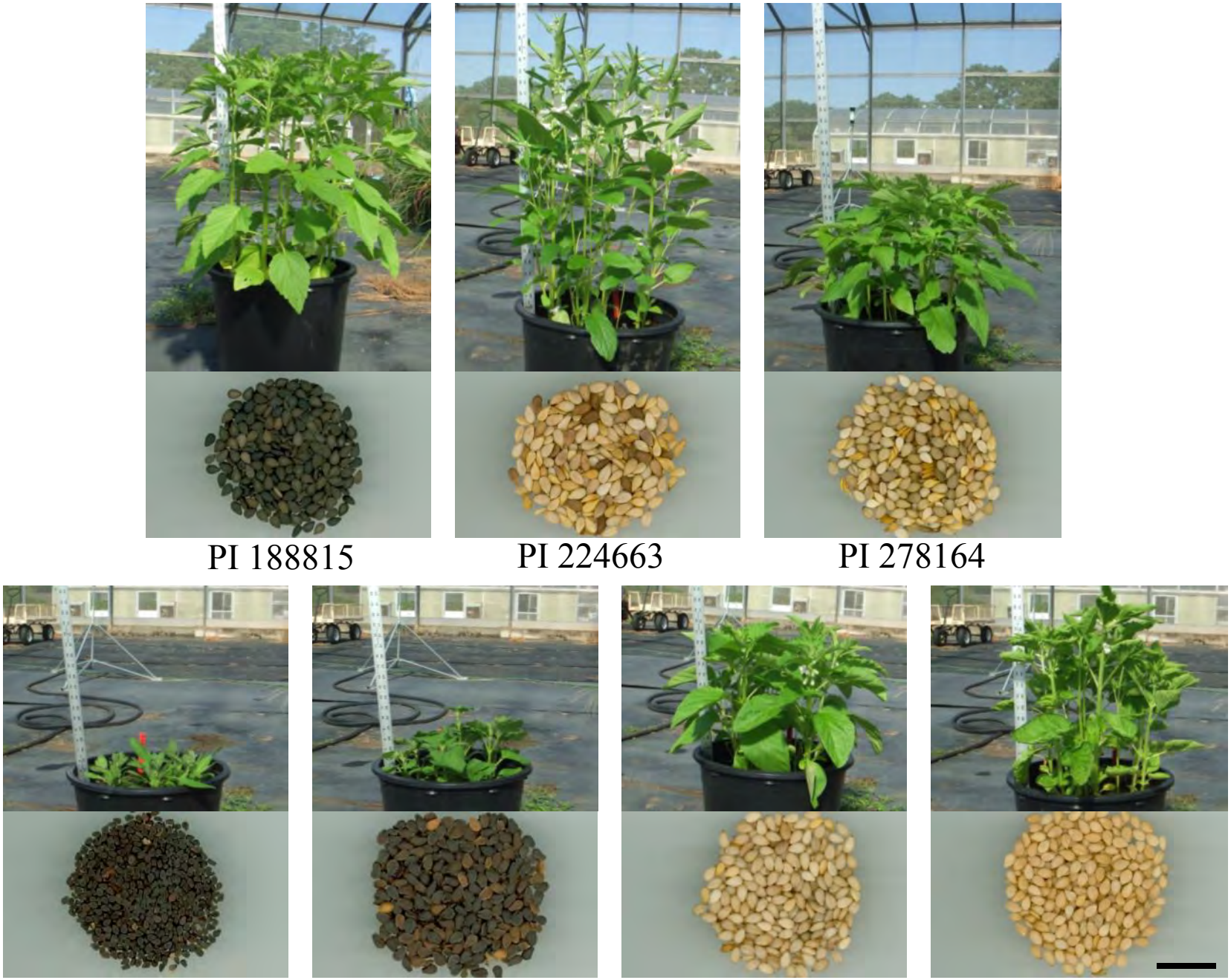
Sesame
55% oil

Classification of the Sesame Germplasm Based on the Level of Oleic Acid



Confirmation of the Results of Oil Content and Fatty Acid Composition by Replanting and Harvesting

PI number	Species	Collection site	Seed weight (g/100 Seeds)	Oil Content (%)	Oleic acid	Linoleic acid	O/L ratio
188815	*S. indicum* L.	Luzon, Philippines	0.185	57.81	30.96	53.22	0.58
224663	*S. indicum* L.	Libya	0.375	55.67	39.4	45.78	0.86
278164	*S. radiatum* Schum. & Thonn. ?	Angola	0.34	60.83	36.91	47.35	0.78
367899	*S. radiatum* Schum. & Thonn.	Tanzania	0.1	29.92	21.07	61.79	0.34
490268	S. indicum L. ?	India	0.225	31.82	27.08	54.5	0.5
599435	S. indicum L.	Texas, United States	0.265	46.76	48.81	34.99	1.4
599442	S. indicum L.	Texas, United States	0.325	49.25	50.07	33.49	1.5


PI 188815
PI 224663
PI 278164
PI 367899
PI 490268
PI 599435
PI 599442

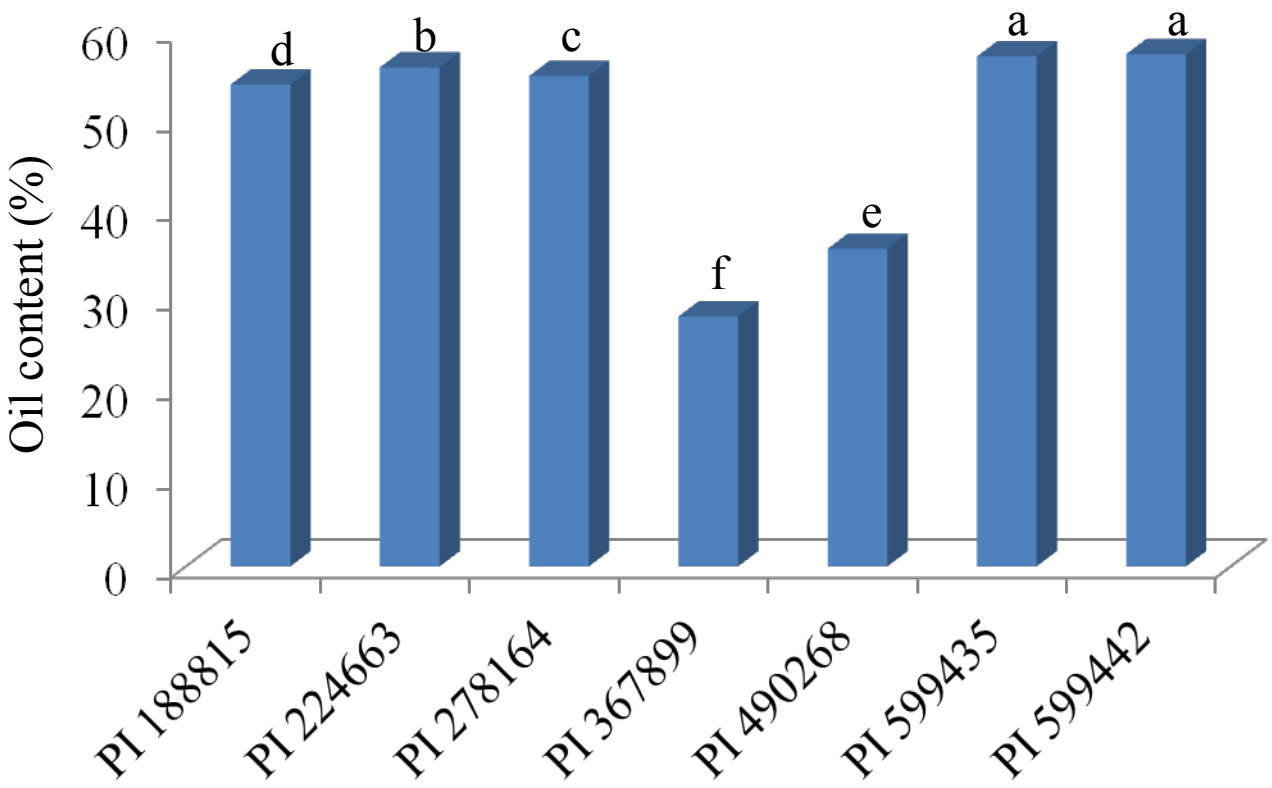
Confirmation by Plant Morphology Observation

PI 367899

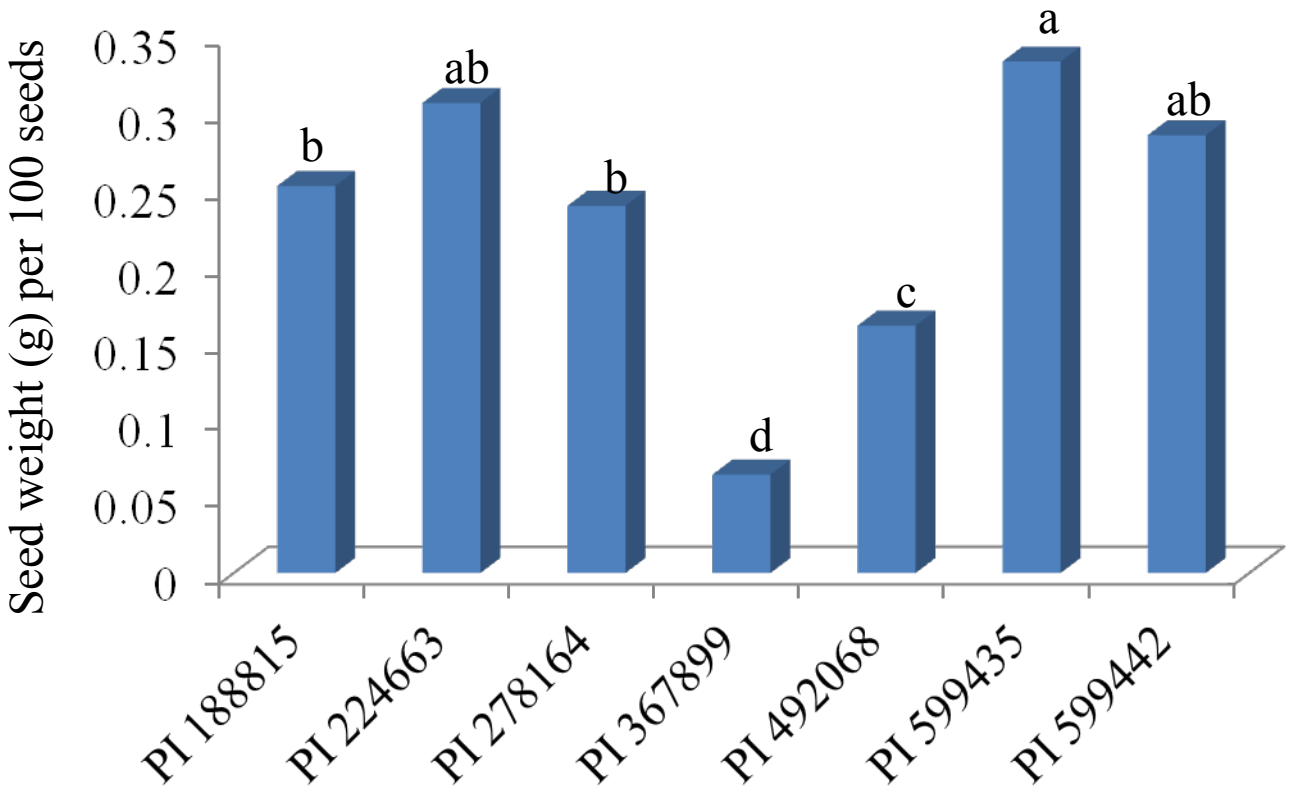
Not cultivated and definitely wild

PI 490268

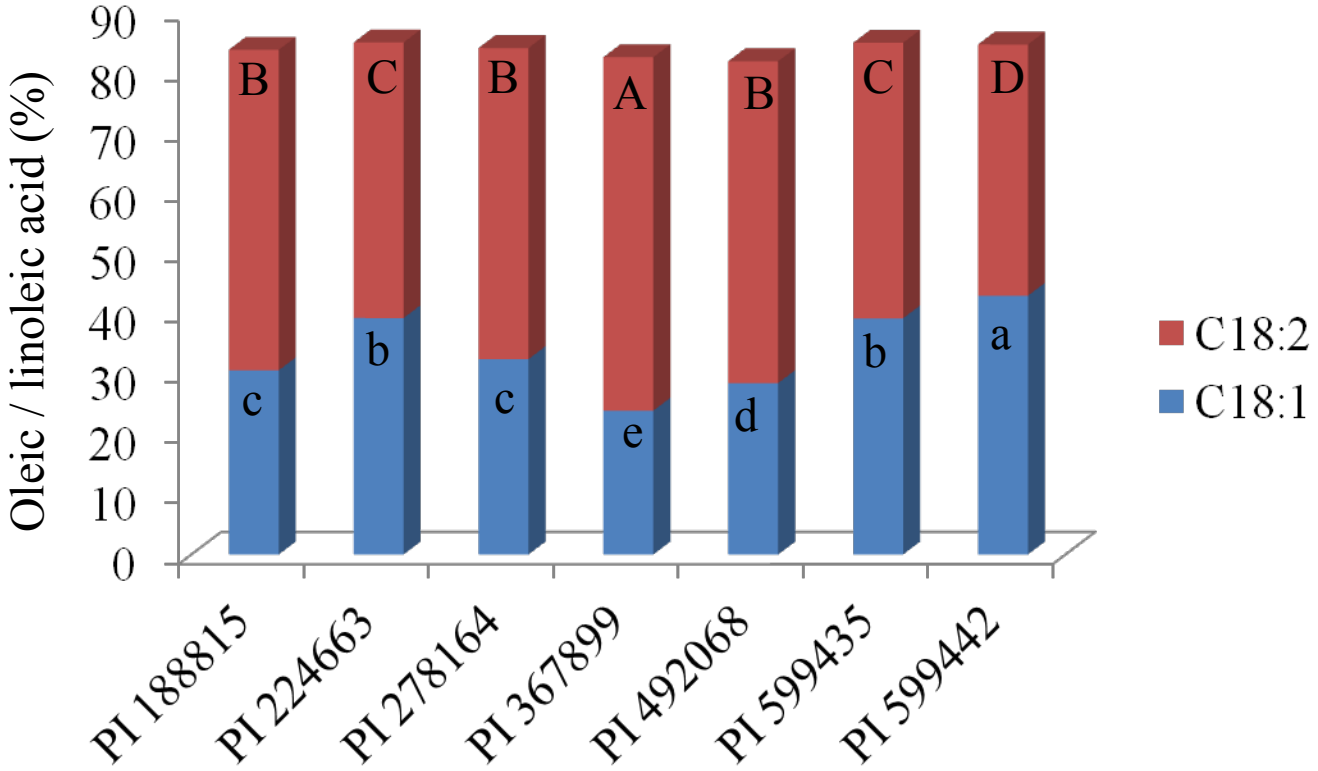
Confirmation by Seed Oil Content



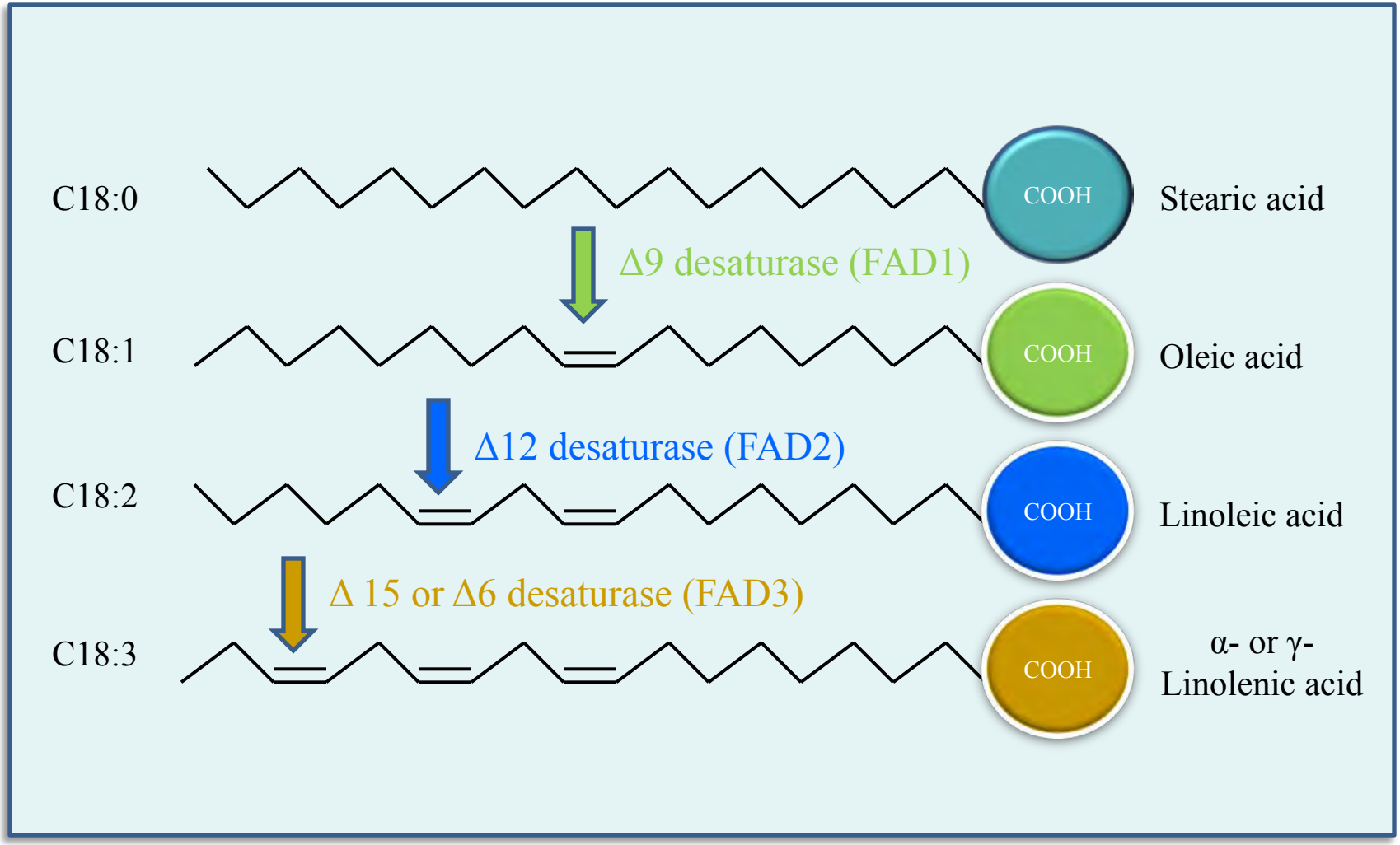
Confirmation by 100 Seed Weight



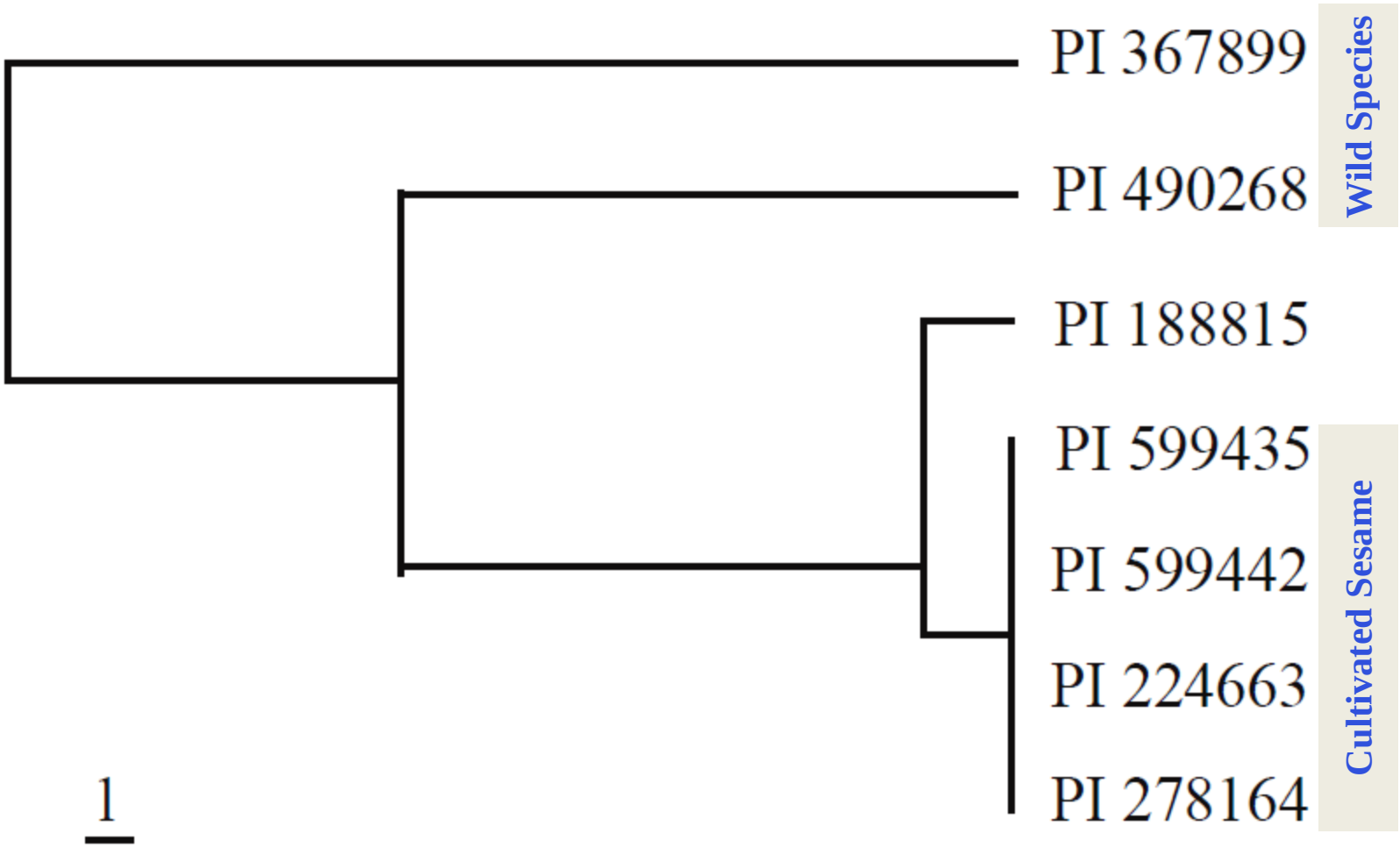
Confirmation by O/L Ratio



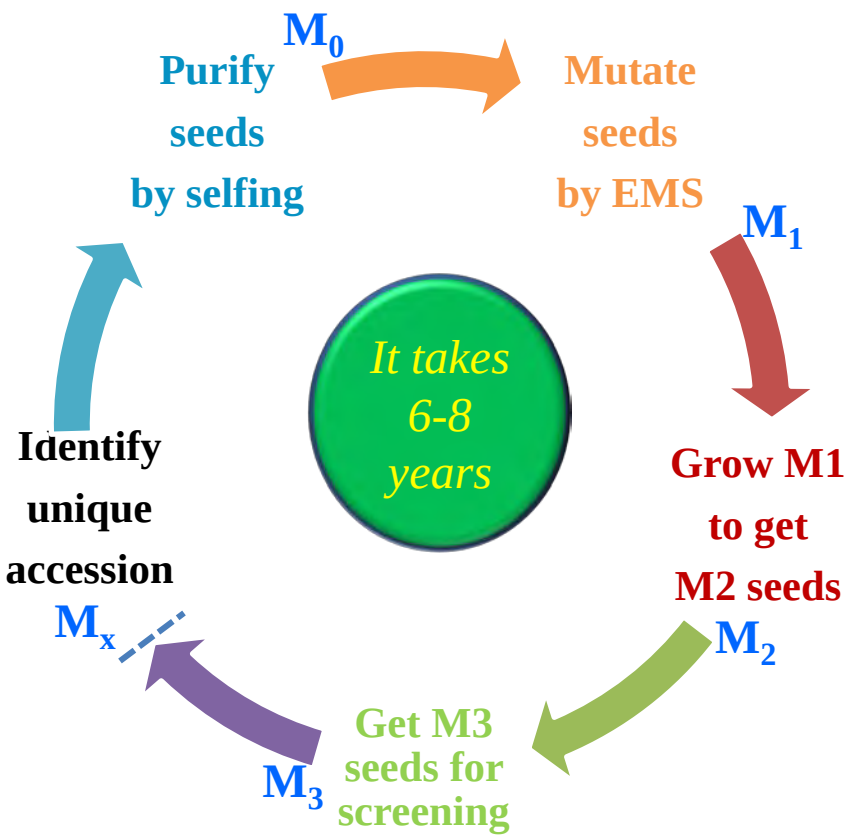
Desaturation of C18 Fatty Acids



Confirmation by Phylogenetic Analysis



Mutagenesis of Unique Line by EMS



Check
(without EMS)

M1 seedlings
(with EMS)

Thin seedlings from 4 to 1 in each pot

Using sticks to support check plants

Using sticks to support M1 plants

Growing 2,400 M1 plants in greenhouse

Setting too many capsules but no mature seeds

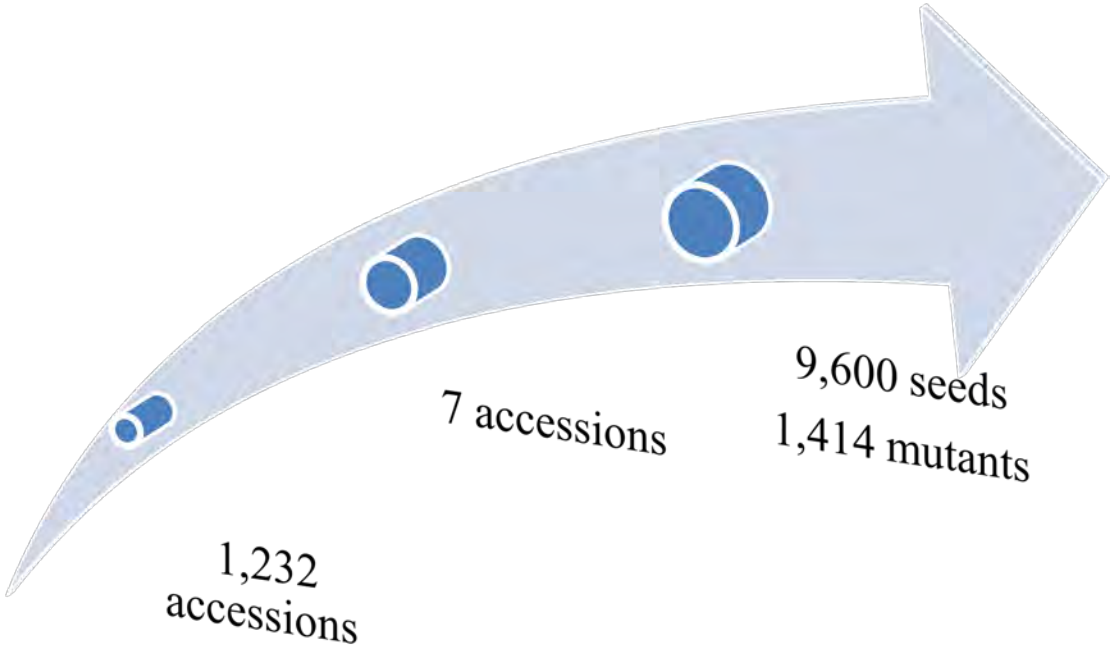
M1 abnormal plant with normal capsule

Planting M2 seeds for collection of M3 seeds

We should see 3:1 (1 A/A : 2 A/a : 1 a/a) segregation ratio

Starvation of M2 plants for collection of M3 seeds

Summary of My Talk



Acknowledgements

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Dr. Zhenbang Chen	University of Georgia
Mr. Ken Manley	PGRCU, UGA
Ms. Angie Lewis	PGRCU, UGA
Ms. Kami Lindberg	PGRCU, USDA-ARS
Ms. Morgan Beckham	Watering Temp
Dr. Noelle Barkley	PGRCU, USDA-ARS
Dr. Gary Pederson	PGRCU, USDA-ARS

Appendix 4

BRANDON TONNIS

ANALYSIS OF PRIMARY AND SECONDARY PLANT COMPOUNDS
AN OVERVIEW OF THE PGRCU CHEMISTRY LAB

ANALYSIS OF PRIMARY AND SECONDARY PLANT COMPOUNDS

AN OVERVIEW OF THE PGRCU CHEMISTRY LAB

GENETICS LAB HAS EXPANDED…

Lab originally designed with intent to measure genetic diversity in crop collections.

- Molecular markers: SNPs, SSRs, AFLPs
- Gene sequencing

Focus has gradually shifted

In the past 10 years, key instrument additions have allowed us to include chemical analysis.

- HPLC-DAD (2004) + RID (2009), FLD (2014)
- GC (2008)
- TD-NMR (2009)

PLANT COMPOUNDS FOR CHARACTERIZATION

Primary compounds

- **Protein:** total crude protein content, amino acid composition
- **Carbohydrates:** sugars, cellulose, hemicellulose
- **Lipids:** seed oil content, fatty acid composition

Secondary compounds

- Polyphenolics
- Tocopherols
- Phytosterols
- Capsaicinoids
- Alkaloids

PLANT PROTEIN CONTENT

Food Science: **Elementar rapid N-cube**

- Sample combustion, protein content calculated based on measured total nitrogen
- Protein measured in peanut mini-core and bamboo collection
- Currently working on additional bamboo samples in collaboration with USDA-TARS in Puerto Rico.

SEED OIL CONTENT BY TD-NMR

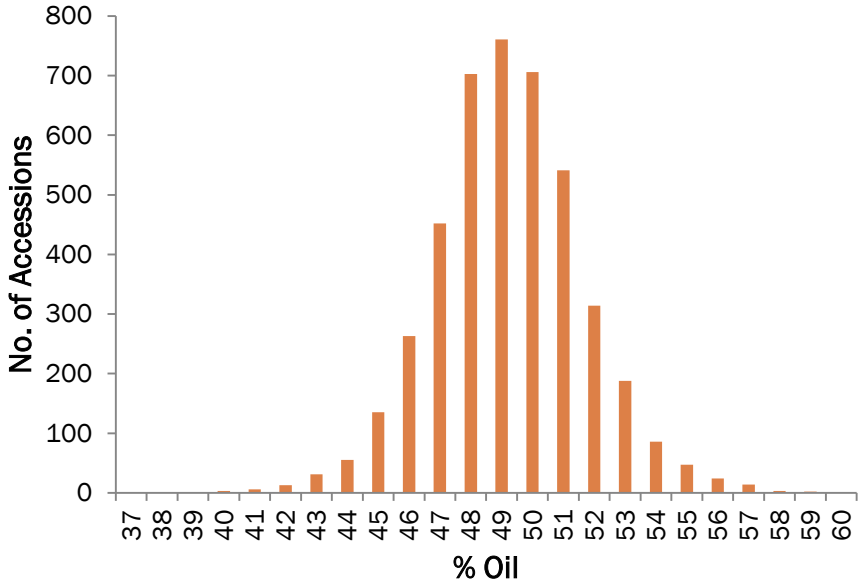
TD-NMR – distinguishes protons in lipids from the surrounding sample matrix and measures signal intensity against a standard curve.

Measurements are:

- fast
- accurate
- inexpensive, essentially no cost
- non-destructive
- repeatable

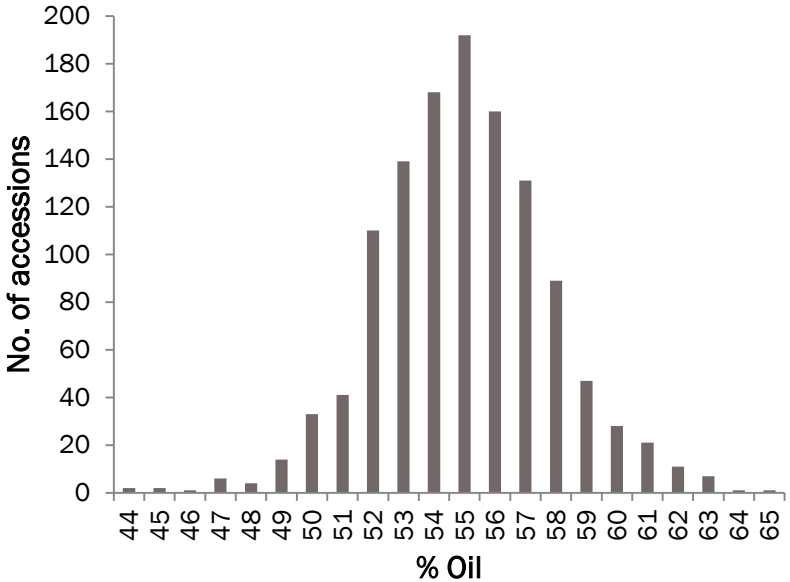
Large amounts of accurate data can be collected on seed oil content

Peanut Accessions - Seed % Oil Distribution



N	4349
Mean	49.219
Max	58.830
Min	37.637

Sesame Accessions – Seed % Oil Distribution



N	1208
Mean	54.919
Max	65.479
Min	28.273

Other crops measured: castor (entire collection), Hibiscus (entire collection), Desmodium, okra, soybeans, watermelon, etc.

GAS CHROMATOGRAPH

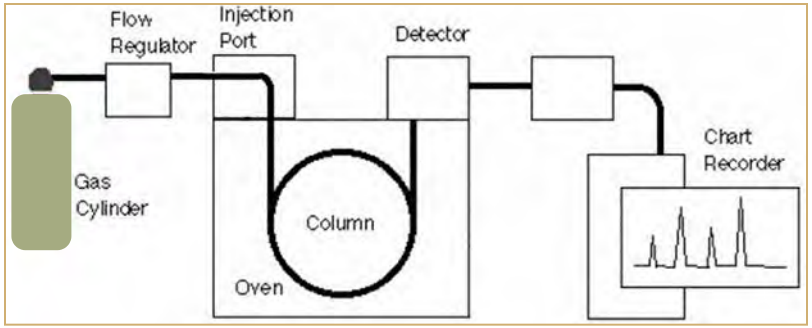
Efficient and quick separation and detection

Compounds must be volatile and thermally stable (20% of compounds)

Relatively high throughput

All carbon-containing compounds are detected

Compounds identified by reference to known standards



http://www.boomer.org/c/p3/c03/c0306.html

FATTY ACID COMPOSITION

Measured using gas chromatography.

Seed triglycerides are extracted in an organic solvent and esterified in the presence of a catalyst (acid or base) to form FAMEs.

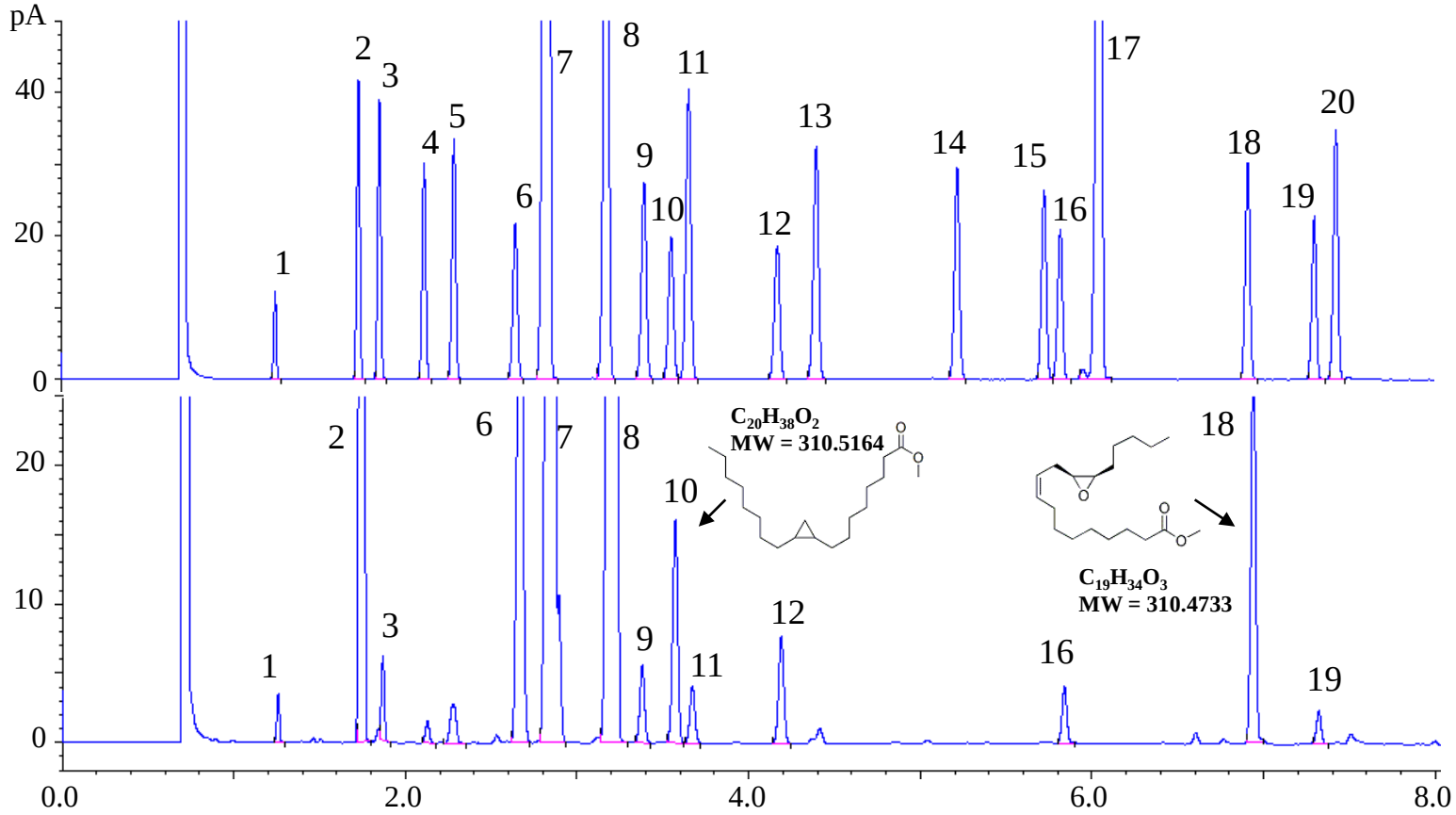
Volatile FAMEs injected, vaporized, passed through column via a carrier gas.

Separated by interaction with the column, exit at specific times.

Compounds identified by reference to known standards.

Limitation – compounds not corresponding to standards cannot be identified. Detectors only give information on retention time.

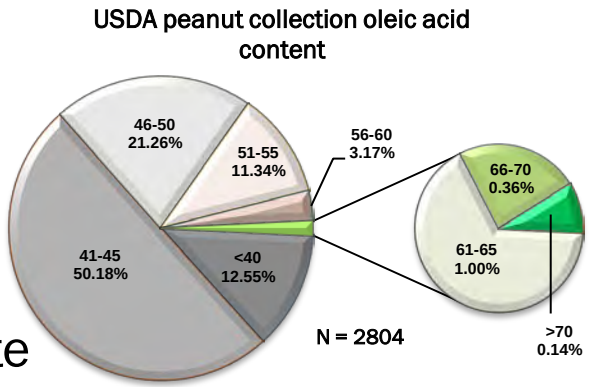
Hibiscus seed fatty acid profile



1.	C14:0	**6.**	C18:0	**11.**	C18:3α	**16.**	C22:0
2.	C16:0	**7.**	C18:1	**12.**	C20:0	**17.**	C22:1
3.	C16:1	**8.**	C18:2	**13.**	C20:1	**18.**	Vernolic acid
4.	C17:0	**9.**	C18:3γ	**14.**	C20:4	**19.**	C24:0
5.	C17:1	**10.**	DHSA*	**15.**	C20:5	**20.**	C22:6

To date, entire collections have been measured for seed fatty acid composition:

- Castor
- *Hibiscus sabdariffa* and *H. cannabinus*
- Sesame (interesting findings being pursued)



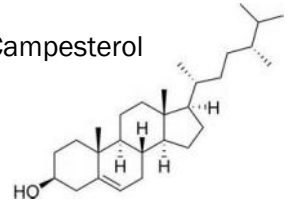
Currently working on peanut collection: ~1/3 complete

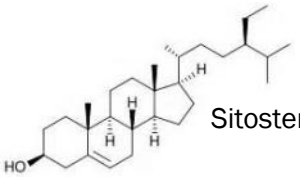
Other species: Desmodium, horsegram, Lablab, Nyger, okra, soybean, Teramnus.

Other compounds: **phytosterols** (lipid-soluble compounds with antioxidant properties) can be derivatized to make them volatile for GC analysis.

Reduce cholesterol
Reduce inflammation
Anti-cancer?

Campesterol





Sitosterol

LIQUID CHROMATOGRAPHY

Liquid mobile phase carrying injected sample extract pumped through column stationary phase.

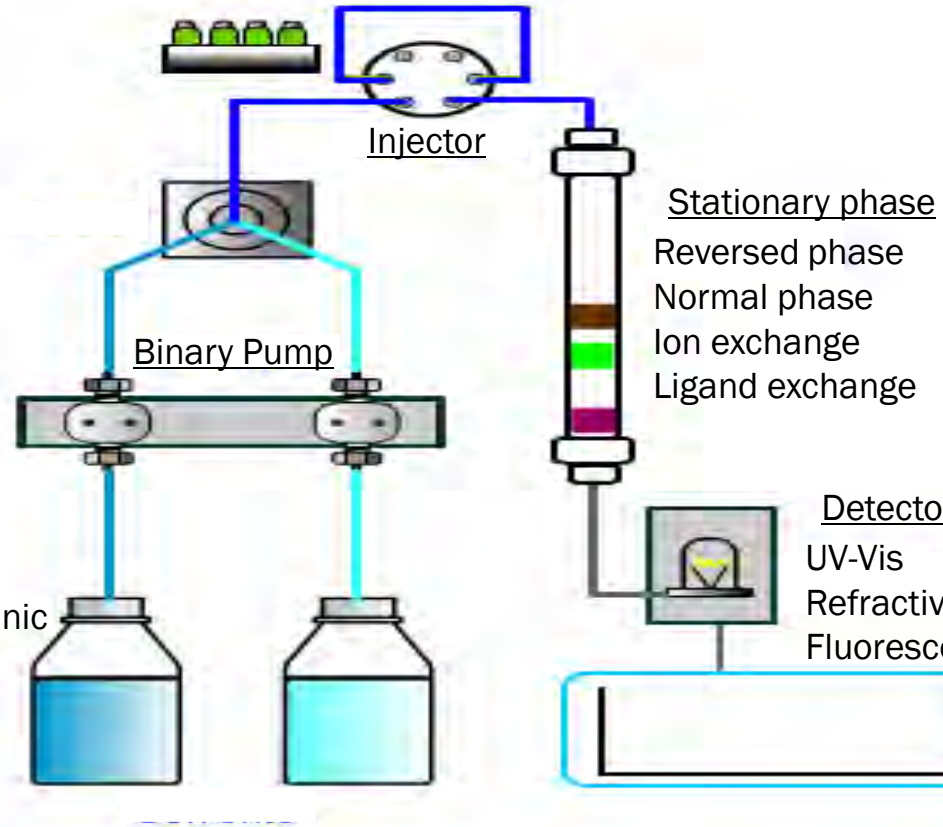
Components of extract separated by their varying interaction with the stationary and mobile phases.

Separated compounds exit column at specific times and are detected by a detector.

Compounds identified by reference to known standards.

Limitation – compounds not corresponding to standards cannot be identified. Detectors only give information on retention time.

HPLC system


Injector
Stationary phase
Reversed phase
Normal phase
Ion exchange
Ligand exchange
Binary Pump
Detector
UV-Vis
Refractive index
Fluorescence
Mobile phase
Polar – aqueous/organic
Non-polar – organic

LIQUID CHROMATOGRAPH

Diode Array Detector – UV-Vis detector

- Most common, good sensitivity
- Molecules with chromophores
- Also provides information on compound absorption spectrum

Refractive Index Detector (RID)

- Universal detection by light refraction
- Sensitivity limits

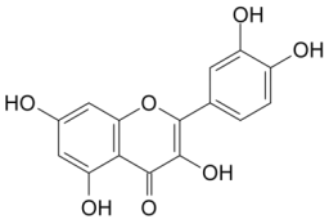
Fluorescence Detector

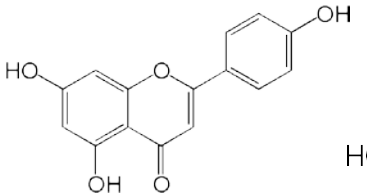
- Light absorbed by fluorophores and re-emitted at longer wavelengths
- High sensitivity
- Limited to molecules with fluorophores

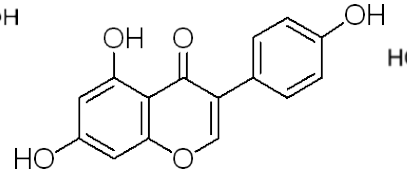
FLAVONOID CONTENT

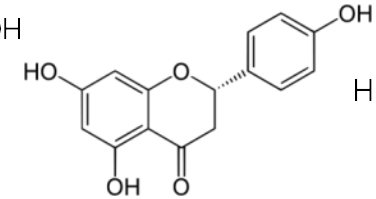
Flavonoids are polyphenolic compounds that are strong antioxidants and have exhibited anti-cancer properties in lab studies.

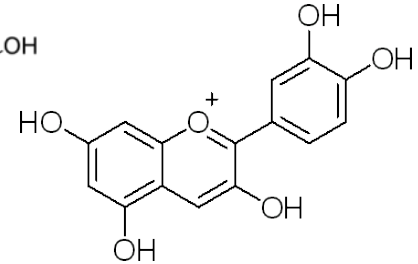
Flavonols, flavones, flavanones, isoflavones, anthocyanins, etc.



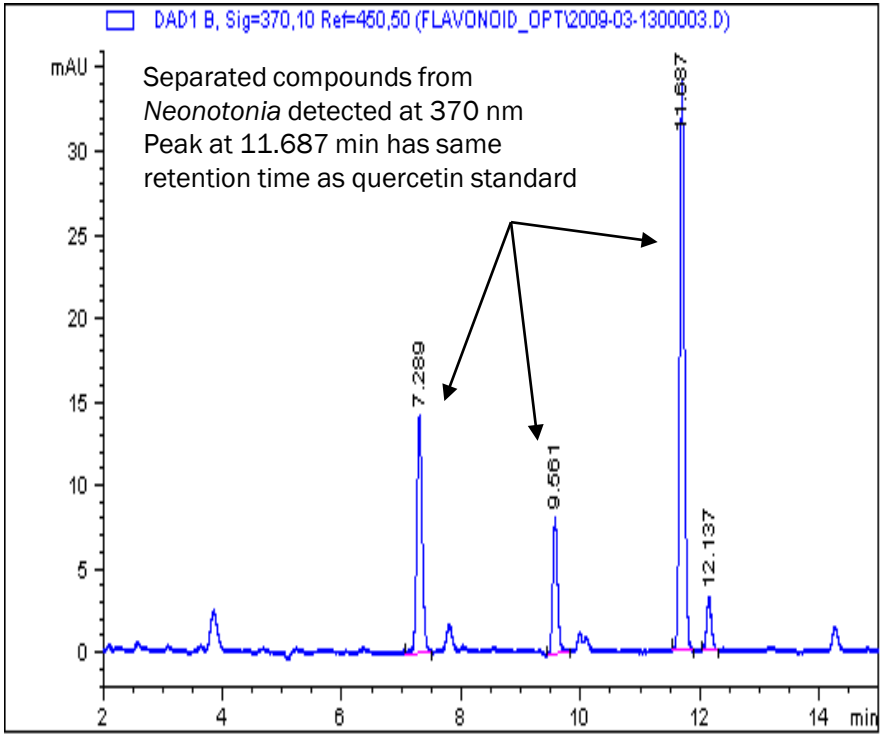


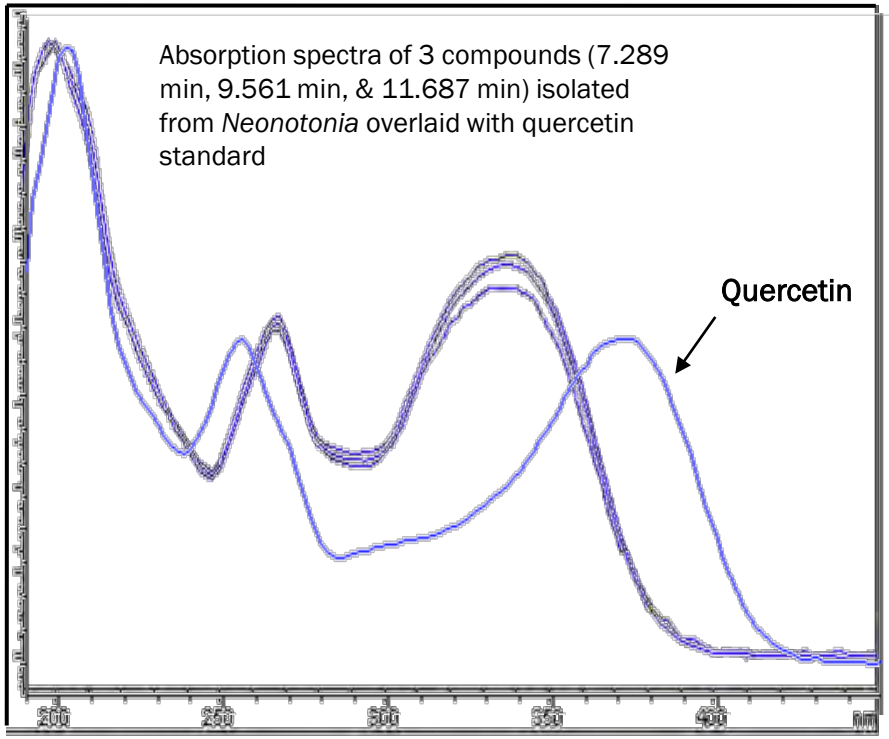






Various flavonoids have been found in several different plant species; in seeds, leaves, pods, calyces, etc.





Detector **limitation:** Peak matches T_R but not absorption spectrum, cannot be positively identified

SUGAR CONTENT

Measured by HPLC with water as mobile phase.

Detected by RID.

Dissolved sugars – sucrose, glucose, fructose – in sorghum or sugarcane juice.

Cellulosic/hemicellulosic sugars – arabinose, glucose, xylose, mannose, galactose, cellobiose – in plant biomass.

AMINO ACID ANALYSIS

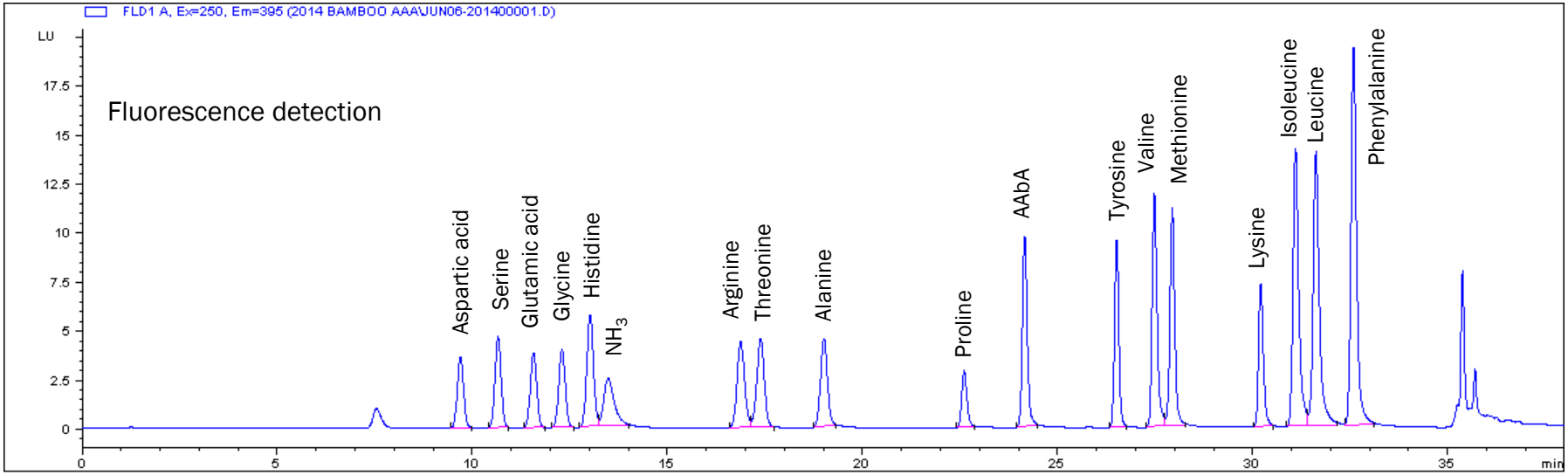
Sample dried, powdered, and proteins hydrolyzed to individual amino acids with strong acid and heat.

Amino acids derivatized to yield compounds containing fluorophores capable of being separated and detected by HPLC-FLD.

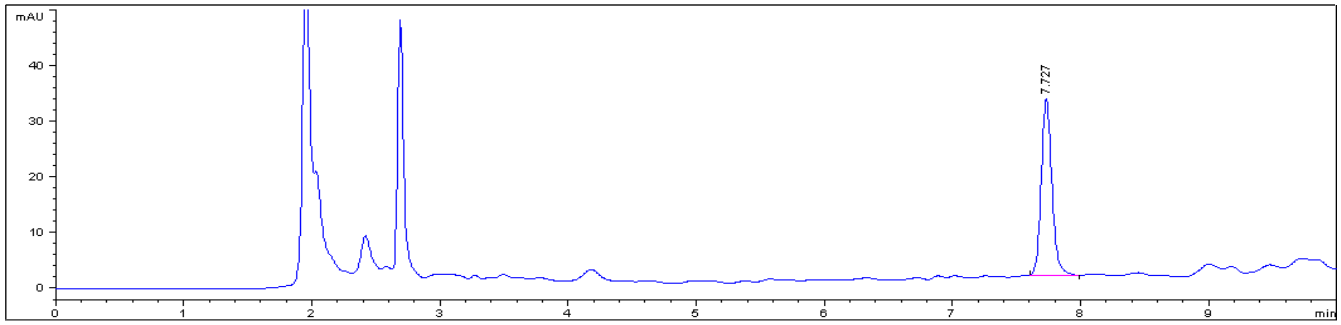
Currently working to optimize procedure for analyzing bamboo samples for amino acid content, complementary research to total protein measurements.

All plant materials have proteins, all potentially suitable for amino acid content analysis.

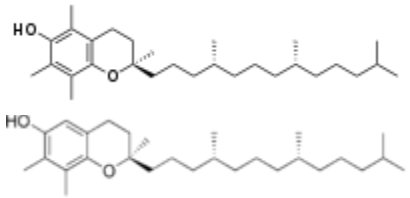
Derivatized amino acid standards

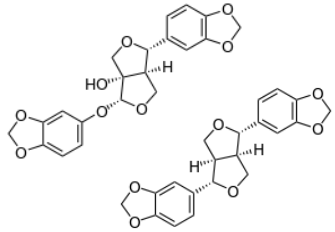


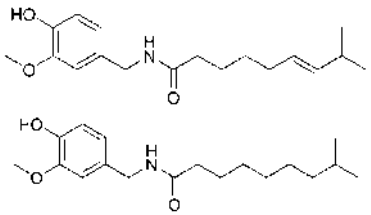
Tryptophan detection
by UV absorption



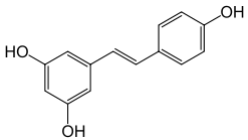
OTHER SECONDARY COMPOUNDS







- **Tocopherols** – measured in sesame and peanut seeds
  - alpha, beta, gamma, delta – vitamin E
  - Separated by reversed or normal phase HPLC
  - Detected by UV absorption or fluorescence (more sensitive)
- **Sesamin** – measured in sesame seeds, related compounds as well
- **Capsaicinoids** – measured in peppers, give them their 'hotness'
  - Separated by reverse phase HPLC
  - Detected by UV absorption or fluorescence
- **Resveratrol** – polyphenol found in grapes, red wine, peanuts, etc.
  - Response to abiotic stresses
  - Anti-oxidant, anti-cancer?



LAST WORD

Using these tools for chemical analysis of plant material should be for generating biologically meaningful data:

-- Differentiation of species

-- Meaningful variation

1. Oil measurements in oilseeds (peanuts 38-59% oil) vs. FA or oil analysis in crops where it is not biologically or economically valuable
2. Old vs. new material
3. Environmental vs. genetic

Our lab uniquely integrates genetic and chemical analysis of our crop germplasm

Appendix 5

DR. ALBERT CULBREATH

RUST RESISTANCE IN PEANUT GENETIC RESOURCES

Rust Resistance In Peanut Genetic Resources

Albert Culbreath and Imana Power, Dept. of Plant Pathology, The University of Georgia, Tifton
Barry Tillman, The University of Florida, Marianna, FL
Noelle Barkley, USDA-ARS, Griffin, GA

Characterizing peanut rust resistance: Determining its mechanisms, and the genetics of the peanut host and *Puccinia arachidis*

Imana Power

Ph.D. Disseration

2014

Peanut

- Important crop in the world for food, feed and oil
- U.S. peanut producers:
  - GA, FL, AL
  - NC, VA, OK, TX
- Georgia 2012:
  - Harvested from 292,000 ha
  - Average yields of 5130 kg/ ha
  - Total yields of 1.5 billion kg
  - Total value > $ 586 million

Peanut rust disease

- Caused by *Puccinia arachidis*
- Yield losses can be as high as 70% alone & 86% combined with leaf spot
- Primarily a disease of the tropics

UGA Tifton Lang farm

Rust resistance

- No single gene for resistance?
- Partial quantitative resistance
- Components of rust resistance
  - Incubation & Latent period
  - Infection frequency
  - Pustule size
  - Percent disease area
  - Spore production
- **Little information on rust resistance in recently developed breeding lines and cultivars in the Western Hemisphere**

Peanut CRSP: UF 150

- Peanut CRSP: UF 150 →
- PI: Dr. Barry Tillman, University of Florida

Albert Culbreath, University of Georgia
Noelle Barkley, USDA-ARS

  - Goal: to develop multiple disease resistant peanut genotypes to be deployed in the Western Hemisphere

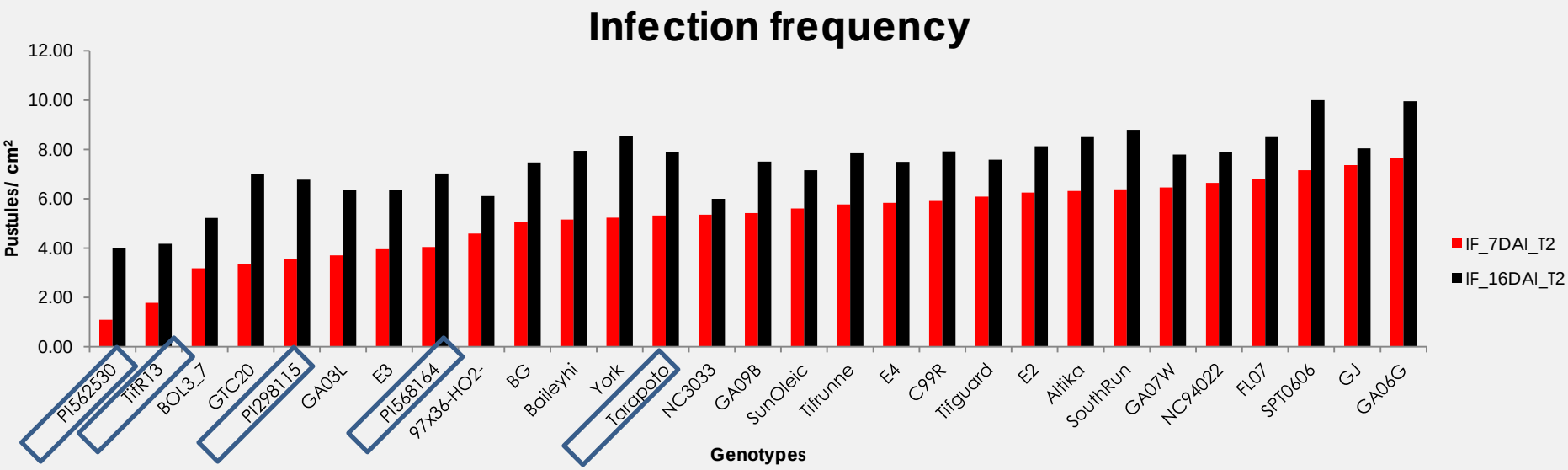
Overall goal

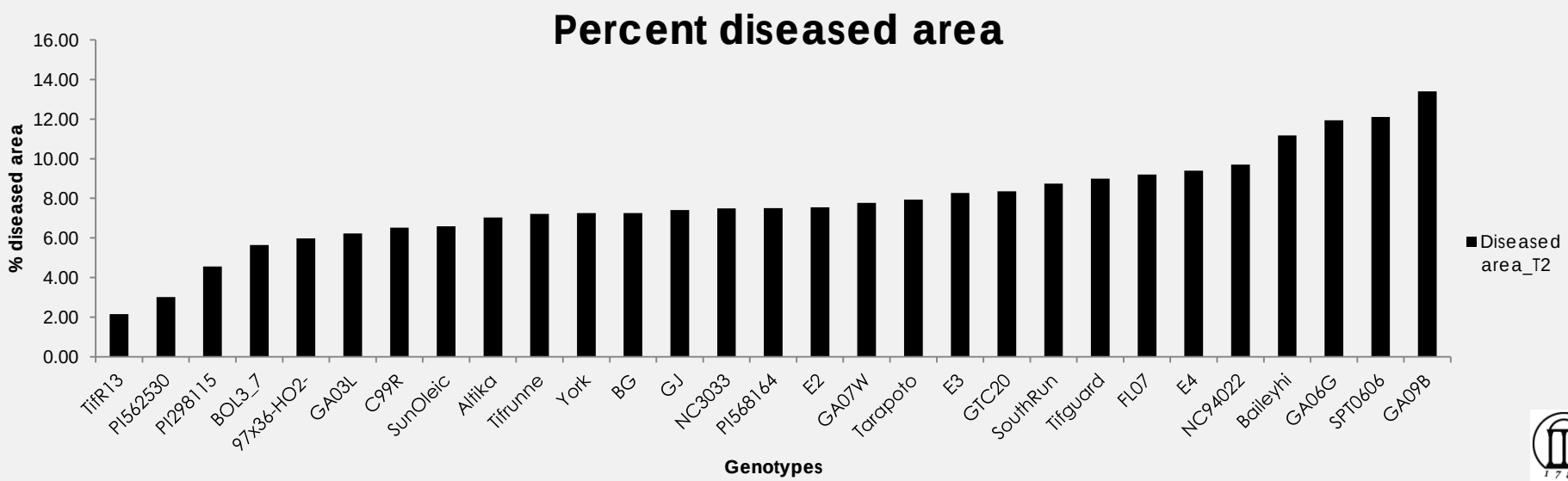
- To manage the peanut rust disease more effectively by screening and characterizing available peanut breeding lines and cultivars for resistance to *Puccinia arachidis*

- Short term → immediate use
- Long term → breeding programs

Greenhouse/growth chamber experiments

Results growth chamber assays

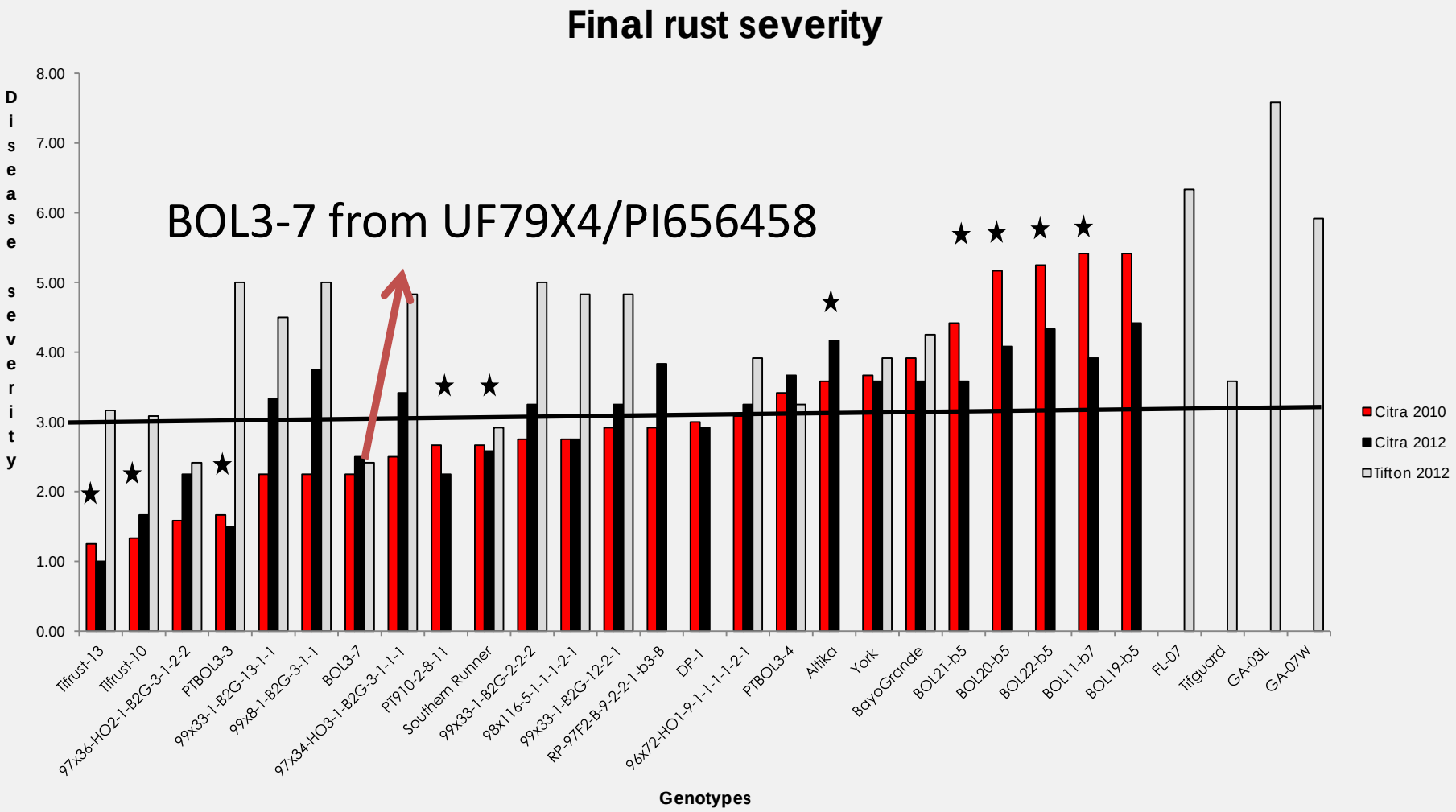




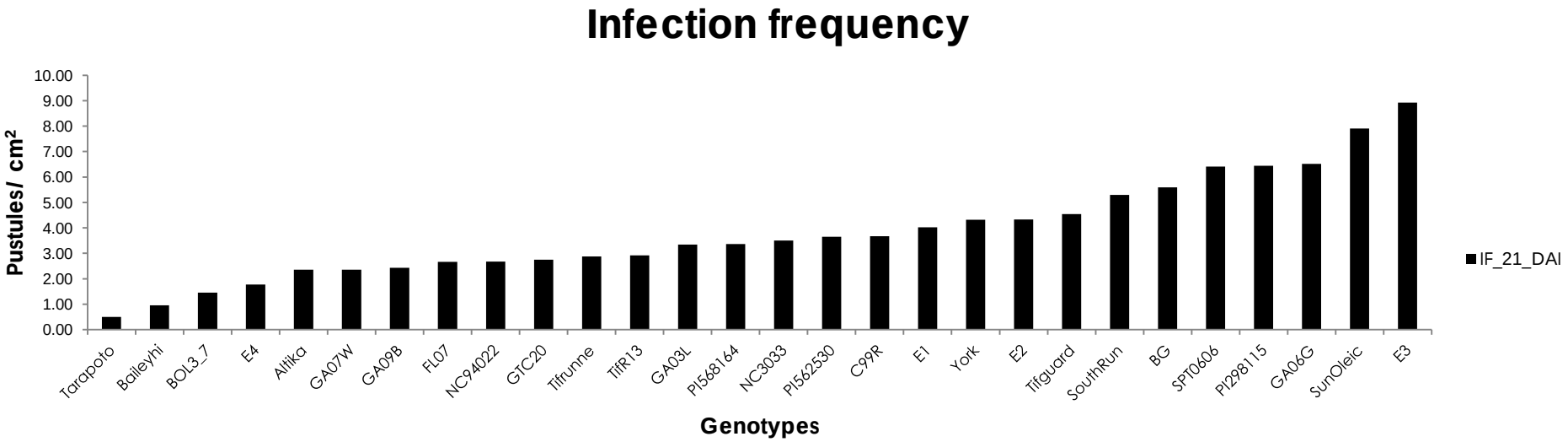
Altura R1
Tarapoto R1

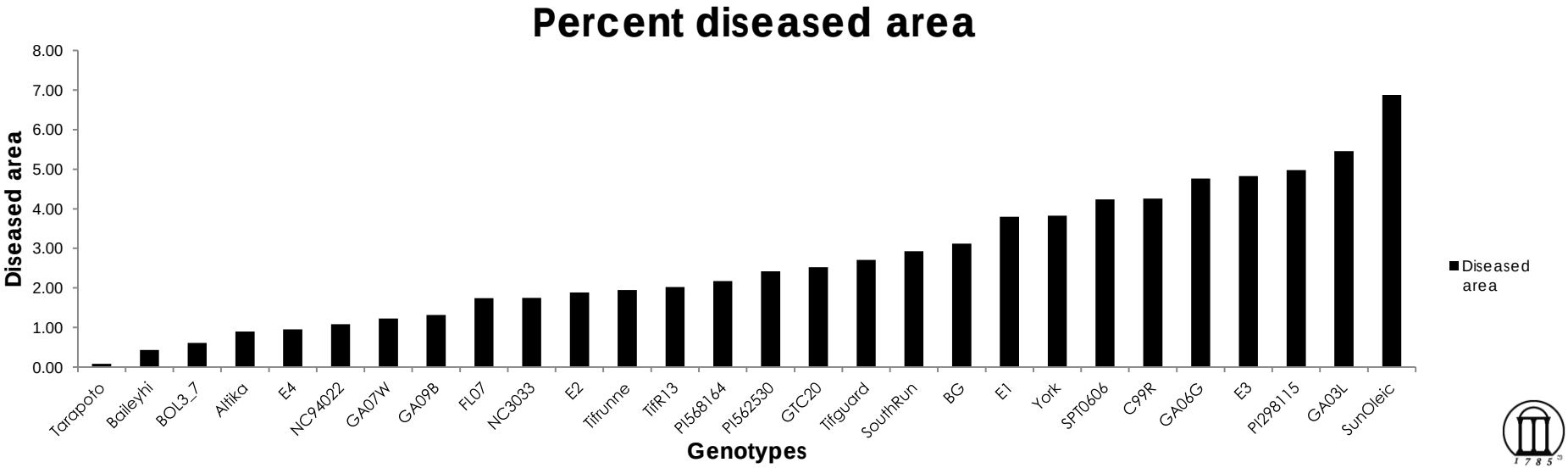
984 R1
949 R1

Results field resistance

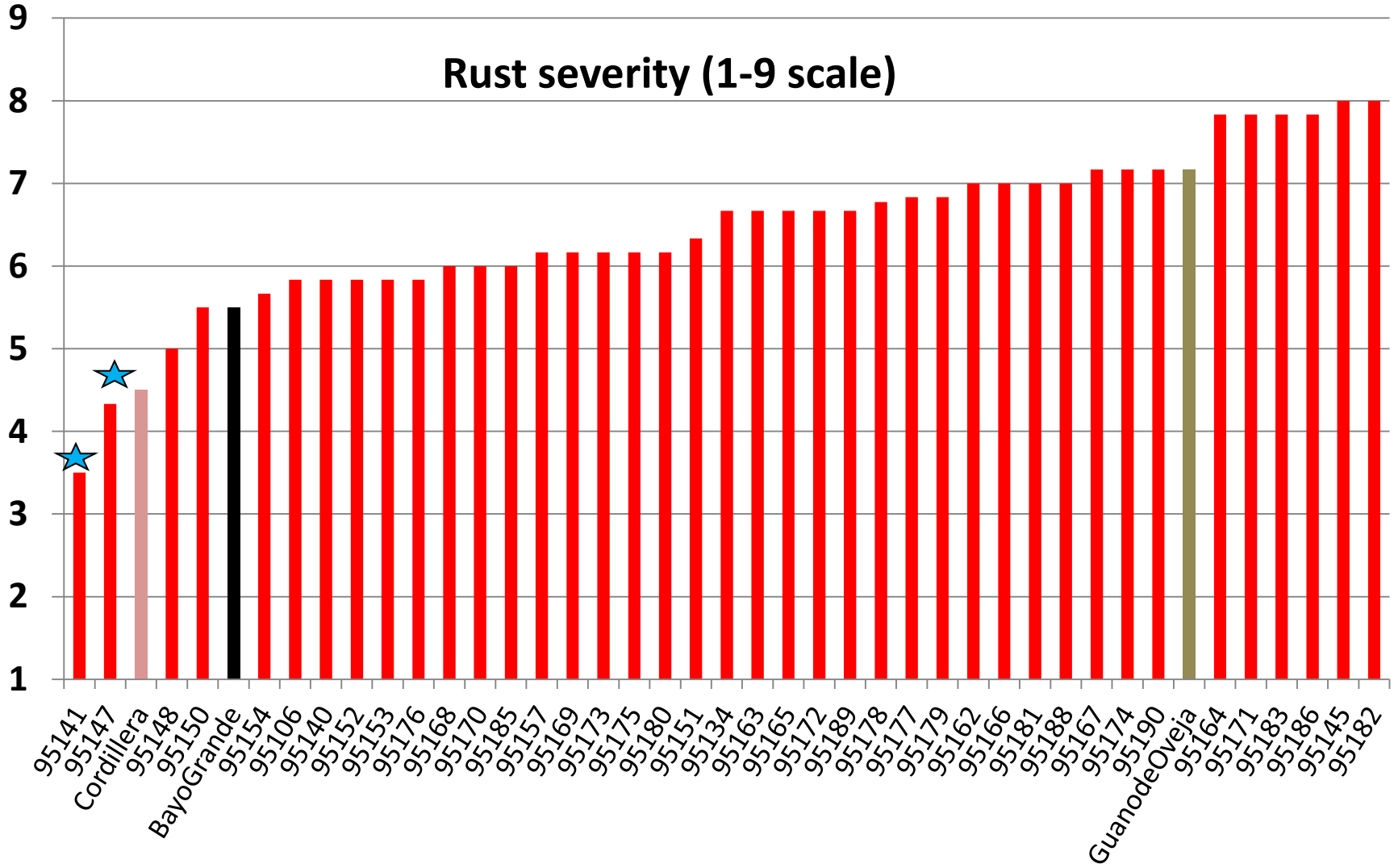


Results growth chamber assays

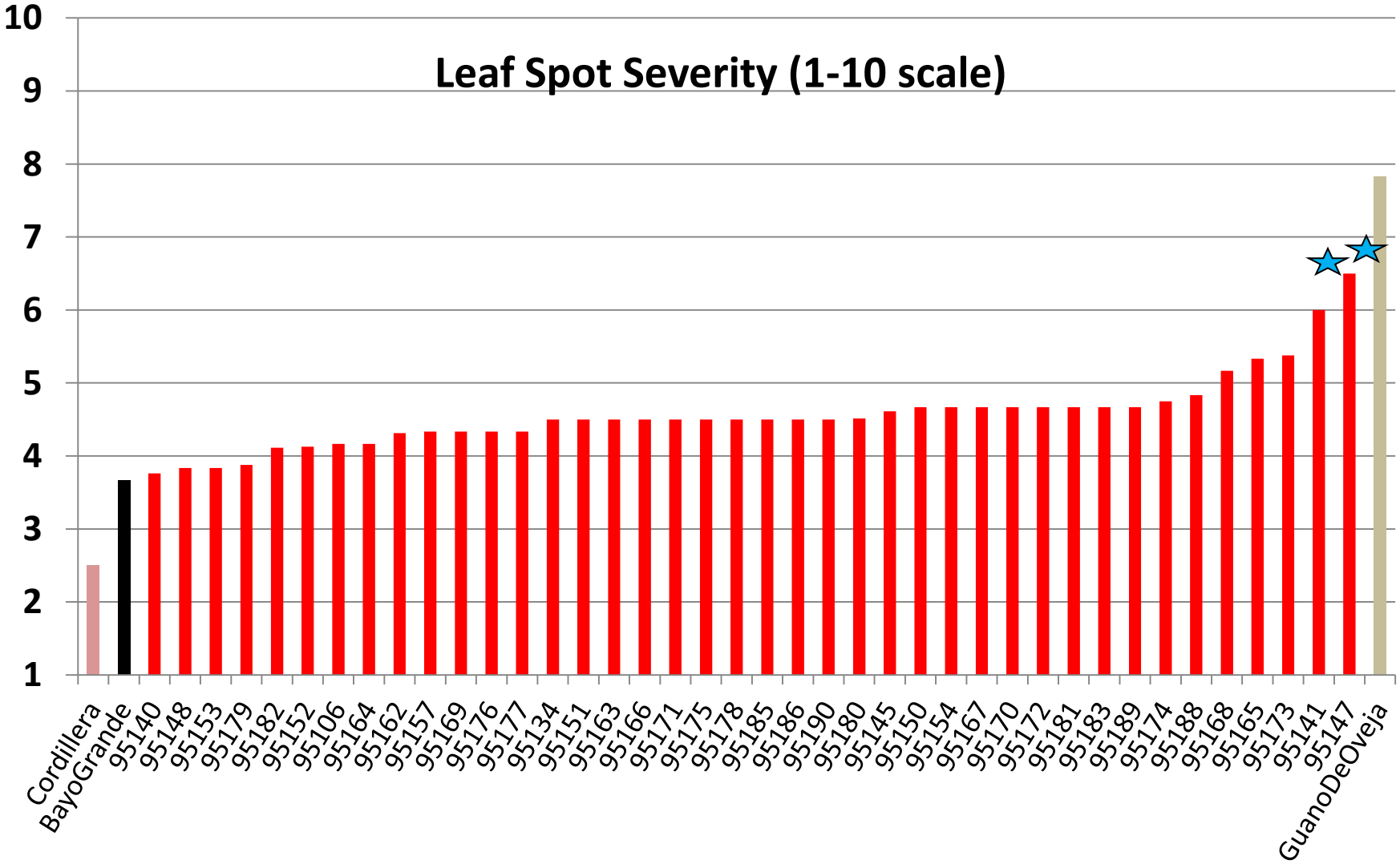




Effect of peanut genotype on rust severity, Saavedra, Bolivia, April, 2012



Effect of peanut genotype on leaf spot severity, 26 de Agosto, Bolivia, April, 2012



04.09.2012

CORDILLERA
04 09 . 2012

Peanut Germplasm Collection

- Provided access to germplasm previously reported to be resistant
- Ex. Tarapoto, Tifrust lines

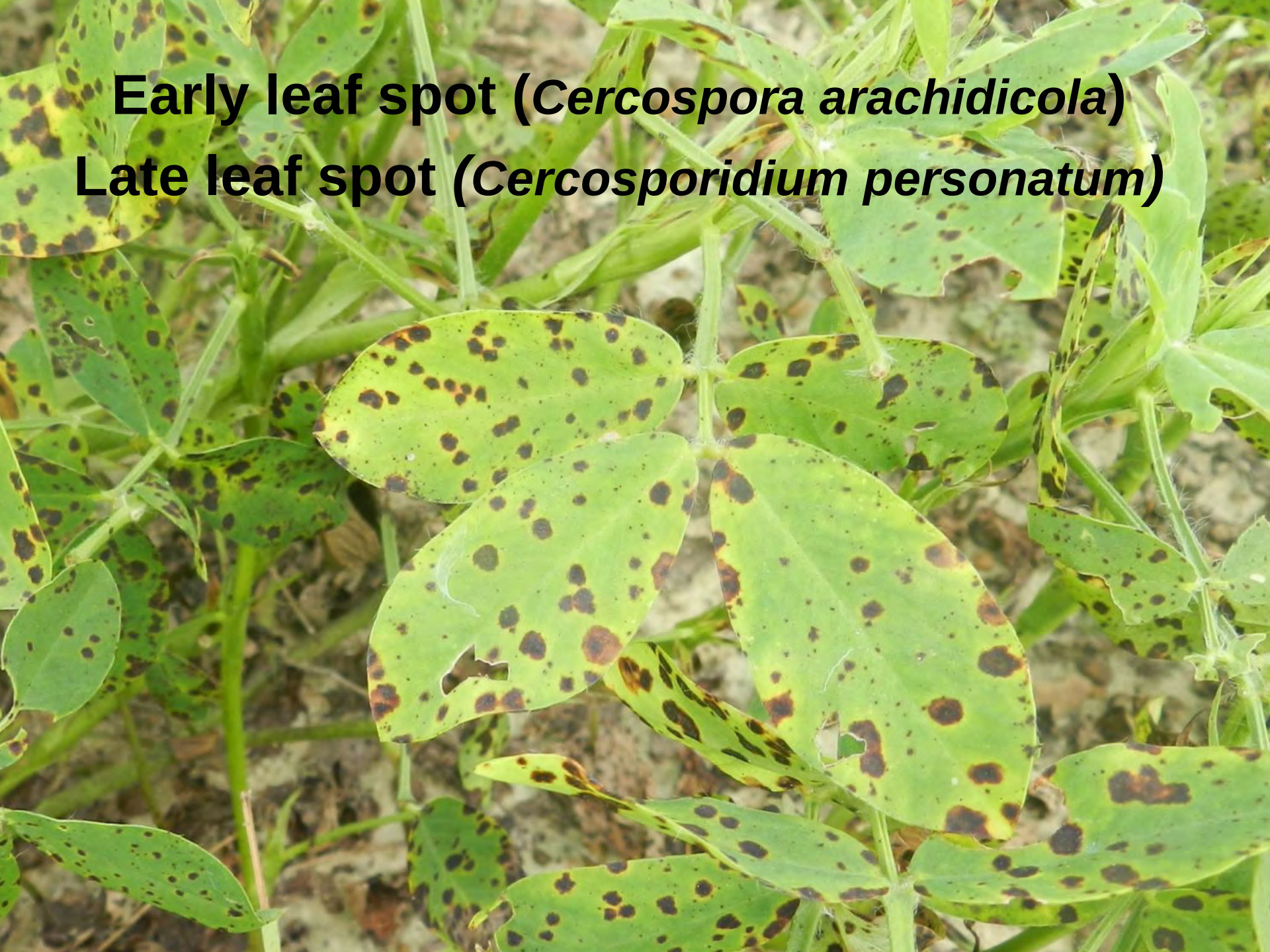
- A source of standards, especially in a new project

Peanut Germplasm Collection

- Provided germplasm for use in crossing that yielded lines resistant to rust and late leaf spot
- Ex. BOL3-3 from the cross of:

UF79X4/PI 656458

PI 656458 Bolivian origin


Early leaf spot (*Cercospora arachidicola*)
Late leaf spot (*Cercosporidium personatum*)

Peanut Germplasm Collection

- Major resource when starting new programs for breeding for pest resistance
- Searching for other sources of resistance to pathogens we already have

Tomato spotted wilt of peanut
Tomato spotted wilt tospovirus
(TSWV)

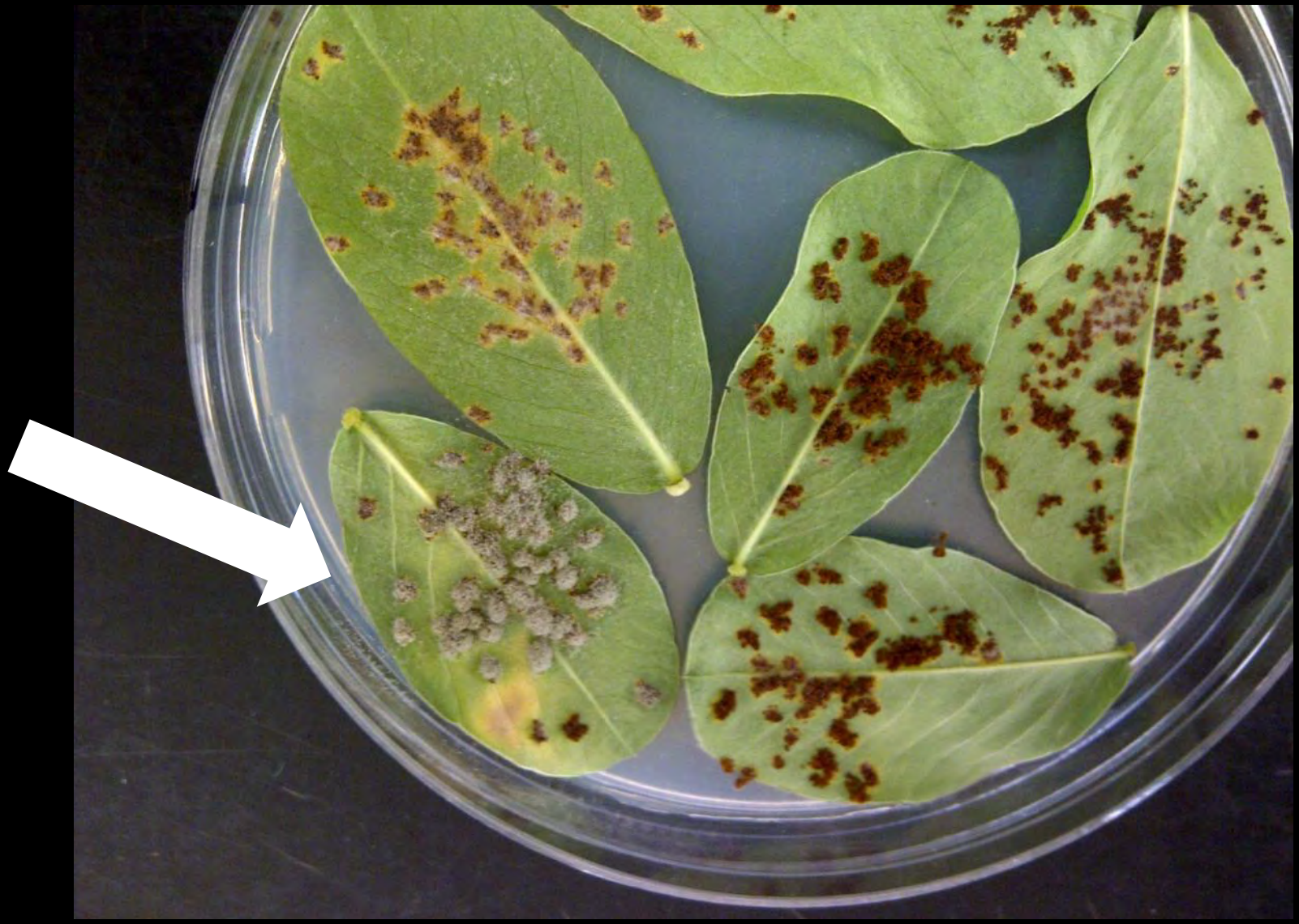
Peanut Germplasm Collection

- Critical for responding to new problems that occur, such as Tomato spotted wilt in the early 1990s.
- PI 203396 likely saved the peanut industry in the southeastern U.S.

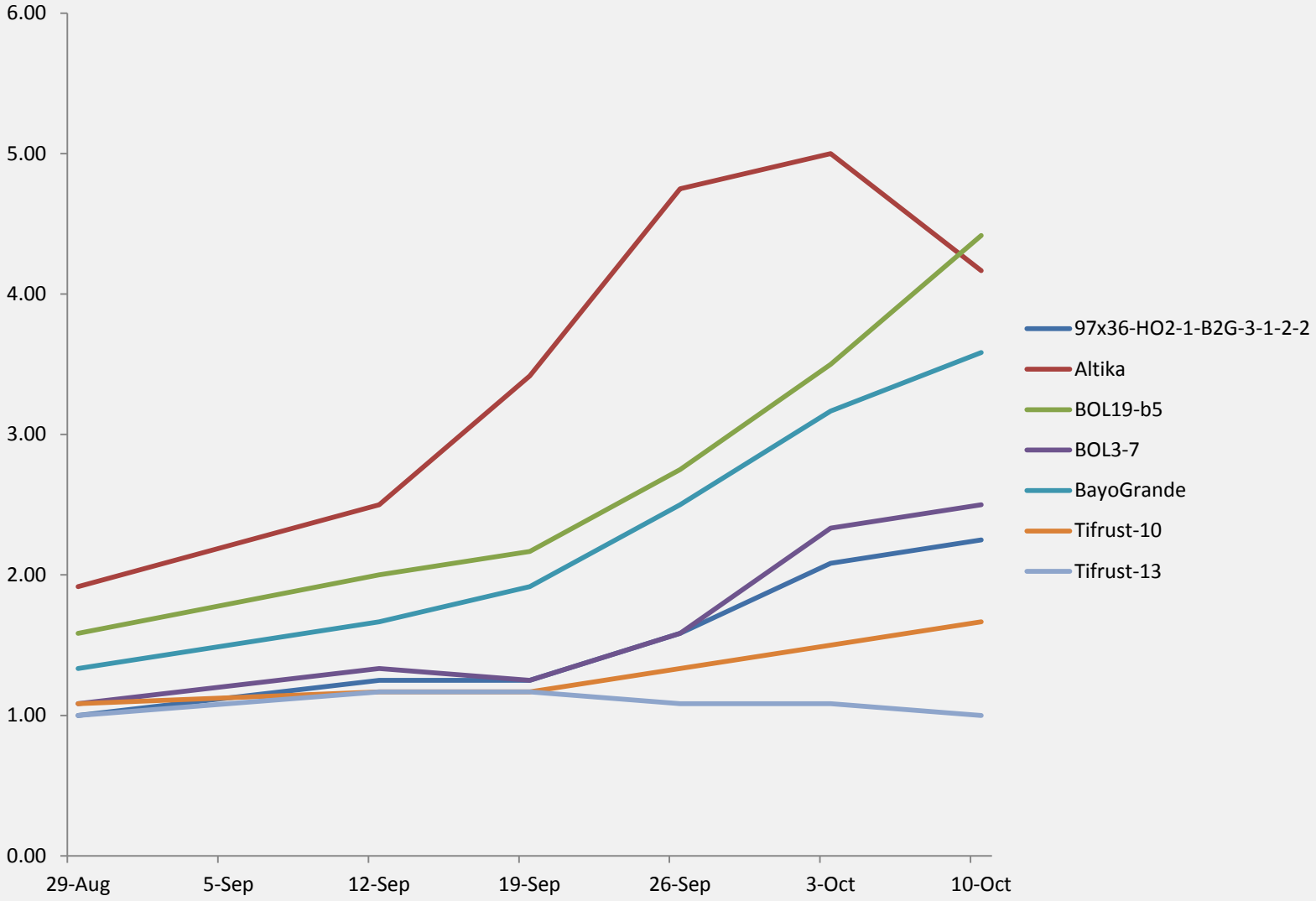
http://merln.ndu.edu/index.cfm?type=section&secid=249&pageid=3

Thank you!
Questions?

Hyperparasite??



DPC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3-Oct
10-Oct
97x36-HO2-1-B2G-3-1-2-2
Altika
BOL19-b5
BOL3-7
BayoGrande
Tifrust-10
Tifrust-13