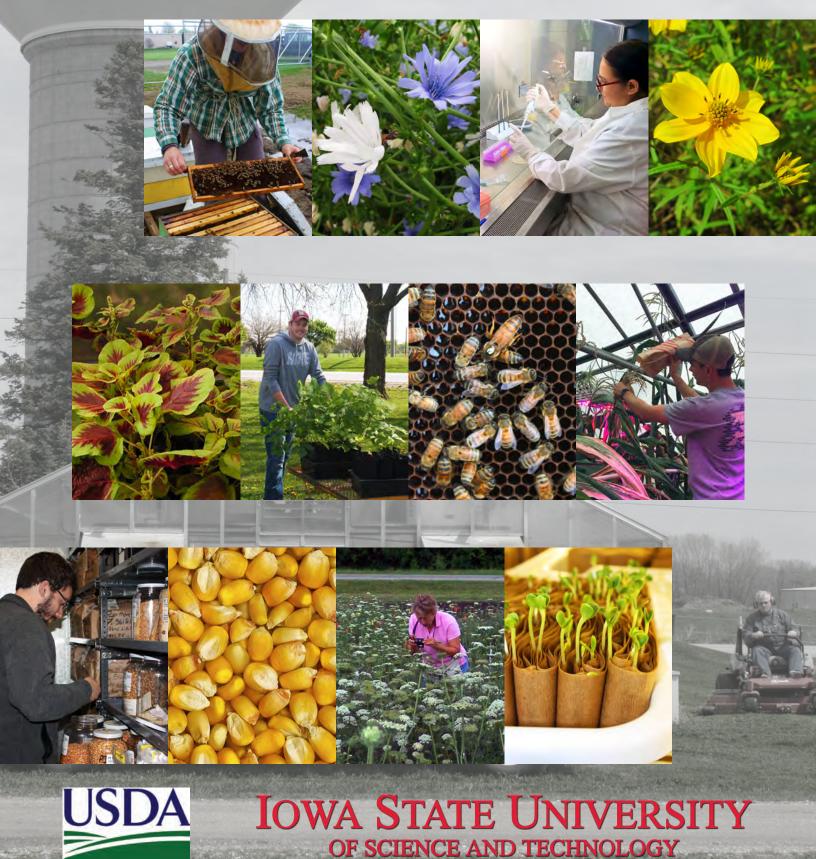


North Central Regional Plant Introduction Station NC7 Annual Report 2016

AMES, IOWA



## NCRPIS ANNUAL REPORT - 2016 TABLE OF CONTENTS

I.	PROJECT TITLE	1
II.	COOPERATING AGENCIES AND PRINCIPAL LEADERS	1
III.	PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS	2
IV.	PROGRESS IN GERMPLASM AND INFORMATION MANAGEMENT, RESEARCH, AND EDUCATION	4
V.	IMPACTS OF GERMPLASM USE BY NORTH CENTRAL REGIONAL RESEARCHERS	11
VI.	SUPPORT TEAM REPORTS	13
	A. FARM	13
	B. INFORMATION TECHNOLOGY AND TELECOMMUNICATIONS	14
	C. INFORMATION MANAGEMENT-GERMPLASM COLLECTIONS	17
	D. ORDER PROCESSING	17
	E. SEED STORAGE	18
	F. GERMINATION	19
VII.	CURATORIAL AND SCIENTIFIC TEAM REPORTS	20
	A. CONTROLLED INSECT POLLINATION SERVICE PROGRAM	20
	B. PLANT PATHOLOGY	29
	C. AMARANTH	33
	D. HORTICULTURE	42
	E. MAIZE CURATION	49
	F. OILSEED CROPS	58
	G. VEGETABLES	65
	H. RESEARCH LEADER ACTIVITIES	74
APPE	ENDIX:	
	TABLE 1       NCRPIS Accessions, Acquired, Available	76
	TABLE 2NCRPIS Accessions Germinated, Regenerated, Made Available, Backed Up .	77
	TABLE 3    EXTERNAL NCRPIS DISTRIBUTIONS	78
	TABLE 4         NCRPIS Accessions Observations in GRIN, Images in GRIN	79
	APPENDIX FIGURE 1	80
	APPENDIX FIGURE 2	81

#### NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION NC-7 ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2016

#### I. **PROJECT TITLE:**

NC-7 "Plant Germplasm and Information Management and Utilization"

#### **II.** COOPERATING AGENCIES AND PRINCIPAL LEADERS (current):

A. <u>Administrative Advisor</u>

\*W. Wintersteen, Iowa

#### B. <u>Regional Coordinator</u>

\*C. Gardner, USDA-ARS, Iowa

#### C. <u>State Experiment Stations Representatives</u>

Voting members:

1. Illinois	E. Sacks	7. Missouri	J. Shannon
2. Indiana	L. Hoagland	8. Nebraska	D. Santra
3. Iowa	T. Lübberstedt	9. N. Dakota	B. Johnson
4. Kansas	M. Stamm	10. Ohio	P. Jourdan
5. Michigan	A. Iezzoni	11. S. Dakota	M. Caffe-Treml
6. Minnesota	A. Lorenz	12. Wisconsin	W. Tracy

#### Non-voting participants:

ron voung participan			
13. California-Davis	R. Karban	26. Missouri	S. Flint Garcia
14. Connecticut	M. Brand	27. Missouri	S. Jose
15. Delaware	R. Wisser	28. Nebraska	C. Urea
16. Illinois	J. Juvick	29. New Jersey	S. Handel
17. Illinois	G. Kling	30. New Jersey	T. Molnar
18. Illinois	S. Korban	31. New York	J. Doyle
19. Illinois	D. Lee	32. New York	M. Gore
20. Iowa	K. Lamkey	33. New York	P. Griffiths
21. Kansas	A. Fritz	34. New York	A. Hastings
22. Kentucky	T. Phillips	35. New York	M. Smith
23. Michigan	R. Grumet	36. Wisconsin	S. Kaeppler
24. Michigan	J. Hancock	37. Wisconsin	N. de Leon
25. Mississippi	S. Popescu	38. Texas	D. Baltensperger

# D.U. S. Department of Agriculture (\*Voting members)1. ARS National Program Staff, Plant Germplasm\*P. Bretting2. ARS Plant Exchange Office\*G. Kinard3. ARS Area Director, Midwest AreaR. Matteri4. Cooperative State Research, Education and Extension ServiceA. Thro5. National Center for Agric. Util. Research\*T. Isbell6. National Center for Genetic Resources Preservation\*S. Greene

## E. <u>North Central Regional Plant Introduction Station, Ames, Iowa</u>

See organizational chart, Figure 1 in the Appendix.

#### III. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

### Personnel changes – May, 2016 – June, 2017:

<u>Departures</u>:

- Charlie Block, USDA-ARS Plant Pathologist, retired July 1, 2016
- Larry Lockhart, ISU Program Manager, retired July 1, 2016
- Robert Stebbins, Germplasm Program Assistant, resigned May, 2016

#### Promotions:

- Lisa Burke, USDA-ARS Biol. Science Tech (Seed Storage manager), transitioned to an ARS Agronomist (IT) position, Seed Inventory Process and Data Management; former position (Biol. Science Technician) to be abolished

#### <u>New Hires</u>:

- Kallie Webber, USDA-ARS Pathways Trainee, Entomology, January, 2016
- Stacey Estrada, USDA-ARS Term Biol. Science Technician, February, 2016
- Heather Kearney, USDA-ARS Germplasm Program Assistant, July, 2016
- David Peters, USDA-ARS Maize Geneticist (GEM Coordinator), July, 2016
- Cole Hopkins, ISU Agricultural Specialist (vegetables), September, 2016

#### Transitions:

- Fred Engstrom, transitioned from a USDA-ARS GEM ASRT position to ISU Program Manager, July 1, 2016
- Adam Vanous completed his Ph.D. program in Plant Breeding & Genetics, December, 2016
- Stacey Estrada, Term Biol. Science Technician transitioned to a permanent Germplasm Program Assistant Position, January, 2017

#### Vacant Positions:

- Agri. Science Research Technician, Oilseeds (vice-Larsen)
- Cat 4 Plant Pathologist (vice-Block)
- Cat 4 Maize Curator (geneticist)
- Term Cat 3 Agronomist (GEM)
- Term Biol. Science Tech (Seed Storage)

Appendix Figure 1 illustrates the organization of the NCRPIS staff and their roles.

#### Management of Federal and ISU Student Temporary Employees:

USDA-ARS resources provided for 19 student part-time temporary positions in FY 2016, primarily via the Research Support Agreement with Iowa State University, and a Ph.D. student (plant breeding and genetics, GEM funds). NC7 resources provided for an addition 0.5 student FTE. The temporary positions support curatorial activities including regeneration, seed processing, viability testing, farm and facilities operations, and IT support. Students were interviewed and selected by ISU Program Managers Larry Lockhart and Fred Engstrom. Marci Bushman and Heather Kearney managed the administrative aspects of all student hires, with support and guidance from Ames ARS HR Specialists Stephanie Hadsall and Sireena Foley, and Admin. Officer Carol Moran.

#### Budget:

We appreciate the support of the Agricultural Experiment Stations of the North Central Region, which have maintained their annual support and continued to provide \$522,980 in Hatch funds. These funds support the salaries of our nine ISU staff members, their professional travel, and some expenses. In addition, Iowa State University's Agricultural Experiment Station provides support valued at over \$400,000 annually that supports infrastructure, administration, and benefits for current NCRPIS-ISU staff members and retirees.

We are grateful that Hatch funding resources were maintained throughout the difficult sequestration period, and hope they continue to be stable or increase in the future. Currently, about 95% of Hatch NC7 funds are devoted to the wages and salaries of the nine permanent ISU employees. In the near future we will be unable to provide incremental salary increases due to Hatch funding constraints. If ISU wage increases of 1.5% are granted in FY18, this figure climbs to 96%; if an additional 1% increase is granted in FY19, 97% of funds are devoted to salaries, barring personnel changes. This will limit professional meeting travel and technical training.

FY2017 USDA-ARS funding was essentially the same as final FY2014 funding, with the PI CRIS funded at \$2.38M (net to location) and GEM CRIS at \$1.2M. Student hiring for summer 2017 was extremely difficult, and we were not able to fulfill the need for 25 summer FTE. We attribute this to both the requirement for all agriculture students to complete internships, and growing disparity in what we can offer for wages versus other local hiring opportunities.

A new GEM Coordinator, David Peters, assumed leadership of the Germplasm Enhancement Program of Maize in July, 2016. Position descriptions for the second maize geneticist (curator) were submitted in November, 2016. Together with the vacant Plant Pathologist, Oilseeds technician, and GEM support scientist positions, these positions remain unfilled and frozen. Until Congress' intent for downsizing and appropriations is clear, this is unlikely to change.

Any reductions in funding will force reduction in student hiring, necessary for executing our genebank's mission. Like many other research units, our ability to cover all aspects of our mission is challenged; our personnel strive to cover all functions and serve the collections entrusted to us and our stakeholders to the best of our ability. Given the high turnover of the past two years, a great deal of time and attention has been paid to recruitment and hiring activities, and will continue given the current seven vacancies.

#### **Construction and Facilities:**

The access security system was converted from an Andover to a Lenel system. The NCRPIS security system is now part of (linked with) with all other Lenel-secured ARS facilities.

Seed storage space is becoming limiting, and needs to be addressed in the next five years. In general, space is extremely tight for all personnel and functions.

Greenhouse pest control continues to be augmented with biological controls such as green lacewings (*Chrysoperla rufilabris*), ladybugs (*Hippodamia convergens*), and a whitefly parasite (*Encarsia formosa*).

Please see the Information Management section of this report for details on upgrades that continue to enhance the NCRPIS' information technology infrastructure. The continued implementation virtual servers and their configuration is noteworthy.

#### Equipment:

The backup server system was replaced in 2016 with a 24 drive, 108 Terabyte capacity machine, which will improve speed as well as capacity. As funding becomes available, we will continue to upgrade greenhouse lighting technology to improve plant productivity and reduce costs. LED lighting is rapidly evolving, and fixtures that provide for improved photosynthetic efficiency will become available at less cost. Acquisition of modern seed sorting technology is desired also.

#### IV. PROGRESS IN GERMPLASM AND INFORMATION MANAGEMENT, RESEARCH, AND EDUCATION (C. GARDNER):

(Part IV. summarizes the accomplishments and progress for calendar year 2016 presented in greater detail in the individual staff reports in the document.)

**Technical Exchange:** An exchange relationship was established between the NCRPIS maize genebank and the CIMMYT maize genebank. In May-June of 2016, Cristian Zavala, lead technician for the CIMMYT genebank who reports to curator Denise Costitch, spent a month at the NCRPIS with the maize curatorial team. Cristian focused on characterization processes and learning how curatorial personnel use GRIN-Global to manage information workflows. In November of 2016, Brady North, Agricultural Research Specialist, spent a month with the CIMMYT technical personnel, traveled to several of their locations at various altitudes, learned their processes, and offered GRIN-Global training. Brady was able to see the Tolucca site, where they have been able to successfully regenerate highland tropical maize the past three seasons. As a result, we will attempt to establish a contract with CIMMYT to regenerate NPGS tropical highland maize.

#### Acquisition and Documentation Highlights:

In 2016, 786 new accessions were acquired (Appendix Table 1). This compares with 229 in 2015, 766 in 2014; 192 in 2013, 470 in 2012, 485 in 2011, 516 in 2010, and 521 in 2009. New ornamentals include a variety of taxa collected by NCRPIS curators, including 355 genomically characterized cultivated *H. annuus* pre-breeding lines from Dr. Loren Rieseberg, University of British Columbia, Vancouver, Canada; 101 new *Daucus* accessions with new species representation, collected in Spain by Drs. David Spooner and Philipp Simon (USDA-ARS, Vegetable Crops Research Center, University of Wisconsin, Madison, WI) and Fernando Martinez-Flores (Universidad de Alicante, Spain); ornamentals collected in Minnesota, Illinois and Iowa and a wide variety provided by numerous cooperators; 43 accessions of *Dalea* species provided by Dr. Douglas Johnson of the USDA,ARS from Logan, Utah; and 110 new maize accessions, including tropical inbred from Dr. James Brewbaker's program in Hawaii,

seed of the B73 lot used for the new assembly of the maize genome, expired PVPS, GEM released lines, and an 'Alaska Synthetic' donated by Dr. Arnel Hallauer that may have cold tolerance genes . (See the curators' reports for interesting detail.)

The U.S. is now a partner to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). How this will be implemented by the NPGS is in development. International collection continues to be challenging as countries adopt variations of the SMTA or other requirements that the NPGS cannot accept. Of ongoing concern is the successful entry of germplasm collected from international explorations into the U.S. It is critical that clean, pest- and pathogen-free seed be shipped or carried in by collectors; sufficient time needs to be devoted to collection sample preparation and sufficient care post-collection. Excellent quantities of seed provided by collectors of many new accessions have made a significant proportion available and distributable immediately.

Original seed samples continue to be scanned in order to provide useful visual references for comparison of regeneration lots with original samples.

#### **Regeneration and Maintenance Highlights:**

In 2016, 1033 accessions were grown for regeneration and 1067 were harvested. This contrasts with 1,627/1,169 in 2015, 1,230 / 1,085 in 2014; 1,184 / 1,048 in 2013; 759 / 954 in 2012; and 1,069 / 1,017 in 2008 (Appendix Table 2). A low 2016 number reflects budget uncertainty at the time when germinations and vernalizations must occur. A large maize winter nursery to regenerate previously unavailable tropical accessions is planned for fall/winter 2017, a high priority. About 889 accessions were made available to the public. Accessions backed up at the NLGRP in Ft. Collins in 2016 numbered 428, compared with 431 in 2015, 1,231 in 2014, 781 in 2013, 799 in 2012, 792 in 2011, 2,388 in 2010 and 1,848 in 2009. The percent of NCRPIS collection holdings backed up at the NCGRP declined by 1% (Appendix Table 2). Overall collection availability is 76%, despite 8% growth in collection size since 2006.

Assistance in regeneration was provided by USDA-ARS staff of Parlier, CA for increase of wild *Helianthus* taxa. *Daucus* regeneration efforts were supported by seed increases from Seminis Vegetable Seeds (R. Yzquierdo), Bejo Seeds (R. Maxwell), and New Mexico State University (C. Cramer).

Assistance for maize regeneration and observation was provided for tropical maize populations by Monsanto (D. Butruille) in Hawaii, and by DuPont Pioneer in Puerto Rico. USDA-ARS staff of Mayaguez, PR (R. Goenaga) and the St. Croix quarantine nursery staff supported regeneration of 21 maize accessions. Raleigh ARS GEM Project Coordinator Matt Krakowsky provided increases of 17 GEM lines and the Ames GEM team regenerated 31 GEM lines. 3<sup>rd</sup> Millennium Genetics in Puerto Rico was contracted to increase tropical populations as well.

Spinach regenerations continue to be supported by cooperative efforts between the USDA-ARS and Sakata Seed America, Inc. in Salinas, CA.

#### **Distribution:**

Approximately 33% of the germplasm distributions were to international and 67% to domestic requestors. Distributions declined in 2015 from 2014 (Appendix Table 3).

Year	# Items	<b># Unique Accessions</b>	<b>#</b> Orders	<b>#</b> Requestors
2016	39,520	18,093	1,254	963
2015	34,188	14,279	1,186	945
2014	41,655	17,558	1,285	993
2013	40,409	17,788	1,523	1,204
2012	45,115	18,811	1,632	1,344
2011	38,402	18,634	1,501	1,180
2010	26,651	13,226	1,183	820
2009	26,904	13,515	1,487	1,081

Non-research requests (home gardeners), which heavily target vegetable germplasm, have largely stabilized. Approximately one-half of all orders to NC7 are cancelled non-research requests; other NPGS sites are also heavily targeted (Appendix Table 6). Home gardeners are redirected to other sources of commercially available materials. Although our resources cannot support maintaining and distributing the collections to home gardeners, we inform these requestors about plant genetic resource conservation and encourage interested individuals to save seeds, conserve them, and share germplasm and associated information. The proliferation of websites instructing non-research requestors how to deceive curators at various germplasm sites in order to get free germplasm continues to be problematic. The careful efforts that go into each and every increase, characterization, imaging, processing, storage, viability testing, and distribution surely make these seeds among the most expensive to provide in the world.

The relative numbers of distributions generally correlate well with the proportional makeup of the collections and vary from year to year, although demand for maize is usually greater than for other crops.

Curator	Collection Size 2016	% of Total Collections	% of 2016 Distributions	% of 2015 Distributions	% of 2014 Distributions
Brenner	9,214	17	18	18	14
Carstens <sup>†</sup>	3,578	7	1	3	<1
Marek	12,621	23	25	26	16
Millard	20,920	39	34	40	35
Reitsma	7,823	14	22	13	28
Totals	54,156	100	100	100	100

†Carstens and Barney collections assumed by Carstens in late 2015

Research demand for our plant genetic resource collections continues to be very high; requests for diversity and relationship analyses, disease resistance, biofuel, and health and nutrition contribute increasingly to these increases, as well as for basic research applications such as photoperiod response, and an array of performance traits. Demand for *Zea mays* inbred lines, vegetables, quinoa, *Helianthus*, Brassicaceae, flax, *Daucus*, and the culinary umbels for evaluation and characterization were particularly high. Maize inbred requests are driven by the continuing publication of information from genomic (genotyping by sequencing) and phenotypic analyses projects.

#### **Evaluation and Characterization:**

Maize technical staff developed a number of maize specific computer applications to capture field observations, ear and kernel lab observations, inventory actions, harvest and seed weights that are captured in MS Access and then loaded into the GRIN-Global database via the drag and drop feature.

In 2016, the NCRPIS utilized 2,556 accessions internally for observation, evaluation and characterization for a wide array of descriptor information, and viability testing n (Appendix Table 2). Appendix Table 4 lists observations associated with traits entered in the GRIN/GRIN-Global database (http://www.ars.grin.gov/npgs/). Other uses include pathogen testing to meet international distribution requirements and back up. A large backlog of images awaits loading to the database, following completion of an attachment loading wizard.

#### Information technology and telecommunications:

The NCRPIS staff continues to provide expertise and leadership for the development of GRIN-Global (GG), the successor to the GRIN system; this has been the sole primary focus of NCRPIS developer Pete Cyr since 2008, and the primary focus of two other NCRPIS staff members, Mark Millard (system analyst) and Lisa Burke (beta testing and training) with substantial time by additional personnel. The Database Management Unit (DBMU) in Beltsville, together with all development team members, the Office of National Program leadership, and the ARS Office of the Chief Information Officer successfully implemented GRIN-Global v1.9.4 in November of 2015. The DBMU is responsible for hosting and maintaining the system, and provides leadership for NPGS implementation. Weekly video training conferences continue to be offered by DBMU personnel (documentation specialist Marty Reisinger) for NPGS site personnel participation, as they have been for the past three years.

Nine national or international genebanks have now implemented GRIN-Global for genebank use, most of these have live public interfaces. Another 17 are in the process of evaluating and/or implementing the system (Appendix Figure 2).

GRIN-Global V1.8 was first released to the international community at the start of 2013. US Development Team efforts then re-focused on gap analysis and programming to address implementation needs of the National Plant Germplasm System (NPGS). CIMMYT was the first to implement and go live with a public website, followed by the Czech Republic. The US NPGS implementation went 'live' on November 30, 2015.

For the past 18 months, the NPGS has utilized a GRIN-Global Advisory Committee (AdCom) as a forum for genebank personnel and developers to identify development needs, prioritize them, test, and approve software for release. The AdCom is chaired by NCRPIS staff member Lisa Burke, and has been highly productive. Within the past three months, and international AdCom has been formulated with participation by key personnel from the Crop Trust, the US NPGS, CIMMYT, CIP, and is still developing. Its first charge is to formalize a process by which international

development products can be checked into branches of the Git vault (maintained by the Trust at CIMMYT)

Please see the IT section for technical details of NCRPIS support activities. We owe IT Specialist Jesse Perrett sincere thanks for his efforts in the conversion of the access security system to Lenel.

#### Germplasm's Viability and Health:

Viability testing was conducted on 11% of the collection accession in 2016; a concerted effort is being made to assure all seed lots 10 years or older have current germination information. Our storage conditions (4 C, 25-35% relative humidity) are very good, and the efforts devoted to seed cleaning ensure storage of very clean seed lots, important to longevity of viability. A field in the GRIN-Global System to differentiates simple viability from 'pure live seed.' Dormant seeds that do not readily germinate should be considered in the context of accession viability.

Pathology ASRT Dr. Narinder Pal and C. Block validated their seed health assay which distinguishes *Pantoea stewartii* from other *Pantoea* species. This assay provides a solution to the problem of false positives from the ELISA test for tropical *Pantoea* isolates. This is important, as international movement of seed corn typically require freedom from the Stewart's wilt organism. It is anticipated that seed health testing for Goss' wilt will become a common requirement in the near future. Dr. Pal's lab carried out all seed health testing and fungicide treatment to support international seed shipments.

Field inspections were made for all crops, and all cucurbit seedlings were screened routinely for presence of Squash Mosaic Virus via ELISA; Outcomes are detailed in the pathology section of this report. A Northern corn leaf blight screening trial of maize germplasm with several known Ht genes was conducted, and resistant germplasm identified; the pathogen races continue to change, and NCLB has again become a significant concern.

In 2016, we continued testing for adventitious presence (AP) of genetically engineered organisms (GEO) in new maize germplasm accessions and germplasm releases. No positives were identified.

#### **Insect management:**

The Entomology staff provided six insect pollinator species to control pollinate 970 accessions. Honeybees continue to be the primary pollinator used in the NCRPIS regeneration program, followed by the Alfalfa Leafcutter Bee (ALC).

Detailed, interesting observations and interpretative information regarding their field pollinator research activities can be found in their extensive section of the annual report for information on their continuing efforts to enhance the pollination program's effectiveness and efficiency. Substantial reporting is devoted to this team's activities because of the uniqueness of this project, limited sources of such information, and relevance to the broader germplasm conservation world. Feedback and suggestions on experimental approaches are welcomed. We continue to consider the impact of the effectiveness of insect pollinators on crossfertilization of caged plantings, and whether the genetic profile of the accessions is maintained during regeneration.

An unfortunate incident was the theft of multiple four-story hives in the fall; hives, bees and honey were all stolen one fall night, valued at \$18,000. The bees now are being monitoring via strategically placed webcams around the property; this cannot prevent such incidents but may help in determining identity of perpetrators.

#### **Enhancement:**

The Germplasm Enhancement of Maize Project (GEM) continues to work with public and private collaborators to adapt exotic maize germplasm to broaden the genetic diversity of temperate U.S. maize production and provide unique, key priority traits. Research and breeding are designed to improve exotic germplasm introgression methods, to provide unique sources of allelic diversity, and to identify traits and genes to support improvement of agronomic productivity, disease resistance, insect resistance, and value-added grain characteristics, including total extractable starch to support ethanol production, and resistant starch – of importance to human health and nutrition.

The Ames and Raleigh, NC GEM Projects and public collaborators have released 300 lines from 2001-2016, representing more than 60 maize races. An important goal is development of a set of inbred lines representative of the diversity inherent to all of the races of maize. In addition to traditional introgression methods, the project is generating doubled-haploid maize lines in partnership with the ISU Doubled Haploid Facility to accomplish this objective, and also with collaboration of private sector partners to accomplish the initial increase of doubled-haploid seeds in Hawaii and Chile winter nurseries. USDA-ARS and ISU jointly released 204 doubled haploid lines in 2014; the next set of lines from the allelic diversity project will be released in January, 2018. These lines have one-quarter exotic, three-quarters temperate background.

Photoperiod sensitive tropical maize often does not flower until September in Ames. GEM and maize curatorial teams have continued to collaboratively develop an effective method for photoperiod control in the field. While successful, it is difficult to achieve the field scale needed to support the number of accessions that require photoperiod control treatment. The sunflower project has used photoperiod control very effectively to induce flowering in certain wild sunflower accessions. Photoperiodcontrol environment capacity on the order of one to three acres would be very useful in maintaining and providing unique genetic resources.

GEM field days, held every September are well attended by scientists, breeders and graduate students. The field days offer a unique opportunity for more molecular-focused researchers to understand the diversity of the materials available for research, and the activities that support germplasm development.

#### **Outreach and Scholarship:**

More than 500 visitors toured the NCRPIS during 2016. Our staff participated in teaching students from grade K to postgraduate level, and provided outreach events

to civic and other organizations about germplasm conservation and management, and the work done at the NCRPIS. Scientific and technical staff members continue to publish scholarly journal articles, make presentations at scientific meetings, and supervise graduate research programs.

#### **Current and future foci:**

Processes involved in regeneration, characterization, and making viable germplasm available are labor intensive. Resources do not allow maintenance and regeneration efforts, including viability testing, to keep pace with demand. We continue to try to improve conservation methods to better use the resources available to us, and to develop labor and resource saving technologies. We continue to evaluate activities that can be reasonably reduced without sacrificing collection health and quality, and to improve efficiency.

Continued emphasis will be placed on communicating with research stakeholders to identify and address development of comprehensive, genetically diverse collections to meet research and development needs. Climate change is forcing researchers to renew efforts to identify superior forage cultivars as well, and interest has increased in collections of suitable species. A 'gap analysis' process is utilized to examine distribution of crops and their wild relatives; information sources include herbarium records, floras of various countries and ecoregions, predictive analyses bases on GIS layers and habitat information, and scholarly publications that cite plant sources, traits, and performance attributes. Wise selection of targets is important to managing collection growth and effective use of resources. The horticulturists' report details how collection priorities have been determined, and how gap analyses affect these priorities.

2016 collecting efforts will be targeted to expand native woody ornamentals, especially *Fraxinus* in advance of the destructive Emerald Ash Borer, continuing to preserve individual mother trees from the populations to support genetic research; and *Helianthus*.

Better characterization information is essential to enable well-targeted use of the collections, especially given the increasing constraints of limited research and conservation resources. Availability of PGR significantly impacts research applications, including taxonomy.

Curator Laura Marek will continue to collaborate with *Helianthus* researchers to understand the genetic basis of multiple important traits.

Software development efforts continue to center on the development and deployment of the successor to the GRIN system, GRIN-Global - its schema, internal and public interfaces, and applications for data capture and transfer. These efforts are facilitated by contributions from germplasm stakeholders in the U.S. and abroad, as we seek examples of use cases and desired features and functionalities of the new system. A formal process has been proposed for US and international users to submit enhancement requests, prioritize development, enlist developers, and to securely share new software applications that will extend the system's functions and features.

## V. IMPACTS OF GERMPLASM USE BY NORTH CENTRAL REGIONAL RESEARCHERS:

#### Impacts of germplasm use by the researchers at the NCR institutions:

A detailed list of examples of germplasm use in research being conducted at NCR institutions was not requested of the RTAC members this year. NC7 Region researchers typically account for nearly half of domestic plant germplasm distributions from the NCRPIS. Requests for germplasm continue to increase for research as well as non-research use. Requests become increasingly better targeted as the quantity and quality of information associated with the collection improves, thus sharing of findings resulting from use of NPGS germplasm, linked with the germplasm's identity and source, is critically important.

The linkage of the GEM Project, the maize curation project, and public and private collaborators throughout the U.S. facilitates the use of exotic maize germplasm by public and private sector maize researchers. This unique partnership offers great potential for diversifying the genetic base of U.S. maize production, the purpose of the GEM Project.

# Linkages among project participants and with other projects/agencies and contributions of the Regional Technical Advisory Committee:

Linkages are driven primarily by common research interests and objectives and by the heritage of the germplasm material utilized for research and education. All states utilize germplasm provided by the NCRPIS and many of the other 19 NPGS sites; the states have a complex array of collaborative research efforts between their institutions, and with the plant genetic resource curators at the NPGS sites.

The Regional Technical Advisory Committee (RTAC) has provided valuable direction in the following areas:

- Requesting and suggesting organizational structure of information needed to determine project impact and provide accountability. This includes advice on useful formats for analyzing and evaluating the nature of distributions, whom they benefit, and how benefits are realized, which are essential for determining the impact and value of the project.
- Identifying needed improvements to the public GRIN interface.
- Providing input from their respective AES Directors to curators, genebank and other administrators.
- Providing guidance to increase the NCRPIS program's relevance to NCR stakeholders.
- Providing technical expertise, particularly in the areas of diversity assessment and taxonomy.
- Providing added breadth in understanding issues at genebanks beyond the NCRPIS.
- Understanding the challenges faced by public researchers partnering with other public institutions' researchers, both governmental and non-governmental. This has provided useful insights for ARS and NCR administrators to guide programmatic decision-making, as well as operational guidance; this function is key because of its direct impact on the public interest as well as the specific research interests of more directly involved stakeholders.

The technical committee gatherings provide an opportunity for the AES Directors' representatives to learn about and understand strategic issues which impact how their institutions operate and how they can cooperate more effectively to address their mission in today's environment, and then provide this information to their Directors. Among the benefits for the representatives are the opportunity for exposure to research in areas outside their own area of expertise, leading to greater understanding and insights, and the opportunity for service to their institutions, to the NPGS, and to germplasm security.

Some of the NC-7 RTAC's specific suggestions and contributions from their 2015 Annual Meeting in Ames, IA include the following: (from the meeting minutes):

- The 2015 RTAC meeting was hosted by Thomas Lübberstedt and the staff of the NCRPIS in Ames, and highlighted the extensive research initiatives related to realizing the value of plant genetic resources for crop improvement. The opportunities afforded by the meetings and field tours are key to establishing the types of collaborative relationships that lead to long-term partnerships for major research and development efforts. Highlights included tours of the University's Agronomy Farm (a world class operation) and the NCRPIS, presentations by ISU researchers and NC7 representations, and an evening presentation by Norman Cloud of Kemin Industries.
- A warm welcome was extended both by ISU Agricultural Experiment Station Dean Wendy Wintersteen and by Manjit Misra, Director of ISU's Seed Science Center and Chair of the NGRAC (Natl. Genetic Resources Advisory Council). Information was provided on perspectives on genetic resources and their relevance for agriculture, nutrition, economic development and societal welfare. The value and importance of multistate committees for regional work and strongly endorsed the work of NC7-RTAC was reiterated.
- The RTAC is concerned that there ARS has no authorized means to recover costs associated with the issue of phytosanitary inspection certificates, seed shipments, and adventitious presence testing for genetically engineered crop accessions. Be it resolved that ARS devote attention to developing a method of recovering costs for these concerns and the authority to do so.
- Five crops have potential for acquisition of accessions that may have transgenes (as their intellectual property protection expires), maize, soybean, sugar beet, canola and cotton. The RTAC believes that providers of these accessions need to provide documentation concerning the transgenes.
- The RTAC is concerned about insufficient back-up generator protection at the NCRPIS, and encourages completion of this project as soon as possible.
- Consideration should be given to increase internal (within each state) outreach for the Plant Introduction Station.
- The committee thanks Dr. Wintersteen, Dr. Lübberstedt and the NCRPIS staff for hosting the 2015 meeting; we look forward to the 2016 meeting.

#### VI. SUPPORT TEAM REPORTS:

#### A. Farm (F. Engstrom, C. Hopkins, B. Buzzell)

We supervised and coordinated daily operations at the NCRPIS farm, including management of all facilities, fields, and greenhouse space. We conducted all pesticide applications in the field and campus greenhouses. We responded to maintenance requests from staff members at the farm and the campus location. We selected, coordinated, and scheduled the student labor force. We coordinated and completed facility construction and upgrades.

#### Labor:

During 2016, 89 applications for hourly employment were received and reviewed. There were 61 interviews, resulting in 55 new or returning hourly employees hired. Currently there are 19.8 (FTE) Biological Science Aides working at the NCRPIS.

#### **NCRPIS Farm Crew Personnel:**

- Larry Lockhart (Program manager II), on staff since 1985, retired July 1, 2016.
- Fred Engstrom started as our new Program Manager II on July 1, 2016. Fred had been at the NCRPIS with the Germplasm Enhancement of Maize Project for the previous 10 years.
- Brian Buzzell (ISU Farm Mechanic) joined the staff in May 2002.
- Cole Hopkins (ISU Agricultural Specialist II) joined the staff in September, 2016, and assists the vegetable project half-time, and facility operations half-time. Cole assisted the Horticulture team while an undergraduate student in years past.

#### Maintenance projects:

During the past year the farm staff initiated and completed the following projects which enhanced the efficiency and safety of the station operations.

- 1. Administered use biological controls for greenhouse pest control.
- 2. Reorganized greenhouse headhouse and soil preparation areas.
- 3. Assisted in the conversion of the facility's access security system.
- 4. Installed felt mat / drip irrigation systems in greenhouse #1, replaced evaporative cooling pads as needed,
- 5. Developed specifications and design for moveable shelving in the GEM Project's cold seed storage building.
- 6. Arranged for repair of compressors, HVAC equipment in multiple areas.
- 7. Replaced a large air compressor that supplies the seed processing area and dust collection system
- 8. Security fencing installed around equipment storage area
- 9. Webcams installed for security at key points, security light install

#### **Purchasing:**

Fred Engstrom coordinated purchasing for the NCRPIS farm: this task included gathering and summarizing requests, writing specifications, and obtaining supplies for the farm.

#### Equipment Purchased:

- 1. Spacesaver moveable shelving for the GEM Project cold seed storage building.
- 2. Tiller with 3-pt hitch
- 3. Power Harrow tiller

- 4. Low temperature refrigerated incubator for pollinator insect (fly pupae) storage
- 5. 330 gallon and 275 gallon water tote tanks, new pressure washer
- 6. New server and 20 desktop computers lifecycle replacement
- 7. Small plot tractor (Tuff-Built)
- 8. Replaced two vehicles with trade, two JD Gators with trade

#### **Tours:**

During 2016 there were more than 500 visitors.

#### **Staff Training:**

We conducted Tractor and Utility Vehicle Safety, Worker Right-to-Know and Worker Protection Standard training sessions for the new staff and student employees as well as updates for existing staff.

#### B. Information Technology and Telecommunications (P. Cyr, J. Perrett)

Jesse Perrett served as the first-line of support for NCRPIS during 2016. Jesse was supervised by Pete Cyr who is dedicated to the GRIN-Global project. The following list outlines the progress made by the IT team during 2016 at NCRPIS.

#### Equipment:

As of December 2016, NCRPIS has 60 desktop and 33 laptop/tablet workstations installed for use by permanent staff members and part-time temporary student help. Most station computers are equipped with solid state drives, have at least 8 gigabytes of memory, and quad core processors. The centralized functions required by the station were supported by 20 physical servers and around 19 active virtual servers including those used for file storage, intranet, backups, and door security systems.

A Cisco ASA 5525X firewall is installed and configured in order to provide enhanced security as well as increased network performance in line with the gigabit network infrastructure. Each server rack is protected by a battery backup. In addition, a station generator system provide power in the event of power grid failures. The generators in conjunction with the individual rack mounted battery backups should limit the possibility of power failure related server issues.

The station continues to implement virtual servers wherever possible in order to better utilize existing server capabilities and improve efficiency. Virtual server hosts use solid state drive tiered storage systems utilizing the technology built into Microsoft Windows Server 2012 R2 in order to enhance storage performance of existing servers at minimal cost.

In 2016, the Lenel system was installed at NCRPIS. This included trenching for new cable and installing a security camera system as well as upgrading the entire door security system with new hardware. The new system allows Lincpass card access as well as being tied into the ePacs network for physical access security. The camera systems consists of 18 cameras recording all building access doors. Lincpass cards were also requested for all non-temporary employees for door access after the completion of the Lenel system upgrade.

A new backup server was installed and configured. The new server has 24 drive slots and is populated with 8 terabyte drives for a total usable space of 108 terabytes. The new server should provide an improvement in both speed and amount of stored backups.

Grin Global was brought online in January of 2016. As a result, Grin Global Curator Tool has been installed on workstations at the site and multiple new versions were deployed in a short period of time using System Center Configuration Manager (SCCM.)

All station labels were updated and reworked for Grin Global. A method of deploying new label files was created using a network folder deployed to all computers with a group policy. New 2D barcode labels were used to improve readability and as a result, new barcode readers had to be deployed.

A new wooden stake printer was installed and configured. Over 4000 wooden stakes were printed for different projects around the station. New labels and methods had to be developed for use with the wooden stake printer.

GRIN Global development continues to be supported by five physical and multiple virtual server systems. The ability to easily and quickly create virtual systems with any operating system and create and restore checkpoints has been invaluable to the development team.

#### Software:

All workstations at NCRPIS are using Windows 7, Windows 8.1, or Windows 10. Microsoft Office 2013, Adobe Acrobat Professional DC, Adobe Creative Suite, Oracle applications for GRIN, and the GRIN Global Curator Tool were installed on systems when necessary. Laptops and tablets were encrypted by bit-locker.

An RSA server is used in conjunction with local RSA SecureID tokens to provide multifactor authentication for administrative access to computer and server systems per USDA and ARS requirements.

During 2016 Symantec Endpoint Protection (SEP) was used for antivirus and firewall management. In addition to SEP, Malwarebytes Anti-Malware and Malwarebytes Anti-Exploit were installed with a centrally managed enterprise version for greater virus and firewall protection. The combination of these security technologies provides much better protection against security vulnerabilities and some protection in the event of zero-day exploits. Frequent updates to anti-virus and anti-spyware definitions in conjunction with regular full system scans help to ensure that these workstations remain vulnerability free.

In 2016, System Center Configuration Manager (SCCM) was used to verify and deploy Windows updates. SCCM is utilized for more control over software deployment and system management for all computers. The station is using Iowa State University SCCM servers which provides the added benefit of ease of management as well as allowing deployment packages for common software installations to be automatically available for use. During 2016 Lincpass cards were issued to State employees and RSA Tokens were issued to the users needing temporary access in order to ensure email multi-factor authentication was available. Users facilitate connectivity to ARSNet e-mail with Junos Pulse software using LincPass or RSA Tokens for multi-factor authentication. Remote users are able to gain access both to email as well as secure internal file shares using Cisco Systems VPN client through the Cisco ASA firewall. Active Directory group policies are used to implement the necessary security policies on all machines. ISU staff await issue of Lincpass credentials.

#### **Documentation:**

Weather station history data was provided via Sharepoint to allow users to download current and past weather data including calculating GDU and CHU data. Continuing to support advanced document management and retention via SharePoint Server 2013 Intranet site. The NCRPIS public webpage was configured using ARS Site Publisher and later after an upgrade, Umbraco. Posted IT support videos and training documents, and information about farm operation, safety, and health to the NCRPIS intranet website. Provided input to the area IT office regarding system/component information for data calls.

#### Plans for 2016:

- Implement monthly windows updates via SCCM.
- Implement Windows 8.1 and Windows 10 where applicable.
- Transition all barcodes and readers to support 2D format.
- Decommission old equipment including outdated servers and workstations.
- Continue to replace NCRPIS workstations on an as needed basis (targeting a 3-5 year lifespan for daily use workstations).

#### **GRIN-Global:**

The GRIN-Global project is a joint partnership between the USDA-ARS NPGS, the Global Crop Diversity Trust and Bioversity International. The project goal was to develop a new genebank information management system to replace the legacy GRIN Germplasm Management System in such a way that it can be deployed on any size computer with a minimum amount of effort and cost. The new Germplasm Resource Information System (dubbed GRIN-Global) supports five different languages, can be configured to support four database systems, and can be installed on a single desktop The development of the GRIN-Global curator desktop computer or a network. applications (which includes the Curator Tool, Search Tool, various Wizards, and all reports/labels) continued throughout 2016 in direct support of NCRPIS daily curatorial operations. There were two enhanced versions of the Curator Tool developed and tested at NCRPIS before being released to the USDA/NPGS and international GRIN-Global partners. The enhancements to the Curator Tool focused on improvements to the Order Wizard and general speed/performance optimizations to assist NPGS sites with low/slow internet bandwidth constraints. NCRPIS development team produced a new Pollinator Tool for creating, tracking, and closing pollinator requests and has been thoroughly tested in advance of the 2017 growing season.

# C. <u>Information Management-Germplasm Collections (L. Burke, H. Kearney)</u>

#### Acquisition:

The North Central Regional Plant Introduction Station (NCRPIS) acquired 591 (750 inventory lots) new accessions in 2016. Of these new accessions, 120 were received from within the National Plant Germplasm System (NPGS) through exploration and transfer (66 Seeds of Success and 54 NCGRP). 146 accessions of *Daucus* and related species were collected in Spain and donated by Phillip Simon, USDA ARS Vegetable Crops Research Unit at the University of Wisconsin. 54 accession of woody landscape accessions were collected by Jeff Carstens, NCRPIS Horticulturist. 26 *Helianthus* were donated by Dylan Burge, Southern Oregon University. 86 *Dalea* donated by the USDA ARS Forage and Range Research Laboratory. 355 *Helianthus* pre-breeding lines were donated by Loren Rieseberg, University of British Columbia.

As new accessions are recorded in the Germplasm Resources Information Network (GRIN-Global) database, we include as much passport information as possible. Typical passport information would include a source history, cooperator records, collection-site description and geographic coordinates for wild collections, pedigree, secondary identifiers, and any other pertinent information provided by the donor.

#### Maintenance:

Curatorial assistance was provided by processing requests for taxonomic reidentifications and nominations of accessions to the inactive file. In total, 25 accessions received taxonomic re-identifications.

Additionally, 101 accessions were assigned PI numbers.

#### D. Order processing (L. Burke, S. Estrada)

During 2016, 2457 orders were entered into GRIN-Global. Of these, 2013 entered the order processing system via the GRIN-Global Public Website.

Order type	# of orders	# of order items	# of order items shipped
DI and OB external	1365	50157	38635
Internal Pathology	44	1077	1077
Germination	115	4933	4031
Internal Observation	20	1218	952
Regeneration	58	1483	1436
NRR	800	10768	398
Backup	18	479	479
Transfer	7	13	13
Repatriation	11	219	130
Total order items	2438	70347	47151
Herbarium	16	199	199
Information only	3	4	0
All orders	2457	70550	47350

#### E. <u>Seed Storage (L. Burke, S. Estrada)</u>

The seed storage area was staffed by two full-time, permanent federal employees (Lisa Burke and Stacey Estrada) and two part-time student employees.

In 2016, we stored 1350 inventory lots, including 229 original seed lots. Of the increase lots, 871 Ames increases and 259 non-Ames increases were stored. Across all stored lots, we reviewed 7721 inventory lots for seed quantity, and any discrepancies were corrected in the GRIN database. Two hundred and eighteen samples were prepared and transferred to the -20C freezer for long-term storage.

We filled 1294 seed orders in 2016, including those for distribution, observation, germination, transfer and backup. NCRPIS distributed 38082 packets to meet distribution and observation requests. There were 479 lots sent to the National Center for Genetic Resources Preservation (NCGRP) for backup, involving both accessions new to NCGRP and additional seed quantities for previously deposited accessions. Thirteen inventory lots were transferred to other NPGS.

2016 saw the continuation of the prepacking program. With the aid of our student workers, we prepacked 68754 packets from 7451 inventory lots. Prepacking affects seed storage operations by keeping the on-hand inventories more accurate and speeding up seed order filling. Prepacking also reduces the need to review total seed counts for individual accessions because distribution lots are continually monitored and only reviewed when order activity is high for a given accession. This year we used the order history to determine the number of prepacks to prepare for future use.

Seed storage personnel continued to maintain the germplasm distribution display in the farm headquarters hallway. New maps were printed at the start of 2015, and destinations for both domestic and international shipments were marked. The maps are a stop on tours of the station and are a good visual to show visitors the national and international destinations for our germplasm orders.

Scanning of original seed samples continues. In 2016, 297 scans were made, mostly of original samples. Some imaged samples were new to the station while others were being pulled for regeneration when the entire sample was needed. Creating a visual reference of seed lots that have been used up for planting is an important tool to allow future comparisons with the increase lots by curators and storage personnel.

Lisa Burke continued to participate in the development of GRIN Global. She served on the GRIN –Global Advisory Committee as chairperson.

Lisa Burke continued as the station's CPR/AED/First Aid instructor. She provided First Aid certification for 22 NCRPIS student workers and two-year CPR/AED/First Aid certification for 9 staff members. Each session was entered into the National Safety Council database and certificates of completion provided for each participant. Cooperative work with campus staff on improving the CPR/AED/First Aid training was continued.

#### F. <u>Germination (L. Pfiffner)</u>

The germination lab was staffed by one full-time federal employee (Lisa Pfiffner) and up to 4 part-time student employees.

In 2016, the germination lab completed germination or Tetrazolium (TZ) testing on 116 orders containing 5,665 accessions.

Type of Order	# Orders	# Accessions
Regeneration/Other	60	1097
Maintenance	52	4541
TZ	4	27

Advances were made in maintenance testing of the following crops, *Daucus* 615 inventory lots tested, *Linum* 700 inventory lots tested, *Brassica* 500 inventory lots tested, Amaranth 499 inventory lots tested and *Zea mays* 515 inventory lots tested. An additional 904 inventory lots were TZ tested after standard germination testing was complete. After the germination test, the remaining ungerminated seeds were tested to verify if they were viable or non-viable, giving a complete look at the state of the inventory lot.

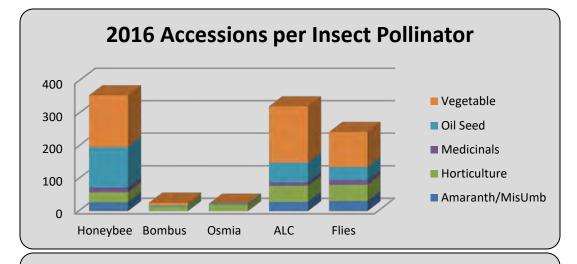
Attended the Annual AOSA meeting, in Portland, OR.

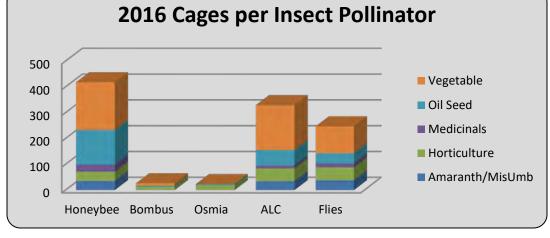
#### VII. CURATORIAL AND SCIENTIFIC TEAM REPORTS:

#### A. <u>Controlled Insect Pollination Service Program (S. Hanlin)</u>

Number of Unique ACCESSIONS per pollinator												
-	Honeybee	Bombus	Osmia	ALC	Flies	TOTAL						
Amaranth/MisUmb	27	0	0	28	31	86						
Horticulture	31	13	19	50	50	163						
Medicinal	15	0	0	11	15	41						
Oil Seed	124	4	4	60	40	232						
Vegetable	159	9	0	173	107	448						
OVERALL	356	26	23	322	243	970						
Number of TOTAL (	CAGE/HIVES	5 per pollir	ator			Number of TOTAL CAGE/HIVES per pollinator						
	noncybee	Bombus	Osmia	ALC	Flies	TOTAL						
Amaranth/MisUmb	35	0 0	Osmia 0	<b>ALC</b> 34	<b>Flies</b> 38	<b>TOTAL</b> 107						
Amaranth/MisUmb Horticulture	•											
	35	0	0	34	38	107						
Horticulture	35 36	0 13	0 19	34 50	$\frac{38}{50}$	107 168						
Horticulture Medicinal	35 36 27	0 13 0	0 19 0	34 50 11	$38 \\ 50 \\ 15$	107     168     53						

#### Summary of Pollinators supplied to 2016 regeneration cages





#### **Progress:**

Caged pollination:

Bee pollinators (minus the alfalfa leafcutting bee) were supplied a single time to 467 cages for controlled pollination of 405 accessions. Alfalfa leafcutting bee and fly-pollinated cages are tabulated and reported separately due to multiple distributions of those insects to the same cages over the pollination season.

Honey bee pollination:

Honey bees were used to pollinate 354 accessions in the field and 2 accessions in a single greenhouse.



Crop Group	Total # of Accessions	# of Genera	# Accessions/Genera
Misc. Umbels	27	9	11 Coriandrum, 6 Petroselinum, 4 Eryngium, 1 Angelica, 1 Foeniculum, 1 Polytaenia, 1 Setaria, 1 Trocdaris, 1 Zizia
Horticulture	31	11	10 Cornus, 6 Diervilla, 4 Prunella, 3 Spiraea, 2 Caragana, 1 Alcea, 1 Althaea, 1 Euonymus, 1 Symphoricarpos, 1 Malva, 1 Physocarpus
Medicinal	15	3	7 Hypericum, 5 Calendula, 3 Echinacea
Oil Seeds	124	3	117 Helianthus, 5 Euphorbia, 2 Brassica
Vegetable	159	5	51 Cucumis, 45 Cichorium, 30 Daucus, 18 Pastinaca , 15 Cucurbita
Total	356	31	

#### 2016 Honeybee Pollinator Deliveries to Regeneration Cages

Overwintering success: 100% of the 23 three story parent colonies, 67% of the 48 two story parent colonies and 66% of the 48 double-story nucleus colonies stored in the 2016 indoor wintering facility survived, comparable to the 58%, 0% and 16% from 2015.

No colonies were left outside during the winter 2015, all colonies and nucleus hives were placed into the overwintering facility. We removed all colonies and nucs from the room starting on March 7, 2016. In the winter of 2016, we placed in the overwintering room 36 two story parent colonies and 88 double story nucleus hives. We left outside and wrapped with thirty pound roofing paper 4 four story parent colonies and 21 three story parent colonies at two locations.

We purchased 30 "Buckfast" three-pound packages and 50 "Buckfast" queens to supplement over-winter losses and to supply spring nucs used for cage pollinations. Because of shipping issues, the packages were again picked up by the "bee crew" in late May at the supplier's location in Texas. The queens arrived USPS in early May and we began nucleus hive production at that time. Because of the late arrival of the purchased queens, we did not start grafting queens until June. The packages were placed into full size hives and given five feedings of high fructose corn syrup (HFCS) and two pollen treatment. The majority of the queens were placed into nucleus boxes with two frames of brood and a single frame of honey and adhering bees. A total of ten queens were used to re-queen either established colonies with weak queens or package queens which did not survive.

In late May we selected queens from resilient, over-wintered parent colonies and set them up as "cell builder colonies" for queen production for nucleus hives during the summer 2016. In the past, we usually begin grafting queens in May, but in 2016 we did not start grafting queens until June 1. During the grafting time we produced an average of 41 queens per week, with nucleus hives being produced until the second week of August. All nucs which were not used in cages for pollination were fed HFCS, an additional super placed below and treated for mites to prepare them for overwintering. Approximately fifteen stronger nucs were placed into full size equipment, given an additional super and over-wintered as a two story parent colony.

Because most of November had higher than normal temperatures and the bees could continue to store syrup, most colonies and nucs going into the over-winter room were

heavier than normal. The mortality rate prior to placing hives into the over-wintering room in December was 7% for the parent colonies and 23% for the nucleus hives. On October 11, a total of 12 parent colonies were stolen from the station location, the makeup of colonies stolen was 6 four stories, 1 three story and 5 two stories.



Mite counts were made using the powdered sugar method in both June and August on 50% of the total colonies and double story nucs. Mite numbers were found to be between 1 to 10 mites per 100 bees which is higher than the documented economic injury levels (EIL) of 5 per 100 bees. In August and September all colonies and nucleus hives were treated with Amitraz (Apivar®). A sugar roll sample was taken in October after the treatment period and observed mite numbers were lower than before the treatment. However in some hives, mites were still present, but a low populations. (See ARS Photo by Scott Bauer)

Starting in March through April 2016, all parent colonies and nucleus hives were given five feedings of HFCS with two feedings being medicated with Fumagilin – B® for the prevention of dysentery (nosema). In late September through November, all hives being prepared for over-wintering were given a total of eight feedings of HFCS with four medicated feedings of Fumagilin – B®. During the summer neither European Foul Brood (EFB) nor American Foul Brood (AFB) were observed, so in 2016 there was no treatment of Terra-Pro for prevention of brood diseases.

For wax moth control during the summer, stored supers with "cleaned" frames were stacked at right angles to each other to prevent adult moth migration in the equipment room. Starting in May through September, the lights in the equipment room were left on during working hours (8 hours; five days). All equipment removed from the field as "dead hives" were stored in the overwintering room at a temperature of 60° F. However for about a month during the summer the air conditioner (AC) stopped working and the temperature increased to ca. 90° F., so ca. 75% of the stored frames were destroyed or damaged by wax moth.

We continue to use our syrup feeding system of a 1,050 gallon polypropylene tank, a 30 gallon poly "mixing" tank and a dish washer for cleaning feeding containers. To prevent crystallization of the HFCS in the large interior storage tank, the contents were circulated daily during the spring, fall and winter. In April 19, 560 gallons of HFCS was purchased for the supplement feeding of bees during the summer and into

the spring of 2017. A new method was used for syrup pick up, with syrup being received using two 275 gallon bulk tanks. The tanks were stored in the maintenance shop until they were needed. Once the syrup supply was depleted in the shop, a tank was taken to the bee shop and the syrup was then pumped out into the main tank. Once again in the spring and fall, once feed containers were placed on hives in the field, the containers were refilled using five gallon buckets rather than filling the individual feed containers in the shop and switching empty containers out.

Because Iowa Department of Agriculture and Land Stewardship (IDALS) was creating a new system for registering bee locations called "Fieldwatch", registering bee yards was not done in 2016. The IDALS registry assists pesticide applicators in locating bee-yards and in obtaining contact information of appropriate beekeepers prior to spraying.

#### Bombus pollination:

Nineteen "mini-research" colonies of *Bombus impatiens* were purchased from a commercial supplier and used to pollinate 26 field cages with 26 accessions. A single *Bombus* hive can be used for pollinating more than one cage with a minimum lapse of 24 hours between sites to prevent pollen contamination. In the *Cucurbita* cages, as in 2016 all *Bombus* hives were paired with a nuc of honey bees so only one hive of each bee was used per cage.



#### 2016 Bombus Pollinator Deliveries to Regeneration Cages

Crop Group	Total # of Accessions	# of Genera	# Accessions/Genera
Horticulture	13	5	7 Diervilla, 2 Spirea, 2 Staphylea, 1 Cercis, 1 Cornus
Oil Seeds	4	1	4 Helianthus
Vegetable	9	1	9 Cucurbita
Total	26	7	



We continued to use 60-quart protective plastic containers to house the cardboard *Bombus* hives while in field cages. Two water-filled quart containers are placed inside as weights to prevent the wind from blowing the container and hive off of the stand. The protective shelter, bottles and hive are placed on a full size honey bee hive body and lid for a stand. The stand prevents the tub and hive from getting flooded in fields which standing water can occur.

<u>Osmia cornifrons/O. lignaria pollination</u>: Osmia were used to pollinate a total of 23 field cages with 23 accessions.



2016 Osmia H	Bee Pollinator	<b>Deliveries</b> to	Regeneration	Cages
	Dec i omnavoi	Don to to	logonoration	Cugoo

Crop Group	<b># of Cages</b>	Total # of Accessions	# of Genera	# Accessions/ Genera
Horticulture	19	19	1	19 Aronia
Oil Seeds	4	4	2	2 Brassica, 2 Linum
Total	23	23	3	

In the 2015 growing season, we obtained an increase of ca. 764 *Osmia* pupae (38 domiciles at 20 bees/domicile) which could be used for pollination and increase during the 2016 pollination season. We purchased an additional 600 commercial cells in the spring of 2016. As in 2015, the pupa were shipped as loose cells. Prior to domicile placement in the field, 25 pupa (15 males and 10 females) were placed into specimen cups and were poured into the domicile just prior to hanging it in the field.

The pupae were used to fill 47 two-inch domiciles and 21 three-inch domiciles. The two inch domiciles were divided in the following manner, 23 were used in pollination cages and 24 were used at three locations at a single "increase" site. The three inch domiciles were all placed at three locations at a single "increase" site.

In the fall of 2016, we collected ca. 360 pupae (18 domiciles at 20 bees) which will be used in the spring of 2017. Additional pupae will be ordered in the spring of 2017 to assure enough pollinators for the spring cages and for placing at "increase" sites.

Through the use of a GPS unit, we plotted and documented the 45 "increase domiciles" at the single location for retrieval later in the summer.

#### <u>Alfalfa leafcutting bee (ALC) Megachile rotundata:</u>



ALC bees were purchased as larvae in leaf cells from a single supplier for use in 2016, arriving in Ames, IA on February 4, 2016. The bee cells were held in refrigerated storage until scheduled for placement in warm incubation and bee emergence boxes. Bees were available weekly throughout the year for use in plant regeneration cages in the field and greenhouse from late December 2015 through early November 2016. The 2016 greenhouse

pollinations started in early December, with the bees being collected from the 2015 supply until February. At this time, the 2015 pupae were supplemented by the new pupae for approximately a month, after which the 2016 pupae was used for the remainder of the year. The 2016 bees will be used into early 2017 and then will be replaced with the newer pupae purchased in 2017.

In 2016, 1989 total ALC deliveries were made to a total of twelve fields and four greenhouses with 329 cages containing 322 accessions. Three greenhouse cages of single accessions of *Bifora*, *Cichorium* and *Cucumis* are still undergoing pollination at the transition from 2016 into 2017.

2010 Infanta Dealeutier I onnation Denverties to Regeneration Cages							
Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period	
Misc.Umbels	74	34	2	28	11	Dec (15) – Oct	
Horticulture	185	50	6	50	11	May – Oct	
Medicinal	45	11	1	11	3	July – Aug	
Oil Seeds	316	60	3	60	6	Feb – Nov	
Vegetables	1369	174	4	173	4	$\operatorname{Feb}-\operatorname{Sept}$	
Total	1989	329	16	322	35	Dec (15) – Nov	

#### 2016 Alfalfa Leafcutter Pollinator Deliveries to Regeneration Cages

Numbers of active ALC-supplied cages and frequency of bee delivery vary seasonally and by cage structure/location and individual accession characteristics. In normal pollination situations, ALC bees/cells are only provided to crops in the field during the summertime months. However at the station, ALC are used outside of the normal time frame. From December 2015 through November 2016, greenhouse cages were supplied weekly with bees in the spring and summer and during the fall and winter twice weekly. 2016 field requests for ALC bees started in late April and the number of weekly active cage increased rapidly through mid-August and then declined with the final field cages being supplied through early November.

In 2016 we received Canadian sourced cells, which have a reduced amount of parasites and parasitoids than found in U.S. cells. In March, the Precision® incubator used for ALC storage needed to be defrosted and all pupal trays were relocated for 72 hours to a comparable incubator at a comparable temperature. There was no observable decline in the emergence of adult bees throughout the summer from this limited relocation.

From October to mid-November, ALC were placed into *Helianthus* and assorted horticulture field cages. Under normal conditions ALC are not the major pollinator of these accessions, however because caged greenhouse plants had not yet begun to bloom and there was a reduced daily supply of adult bees, they were placed into cages for possible pollination rather than discarding them.

#### Flies (Blue Bottle Flies and Houseflies):

Fly pupae of two species (Calliphoridae and *Musca domestica*) were purchased from two suppliers and incubated for weekly use from January 2016 through October 2016 for greenhouse and field pollinations. From late May through August, twenty-eight orders of 10,000 house fly pupae were purchased and from January through September, 247 cups of blue bottle fly pupae was purchased. Starting in late November through December



an additional total of 6 cups of blue bottle pupae was purchased. In 2016, 1165 fly deliveries were made to thirteen fields and three greenhouses with 247 cages containing 243 accessions representing 35 genera.

Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period
Misc.Umbels	204	38	2	31	12	Jan – Oct
Horticulture	142	50	7	50	12	May-Oct
Medicinal	84	15	1	15	3	July – Oct
Oil Seed	149	40	5	40	4	$\operatorname{Feb}-\operatorname{Oct}$
Vegetables	586	104	4	107	4	$\operatorname{Feb}-\operatorname{Sept}$
Total	1165	247	19	243	35	Jan – Oct

2016 Fly Pollinator Deliveries to Regeneration Cages

An average of 8 greenhouse cages and an average of 34 field cages received flies weekly from January through early-October. One greenhouse cage of *Bifora sp.* began getting blue bottle fly pupae starting in early December and transitioned into 2017.

Because blue bottle flies work better at cooler temperatures and a greater number of cage requests were in a cooler greenhouse, only blue bottle flies were distributed

weekly during the winter and spring. During the summer, both blue bottle flies and houseflies were distributed weekly to both greenhouse and field cages for pollination. Adult flies are re-supplied weekly to cages to ensure continued pollinator presence and if appropriate and available, bee pollinators may be present in the same cages that received flies. Throughout the summer several accessions lacking favorable flowers received fly pollinators. This decision was made by curators and the entomology staff to possible increase pollinations and to prevent the discarding of unused fly pupae.

#### **Tests:**

#### Field testing of improved screen bottom boards:

In the winter of 2015, 31 screen bottom boards were improved by strengthening the joints and placing a center support so that the hand cart could be used in transporting and relocating the colonies. However in the summer of 2015 these bottom boards were never tested in the field for strength or the possibility that the center support could help in harboring mites. In the summer of 2016, several colonies at five locations were switched in the spring from solid bottom boards to a screened bottom board. Also during the summer, several colonies on screen bottom boards had to be relocated with no observable damage occurring to the screen on the bottom and no real problem moving the colony with the hand cart. When the colonies were tested during the summer for mite populations using the powder sugar roll, mite numbers did seem lower when compared to colonies on "normal" bottom boards. So it was determined that the screen bottom did assist in mite control and that the center support probably did not allow mites to not drop down to the ground. In the fall when the screened bottom boards were removed, there was some accumulation of wax and bee part residue on the center support, but it did not appear to cause any health issues with the colony. We will continue to place screen bottom boards on hives and will assemble additional bottoms in the coming winters.

#### Winter feeding of OW bees:

Starting on January 28, 25 double story nucleus hives were given a 2" X 8" "winter patty" which was placed directly over the cluster of bees. In 2015, when patties were placed on nucs the surrounding wax paper was not removed from the patties and very little of the patties were consumed. So in 2016 the majority of the wax paper was removed to give easier access by the bees to the patty. The patties were checked weekly for consumption and a second patty was placed on the majority of the nucs on February 24. Because of adequate weight, no patties were placed on colonies of bees while in the over-winter room, however once the colonies were removed in March, full patties were placed on weaker colonies. It was found that in the over-winter room, the bees consumed very little of the patties, however once the hive was placed outside the patties were completely consumed in a week or two. The only explanation for this is that because of the increased bee activity once outside that more energy was needed. In comparing nucleus hives to colonies and fecal material on the fronts of hives it did not appear that feeding the winter patties promoted any greater dysentery (nosema) than if the bees are feeding on honey/HFCS stores. It was determined based on the slow consumption of the winter patties while in the over-winter room that it may assist slightly with survival of the hives, however maybe feeding the hives HFCS would be of more benefit to the bees' health.

Creating and using new devices and data sheets with GRIN-Global (GG):

In late January, with the assistance of K. Reitsma and P. Cyr the 2015 pollination records were uploaded to the "GG-trainer" so initial practicing and troubleshooting issues could be determined. In early February, the 2015 records were moved from the "GG-trainer" to "GG-1" which is a local site so that "pollination inventory actions" could be tested and all changes could be done more quickly. In early March, the "inventory actions" were sent to be added to the working version of GRIN-Global (GG) and 2016 pollination records were entered into the system. Starting in May, all projects which request pollinators were using GG for making requests for pollinators and all earlier records had been migrated over. Throughout the summer a hand held device was not available for making requests, so all records were made in excel and placed into GG. In the spring, K. Reitsma and S. Hanlin worked with the several other projects in assisting in creating the pollination tables both in excel and on GG. Starting in November, S. Hanlin began checking summer records and updating any incomplete or missing records. In the past, these records were done in an excel spread sheet which was created by S. Hanlin and then sent to a curator to be loaded on to GRINclassic. This checking and entering of pollination records took an extended amount of time to complete by both the pollination staff and the curators. With the present system of GG these records were checked and automatically loaded into the GG system without taking time from the curator and taking less time by the entomology staff. A future improvement is to utilize a hand held PC to obtain accession information via a barcode, create a pollination request, and load it directly to GG where it can be downloaded to the entomologist's handheld PC and filled.

#### Safety:

#### Chemical Inventory and Lab Inspections:

On February 19, S. Hanlin updated the Entomology chemical inventory. On February 16, S. Hanlin met with Paul Hokinson of EH&S for inspections for room 112 (Equipment Rm). It was decided that this is not a lab and does not need an annual inspection.

#### **Defensive Driving**:

Because of the amount of time that the bee crew is off site and the amount of cumulative miles during the summer, annual driving training is needed to refresh good driving habits. Several courses were taken on AgLean to fill this requirement. On January 27, S. Hanlin completed "Urban Driving", on January 29 "Distracted Driving" and on February 10, "Defensive Driving Fundamentals". K. Webber completed the following AgLearn defensive driving modules "Collision Avoidance" on February 11 and "Defensive Driving Fundamentals" on February 17.

#### <u>Epi-pens</u>:

On March 28, S. Hanlin sent out a link to the Epi-pen website and "completion document" to all permanent PI staff. Prior to sending staff this information, K. Grooms (nurse at ISU Occupational Medicine) was contacted for her approval of the new training site which contained "the signs of anaphylactic shock and the correct use of Epi-pens". On April 12, K. Grooms was contacted to order replacement pens and On May 3 all completed forms and expired Epi-pens were exchanged for new pens. The Epi-pens are available at four NCRPIS locations as prevention of anaphylactic shock caused by bee stings or other allergic reactions.

#### **Presentations and Outreach:**

In late May, S. Hanlin instructed both G. Welke and N. Ouellette on "The color coded flag system based on type of pollinator needed and where to place flags on cages".

On May 12, K. Webber and S. Hanlin talked to approximately 160 sixth grade students about honey bees in eight half hour presentations at the Squirrel Hollow Outdoor Classroom in Jefferson, IA.

On December 8, S. Hanlin visited via phone with M. Stamm at KSU about the pros and cons of honey bees, ALC and osmia to be used for greenhouse canola pollination.

During the summer of 2016, S. Hanlin and the bee crew assisted M. O'Neill and A. Toth (both ISU Entomology staff) on a study to determine how critical honey bees are to the pollination of soybeans. The bee crew was responsible for suppling the honey bees throughout the summer to a one acre plot of soybeans and ISU staff was responsible for caging plants and making bloom observations.

S. Hanlin spoke to the Gilbert FFA (Future Farmers of America) about getting started in apiculture and what supplies would be needed. The school had been donated two hives from a supporter and had student interest in starting beekeeping during the summer of 2016.

#### Plans for 2017:

#### Winter feeding of over-wintered bees:

In the past two winters we have tried feeding the inside stored hives a commercial "winter patty" with limited success based on consumption of the patties. A method used by several local beekeepers for spring outside feeding is to place either sugar syrup or HFCS into a gallon plastic bag. The method consists of filling a the plastic bag with "syrup", placing it over the cluster of bees and making a small slit in the top of the bag which allows bee access to the liquid feed. In February, all hives will be checked and lifted for weight, if the hive is light, a bag of HFCS will be placed on top of the cluster and monitored for consumption. In the case of a nucleus hives, all surviving hives will be fed using a sandwich bag of syrup and for the 'parent colonies", a gallon bag will be used. We will determine syrup consumption with this method and the amount of cleanup needed once the hives are removed from storage.

#### Creating and using a hand held device for GRIN-Global requests:

In 2016 we were able to create a list of "pollination inventory actions" and created a folders in GG for making pollination requests and fulfilling them. However, the requests were completed/closed by the entomology project and curators by creating an excel file and then copy/dragging the requests into the GG folders. To fill the request the pollination information was taken from GG and placed into an excel folder, a hard copy list of requests was made and requests were filled. The request was completed by coming back to the office and placing a "completed date" into GG. The entomology project were also responsible for creating all "Ongoing" requests in for both ALC and flies by creating a request in an excel folder using the original curatorial information, changing the action and date and then dragging it into GG and saving it. These activities took time for all individuals at the end or beginning of a day and was somewhat labor intensive. Starting in the spring of 2017 working with the IT staff and several curators we will need to create files on a hand held device which we can

take to the field, scan the label barcode, make a request using drop down menus and complete the action by syncing with GG and the information upload. This will be less labor intensive and cause less data mistakes that need to be fixed in the fall.

#### B. <u>Plant Pathology (C. Block, N. Pal)</u>

#### Personnel changes:

Dr. Charlie Block, Plant Pathologist joined the Seed Science Center at Iowa State University in July, 2016.

#### **Research:**

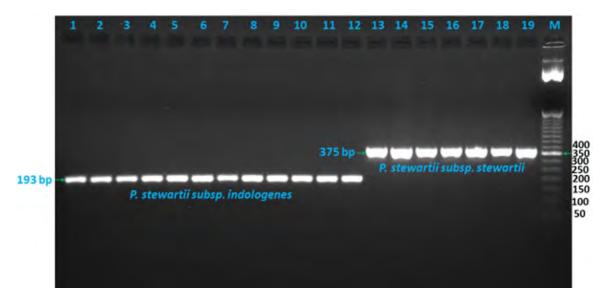
Characterization of unknown Pantoea isolates from maize seed of tropical origin:

Stewart's wilt caused by Pantoea (syn. Erwinia) stewartii subsp. stewartii, is a bacterial disease of major phytosanitary importance. Testing for Stewart's wilt depends mainly on ELISA and published PCR assays. The unknown *Pantoea* isolates occasionally present in tropical maize seed as part of the resident bacterial population, are particularly troublesome because they yield false positives on the Stewart's wilt ELISA. We characterized these unknown *Pantoea* spp. isolated from maize seed of tropical origin by biochemical (motility agar, gram staining, indole reaction, esculin hydrolysis, oxidation-fermentation, ELISA), molecular (PCR using published primers) and pathogenicity tests. And compared them with known isolates of Pantoea stewartii subsp. stewartii, P. stewartii subsp. indologenes, P. agglomerans, P. ananatis and various Erwinia species. Unlike P. stewartii ssp. stewartii, the tropical Pantoea spp. were esculin-positive, indole-positive, motile and non-pathogenic when inoculated onto susceptible maize varieties. The atypical biochemical reaction and nonpathogenicity of tropical Pantoea spp. indicated that they were not Pantoea stewartii ssp. stewartii. Yet they tested positive with the Stewart's wilt ELISA and published PCR methods. Such false positive test results can lead to unnecessary restrictions on international movement of maize seed.

Because ELISA and the published PCR assays fail to distinguish between the closely related *Pantoea stewartii* subsp. *stewartii* (pathogenic on maize) and *P. stewartii* subsp. *indologenes* (non-pathogenic on maize), we designed new primers from the *cpsA-cpsB* intergenic region of the *cps* gene cluster (required for synthesis of exopolysaccharide, stewartan) for the specific detection of *Pantoea stewartii* subsp. *stewartii* in a qPCR assay and for distinguishing the two subspecies in conventional PCR by size differentiation of PCR products. When tropical *Pantoea* spp. were tested by our qPCR and conventional PCR assay, they all tested negative by qPCR as expected and yielded 193 bp PCR product (Figure 1) in the conventional PCR. Sequencing of the 193 bp PCR product and sequence alignment in NCBI BLAST confirmed that the unknown *Pantoea* spp. isolated from maize seed of tropical origin were *P. stewartii* subsp. *indologenes*.

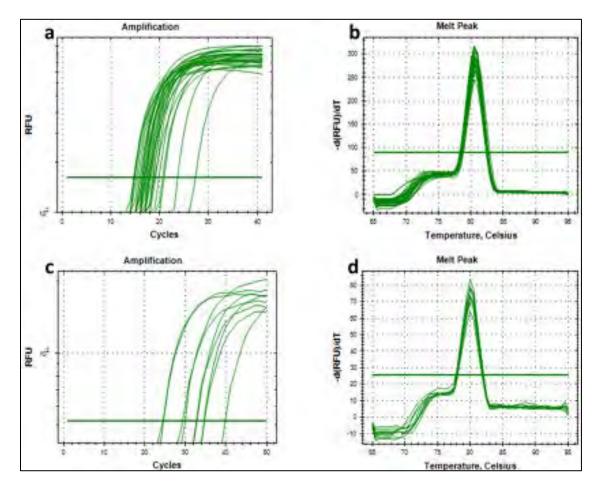
**Figure 1.** 2% agarose gel showing size differentiation of PCR products for *P. stewartii* subsp. indologenes (193 bp) and *P. stewartii* subsp. stewartii (375 bp) in conventional PCR using CpsA-B 1F + CpsA-B 1R primer pair. Lanes 1 to 3, *P. stewartii* subsp. indologenes isolates from sudangrass of California; Lanes 4 to 6, *P. stewartii* subsp. indologenes isolates from the USDA-ARS NRRL culture collection (B-1061) and the American Type Culture Collection (ATCC 35397 and ATCC 51785); Lane 7, *P.* 

*stewartii* subsp. *indologenes* isolates from maize seed of Hawaii origin; Lanes 8, 10 to 12, from maize seed of Puerto Rico origin; Lane 9, from maize seed of Brazil origin; Lanes 13 to 19, known *P. stewartii* subsp. *stewartii* isolates; Lane M, size marker (50 bp Track it DNA ladder, Invitrogen).



Development of a novel seed health assay for diagnosis of Stewart's wilt in maize: Phytosanitary testing for Stewart's wilt, caused by the bacterial pathogen *Pantoea stewartii* subsp. *stewartii*, relies on ELISA and published PCR assays which occasionally generate false positive results. We developed a novel SYBR-green qPCR assay (Figure 2) for specific detection of *Pantoea stewartii* subsp. *stewartii* using primer pair from the *cpsA- cpsB* intergenic region of the *cps* gene cluster. The assay successfully detected the Stewart's wilt bacterial pathogen directly from the seed wash without grinding seeds, isolating the pathogen or extracting DNA.

**Figure 2.** Detection of *P. stewartii* subsp. *stewartii* by qPCR assay. Representative amplification plots (log scale) and melt curves from qPCR using (a, b) DNA from pure cultures of known *P. stewartii* subsp. *stewartii* isolates; (c, d) direct seed wash from maize seed of accessions for which parent plants had shown typical Stewart's wilt disease symptoms in the field during the growing season and the harvested seed lots were positive for the Stewart's wilt ELISA.



Identification of new bacterial pathogen on maize in the United States:

A new bacterial leaf streak disease was reported on maize in the United States from Nebraska, Kansas and Colorado counties during the years 2014 and 2015. In collaboration with researchers from University of Nebraska, Lincoln and Colorado State University, Fort Collins, we isolated the pathogen from the symptomatic leaves, confirmed Koch's postulates on the greenhouse-inoculated maize plants and performed PCR on 16S rDNA and 16S-23S ITS regions. By sequencing of 16S rDNA and 16S-23S ITS regions in our laboratory and multilocus sequence analysis of partial 16S rDNA, *rpoD*, *dnaK*, and *gyrB* genes at Colorado State University, the causal agent of bacterial leaf streak disease on maize in the United States was identified as *Xanthomonas vasicola*.

#### Bacterial Fruit Blotch (BFB) disease in Cucumis melo:

Single seed washes from 50 seeds of each of four *Cucumis melo* accessions known to be contaminated with the BFB pathogen, *Acidovorax citrulli* were tested by SYBR-green qPCR using the published primer pair WFB1+WFB2 as a prescreening method.

The objective was to identify healthy seeds that could be used for seed increase. The prescreening seemed to be quite capable of identifying contaminated seeds, but the percentages were very high in some seed lots (e.g. 48 of 50 seeds). Because the assay could not distinguish live vs. dead bacteria, the approach did not seem practical without creating a potential genetic bottleneck.

#### **Disease Observations on Seed Increase Crops:**

The plant pathology team provides support for curators and technicians on plant health questions and disease diagnosis. Plant health monitoring continued with field inspections of seed parent plants for maize (curation and GEM), sunflowers, and cucurbits.

#### <u>Maize</u>:

During July and August, we made field inspections of 375 maize seed increase plots recording both presence and relative disease severity. Plots were inspected for gray leaf spot, Stewart's wilt, Goss's wilt, northern and southern corn leaf blight, eyespot, crazy top, common rust, common smut, head smut, sorghum downy mildew and wheat streak mosaic virus. Similarly, 543 GEM plots were inspected for the same group of diseases. The incidence and severity of fungal leaf diseases found in 2016 was far less than what we saw during the year 2015 probably due to infrequent rains. The dominant diseases in terms of incidence of infected plants were gray leaf spot (*Cercospora zeae-maydis*), common rust (*Puccinia sorghi*) and northern corn leaf blight (*Exserohilum turcicum*). In terms of common diseases of phytosanitary concern, none were found – no Stewart's wilt, Goss's wilt, head smut, crazy top or other downy mildew diseases were observed. The last time Stewart's wilt was found at the Station was in 2007.

#### Sunflower:

Two field inspections were carried out for downy mildew (*Plasmopara halstedii*), viruses, and phytoplasmas. No downy mildew (the main phytosanitary issue) was present and no other unusual disease problems were observed except that Phomopsis (*Phomopsis helianthi*), Charcoal rot (*Macrophomina phaseolina*) and Verticillium wilt (*Verticillium dahliae*) were found on one or two accessions.

#### Cucurbits:

Routine disease testing for squash mosaic virus was conducted on all cucurbit seedlings prior to transplanting; annual testing has been done since 1993. One hundred and six accessions and 1810 plants were sampled and tested by ELISA. Test results are summarized in Table 1. Seven SqMV-infected plants were identified from one *Cucurbita pepo* accession. Field plantings were scouted every 2-3 weeks to monitor disease development. The seedling screening combined with cage screening was successful in keeping SqMV out of the seed increase planting.

Species	Accessions tested	Accessions with infected plants	Plants tested	# of SqMV infected plants
Cucumis spp. (melo, sativus)	87	0	1486	0
Cucurbita pepo	18	1	279	7
Cucurbita maxima	1	0	45	0
Total	106	1	1810	7 (0.39%)

Table 1: Squash mosaic virus testing results for 2016

We conducted multiple disease inspections of the cucurbit cages during July and August. Powdery mildew (*Podosphaera xanthii*) was a problem and regular fungicide sprays were needed. Anthracnose (*Colletotrichum orbiculare*), Cercospora leaf spot (*Cercospora citrullina*), Bacterial wilt (*Erwinia tracheiphila*), Bacterial leaf spot (*Xanthomonas cucurbitae*) were found on one or two cucurbit accessions. One accession of *Cichorium intybus* was found to have aster yellows.

#### Seed Health Testing/Seed Treatment:

We carry out a seed health testing and fungicide seed treatment program to support international seed shipments -1766 laboratory tests were run, 1363 for maize (primarily for Stewart's wilt), 396 for sunflower, 2 for parsley and 5 for marigold. Results were added to the GRIN database. Phytosanitary documentation, i.e. freedom from specific pathogens, was provided to support seed shipments for 120 international seed orders.

915 maize accessions were tested for Stewart's wilt, Goss's wilt, maize chlorotic mottle virus, wheat streak mosaic virus, six fungal pathogens or nematode (*Ditylenchus dipsaci*). Ninety-nine sunflower accessions were tested for four fungal pathogens (*Verticillium dahliae, Septoria helianthi, Sclerotinia sclerotiorum, Leptosphaeria lindquistii*) and one bacterial pathogen (*Pseudomonas syringae* pv *tagetis*). Two parsley accessions were tested for Phytoplasma and five marigold accessions were tested for bacterial pathogen (*Pseudomonas viridiflava*). In total, 1079 laboratory seed health testing records were uploaded to the GRIN database.

#### Meetings attended:

National Sunflower Association research forum. Fargo, ND (Jan. 11-13, 2016).

#### **Publications:**

Korus, K., Lang, J.M., Adesemoye, A.O., Block, C.C., Pal, N., Leach, J.E., and Jackson-Ziems, T.A. 2017. First Report of *Xanthomonas vasicola* causing Bacterial Leaf Streak on Corn in the United States. Plant Disease. 101(6):1030.

Pal, N., and Block, C.C. 2017. A real-time PCR assay for the differentiation of *Pantoea* stewartii subsp. stewartii from *P. stewartii* subsp. indologenes in corn seed. American Phytopathological Society Annual Meeting, August 5-9, 2017, San Antonio, Texas. (Abstract accepted)

#### C. <u>Amaranthus, Celosia, Chenopodium, Coronilla, Dalea, Echinochloa,</u> <u>Galega, Marina, Melilotus, Panicum, Perilla, Setaria, Spinacia and</u> <u>miscellaneous Apiaceae and Poaceae (D. Brenner, S. Flomo)</u>

#### Acquisition and inactivation:

We acquired 54 new accessions, including 1 *Chenopodium*, 1 *Portulaca*, and 9 *Pseudorlaya* that became *Daucus* in 2017, and 43 *Dalea*.

The *Chenopodium* is a rare Hawaiian species *C. oahuense* (Ames 33359) donated by the USDA-NRCS Plant Materials Center in Hawaii. Unfortunately the seed viability is poor and is to be improved when the donor sends a better seed lot.

An accession of *Portulaca amilis* (PI 677126) was segregated from an older *Portulaca pilosa* accession (PI 677125). The *P. amilis* segregate was a contaminant unknowingly collected during a 2013 American Society of Agronomy meeting in Tampa, Florida by David Brenner in a mixed population of very similar weedy *Portulaca* species.

The nine *Pseudorlaya* that became *Daucus* were donated by a plant exploration to Spain led by David Spooner and sponsored by USDA-ARS Plant Exploration office. They became *Daucus* in early 2017 because of a recent taxonomic revision (Banasiak et al. 2016) that combined the genus *Pseudorlaya* with *Daucus*.

Dr. Douglas Johnson of the USDA, ARS in Logan, Utah donated 43 accessions (Ames 33244 to Ames 33286) of *Dalea ornata* and *Dalea searlsiae*. These are perennials with attractive flowers that are native in the Great Basin, and used in the Great Basin for revegetation.

#### Maintenance:

One-hundred-sixty-eight increased seed lots grown from 2013 to 2016 of our priority crops were stored in 2016, mostly *Amaranthus*. See Table 2 for specifics. Our priority crops are very diverse, they include 446 taxa, in 96 genera, in 6 plant families.

#### <u>Amaranthus</u>:

We had an unfortunate greenhouse overheating in room 134 of the campus greenhouse due to a faulty pneumatic temperature control hose. The temperature was about 40°C (100 °F) for several days in late spring. The amaranth plants in the room survived and were mostly healthy, but seeds on the plants began germinating, so in about six seed lots, viability was poor and the seed cleaning was difficult. The damaged seed lots will be replaced by new regenerations in 2017. In the future we will have more spare parts on hand for quick repairs of the pneumatic ventilation control hoses to reduce the chance of recurrences.



An inadvertent setting of the greenhouse lights to long-days caused this amaranth to revert from flowering to vegetative growth in spring 2017. Later when the lights were re-set to short-days flowering resumed. So there is an unusual interruption of flowering by foliage on this stem.

# Chenopodium:

The wild *Chenopodium* germplasm is prone to poor germination due to seed dormancy. However, an improved protocol for germinating wild *Chenopodium* is in development. Seeds of *Chenopodium berlandieri* (Ames 29759) from a 2014 harvest were planted in both the warm campus greenhouse, and in our cool with fluctuating temperatures, greenhouse, and observed for 4 months from winter to spring. In the campus greenhouse there was no germination, but in the cool greenhouse 36% germination. On the basis of this observation we are planting wild *Chenopodium* in our cool greenhouse and in most cases potting them up for seed production in the warmer greenhouse where growth is faster and there is more space.

# <u>Melilotus</u>:

Sixty-three newly regenerated seed lots were stored in 2016. We regenerated two accessions of mutant *Melilotus albus* genetic stocks.



Folded leaflet PI 557515 mutant Melilotus albus.

# Miscellaneous Apiaceae:

<u>Parsley seed-set failure</u>: A *Petroselinum* (parsley) increase in the field resulted in poor harvests of four of the five accessions, and a good harvest of the fifth accession. The accessions were planted in a greenhouse on July 22, 2015, were kept in a cool greenhouse over the winter, and established well in the field after transplanting on April 13, 2015. All the accessions flowered well and were cage-pollinated by both honey bees and flies. The poor harvest were from healthy plants that did not set seed. Because of a little yellowing and slightly serpentine stems, we suspected the presence of aster yellows phytoplasma disease in these plants. Our Pathologist Narinder Pal performed PCR for 16S ribosomal DNA (rDNA) and 16S-23S intergenic transcribed spacer (ITS) region using universal and phytoplasma-specific primer pairs. Sequencing of PCR products did not detect phytoplasma, which eliminates one hypothetical explanation. So we still do not know what went wrong.

<u>Angelica seed viability</u>: A decline in <u>Angelica seed viability</u> of approximately ten-year old seed lots was discovered by Lisa Pfiffner's seed viability group. Some of the decline could be due to changing viability testing methods; but we need to better understand seed viability in numerous small-collection Apiaceae genera. They may need storage at less than the current 4 °C for an ideal storage life spans.

# <u>Perilla</u>:

*Perilla* seeds have a short storage life and some of the old seeds lots are expiring. Four *Perilla* accessions were regenerated in 2016, to get new seeds and replace older seeds with new higher viability seeds.

# <u>Spinacia</u>:

Two wild spinach accessions were regenerated in our Ames facilities, and two more were planted for harvesting in 2017.

# GRIN database management:

New PI numbers were assigned to 97 temporary-numbered accessions in these crops. One-hundred-fifty-eight literature citations were loaded in GRIN. David Brenner added 22 secondary identifiers to old accessions including 8 quinoa identifiers used by the FAO, and provided by Didier Bazile that allow new cross-linking to published research (Bazil et al. 2016).

# Characterization/evaluation/taxonomy:

# <u>Amaranthus</u>:

There is a taxonomic controversy involving some and probably most of our 109 *Amaranthus caudatus* accessions from India. On a morphological basis they appear to be *Amaranthus caudatus* as determined by David Brenner during greenhouse growouts. But, researchers using molecular methods identify them as *Amaranthus hypochondriacus* and have published about the discrepancy (Chaney et al. 2016, Clouse et al. 2016). In 2017, PI 481125 was grown for confirmation, and was determined to have the flower morphology of *A. caudatus*, so it is a good standard reference accession to study this discrepancy with. One plausible explanation is that *A. hypochondriacus* accession in India evolved traits resembling *A. caudatus* under selection by farmers since the *A. caudatus* flower traits are less prickly, and therefore easier on the hands of farmers than *A. hypochondriacus* flower traits.

A multi-year effort to disentangle the *Amaranthus quitensis* and *A. hybridus* accessions from South America is reaching a conclusion after many taxonomic reidentifications and some re-growing to purify. Four accessions of relatively rare South American *A. hybridus* have new purified distribution seed lots (PI 490694, PI 490696, PI 490703, and a yet-to-be-segregated seed lot of PI 667156).

A small experiment was conducted with severely trimming amaranth inflorescences of field-grown plants on August 10, 2016, early in their development. As compared to un-trimmed, trimmed inflorescences grew fewer but thicker arms with larger glomerules. In conclusion, it appears that some of the tremendous variability in *Amaranthus* inflorescences is due to how plants partition photosynthate to available

primordia. A comparable project (Vargas-Ortiz et al. 2015) but with trimming leaves instead of inflorescences determined that once flowering starts the plants were unable to develop more primordia to replace leaves lost to defoliation.

	Inflor	rescence Measuremen	ts
Arm Thick	ness	Goumerule Width	Flowers per Glom.
Trimmed	4  cm	$4.5~\mathrm{cm}$	Approx. 100
Un-trimmed	$2~{ m cm}$	1 cm	Approx. 20

Inflorescence Measureme	nts
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An example of how grain amaranth inflorescences grow fewer but thicker inflorescence arms out after severe trimming early in development. An un-trimmed inflorescence is on the left and a trimmed example of the same accession is on the right.

# Millets:

We assume that most accessions of *Panicum sumatrense* are un-adapted to temperate, Iowa conditions. However, an accession (PI 463720) of little millet was grown in the 2016 field and was found to be adapted for seed production here, so there is some adaptation.

# Taxonomy:

In 2016, David Brenner made 11 taxonomic re-identifications, involving six genera.

# **Research products publications and presentations:**

The new vibratory deck seed cleaner (VIB) was tried experimentally on seed lots of several crops. Generally, if the air column separators (blowers) work they are a more

efficient choice even though the VIB would work. However the VIB was helpful for cleaning *Melilotus*, and *Origanum* seeds because they combine smooth seed surfaces and rough-surfaced chaff that does not blow out in the air column separators.



A vibratory deck seed cleaner with newly constructed wooden and plywood gear built by David Brenner.

# Presentations:

David Brenner gave a PowerPoint presentation: Medical oxygen concentrators for releasing seed dormancy, at the June 14-16, 2016 Joint National Plant Germplasm System Meeting in Fort Collins, Colorado (Brenner 2016a); and a presentation: *Amaranthus* research trends, at the August 3-5, 2016 Amaranth Institute Conference, at Tennessee State University (Brenner 2016b). David also moderated a session of oral papers at the 2016 American Society of Agronomy conference.



Scientists among a research planting of amaranths from the NCRPIS during a tour at the Tennessee State University, Amaranth Institute Conference.

David Brenner led the Iowa State University NREM 305 Wild Edibles Class on a tour of the campus greenhouse on March 10, 2016. The tour featured edible wild plant species growing in the greenhouse. The class was also shown a demonstration of popping (like popcorn) amaranth seeds in a skillet on a hot plate.

<u>Manuscript Review</u>:

David Brenner reviewed three manuscripts for scientific journals.

### Crop Germplasm Committee reports:

Written progress reports were prepared for the Clover and Special Purpose Legumes, Forage and Turf Grass, Leafy Vegetable, and New Crops, Crop Germplasm Committees (CGCs).

# Service:

David serves on the Board of the Amaranth Institute. Also in early 2016 David was elected as Crop Science Society of America, Division C-8 Chair-Elect which progresses to service as Division Chair-Elect in 2017, Division Chair in 2018, and Past Chair in 2019.

# Conclusions and Plans for 2017 and 2018:

We are developing a temperate-adapted cytoplasmic male sterile (CMS) system in *Amaranthus hypochondriacus*. CMS is helpful in many crops because, with no male fertility, all the progeny from crossing with male fertile plants are hybrid, and are therefore vigorous  $F_1$  hybrids. CMS amaranth lines would aid plant breeding and laboratory research as emphasized in a recent publication by Stetter et al. (2016). There is precedent, Peters and Jain (1987) documented CMS in *Amaranthus* using germplasm from India that was later donated to the NPGS (PI 576485 to PI 576489). In 1993 David Brenner observed CMS inheritance in a line designated as DB 199313 from a cross of PI 568125 X PI 568179. We replicated DB 199313 in 2016, and will attempt additional crosses to demonstrate fertility restoration.



This picture shows a row of male sterile DB 199313 in the 2016 NCRPIS observation field planting. The pink ribbons indicate that male sterility was confirmed visually.

We are developing a descriptor for variation in the frequency of male amaranth flowers. The male flower frequency is known to vary between accessions. A committee of 3 amaranth breeders was formed to work on the project. We will exchange drafts and observations in 2017. Hypothetically lines with high male flower frequency would be ideal male fertility restorer lines in a hybrid seed production system.

Our *Angelica* seeds are losing viability in storage. An effort will be made to consult with seed storage experts at other world seed banks regarding *Angelica* seed storage. We may be able to find out about successful methods that we can adopt.

Our little millet (*Panicum sumatrense*) accessions are thought to be mostly unadapted for seed production in our Iowa temperate climate, because of flowering too late. We want to know which are adapted. All 205 available little millet accessions will be planted in short field rows near the beginning of May 2017 to evaluate for temperate adaptation.

David is working with others to write a book chapter on North American crop wild relatives of the cereal and pseudo cereal crops. Most of the chapter was written in 2016 and the final revision will be in mid-2017.

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# D. <u>Horticulture (J. Carstens, N. Ouellette)</u>

The Horticulture project currently holds 3,680 accessions representing 201 genera (Table 1.0). Significant NC7-medicinal collections includes: Actaea (40), Agastache (69), Echinacea (180), Calendula (83), Hypericum (224), Monarda (81), Prunella (52), and Tanacetum (53). Significant NC7-ornamentals collections includes: Alcea (34), Malva (53), Phacelia (52), Potentilla (106), Sphaeralcea (90), and Thalictrum (52). Significant NC7-woody.landscape collections includes: Aronia (100), Betula (149),

Cornus (196), Euonymus (59), Fraxinus (448), Gymnocladus (88), Rhus (75), Salix (60), Spiraea, (92), Staphylea (42), and Ulmus (39). Jeff Carstens is serving as curator with Nickolis Ouellette serving as horticulture technician. Jeff Carstens focused efforts on continued collaboration with the Brenton Arboretum in assembling a comprehensive collection of Gymnocladus; coordinating deposition of select Fraxinus spp. into cryo at the National Center for Genetic Resources Preservation (NCGRP) in Ft. Collins, CO; research on flowering of Prunella vulgaris; and establishment of a common garden evaluation of NCRPIS Betula nigra accessions. Carstens spent significant time in 2016 assigning Ames accession numbers (528) for all NC7 projects (vacant germplasm program assistant position) and also training new employees on loading passport data to GRIN.

Management group	Genera	Accessions
NC7-medicinals	34	974
NC7-ornamentals	62	735
NC7-woody landscape	105	1971
Total	201	3680

Table 1. Active accessions maintained in the NC7 horticulture collections (medicinals, ornamentals, and woody landscape) as of June 13, 2017

# Acquisitions:

During 2016, we acquired a total of 87 accessions including 18 medicinal, 6 herbaceous ornamental, and 63 woody landscape accessions to the horticulture collections.

Collection trips were completed by Jeff Carstens in Iowa sampling *Monarda punctata* (6) and *Ulmus thomasii* (1), Minnesota sampling *Gymnocladus dioicus* (4), and Missouri sampling *Cornus alternifolia* (2). Nickolis Ouellette completed a collection trip in Illinois sampling *Betula nigra* (1) and Iowa sampling *Maclura pomifera* (1).

Significant contributions from other cooperators included *Betula* (3) and *Fraxinus nigra* (1) from Joseph Zeleznik (North Dakota State University); Aronia (10) from Mark Brand (University of Connecticut); *Ulmus* (3) from M. Eristavi and T. Kurdadze (Republic of Georgia); *Hypericum* (2), *Fraxinus* (4), and *Physocarpus* (1) from Kevin Conrad (Woody Landscape Crop Germplasm Repository); *Monarda* (1) and *Rudbeckia* (1) from Susan Farrington (Missouri Department of Conservation); *Monarda punctata* (2) from Greg Houseal (University of Northern Iowa), and *Fraxinus* (6) from Michael Dosmann (The Arnold Arboretum); *Fraxinus* (1) from Joseph Rothleutner (Lincoln Park Zoo, formerly The Morton Arboretum); and *Malus angustifolia* (3) from Bruce Henry (Missouri Department of Conservation).

### Maintenance:

We currently maintain 31 *Ulmus* accessions as plants in permanent plantings, typically representing only 1-3 clones. Many of our older (40+ years) *Ulmus* accessions are in decline. In fall of 2016, we initiated the establishment of a new *Ulmus* field by transplanting a total of 6 accessions to the new field. We attempted clonal propagation via shoot cuttings for 16 additional accessions. The goal is to establish 3 specimens of each *Ulmus* accession in the new field.

### Regeneration:

Existing plantings that mostly consisted of *Aronia, Calendula, Cornus, Diervilla, Euonymus, Hypericum, Prunella, Spiraea, Staphylea* were harvested via controlled pollinations. A total of 83 accessions were harvested.

We attempted germination for future regeneration on 58 accessions focused on *Alcea*, *Calendula*, *Cotinus*, *Echinacea*, *Euonymus*, *Malva*, and *Ulmus* accessions.

A total of 70 accessions were transplanted to the field mostly focused on *Alcea, Betula, Alcea, Calendula, Echinacea, Hypericum,* and *Malva.* 

Over the last few years, insight has been gained in looking at seed production or yield of wild collections. Although these are preliminary observations, it seems apparent that accessions arising from single plant collections as seeds are extremely difficult to increase likely due to existing self-incompatibility systems. As an example, recent *Diervilla lonicera* harvests from accessions arising from single versus numerous plant collections produced approximately 0.2 grams and 1.0 grams of seeds per plant, respectively. Time is being spent instructing potential collectors on the importance of sampling from numerous specimens and also from the edges of a population.

### Availability and Backup:

Currently, approximately 87% of the medicinals, 73% of the herbaceous ornamentals, and 56% of the woody landscape accessions are available. These percentages are higher for the medicinals and woody landscape accessions compared to last year by 15% and 4%, respectively. The large increase in availability for the medicinals was due to the storage of both 2015 and 2016 increases shortly after last year's reporting. The availability of the herbaceous ornamentals dropped 2%.

Currently, approximately 75% of the medicinals, 77% of the ornamentals, and 43% of the woody landscape accessions are backed up at the National Center for Genetic Resources Preservation in Ft. Collins, Colorado.

### Viability Testing:

A total of 168 seed viability assessments were made for the horticulture collections including maintenance (32), increase (38), original (89), and research (9).

We noted 4 *Monarda fistulosa* accessions (PI 561065, PI 561066, PI 561068, and PI 561069) that were last tested in 2011 were recorded with percent normals of 0, 4, 1, and 0% respectively. After visual inspection, many of these 1991 inventory lots had over 10,000 seeds and majority (50% or more) of the seeds were empty. Lots (PI 561065, PI 561066, PI 561068, and PI 561069) were recleaned and retested resulting in 62, 85, 88 and 36% percent normals, respectively. These 4 samples indicate that the viability of *Monarda* under 4C conditions is reasonable after 25 years in storage and that proper cleaning and assessment before storage is extremely important. Storage of *Monarda* spp. under -18C would likely result in preservation of viable seeds over a much greater time period.

Tetrazolium testing was completed on 6 samples of *Euonymus* looking at freshly collected samples (2 accessions), -18C (3 accessions– stored in 2003, 2004, and 2009), and 4C (1 accession – stored in 2009). According to our germination laboratory

manager, Lisa Pfiffner, all seed lots appeared viable, however a definite answer could not be determined because of green tissue present in the embryos. No difference was detected between the accessions of Ames 30360 that was split during storage and placed in both 4C and -18C. Samples of Ames 27325 was sown in the greenhouse to achieve actual germination. Normal viability tests are not executed on woody landscape accessions that require numerous weeks of warm followed by cold stratification.

John Ball and Richard Kisor (1985), reported that germination of a Minnesota sourced  $Gymnocladus \ dioicus$  seed sample did not benefit from acid scarification. We tested the germination of 4 accessions (Ames 29376 – Iowa; Ames 30371 – Illinois; Ames 29848 – Minnesota; and Ames 32894 – Oklahoma) by soaking them in sulfuric acid for 0, 1, 2, 3 hours. Results determined that seeds from the Minnesota source germinated (25%) in the control (0 hours), which concurred with results of Ball and Kisor. Both Ames 29376 and Ames 32894 samples did not germinate in the control and only 5% germinated with Ames 30371. These results confirm that acid scarification requirements vary based on source. It is suspected that possibly a longer growing season in southern latitudes may result in sufficient hardening of the seed coats and thus germination without scarification.

**Figure 1.** Germination of Kentucky coffeetree after scarification in sulfuric acid for 0 (top left), 1 (top right), 2 (bottom left), and 3 (bottom right) hours.



Ames 29376 – Iowa source

Ames 29848 – Minnesota source

We continue to collaborate with the NCGRP, sending approximately 10,000 or more seeds from three accessions of woody taxa with orthodox seed type for for long term viability testing. To date a total of 17 taxa (33 samples) including *Abies balsamea* (1), *Aronia arbutifolia* (3), *Betula alleghaniensis* (2), *Betula papyrifera* (1), *Betula pumila* (1), *Betula nigra* (3), *Cephalanthus occidentalis* (3), *Cornus alternifolia* (2), *Diervilla lonicera* (3), *Euonymus atropurpureus* (1), *Euonymus europeaus* (2), *Fraxinus quadrangulata* (1), *Gymnocladus dioicus* (1), *Hydrangea quercifolia* (3), *Physocarpus opulifolius* (2), *Pinus strobus* (3), and *Ulmus parvifolia* (1) have been deposited.

# **Distribution:**

Distribution figures for the horticulture collections are summarized in Table 2 and 3, below, and Appendix Table 3. For the combined horticulture program, we distributed 166 external orders to 132 requestors totaling 473 items from 328 accessions. We

cancelled 264 orders from 205 requestors representing 1,171 items. Most of the orders were cancelled because they were requested for home gardening or other non-research use and/or commercial sources could meet the needs of the request.

Most distributed (greatest to least) Taxa Medicinals Echinacea Origanum Calendula Monarda Hypericum Ornamentals Malva Plectranthus Glebionis Caltha Sanvitalia Woody landscape Betula\* Fraxinus Spiraea *Gymnocladus* Aronia

Table 2. Taxa most distributed from the NC7 horticulture program in 2016

\* During 2016, numerous *Betula* accessions were regenerated resulting in sufficient quantities of seedlings which were subsequently made available on GRIN.

N	URPIS no	rticultui	<u>re program d</u>	uring 2016	
		No. of	No. of	No. of Items	No. of Accs
Сгор	Year	Orders	Recipients	Distributed	Distributed
Medicinals*	2012	32	29	166	97
	2013	31	30	150	94
	2014	21	18	133	87
	2015	39	39	218	174
	2016	36	33	99	99
	Average	32	30	153	110
Ornamentals*	2012	49	48	106	86
	2013	45	41	190	154
	2014	41	40	186	160
	2015	21	20	78	74
	2016	33	30	72	61
	Average	38	36	126	107
Woody Landscape	2012	47	43	166	131
	2013	76	63	265	186
	2014	73	58	230	139
	2015	95	66	335	191
	2016	97	69	302	168
	Average	78	60	260	163

Table 3. External domestic and foreign germplasm distributions for the
NCRPIS horticulture program during 2016

\* During 2015, all NC7-mints accessions were reassigned to either the NC7-medicinals or NC7ornamentals collections. During 2016, numerous *Betula* accessions were regenerated resulting in sufficient quantities and thus made available on GRIN. An announcement via email was sent to horticulture professionals indicating the availability of small liners representing well-documented collections. This resulted in an additional 26 orders representing 68 items.

Leaf tissue of *Gymnocladus dioicus* accessions was distributed to Dr. Joanna Freeland (Trent University). Their plans are to investigate the causes of low recruitment, determine the limitations of reproduction, and develop molecular markers to characterize georeferenced materials.

### Characterization/taxonomy:

During 2016, no horticulture accessions were renamed based on morphological characteristics. No PI numbers were assigned.

### **Evaluation:**

In 2016, observation data collected included seed diameters on *Gymnocladus dioicus*, Omernik Ecoregion and USDA Cold Hardiness Zone for *Gymnocladus dioicus*, soluble solid contents of *Aronia* berries, and nutlet measurements of *Betula nigra* totaling 158 observations loaded to GRIN. Ploidy level data determined by Dr. Mark Brand (University of Connecticut) for *Aronia* (11 accessions) and Dr. Alan Whittemore (USDA-ARS National Arboretum) for *Ulmus* (1 accession) were also loaded in GRIN.

A common garden study/evaluation plot of select *Betula nigra* accessions was established in 2016. This evaluation plot includes 5 wild collected accessions from Iowa with 5 mother trees from each accession, 2 commercial seed sources, and 2 cultivars currently in nursery trade, replicated 5 times totaling 145 trees. This evaluation plot has also been duplicated in Haysville, KS. Observation data that was captured in 2016 included caliper, plant height, peak fall color date, and chlorophyll readings (SPAD meter), but has not been loaded to GRIN. The main goal is to potentially identify *B. nigra* germplasm that performs in higher pH soils. Most Midwest and far West soils are inherently more alkaline than soils of the East and Southeast where likely majority of *B. nigra* germplasm is obtained for the nursery industry. Germplasm acquired from acidic soils typically display symptoms of nutrient deficiencies (e.g. chlorosis) when subjected to alkaline soils. Evaluation of these B. nigra genotypes that are adapted to alkaline soils may provide genetic resources with increased adaptability to our typically higher pH, Midwestern soils. Currently research at Kansas State University under Dr. Jason Griffin, is screening our NCRPIS B. nigra genotypes in vitro for rapid and efficient identification of pH tolerance.

# **Enhancement:**

A second generation grow out of *Quercus prinoides* (Ames 23752) was established in 2016 totaling approximately 150 seedlings. Seedlings were grown from select specimens (12) that were selected for form and mildew resistance. The goal is create a seed orchard that will produce progeny that are consistent in form and mildew resistance.

# **Coordination of the NC-7 Regional Ornamental Trials:**

In 2016, Jeff Carstens distributed 101 plants of four *Carya laciniosa* accessions to nine sites for long-term evaluation.

### **Posters, Presentations, and Seminars:**

In spring of 2016, Jeff Carstens hosted a tour of the NCRPIS and gave a presentation on viability testing to the Iowa State University Horticulture 322 (Plant Propagation) class to approximately 39 students. In May, Jeff Carstens gave a presentation at the Gene Conservation of Tree Species workshop in Chicago, IL that was attended by approximately 200 individuals from throughout the world.

### **Conclusions and Plans for 2017:**

The 2016 growing season was generally productive in terms of overall regeneration of the horticulture collections. Progress continued in the acquisition and curation of *Gymnocladus, Aronia, Spiraea,* and *Betula* woody landscape germplasm. Future acquisitions will switch to sampling of western *Fraxinus* species, *Monarda* spp., Midwest *Aronia,* collaboration with Kevin Conrad to sample *Cladrastis kentukea,* and also exploring the potential to successfully store seeds on N. American *Ulmus* spp. Historically, *Ulmus* has always been accessioned clonally as we know that seeds are short-lived (10 years or less) under 4C conditions. Use of cryo and -18C may result in successful preservation of *Ulmus* seeds.

### **Curation:**

For 2017, we will attempt to obtain seed increases from 20 medicinal and ornamental accessions. We will attempt to obtain seed increases from approximately 50 NC7-woody landscape accessions. Historically, our station has utilized a number of different cage sizes ranging from 20x5x5 (5' cages); 20x7x7 (7' cages); and 20x10x10 (10' cages). For regeneration purposes on woody ornamentals, even our 10' cage size is rather small for trying to accommodate large shrubs e.g. *Cornus* spp, *Viburnum* spp., *Staphylea* spp., *Rhus* spp., where typically only 10 large shrubs can be planted.

As an example, some of our *Staphylea trifolia* 10' cages have been in production for 10 years. Average quantity of seed production per plant per harvest is 100 seeds. Recently we've constructed four large (50x30x15) pollination cages (image below) which are able to accommodate approximately 75 large shrubs. In order to reach a goal of 10,000 seeds for a single accession, using a 10' cage or a large hoop house cage will take approximately 10 and 2 years, respectively.



The *Fraxinus* accessions obtained in winter of 2015-2016 via Conrad, Dosmann, and Rothleutner were accessions that originally originated from Japan or China acquired in the late 1970's or early 1980's. These are species that are uncommon in North American collections and were sent directly to NCGRP for depositing into cryopreservation. The ultimate goal is to look at the feasibility of storing these species under cryo and also to establish specimens at NCRPIS for future seed regenerations. The collaboration with NCGRP is extremely beneficial to all parties. Their research on cryopreservation also includes research on *Ulmus* and *Quercus*, which we have and

will continue to provide propagules in order to investigate the ways to potentially and safely preserve recalcitrant species.

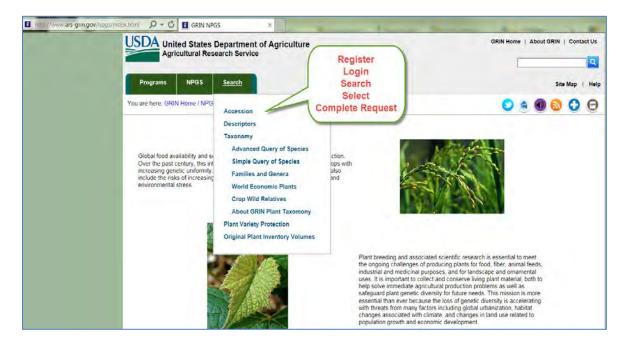
# E. Maize Curation (M. Millard, B. North, D. Zimmerman)

# **Personnel:**

The maize curatorial tech team is fully staffed. David Zimmerman joined the staff in March 2015 as an Iowa State University Ag. Specialist II. Brady North returned to the maize project on November 30, 2015 as a Federal Biological Technician. The team now has enough experience to manage regeneration nurseries and data acquisition on those nurseries efficiently. The maize project has a number of maize specific computer applications. The GRIN development team has not yet had time to develop crop Brady and David developed computer data acquisition specific applications. applications for field observations, ear and kernel lab observations, inventory actions, harvest and hundred weights that are captured in Microsoft Access and then indirectly loaded into GRIN-Global via the drag and drop feature. In time, these applications will be converted GRIN-Global applications that will load the data in real time. Brady North went to CIMMYT at the end of October 2016. He worked alongside CIMMYT Maize Assistant Research Associate Christian Zavala and his team harvesting maize at low, middle, and high elevations. This was a very good exposure to tropical germplasm for Brady because he has only seen ears of many of these tropical races so far during his career. He learned how CIMMYT is using GRIN-Global and provided training on how they could use additional features of GRIN-Global.

# **Research Progress:**

The old GRIN-Classic database has been decommissioned. The NPGS version of GRIN-Global was implemented on November 30, 2015. Go to http://www.ars-grin.gov/npgs/index.aspx as the entry point for GRIN-Global and your old favorites.



To request germplasm, the user should register, login, search, make selections and place the order. This will allow much faster processing of the request. By creating a login, the requestor user will be able to monitor the status of requests, see a history of their requests, mark favorites, etc.

Users are encouraged to use the 'Contact Us' button to send feedback directly to the development team. Not all suggestions for improvements will initially be incorporated in GRIN-Global live, but all suggestions will be logged and addressed as the website is continually improved.

Two upgrade releases have been made since NPGS GRIN-Global went live. The first addressed some speed issues that could really only be seen when the production version had the number of users that the previous version of GRIN had. This was only going to occur after the old system was retired. Additionally, the USDA-ARS was consolidating networking during the same timeframe and requiring VPN access by USDA-ARS employees and contractors to many USDA computer systems including email. This made it apparent that enhancements for speed efficiencies were necessary.

The second release addressed several GRIN public interface enhancements. The GRIN-Global Advisory committee was heavily involved in recommending changes. The most visible enhancement was to change the initial login and search page from this minimalist approach seen in the first image below where the "Search Options" or "Advanced Search" had to be click on to see search features beyond the simple text box.



This is the new GRIN public presentation of the login and search page which shows up front many of the options for search that needed a click above to show. In my experience as the maize curator of over thirty-five years, these options are much more inviting to the new user than the above. There are many other ways to search the database that one should investigate for their particular needs. Searches can be select that will allow queries of taxonomy, descriptors, and passport data all at the same time. More queries will be added over time.

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✓	<u>PI 213706</u>	Osterland Reids Yellow Dent	Zea mays subsp. mays	United States, Iowa	Seed	<u>NC7</u>	Add to Cart
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✓	<u>PI 222319</u>	Red Meadowbrook Reid	Zea mays subsp. mays	United States, Nebraska	Seed	<u>NC7</u>	Add to Cart
✓	<u>PI 452058</u>	Reids Yellow Dent	Zea mays subsp. mays	United States, Illinois	Seed	<u>NC7</u>	Add to Cart
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The USDA-ARS GRIN-Global team won awards for the GRIN-Global work at the ARS regional level, the ARS level, and the USDA level in FY '16. Agriculture Secretary Tom Vilsack and U.S. Department of Agriculture (USDA) Department of Administration (DA) Assistant Secretary Gregory Parham presented the Abraham Lincoln Honor Award to USDA's Agricultural Research Service (ARS) Germplasm Resources Information Network-Global Team leaders Gary Kinard, Ph.D. and Peter D. Cyr at USDA's 2016 Abraham Lincoln Honor Awards Ceremony in Washington, D.C. on Tuesday, Sep. 13, 2016. The Germplasm Resources Information Network-Global Team leaders Gary Kinard, Ph.D. and Peter B. Cyr at USDA's 2016 Abraham Lincoln Honor Awards Ceremony in Washington, D.C. on Tuesday, Sep. 13, 2016. The Germplasm Resources Information Network-Global, an innovative, state-of-the-art information management system for plant genebanks. The maize curator as part of the GRIN-Global development team continues to provide advice on solutions to GRIN-Global issues including those presented by testing at all NPGS facilities. Six international / national genebanks have also implemented, and eight others are evaluation or their implementations are in progress.



**The USDA-ARS GRIN-Global Development team.** Jeanette Duncan (OCIO); Beltsville DBMU -Marty Reisinger, Laura Gu, Kurt Endress, G. Kinard (missing Q. Sinnott); Ames PIRU – Candice Gardner, Pete Cyr, Mark Millard, Lisa Burke; ONP – Peter Bretting

A paper was published in FY '16: Kurtz, B., Gardner, C. A. C., Millard, M. J., Nickson, T. & Smith, J. S. C. 2016, 'Global Access to Maize Germplasm Provided by the US National Plant Germplasm System and by US Plant Breeders', Crop Science, vol. 56, no. 3, pp. 931-941. I provided updated statistics from the GRIN-Global database for final publication.

# Acquisition:

In 2016, 110 new accessions were obtained. These included 27 GEM accessions. We received 20 tropical inbreds from Dr. James L. Brewbaker in Hawaii. We received seed of the sequenced inbred used for the new assembly of the maize genome (B73 RefGen\_v4). The accession is called "B73 RefGen\_v4" (PI 677128) and was reproduced by Dr. Michael D. McMullen from the NCRPIS B73 standard (PI 550473). There were 39 expired or soon to be expiring PVPs received. Dr. Hallauer donated an "Alaska Synthetic" he held in his collection. This synthetic appears to be as early as many of the earliest populations in the collection and may have cold tolerance genes.

### **Regeneration:**

The Zea program had 442 regeneration attempts in 2016 (2.1% of the collection compared with 555 (2.7%) in 2015 and 555 accessions (2.7% of the collection) in 2014.

For perspective, maize accessions store for about 30 years in the intermediate cold storage conditions at Ames. We should be regenerating at least 695 accessions (3.3%) just to address viability deterioration. The breakdown of the regeneration nurseries are as follows:

1. The Ames summer nursery consisted of 267 accessions in 2016. This compares to 259 in 2015 and 247 in 2014 (3,110 vs. 2,674 vs. 2,860 25-foot rows). The nursery was composed of 60 expired or soon to be expired PVPs compared to 43 in 2015. There were 103 other non-PVP inbreds in the nursery. There were 4 GEM and 100 other populations. Several inbreds in high demand such as the NAM parents were grown at the 20 or 40 rows per accession level rather than the standard 10 rows.

After an average two planting dates in early May, warm weather in May and June rapidly developed the crop. Pollinations started during the last week in June and drip irrigation started at that time. After that initial irrigation, no further irrigation was required. The nursery did contain a larger proportion of PVPs with average corn-belt maturity causing a heavy peak in mid-July. After that peak, the pace rapidly relaxed as later maturities were well distributed throughout the latter half of the season. Harvest followed the same non-pressured cycle assisted by a long fall that we have experienced the last three years. The Ames summer nursery increased to 259 accessions in 2015. This compares favorably to 247 in 2014 and 141 (2,674 vs. 2,860 vs. 1,612 25-foot rows). The nursery was composed of 43 expired or soon to expire PVPs, 159 non-PVP inbreds, 1 GEM, and 56 populations. Several inbreds in high demand such as the NAM parents were grown at the 20 or 40 rows per accession level rather than the standard 10.

Stewart's wilt was not observed in any increase plots in 2016, as in every year between 2010 and 2016. ELISA testing is still necessary on Ames increase lots to meet phytosanitary requirements because the state cannot be declared Stewart's wilt free. Evidently, the 2015-16 winter that was warmer than recent cold winters in central Iowa did not significantly increase flea beetle populations, keeping Stewart's wilt under control. Seasonable weather during almost all of the growing season and a long fall is giving good quality seed especially on longer season accessions. This summer's regeneration is rated as above average.

- 2. Eighteen GEMs were regenerated by the Ames GEM team for the maize collection.
- DuPont/Pioneer Hi-Bred planted, pollinated, harvested, and quality tested seed from a nursery of 42 tropical populations planted on Puerto Rico in winter 2015-16. The nursery targeted 100 females per population. Many thanks to all at DuPont Pioneer who assist in these large tropical nurseries.
- 4. A nursery of 100 tropical accessions was received from Monsanto in 2016. Monsanto planted, pollinated, harvested, and quality tested the nursery. The nursery targeted 50 females per population of mainly lowland tropical adaptation. Many thanks to all at Monsanto who assist in these large tropical nurseries.
- 5. In May 2016, a nursery of 24 tropical accessions was planted on St. Croix, then pollinated and harvested. The nursery was received from St. Croix in October

2016 and was managed by Research Leader Dr. Ricardo Goenaga, USDA-ARS, TARS at Mayaguez, PR. Adolfo Quiles retired at the beginning of 2014 and the position has not yet been refilled as of December 2016, therefore this nursery was small.

- 6. 3rd Millennium Genetics was contracted in September 2015 to plant, pollinate, and harvest 70 tropical inbreds. These accessions were planted on October 30, 2015, and received March 30, 2016, and have been processed.
- 3rd Millennium Genetics was contracted in September 2016 to plant, pollinate, and harvest 67 tropical populations. These were planted on October 8, 2016. Lowland tropical accessions from Central and South America from the Goodman racial collection were targeted 3rd Millennium.
- 8. Nine GEMs regenerated by the Raleigh GEM team in 2016 were received and have been or are being processed at the end 2016
- 9. Ames greenhouse increases consisted of thirteen accessions planted at the end of 2015 and harvested in 2016. Two southwestern U.S. accessions grown in the greenhouse during the summer of 2016. Seven accessions were planted in the fall of 2016 for a spring 2017 harvest.

### Maintenance:

There were 20,819 accessions of *Zea* held at the NCRPIS as of December 31, 2016. This compares with 20,744 in 2015 and 20,694 in 2014. GEMs and expiring PVPs made up the majority of the additions. The maize curator maintains an additional 101 accessions from the *Coix* and *Tripsacum* genera.

There were 14,376 available Zea accessions held at the end of 2016 (69% of the total). This compares with 14,144 (68%) in 2015 and 13,876 (67%) in 2014. Improvement in the number of available accessions continues even though the average age of distribution lots in the collection continues to increase. Progress would not be possible without in kind regeneration assistance of Monsanto, DuPont Pioneer, and GEM programs at North Carolina and Iowa.

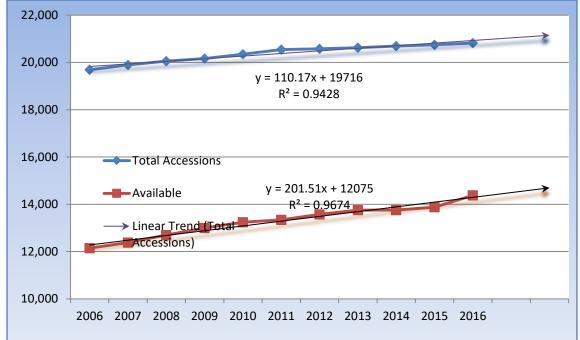


Figure 1. Maize Collection Holdings and Availability Statistics, December 31, 2016

This table indicates		Yearly	Accession .	Availability	
that Zea accession	Year	Total Accessions	Available Accessions	% Available	New Accessions
availability continues to	2007	19,894	12,699	63.8%	124
maintain though the	2008	20,057	12,997	64.8%	150
collection grows and	2009	20,166	12,239	65.7%	105
accessions become	2010	20,347	13,338	65.6%	178
unavailable. Efforts	2011	20,540	13,572	66.1%	180
in recent years have	2012	20,579	13,753	66.8%	39
been focused on	2013	20,624	13,757	66.7%	39
increasing inbreds	2014	20,694	13,876	67.7%	98
and expired PVPs to	2015	20,744	14,144	68.2%	53
meet demand.	2016	20,819	14,144	69.1%	110

Viability testing continued at approximately 2015 levels of about 1,000 accessions.

Fifty-six accessions were backed up at the NLGRP in 2016 compared to one hundred and one accessions in 2015 and seventy-one accessions in 2014. The percent of the collection backed up held at 73%.

# **Distribution:**

Orders for all accessions that are maintained by the maize curator including those of the genera *Tripsacum* and *Coix* increased 15.7% compared to a decrease of 12.4% in 2015 and a 20% decrease in 2014. Almost all orders are now entered by requestors in GRIN-Global. Expired PVP-lines continue to be a major maize distribution category followed by NAM inbred parents, the Goodman-Buckler inbred diversity set, and all other inbred lines. Foreign requests continue to increase on inbred line groups as more is published on them.

			Annı	ual Distri	ibution ]	Data		
Year	Total Packets	Foreign Packets	Total Accs	Foreign Accs	Orders	Foreign Orders	Requestors	Foreign Requestors
2012	20,086	3,412	4,476	1436	814	100	621	88
2013	13,786	3,811	3,573	1,582	755	93	593	83
2014	15,136	4,456	3,556	1,900	603	102	477	93
2015	13,860	3,427	4,444	2,008	528	87	414	80
2016	13,541	4,252	4,572	2,707	611	121	473	106
Averages 2012-16	15,282	3,872	4,124	1,927	662	101	516	90

Expired PVP-lines continue to be a major maize distribution category followed by NAM inbred parents, the Goodman-Buckler inbred diversity set, and all other inbred lines.

Orders for expired PVPs were sent to 168 requestors (33% of all Zea requestors). Expired PVPs made up some portion of 37% of all Zea orders shipped.

	Expired <b>F</b>	<b>PVP Annual D</b> i	istribution D	ata
Year	Total Packets Distributed	Total Accessions Distributed	Orders Processed	Individual Cooperators
2012	6,788	303	270	169
2013	6,830	340	269	170
2014	5,125	346	198	149
2015	3,703	372	188	144
2016	4,017	403	227	168

# **Characterization:**

There were 9,715 data points loaded into GRIN in 2016 on 715 accessions. This compares with 11,791 data points loaded into GRIN on 346 accession in 2015 and 5,099 points on 332 in 2014. More inbred lines were characterized in 2016 than in the previous 2 seasons. More uniformity represents fewer data points.

We imaged 527 accessions in 2016 compared to 420 in 2015 and 365 in 2014. Image loading to GRIN awaits a new process in GRIN-Global in 2017.

# **Evaluation:**

Two disease-screening nurseries were distributed in 2016. Rick Blum, DuPont Pioneer Hi-Bred, screened 272 accessions for northern leaf blight resistance at Johnston, Iowa. Dr. Marc Mancl screened 386 accessions for head smut resistance. This will be the first head smut screening data received for the GRIN database. Many thanks are extended to DuPont Pioneer Hi-Bred for this long-term, continuing contribution. Dr. Charles Block, USDA-ARS pathologist, left the NCRPIS in 2016 to take a job with the Iowa State University Seed Science Center; therefore, no disease screening was done in 2016 on the station. Pathology technician Narinder Pal observed all nurseries in Ames for disease occurrence during the growing season for phytosanitary purposes.

# Plans for 2017:

In 2017 as in recent years, attending to regenerations and regeneration processing will need to take precedence. Regeneration remains my first priority because without viable seed, distribution and resulting research cannot be done.

We will start transferring seed of the tropical increases initiated by Major Goodman in the 1990's and only deposited at the NGRL at Fort Collins for distribution and management at Ames.

We will be funding at least two tropical increase nurseries of ~500 rows each.

During 2017 additional tools will be made available in GRIN-Global and will utilized to enter new and pending data.

NSL and Ames numbered accessions will be reviewed and PI numbers assigned. Over 1,200 Ames-numbered accessions and 400 available NSL-numbered accessions could be assigned permanent PI numbers in 2017. GRIN-Global should greatly assist in this PI assignment project.

We will continue acquiring germplasm from public collections.

I will co augment the collection of images currently on GRIN of 5,000 accessions with images of additional accessions in 2017. An image loader (wizard) is anticipated sometime in 2017 from the developer.

A second maize curator will be recruited in 2017 for additional support, barring resource decreases.

# F. Oilseed Crops (L. Marek, G. Welke)

## **Project management:**

Curator Dr. Laura Marek is assisted by full time staff, Grace Welke, ISU Agronomy Assistant Scientist. Full time staff Irvin Larsen, USDA Research Technician, retired on March 31, 2016 and the position remains unfilled. Part time curation support staff, Lloyd Crim, ISU Farm Equipment Operator, retired at the beginning of May and his replacement was reassigned to support the Vegetable curation project. John Reinhardt, ISU Farm Equipment Operator, works full time for the Oilseed Project November through March. The project is also supported by a team of hourly student workers. Due to the vacant position for the oilseed project, collection maintenance (primarily regenerations) was simplified (limited species to decrease the range of activities required) and reduced for 2016 and some data loading to GRIN-Global delayed.

# Acquisitions:

The oilseed project received 437 new accessions in 2016.

### Brassicaceae:

One new *Camelina arvense* accession, a sample of a known winter type cultivar, was received from Dr. Russ Gesch, MN and three new wild collected *Thlaspi arvense* accessions were received from Dr. Jim Davis, Idaho.

### <u>Helianthus</u>:

Three hundred fifty five cultivated *H. annuus* pre-breeding lines were received from Dr. Loren Rieseberg, University of British Columbia, Vancouver, Canada. The lines contain introgressions from eleven different crop wild relative annual *Helianthus* taxa

crossed in a standard cultivated breeding line background and were developed by Dr. Greg Baute. All of the lines have been genomically characterized and the linkage group location of all introgressions is known. The genomic information will be entered into GRIN.

Nine cultivated *H. annuus* accessions were received from either NCGRP, Ft Collins, CO (5) after expiration of plant variety protection or from their developers in Fargo, ND (4).

Seven new wild annual sunflower accessions were received from Dr. Dylan Burge as he continued to fill geographic gaps in the wild collection as part of a comprehensive search for accessions in drought habitats in the southwest directed and funded by Dr. Loren Rieseberg at the University of British Columbia.

### BLM Seeds of Success:

The Oilseed Project received fifty-six accessions as transfers from W6 and the BLM Seeds of Success program. The 2016 transfer group included 17 Brassicaceae, primarily accessions of several *Lepidium* taxa, 19 wild *Helianthus* accessions (samples of 11 annual and eight perennial wild species populations), 15 miscellaneous asters and five wild flax accessions.

# **Collection Maintenance:**

General statistics about availability and management of the collections are presented in Tables 1 and 2 in the appendix. Selected details for oil seed accessions increased during 2016 are noted below. Due to reduced Oilseed Project staffing, regenerations and their complexity were reduced.

### *Helianthus*, Ames regenerations:

The summer weather in 2016 was not particularly favorable for sunflower with frequent rain, cloudy days, and lack of heat. For example, the cultivated accessions of the UGA-SAM1 population flowered as much as two weeks early and seeds were smaller than in previous years.

Cultivated *H. annuus* accessions are 95.2% available for distribution, including two specialized subsets, the association mapping population UGA-SAM1 and the prebreeding lines from Canada, both of which are currently only available with curator permission until after the 2017 growing season. We are managing regenerations to maintain a high level of availability and to ensure that core collection accessions and other accession groups of interest to specific stakeholder groups are available. In 2016, 109 cultivated *H. annuus* accessions were regenerated in the field. Eighty-nine accessions of the UGA-SAM1 association mapping population were grown in screened seven foot (60) or twenty foot (29) cages. The remaining 20 cultivated accessions were grown in plots of four 20 foot rows with head bagging and sib pollination.

Seed was harvested from all of the cultivated sunflower plots and processing of the harvest is under way.

Wild annual *Helianthus* accessions are 86% available and wild perennial accessions are 82% available. Both the wild perennial and annual collections have new material pending establishment of distribution lots which will increase availability

substantially once they are stored. No new wild sunflower regenerations were started in 2016. Twenty-seven previously established perennial plots were re-caged and seed was harvested from 25 of the populations. Processing of the wild sunflower harvests is underway.

Helianthus, Parlier alternate grow-out site regenerations:

We continue to partner with National Arid Lands Plant Genetic Resources Unit (NALPGRU), Parlier, CA personnel to regenerate wild taxa requiring longer growing seasons than are reliably obtained in Ames. The Parlier environment also provides a valuable alternative for growing mountain and desert species that do not grow well in mid-western humidity and heavy soils. The Parlier location uses sunflower cages purchased by NCRPIS, and can grow up to 40 accessions per year. We germinate seeds in Ames and ship live seedlings to Parlier in late March or early April. The Parlier staff transplants seedlings and manages plant growth. As in Ames, plots are caged before flowering, pollinator insects are introduced, and seed heads are harvested as they mature. Ames funds the pollinator services. Harvested material is shipped to Ames for threshing and processing.

In 2016, 29 small cultivated sunflower accessions were shipped to Parlier in addition to seven wild annual accessions. Heads were harvested from all 36 accessions and shipped to Ames for processing by mid-September much earlier than average.

Typically the curator and technician travel to Parlier to record standard descriptor data for which the Parlier staff do not have time to measure. However, all accessions flowered much earlier than expected and due to reduced staffing in Ames and the fact that flowering in CA coincided with busy field time in IA, meant that we did not visit the CA regeneration plots in 2016. Because of the reduced complexity of the material we sent (only one species) and its early and coordinated flowering, the NALPGRU staff was able to record data for the most important descriptors.

We have an excellent partnership with the NALPGRU staff, ensuring successful regenerations of many sunflower taxa. We are most grateful for the dedicated efforts of Dr. Claire Heintz, new curator with the arid lands location since March 2016, Mr. Jerry Serimian, the Parlier field technician, and his crew.

### Brassicaceae regenerations:

Brassicaceae accessions are 91% available. The Brassica genus encompasses species with a range of growth habits required for successful flowering and seed production. Both *Brassica napus* and *B. rapa* have spring, winter and partial winter types making regenerations of many accessions challenging. In fall 2015, three spring planted Brassica napus accessions which did not flower in the field despite being vernalized before transplanting, were moved into the NCRPIS Farm Greenhouse 2 (FGH-2). All three accessions were successfully harvested in 2016.

In fall 2015, seven *Brassica napus* accessions were direct seeded in the field to overwinter as young plants and vernalize in the field. Two of the accessions overwintered with high survival and were harvested in spring 2016.

In fall 2016, eight *Brassica napus* accessions were direct seeded in the field to overwinter as young plants and vernalize in the field. Three of the accessions had low

emergence rates. Because overwintering success is in part dependent on having a good plant stand going into the winter, the plants of these three accessions were transplanted to FGH-2 before the start of winter. One accession with poor field emergence in 2015 was germinated in germ boxes and the one seedling which emerged from 1200 seeds was kept in the greenhouse.

FGH-2 is managed to provide conditions that approximate a Mediterranean climate allowing us to regenerate brassicaceae accessions native to that region and other brassicaceae taxa which flower very early in the growing season. Because of the interest in *Thlaspi arvense* as an alternate crop for biofuel production, we have been focused on making all accessions of that species available. *T. arvense* flowers very early in the season in Iowa and is present in all NCRPIS farm fields as a weed. To get reasonable regenerations and to ensure the genetic integrity of each accession, we have been increasing *T. arvense* in FGH-2. Five *Thlaspi arvense* accessions planted in fall 2015 were harvested in late spring 2016. One *Crambe glabrata*, one *Isatis costata*, one *Lepidium sativum*, six *Mathiola incana*, and one *T. arvense* accessions were started in fall 2016 for winter 2016-2017 greenhouse regeneration.

### <u>Linum</u>:

Cultivated flax accessions are 99% available. Wild flax accessions are 81% available. The cultivated flax collection was transferred to Ames in 1998 with uniform seed age. The viability group in Ames determined that viability has started to decline in some seed lots and increased their maintenance germination efforts. Based on their data, we are regenerating some accessions of cultivated flax every year, first to ensure all accessions have viability above 50% and then, over the next few years, that viability exceeds 70% for all accessions. Fifteen cultivated flax accessions were successfully regenerated in 2016.

# <u>Cuphea</u>:

No *Cuphea* regenerations were attempted in 2016. Seeds are available for 94% of the accessions of seven species (*Cuphea calophylla*, *C. carthegenensis*, *C. lanceolata*, *C. lutea*, *C. tolucana*, *C. viscosissima*, *C. wrightii*) and the *Cuphea* hybrid accessions that have been part of the agronomic development efforts by members of the National Cuphea Consortium. Thirteen accessions of *Cuphea* are maintained as clones in FGH-1 and distributed as vegetative cuttings. Over all, the *Cuphea* collection is 80% available.

### Miscellaneous asters:

The miscellaneous asters are 34% available. No miscellaneous aster accessions were attempted in 2016.

### <u>Euphorbia</u>:

The *Euphorbia* collection is 49% available. *E. lagascae* is the taxon within this genus of greatest interest for seed oil production and it grows reasonable well during an average Iowa growing season. Accessions of this species are now 88% available. Five accessions of *Euphorbia lagascae* were successfully regenerated in the field during 2016.

### **Distributions:**

General statistics about oil seed collection distributions are presented in Appendix Table 3.

### <u>Helianthus</u>:

In 2016, 5234 sunflower items were distributed to 78 requesters in 102 orders, 41 of which (58% of the items) were sent internationally. Roughly 43% of the distributed items were packets of the 288 line UGA-SAM1 mapping population sent in eight complete and four partial distributions to eight researchers at seven different research institutions for evaluations of drought, salt and flooding tolerance as well as to a researcher investigating heliotropism and circadian rhythm in cultivated sunflower. Other distributions of more than 100 accessions each included requests filled for plant breeders in Spain (2), Zimbabwe, Thailand (evaluating sunflower sprouts for the fresh market) and India, for a pathologist in Serbia evaluating for mildew and *Orobanche* resistance and from a scientist in China seeking to clone salt tolerance genes from salt tolerant wild sunflower species.

### Brassicaceae:

In 2016, 92 orders containing 4344 packets of Brassicaceae germplasm were sent to 79 researchers around the world; 26 international distributions contained 48% of the packets. Forty-seven of the orders contained only accessions of the genus *Brassica*, 42 orders contained only samples of other Brassicaceae genera, and 3 orders contained samples of both *Brassica* and other genera in the family. The three largest distributions were all *Brassica napus* accessions, totaling 1623 packets, sent for varietal development in Turkey, screening for *Orobanche* resistance in Spain and screening for fatty acid metabolism genes in Canada. A researcher in Idaho received 402 *B. juncea* accessions for genetic profiling related to evaluation of blackleg resistance and the *Eruca* collection was sent to a researcher in Nebraska for evaluations of biotic and abiotic stresses. The diversity present in the Brassicaceae collection (262 taxa from 21 genera) supports a wide range of research purposes.

### <u>Linum</u>:

Twenty-five orders containing 577 flax packets were distributed in 2016 to 22 researchers; 82% of the packets were sent to 11 international researchers. The largest distribution, 239 packets, was sent to a researcher in China studying germplasm diversity in flax lines developed in different geographic regions. Other requesters indicated a focus on varietal development and taxonomic investigations.

### Cuphea:

Two orders for varietal development containing 11 cuphea accessions were distributed in 2016, one to a domestic location and one international.

#### Euphorbia:

Two orders containing 4 accessions of *Euphorbia* were distributed to international locations, one for breeding and one to be used in a national seed herbarium for seed analyst training.

### Miscellaneous asters:

Seven orders of miscellaneous aster accessions from seven requesters were filled in 2016; four were sent to domestic destinations (10 accessions) and three to

international locations (eight accessions). Stated research purposed included genetic and anthropological research, seed herbarium specimens and development of a molecular phylogeny.

## **Research Activities:**

General statistics about observations and images recorded for the collections are presented in Appendix Table 4.

# <u>Helianthus</u>:

<u>SAM evaluations/Pre-breeding lines seed supplier</u>: We cooperate with the NSF and Genome Canada grant supported UGA-SAM1 association mapping population and pre-breeding line evaluations managed by Dr. John Burke and Dr. Lisa Donovan, UGA, USA and Dr. Loren Rieseberg, UBC, Canada. We are providing Iowa observations and seeds for evaluations in a drought environment in southern California, a flooding environment in North Dakota, a salt environment in central Canada and a nutrient stress environment in Israel. Additional future environments include Uganda, Chile, India and Argentina.

<u>Cultivated sunflower self-fertility evaluations</u>: We continued to partner with Dr. Jessica Barb, ISU Agronomy Department, who is examining self-fertility in cultivated sunflower using the UGA-SAM1 association mapping population which we maintain and distribute. We helped plant the entire 288 line UGA-SAM1 association mapping population in replicated 10 foot single row plots, managed regenerations for a select subset of the population, and assisted in recording observations. Data from the 2014 and 2015 seasons were presented at the National Sunflower Association Research Forum, Fargo, ND in January 2016.

# **Professional Activities:**

### Meetings and Presentations:

January: I attended the Plant and Animal Genome XXIV Conference in San Diego and, by invitation, a meeting of the Genome Canada group working with sunflower (including the UGA-SAM1 association mapping population).

March: I attended the RF Baker Plant Breeding Symposium, ISU.

May – June: I attended the 19th International Sunflower Association Conference in Edirne, Turkey May 29 – June 2, 2016 as the invited plenary speaker for sunflower genetic resources. The ISU College of Agriculture kindly supported my travel.

June: I attended the NPGS PGOC meeting Curator workshop in Ft Collins, CO June 14-16, 2016.

September: I traveled to the University of Memphis to present the Biology Department's fall semester opening seminar "USDA Genetic Resources for Agriculture: your favorite foods and why you care about genetic resources" on September 1.

November: I attended the CSSA/ASA/SSSA annual meetings, November 6-9, 2016 and presented a talk "Sunflower Germplasm from the Southwest" as an invited speaker for the C8 Symposium Native Crops and Genetic Resources from the Southwest. A New Crops CGC report was made in absentia because the CGC meeting exactly overlapped with the C-8 symposium during which I was presenting.

### Training:

Throughout the year I completed safety trainings as required including Epipen, Fire Extinguisher and assorted AgLearn modules.

### **Publications/Posters:**

Prasfika, J.R., Marek, L.F., Lee, D.K., Hahn, V., Bradshaw, J.D. 2016. Effects from early planting of late-maturing sunflowers on damage from primary insect pests in the United States. Helia. 39(63):45-56.

Barb, J., Marek, L., Welke, G. 2016. Maximizing self-autonomous pollination in sunflower. 38th National Sunflower Association Research Forum, January 12-13, 2016, Fargo, ND. Available:

 $https://www.sunflowernsa.com/uploads/research/1271/maximizing.pollination\_barb.et.al\_poster-2016.pdf$ 

Olson, T., Kontz, B., Mathew, F., Marek, L. 2016. A diagnostic assay to detect the Phomopsis stem canker pathogen. 38th National Sunflower Association Research Forum, January 12-13, 2016, Fargo, ND. Available:

 $https://www.sunflowernsa.com/uploads/research/1283/assay.phomopsis_olson.etal\_paper-2016.pdf$ 

Humann, R., Gulya, T., Marek, L., Jordahl, J., Meyer, S., Acevedo, M., Markell, S. 2016. Evaluation of *Helianthus* germplasm for resistance to *Plasmopahra halstedii* (downy mildew) and *Puccinia helianthi* (rust). 38th National Sunflower Association Research Forum, January 12-13, 2016, Fargo, ND. Available:

 $http://www.sunflowernsa.com/uploads/research/1280/evaluation.helianthus\_humann\_paper-2016.pdf$ 

Seiler, G.J., Marek, L.F. 2016. Collection of *Helianthus anomalus* (Sand Sunflower) from the Southwestern United States 38th National Sunflower Association Research Forum, January 12-13, 2016, Fargo, ND. Available:

http://www.sunflowernsa.com/uploads/research/1270/collection.helianthus\_seiler.ma rek\_poster-2016.pdf

Mandel, J.R., Hubner, S., Nambeesan, S.U., Bowers, J.E., Marek, L., Rieseberg, L.H., Burke, JM. 2016. The public sunflower association mapping population. In: Proc. 19th Intl Sunflower Conference, Edirne, Turkey, Intl Sunfl Assn, Paris, France. p. 489.

Marek, L.F. 2016. Sunflower genetic resources. In: Proc. 19th Intl Sunflower Conference, Edirne, Turkey, Intl Sunfl Assn, Paris, France. p. 31-44.

Seiler, G., Marek, L. 2016. Collection of wild *Helianthus anomalus* and *deserticola* sunflower from the desert southwest USA. In: Proc. 19th Intl Sunflower Conference, Edirne, Turkey, Intl Sunfl Assn, Paris, France. p. 253-262.

# Active Grants:

FY 2012 - 2017 US Helianthus collection trip funding pending 2017 travel. Role: PI.

# Service Activities:

Journal peer review:

I served as a peer reviewer for submissions to Breeding Science, Theoretical and Applied Genetics and the Journal of Plant Registrations.

# PGOC:

I serve as a member of the GIS and Geo-referencing Subcommittee and the Molecular Subcommittee. The GIS and Geo-referencing Subcommittee continues to work with the GRIN Global development team, primarily through trainer M. Reisinger, to ensure that all descriptors including new fields recommended by the committee are included in a useful way in the new database.

# CSSA:

I took over as chair (2017) of the C8 division Plant Genetic Resources of the Crop Science Society of America.

# G. <u>Vegetables (K. Reitsma, L. Clark, C. Hopkins)</u>

Collections curated by the Vegetable Project include *Cichorium* (NC7-chicory), *Cucumis sativus* (NC7-cucumis.cucs), *Cucumis melo* (NC7-cucumis.melo), *Cucumis* species (NC7-cucumis.wilds), *Cucurbita pepo* (NC7-cucurbita), *Daucus* (NC7-daucus), *Ocimum* (NC7-ocimum), and *Pastinaca* (NC7-parsnips). Statistics for accession numbers and availability for each site crop are found in the appendices in "Table 1: NCRPIS Accessions (Accs), Acquired, Available."

# Acquisition:

Two *Cucumis sativus* cultivars and two *Cucurbita pepo* expired PVPs were received from NLGRP (formerly NCGRP) to be incorporated into the NCRPIS collections; three *Cucumis spp.* were transferred from Griffin, Georgia due to reidentification from *Citrullus naudinianus*; and 101 new *Daucus* accessions were collected in Spain by Drs. David Spooner and Philipp Simon (USDA-ARS, Vegetable Crops Research Center, University of Wisconsin, Madison, WI) and Fernando Martinez-Flores (Universidad de Alicante, Spain). The Spain *Daucus* collection included the following new species for the NCRPIS collection: *D. arcanus* (1), *D. carota subsp. majoricus* (13), *D. minusculus* (2), *D. pumilus* (7), *D. setifolius* (6), and *D. hybrid* (*D. carota* subsp. *majoricus* x *D. carota* subsp. *maximus*).



Daucus setifolius Ames 33892 from Cudad Real, Spain is a perennial and a new species in the NCRPIS collection. Images courtesey of Fernando Martenez-Flores, Universidad de Alicante, CIBIO-Departamento de Ciencias Ambientales, Alicante, Spain.

### Maintenance:

Data for vegetable crop regenerations attempted and number of accessions harvested in 2016 are summarized in the appendices in "Table 2: NCRPIS Accessions (Accs) Germinated, Regenerated, Made Available, Backed Up."

*Cichorium* increases focused primarily on accessions having low or declining seed viabilities based on recent maintenance germination testing results. Fifty-seven accessions were planted and harvests were made on 47. Non-bolted plants of six accessions were dug and transplanted to pots in the greenhouse for increase. Only a few plants of one accession bolted and flowered in the greenhouse, so these plants will be transplanted to field cages in the spring of 2017 for regeneration. Resulting seed increases will be bulked with seeds harvested from the 2016 cage increases. Seed viability testing will occur once processing and cleaning are completed.



Cichorium intybus PI 255566 regeneration cage.

Cucumis increases included both greenhouse and field regenerations of 102 accessions, primarily focusing on accessions with low seed quantities or distribution lots 20+ years old. Regenerations of three C. melo accessions were attempted - one accession was harvested but had a low seed quantity, two accessions failed to set fruit. Of the 63 C. sativus accessions attempted, ten failed to germinate and five flowered very late resulting in low or no fruit development. Thirty-five accessions of Cucumis spp. were planted for field and greenhouse regeneration in 2016. Harvests were made on 20 accessions (five of which had low seed quantities), 14 failed to flower, and one failed to germinate. Tentative taxonomic identifications to Cucumis metuliferus were made on 14 of these accessions based on leaf, flower/ovary, and seed characteristics. We will regenerate these accessions in the greenhouse in 2017 and 2018 and verify the taxonomy. The 2016 regeneration seed lots will be stored and made available for distribution after viability testing in April 2017.

*Cucurbita pepo* field regenerations focused on accessions with low seed quantities or distribution lots 20+ years old. Sixteen of 21 accessions were successfully regenerated, two failed to germinate, and three had low fruit/seed production. The increase lots will be inventoried and stored after viability testing. Five late-maturing accessions were regenerated in the greenhouse through hand pollination.

NCRPIS *Daucus* regeneration efforts focused primarily on Ames-numbered wild, annual species and on old PI-numbered accessions having lower seed quantities. Forty-nine accessions were planted for regeneration and seeds were harvested from

34 accessions (22 biennials, 12 annuals). Interest continues for the newer collections of wild *Daucus* germplasm for use in carrot pre-breeding programs as sources of disease and pathogen resistance, and heat/drought tolerance. In addition to the Ames, IA *Daucus* 2016 regenerations, we received seed increases of six accessions from Rosa Yzquierdo, Seminis Vegetable Seeds, Idaho and five accessions from Rob Maxwell, Bejo Seeds, Idaho. Dr. Chris Cramer (New Mexico State University, Las Cruces, NM) attempted regeneration of two *Daucus pusillus* (from California and Texas), but the accessions had high dormancy resulting in small populations which failed to thrive and produce seed.

*Pastinaca* regenerations focused on 20 accessions with low viability. Harvests were made on 18 accessions and seed quantities appear to be sufficient for distribution and to provide new backup lots to NLGRP. Two accessions failed to germinate and will be replanted using alternative parent seed lots for regeneration in 2018. Increase seed lots will be inventoried and stored after viability testing.

The Vegetable Project continued the use of biologicals to control insect pests inside greenhouse and field regeneration cages. We have seen improved control of thrips, whiteflies, and aphids with the use of Nemasys Beneficial Nematodes, Encarsia formosa, and lady bugs. We plan to continue to use these biologicals in 2017 and also look for additional/alternative options and applications in our program.

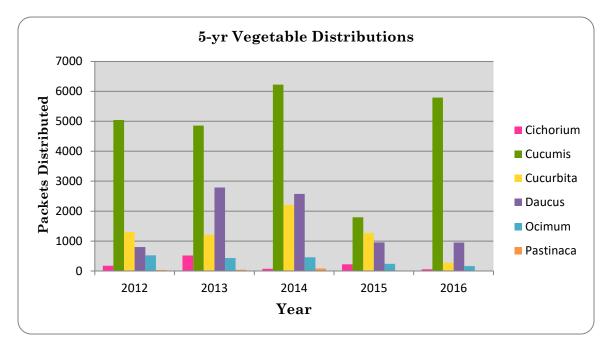
As NCRPIS accessions are regenerated, backup seed samples are sent to NLGRP in Ft. Collins. Overall, 84% of the accessions in the vegetable collections are backed up. Six of eight vegetable site-crops have 80% or more of their accessions backed up at NLGRP (Appendix Table 2). We sent two *Cucumis melo*, 42 *Cucumis sativus*, six wild *Cucumis* species, four *Cucurbita pepo*, and 65 *Daucus* (119 accessions total) for backup to NLGRP in 2016.

In 2016, 1515 vegetable accessions were tested for viability (Appendix Table 2), with the majority of the testing attributed to maintenance germinations on distribution lots. While the GRIN-Global developers were working on the viability wizard for the database, viability data has not been available in a format that allows analysis of the new data which will be loaded to the database in 2017. A cursory assessment of the data shows viabilities continue to remain steady on the cucurbit accessions and *Daucus*, but there was a significant decline in the germinations in the *Cichorium* and *Pastinaca* collections as was reported in last year's annual report; thus, the increased regeneration efforts on these two collections in 2015 and 2016.

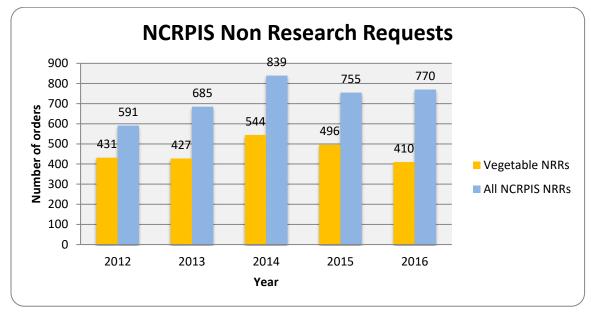
# **Distribution:**

Packet and accession distributions for research and education for the vegetable collections are summarized in the appendices in "Table 3A: External NCRPIS Distributions". In 2016, 7245 seed items (packets) involving 4469 accessions were distributed to fulfill 154 orders (96 domestic, 58 foreign) equaling 126 recipients. The number of items/accessions distributed in 2016 are significantly higher than 2015's distributions (4498 items/2811 accessions). This increase in distributions is due in part to requests for Cucumis germplasm for evaluations associated with the recently funded CucCAP Project (Cucurbit Coordinated Agricultural Project), and preliminary work associated with the *Daucus* Specialty Crop Research Initiative (*Daucus* SCRI). Vegetable research requests received in 2016 specified objective topics such as disease

evaluations, breeding for specific traits and disease resistances, genetic and molecular studies, and diversity assessment for biotic and abiotic stress tolerance. A five-year distribution history of the vegetable crops is shown in the following chart.



Non Research Requests (NRR), i.e., home gardener requests, continue to make up a significant portion of the Vegetable Project requests as shown in the "NCRPIS Non Research Requests" chart below. In 2016, 410 NRR orders were received for accessions maintained by the Vegetable Project. Accessions were distributed for only six of these NRR orders because the requested germplasm was not available from alternative sources, and because of the merit of the objectives of the requests. The remaining 404 NRR orders were cancelled. The number of NRRs received at the NCRPIS continues at a high volume for the vegetable crops even though most of these orders are being cancelled.



# Germinations:

There were 17 seed orders for maintenance viability testing on 1515 seed lots of *Daucus, Cucumis,* and *Cucurbita* performed in 2016. There were also 16 seed orders for viability testing of 249 regeneration seed lots of *Cucumis, Cucurbita, Cichorium, Daucus,* and *Pastinaca* increases in 2016.

# **Characterization and Taxonomy:**

Digital images and basic notes for taxonomic identification and accession characterization were recorded during regeneration. Data for approximately 17 descriptors (primarily fruit descriptors) were recorded at harvest for *Cucumis* and *Cucurbita*. Plant habit, flowering dates, and life-cycle notes were recorded for *Daucus*. Images taken of vegetable accessions in 2016 will be loaded to GRIN. Images are taken to document plant, leaf, flower, fruit, or root characteristics.

Taxonomic identities are reviewed and confirmed as each accession is regenerated or grown in observation plots. The 2016 reidentifications included 3 *Citrullus naudinianus* accessions transferred from Griffin, Georgia as *Cucumis myriocarpus* and *Cucumis spp.*; two *Cucumis spp.* re-identified to *C. melo* subsp. *agrestis* and *C. africanus*; and 27 *Daucus broteri* were reidentified to *D. guttatus* due to nomenclature name changes within the genus *Daucus*.

# **Evaluation/Utilization:**

We continue to screen all *Cucurbita* and *Cucumis* seedlings grown for regeneration for the presence of squash mosaic virus, using ELISA protocols before seedlings are transplanted to the field cages. Seedling screening has been conducted since 1993. All cucurbit field plantings are visually inspected for disease during the growing season. Seed-borne diseases are of specific interest, with bacterial fruit blotch (*Acidovorax citrulli*) in *Cucumis melo* being of particular concern. Phytosanitary issues have prevented the distribution of *Cucumis* germplasm to some countries. The Vegetable Project is working with Dr. Narinder Pal (NCRPIS Pathology Project) and Dr. Charles Block (Iowa State University, Seed Science Center) to develop a method to prevent seed transmission of the bacterial fruit blotch pathogen during the regeneration process which will enable us to secure disease-free seed lots for distribution. Please refer to the Plant Pathology Project section of this report for more information.



Seed transmission of bacterial fruit blotch (Acidovorax citrulli) in Cucumis melo.

As mentioned previously in the Distribution section of this report, there are two important evaluation projects related to NCRPIS vegetable collections, the CucCAP and *Daucus* SCRI projects.

The CucCAP Project has three objectives: develop genomic approaches and tools for cucurbit species, perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars, and perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control. NPGS crop specific curator participation in the project is to provide information and guidance with regard to the germplasm collections and the NPGS. For the NCRPIS germplasm collections, the CucCAP evaluations will focus on disease resistance in *Cucumis sativus* (downy mildew, Phytophthora), *Cucumis melo* (powdery mildew, Fusarium, Cucumber Yellow Stunting Disorder Virus, Cucumber Mosaic Virus), and *Cucurbita pepo* (powdery mildew, Phytophthora, Papaya Ring Spot Virus, Cucumber Mosaic Virus). All data generated in the evaluation process will be referenced in or made available via the GRIN-Global database, and enhanced lines developed through the process may be made available through the NPGS.

The *Daucus* SCRI Project has a similar goal to the CucCAP Project using applied genomics to develop enhanced *Daucus carota* breeding lines based on evaluations of the NPGS cultivated *Daucus carota* accessions for nematode resistance, heat tolerance, flavor and diversity analysis, stand establishment, cavity spot evaluation, and bolting. Additional evaluations are anticipated for later in 2017 and 2018.

# **Publications/Posters:**

Martínez-Flores, F., Arbizu, C.I., Reitsma, K., Juan, A., Simon, P.W., Spooner, D.M., and Crespo, M.B. 2016. Lectotype designation for seven species names in the *Daucus guttatus* complex (*Apiaceae*) from the central and eastern Mediterranean basin. Syst. Bot.

C. Arbizu, P.W. Simon, H. Ruess, F. Martinez-Flores, M. Crespo, and D. Spooner "Integrated Molecular and Morphological Studies of the *Daucus guttatus* Complex." Systematic Botany (2016) 41(2):pp. 479-492

### Plans for 2017:

### Regenerations:

In October 2016, 49 biennial *Daucus* and 20 *Pastinaca* accessions were planted in the greenhouse for regeneration in field cages during the 2017 summer. Approximately 50 accessions of *Cucumis* and 18 accessions of *Cucurbita* will be regenerated in field cages in the summer. Regenerations of wild *Cucumis* species and hard-to-handle *Cucumis* will continue in the greenhouse as time, space, and other resources permit.

# Characterization:

Image loading to GRIN "Classic" was suspended in 2013 in preparation for the launch of the new GRIN-Global database. The Vegetable Project has numerous images taken from 2012 to the present for loading – images acquired as part of the regeneration process, images from the 2013 *Daucus* observation planting, images provided by cooperators for cucurbits and Ocimum, and images taken during the 2016 *Daucus* collection trip in Spain.

Review of accession passport data will continue on the cucurbit and *Daucus* collections in preparation for assigning PI numbers to many of the Ames-numbered accessions in the collections (414 *Cucumis*, 88 *Cucurbita*, and 99 *Daucus*).

## Evaluation:

We are awaiting receipt of evaluation and characterization data resulting from the NPGS funded proposal "Phenotypic and molecular marker evaluation of carrot and wild *Daucus carota* germplasm recently added to the NPGS" submitted by Drs. Philipp Simon and David Spooner (USDA-ARS, Madison, WI) through the Root and Bulb Vegetable Crop Germplasm Committee (RBV-CGC) in 2014. Phenotypic evaluation for key carrot descriptors (storage root shape and color, annual - biennial flowering behavior, other RBV-CGC approved descriptors), and Alternaria leaf blight susceptibility will be collected on the 167 wild and domesticated carrot germplasm accessions collected for the NPGS from 2007 to 2013. Genotyping-by-sequencing (GSB) will be used to characterize the genetic diversity of the germplasm. These data will be integrated with other genomic data to study carrot genetics, domestication, speciation, and evolution. All phenotypic data collected will be loaded into GRIN-Global.

Data generated by the CucCAP (Project Director: Dr. Rebecca Grumet, Professor, Dept. of Horticulture, Michigan State Univ., East Lansing, MI) and the *Daucus* SCRI (Project Director: Dr. Philipp Simon, USDA-ARS, Vegetable Crops Research Unit, Madison, WI) will be loaded to GRIN-Global by the completion of the projects.

# **Observation**:

A Daucus observation planting including 62 accessions collected in Spain in 2016, will be planted in the field the summer of 2017. Data for approximately 39 descriptors will be recorded on plant, leaf, flower, and seed characteristics to support David Spooner's (USDA-ARS, Madison, WI) work on the taxonomic revision of the genus *Daucus*. All data and images collected will be made available to the public through the GRIN-Global database.

Site Crop (Maintenance Policy)	Number Accs	Number Accs Acquired	Number Available	Percent Available	Percent Avail Last Year	PI Numbered Accs	Ames Numbered Accs	NSL Numbered Accs	Backup	Percent Backed up NCGRP	Svalbard
NC7-chicory	279	0	210	92	76	230	23	26	244	87	114
NC7- cucumis.cucs	1388	2	1320	96	95	1230	143	15	1320	96	796
NC7- cucumis.melo	3208	0	2011	63	64	2900	279	25	2592	81	551
NC7- cucumis.wilds	319	3	192	60	58	247	73	0	196	61	48
NC7-cucurbita	976	2	728	74	75	883	96	5	823	84	298
NC7-daucus	1381	101	1149	78	79	996	447	30	1229	83	439
NC7-ocimum	104	0	98	94	94	91	13	0	98	94	76
NC7-parsnips	73	0	50	17	68	52	19	2	50	68	33
Totals	7728	108	5758	74	74	6599	1087	103	6552	84	2355

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# H. <u>Research Leader Activities (C. Gardner)</u>

# Administration and Leadership Activities:

C. Gardner administers the five-year project plan objectives for the USDA-ARS Plant Introduction Research Unit's two CRIS Projects, Plant Introduction Research and the Germplasm Enhancement of Maize (GEM) Project, and contributes to the coordination and execution of activities which support those objectives. Gardner serves as the Coordinator of the Hatch-funded Multistate NC7 Project. Budgetary anomalies due to changing Congressional and Agency priorities continue to command more time and resources. Because of delays in release of funds to the management unit, each year we deal with uncertainty. Making timely decisions for work plans for many taxa that require germination and vernalization treatments in the winter is challenging under these circumstances. The GEM Project CRIS continues to be leveraged to support maize curatorial activities as well, not a permanent solution.

Gardner served in a collaborative group effort to develop ARS' policies and procedures document for sampling for adventitious presence of genetically modified organisms (contamination) in the NPGS collections, and remediation guidelines if contamination is identified.

Gardner was asked to serve on the International Maize Genetic Resources Advisory Committee (IMGRAC) and traveled to CIMMyT in February 2016 for the first meeting of this group.

About 10% of her time was devoted to assisting GRIN-Global System development team members, about 40% to the GEM maize geneticist/Coordinator transition, and about to genebank issues and writing in the past year.

# **Research Activities:**

Graduate student Adam Vanous completed his Ph.D. program in 2016 that deals with phenomena associated with generating haploid and doubled haploid (DH) lines from exotic maize, and with understanding genetic changes that occur during the process of adaptation maize to temperate environments. He also worked with Ag Biosystems engineer to develop software to capture morphometric images from maize ears, which works very well.

As an outcome of Vanous' MS project dealing with methods to double chromosome numbers of haploid lines, thousands of doubled haploid lines were generated from B73 and from Oh43. In 2012 and 2013 we grew these lines for observation, curious about whether DH lines derived from an inbred line would vary. A significant number of lines showed phenotypic variation for morphology, plant and ear height, flowering date, and kernel traits. A new series of questions has been generated about inherent variability in conventionally derived inbred lines, whether the haploid or induction processes are responsible for genetic or epigenetic changes, etc. These lines were topcrossed and were evaluated in yield trials in 2015, and will be again in 2017.

# 2017 Plans:

We will continue to focus on recruiting and filling vacant NCRPIS positions with outstanding individuals and facilitate smooth transitions, and to assist graduate students in completion and publication of their work. We will be looking at the ORISE program to potentially hire contract employees to cover some aspects of our activities.

A course is being developed in collaboration with Dr. Stephen Smith (collaborative ISU Agronomy faculty, retired DuPont/ Pioneer), Dr. Bill Beavis (ISU Agronomy), and other individuals to train individuals interested in plant genetic resource conservation and utilization.

# **Publications:**

Kurtz, B., Gardner, C.A., Millard, M.J., Nickson, T., and Smith, J.S.C. 2016. Global Access to Maize Germplasm Provided by the US National Plant Germplasm System and by US Plant Breeders. Crop Science. 56(3):931-941.

Smelser, A., Gardner, C., Blanco, M., Lübberstedt, T. and Frei, U. 2016. Germplasm enhancement of maize: a look into haploid induction and chromosomal doubling of haploids from temperate-adapted tropical sources. Plant Breeding. 135(5):593-597.

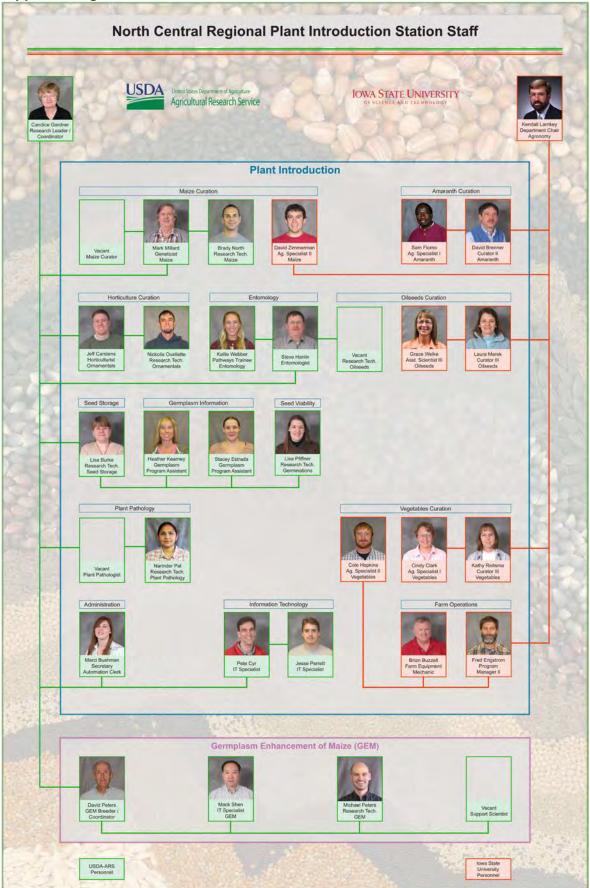
	Table 1.		NCRPIS Acc	essions (A	ccs), Acqui	red, Availa	able
01/01/2016 to	12/31/2016	Number	Number Accs	Percent	Number	Percent	Percent Avai
CURATOR	GENUS_CROP	Accs	Acquired		Available		Last Year
Brenner	NC7-amaranth	3336	0	- 0%	3225	97%	96%
	NC7-celosia	58	1	2%	38	66%	61%
	NC7-echinochloa	306	0	0%	278	91%	92%
	NC7-grasses	133	1	1%	85	64%	64%
	NC7-legumes	294	44	15%	118	40%	47%
	NC7-melilotus	1004	0		891	89%	83%
	NC7-panicum	936	0		912	97%	97%
	NC7-perilla	25	0		18	72%	96%
	NC7-portulaca	11	0		9	82%	70%
	NC7-quinoa	424	1		295	70%	82%
	NC7-setaria	1077	0		1008	94%	93%
	NC7-spinach	413	0		404	98%	98%
	NC7-umbels	1197	13		757	63%	64%
	Total:	9214	60	1%	8038	87%	88%
	NC7-medicinals	971	19	2%	685	71%	73%
	NC7-ornamentals	719	13	0%	529	71%	52%
Carstens	NC7-woody.landscape		55	3%	977	52%	51%
Carstells	Total:	3578	75	2%	2191	61%	55%
Marek	NC7-asters	432	15	3%	133	31%	26%
Marek	NC7-brassica	2009	2	0%	1863	93%	93%
			0		1005	93%	
	NC7-brassica.pvp NC7-crucifers	6	21		-		0%
		1267	0	2%	1107	87% 0%	87%
	NC7-crucifers.pvp	1		-	0		0%
	NC7-cuphea	639	0		498 88	78% 42%	79%
	NC7-euphorbia NC7-flax	209	0				39%
		2834	0	-	2815	99%	100%
	NC7-flax.wilds	147	5		115	78%	82%
	NC7-sun.cults	2236	368	16%	1843	82%	96%
	NC7-sun.cults.SAM	288	0	0%	1 100	0%	100%
	NC7-sun.wilds.ann	1665	24		1433	86%	96%
	NC7-sun.wilds.per	886	8		733	83%	81%
	NC7-sun.wilds.sp	2	0		0	0%	0%
	Total:	12621	443	4%	10629	84%	90%
Millard	NC7-corn.kin	101	0		10	10%	11%
	NC7-maize.gems	267	27			92%	87%
	NC7-maize.inb	2593			2155	83%	79%
	NC7-maize.pop	17117	1	0%		67%	66%
	NC7-maize.pvp	403			401	100%	92%
	NC7-maize.wilds	439			82	19%	19%
	NC7-zea.totals	20819			14376	69%	67%
	Total:	20920	110	1%	14386	69%	67%
Reitsma	NC7-chicory	279				75%	76%
	NC7-cucumis.cucs	1388				95%	95%
	NC7-cucumis.melo	3208			2013	63%	64%
	NC7-cucumis.wilds	320			192	60%	58%
	NC7-cucurbita	978		0%	735	75%	75%
	NC7-daucus	1473	92		1149	78%	78%
	NC7-ocimum	104	0	0%	98	94%	88%
	NC7-parsnips	73	0			77%	68%
	Total:	7823	98	1%	5773	74%	74%

CURATOR GENUS.CROP Brenner NGT-amaranth NGT-echimochoa NGT-fegrasses NGT-fegrasses NGT-fegrasses NGT-genicum NGT-perilla NGT-perilla NGT-perilla NGT-perilla NGT-senarch NGT-amonatals NGT-amonatals NGT-amonatals NGT-amonatals NGT-amonatals NGT-amonatals NGT-amonatals NGT-eruphea NGT	Total: Total: Total:	second se	Number Percent Accs Accs Germed Germed		1. I. I.	Number		Number	with the second		Number Accs	Number Aces	Number Accs Backed Up at		Percent
	vi de la	3336 58 58 306 133 294 1004 936 936 25 11		P	Attempted Regen	Harvested Regen	Permin	Perennial Harvested (Vegetative)	Aces Growing	Accs Made Available	Backed Up at NLGRP for YR	Backed Up at Svalbard for YR	Other Locations for YR	Number Accs Backed Up	Accs Backed Up
	oa Potal: Las ndscape Total:	58 306 133 294 1004 936 25 11	608	18%	165	85	0	0	1.00	11	60	0	0	3255	686
	oa Total: Lis tals ndscape Total:	306 133 294 1004 936 25 11	0	960	5	1	0	0		0	0	0	0	42	729
	Total: ls ndscape Total:	133 294 1004 936 25 11	233	76%	9	00	0	0		0	0	0		275	%06
	Total: ls dscape Total:	294 1004 936 25 11	0	960	60	1	0	0	0	0	0			06 (	68%
	Total: ls tals ndscape Total:	1004 936 25 11	63	21%	4	1	0	0		0	0				61%
	Fotal: Is tals decape Total:	936 25 11	0	960	4	2	0	0		62	49			929	93%
	Total: ls datage Total: Popp	11	0	%0	9		0	0		9	4			916	68%
	Total: ls tals fotal: Total:		0	9%0	5	4	0	0	0	0	0	0		24	68%
	Total: ls tals date Total: Pvp		4	36%	0	0	0	0		4	3	0		6 (	82%
	Total: ls tals dscape Total: pyp	424	11	17%	32	19	0	0		0	8	0			75%
	Total: ls ndscape Total: pvp	1077	1	1%	8	4	0	0		10	6	0			%06
	Total: ls tals ndscape Total: pvp	413	0	9%0	0	61	0	0		0	0	0	0		%16
	Total: ls tals ndscape Total: Pvp	1197	100	8%8	26	35	0	0	0	0	0	0			64%
	ls tals Total: pvp	9214	1080	12%	264	160	0	0		159	133	0		æ	89%
	tals ndscape Total: pvp	116	16	2%	12	40	0	0		1	0	0			76%
	Indscape Total:	719	1	960	17	2	0	0		0	0	0			%LL
	Total: pvp	1888	24	1%	29	115	34	12		17	27	0			44%
	dvq	3578	41	1%	58	160	34	12		18	27	0		6	29%
NC7-brassica, NC7-brassica, NC7-crucifors, NC7-crucifors, NC7-cuphorbia NC7-flax, wilds NC7-flax, wilds NC7-sun, cults NC7-sun, cults NC7-sun, wilds NC7-sun, wilds NC7-sun, wilds	dvq	432	4	1%	0	0	0	0		0	0	0			36%
NC7-crucifors NC7-crucifors NC7-crucifors NC7-cuphea NC7-thax NC7-thax NC7-thax NC7-sun.cults NC7-sun.cults NC7-sun.vulds NC7-sun.vulds NC7-sun.vulds	dad	2009	109	30%	x x	9	4	0		17	H	0		198	%66
NC7-cruciferst NC7-cruciferst NC7-crubbea NC7-fax NC7-fax NC7-sun.cults NC7-sun.cults NC7-sun.cults NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds		9	0.	0%0	0.0	•	0	0		0.4	0	0.0			100%
NC7-cuphea NC7-cuphea NC7-flax wids NC7-flax wids NC7-sun.cults NC7-sun.cults NC7-sun.wids NC7-sun.wids NC7-sun.wids	u.u.u	1201	+ U	170		e			0 0	00	+ 0			1133	237% 2001
NC7-cuptorbia NC7-flax NC7-flax NC7-sun.cults NC7-sun.cults NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds	And	630		200			0	13			c			583	01%
NC7-flax NC7-flax.wilds NC7-sun.cults NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds		209	00	4%	0.10	2 10	0	9 9		0	0	0			42%
NC7-flax.wilds NC7-sun.cults NC7-sun.cults NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds		2834	703	25%	15	15	0	0		0	0	0		2832	100%
NC7-sun.cults NC7-sun.cults. NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds	15	147	0	9%0	0	1	0	0		1	0	0			84%
NC7-sun.cults. NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds		2236	346	15%	17	17	0	0	0	53	19	0		182	81%
NC7-sun.wilds NC7-sun.wilds NC7-sun.wilds	.SAM	288	0	%0	106	35	0	0	0	0	0	0			%0
NC7-sun.wilds NC7-sun.wilds	s.ann	1665	201	12%	L	20	0.0	1		12	61	0			86%
NC/-sun.wilds	s.per	9980	0.4	1%0	0 0	1.7	0.0	0	0	42	32	0		10	80%
	Total.	19891	1978	15.00	0 158	0 UDI		0.0		0 140	0 St	•		10858	0.40
Millard NC7-com kin	TOMOT	101	1	1961	6	6		0		0	0				7661
	ems	267	25	966	10	101	00	0		48	0	o			27%
NC7-maize.inb	qu	2593	675	26%	104	171	0	0		181	44	0		15	61%
NC7-maize.pop	do	17117	226	1%	148	101	0	0		124	1	0		-	%LL
NC7-maize.pvp	dA	403	43	11%	60	59	0	0		41	11	0			100%
NC7-maize.wilds	vilds	439	0	960	0	0	0	0	0	0	0	0		44 (	10%
NC7-zea.totals		20819	989	40%	322	341	0	0	0	394	56	0			73%
	Total:	20920	986	5%	324	343	0	0		394	56	0		15262	73%
Reitsma NC7-chicory		279	0	9%0	22	47	0			0	0	0			87%
NC7-cucumis.cucs	cucs	1388	769	55%	64	48	0	0		73	4	0		1320	92%
NC7-cucumts,meto	meio	0070	100	206	1 06	1 36				N F	2				01.0
NC7-cucurbita		978	16	2%	22	61	0			14	4	0			84%
NC7-daucus		1473	192	52%	44	46	0	0		84	65	0		1	83%
NC7-ocimum		104	0	960	0	0	0	0		0	0	0		98	94%
NC7-parsnips		73	90 I	11%	12	18	0	0.0	0	8	so 1	0	0		68%
TODATO R. I.	TOTAL	1020	1611	02.77	R77	412	0	0	9	100	121			1000	2.60

			anya	External Dome	Domestic Distributions	suom		LOTELDU DISUTIDUTION	TOTOTOTOTOT	an an	TIMITIMET	un onestic an	a roreign.	nonnestic and roteign Distributions
CURATOR	t GENUS_CROP	Number Accs in Collection	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients
Brenner		3336	276	184	33	26	1110	982	14	14	1386	1085	47	4(
	NC7-celosia	58	9	0	5	5			1	1	90	7	9	
	NC7-echinochloa	306	9	9	5	9			3	3	6	7	8	~
	NC7-grasses	133	5		3	3	1	I	1	1		9	4	
	NC7-legumes	294	20		9	5				0		20	9	5
	NC7-melilotus	1004	158	158	5	5	28			4	186	178	6	6
	NC7-panicum	936	19	16	13	11	763	704	2	2	782	706	15	13
	NC7-perilla	25	12	10	2	2	0	0	0	0	12	10	2	
	NC7-portulaca	11	16		9	2	10	2	4	4	26	6	10	~
	NC7-quinoa	424	352	21	40	33	18	283	32	28	2168	295	72	61
	NC7-setaria	1077	325		36	34			2	2		239	38	30
	NC7-spinach	413	1540		11	80	44	39	6	8	-	404	20	10
	NC7-umbels	1197	131		23	19	18			5		126	28	6
	Total:	9214	2866	1	148	119	41	24	9	56	7065	3092	214	175
Carstens	NC7-medicinals	126	62		25	19			9	20	126	92	31	2
	NC7-ornamentals	719	44		22	20			4	4	63	59	26	2
	NC7-woody.landscape	1888	298	-	16	67	39			4	337	167	16	7
	Total:		404		132	66	1	I	16	13		318	148	11
Marek	NC7-asters		10		9	5			8	3	16	12	8	
	NC7-brassica.pvp	9	0	0	0	0	0	0	0	0	0	0	0	
	NC7-brassica	2009	1214	1032	35	30	1865	804	18	17	3079	1558	53	4
	NC7-crucifers	1267	625	568	35	33	276	232	6	6	106	603	44	4
	NC7-crucifers.pvp	1	0		0	0	0	0	0	0	0	0	0	
	NC7-cuphea	639	5		1	1	4		I	1	6	80	61	
	NC7-euphorbia	209	4		1	1	2		4	3	11	10	0	
	NC7-flax	2834	22		8	8	360		5	5	382	357	13	1
	NC7-flax.wilds	147	82		E.	L	111	99	× ×	E E	193	95	15	4
	NC/-sun.csr	I	0		0	0			1	I are	I	I	1	
	NC7-sun.cults	2236	239		36	27			29	27	1692	1035	65	54
	NCI-sun.cuits.SAM	288	908		1.	4	1301		0	4	Rezz	288	12	
	NC7-sun.wilds.ann	6001 200	900	956	10	61	151	821		11	001	010	40	4
	NPT sun milds on	6	070		0	91					OFF	ere .	OT OT	
	Total:	1262	4056	297	145	113	5682	295	82	75	973	4902	227	188
Millard	NC7-corn.kin		6		4	4	6		2	2		10	9	
	NC7-maize.gems	267	804	247	29	24	342	22	7	7	11	247	36	31
	NC7-maize.inb	2593	3461	866	234	200	789	455	75	99	4250	1108	309	266
	NC7-maize.pop	11117	2272	1539	173	143	1635	1561	29	24	3907	2729	202	167
	NC7-maize.pvp	403	2662	4	185	130	1	C.D	42	38	4017	403	227	168
	NC7-maize.wilds	439	81	38	25	24	122		11	п	203	85	36	
	NC7-zea.totals		9280		486	363	4243			106	13523	4572	209	469
a farmen	NCT Aliant	02602	897A	3221	404	996	4202	01/2	122	10/	13041	4082	110	4/3
Neutsma	NC7-enermis enes	1388	803	9	18	4	1950	193	95	16	9753	1991	43	38
	NC7-cucumis.melo	3208	2190	2	29	24	120		14	13	2310	2048	43	37
	NC7-cucumis.wilds	320	207	206	2	2	306	170	8	80	513	208	10	10
	NC7-cucurbita	978	161	150	23	20	16	83	4	4	282	220	27	24
	NC7-daucus	1473	1896	1285	19	14	9	547	10	10	2560	1286	29	2
	NC7-ocimum	104	152	55	12	12	64	21	2	61	174	98	14	1
	NC7-parsnips		0		0	0			0	0	0	0	0	
	Total:	7823	5495	4450	66	74	2175	1210	10	6F	O-LOO	0012		

Mutuality         Mutuality <t< th=""><th>01/01/2016 to 12/31/2016</th><th>0102/12/21 00</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></t<>	01/01/2016 to 12/31/2016	0102/12/21 00													
Terranticity 36 (3) (3) (3) (3) (3) (3) (3) (3) (3) (3)	CURATOR		Number of Accs in Collection		Number of Obs in GRIN for Year		Number of Obs In GRIN Last Year	Number of Accs with Obs In GRIN Last Year	Number of Obs in GRIN (all years)	Number of Acc with Obs in GRIN (all years)		Number of Accs with Images in GRIN for Year	Number of Images in GRIN for Year		Number of Images in GRIN (all years)
Size         1         0	Brenner	NC7-amaranth	3336		363		10392	3335	48035		0	0	0	743	116
(5)         (1) <td></td> <td>NC7-celosia</td> <td>58</td> <td></td> <td>0</td> <td></td> <td>0</td> <td></td> <td>164</td> <td>56</td> <td>0</td> <td></td> <td></td> <td>1 16</td> <td>ŝ</td>		NC7-celosia	58		0		0		164	56	0			1 16	ŝ
Cryptome133000013410000241Cryptome130000000000000Cryptome1300000000000000Cryptome1300000000000000Cryptome1300000000000000Cryptome1300000000000000Cryptome1300000000000000Cryptome130000000000000Cryptome1300000000000000000Cryptome13000000000000000Cryptome13000000000000000Cryptome1300000		NC7-echinochloa	306	1	E .	1	0		1158						130
Cheme39000 <td></td> <td>NC7-grasses</td> <td>133</td> <td></td> <td>0</td> <td></td> <td>1</td> <td>1</td> <td>281</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>2</td>		NC7-grasses	133		0		1	1	281						2
Conditioned100400<		NC7-legumes	294		0		0		547						41
Cpention         56         1         4         4         0         50         1         4         4         0         50         1         0         0         10         0         10         0         0         10         0         10         0         10         0         10         0		NC7-melilotus	1004		347		0		7235						244
Cypenila         II         II         II         II         III         IIII         IIII         IIII         IIII         IIII         IIII         IIII         IIII         IIIII         IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		NC7-panicum	936	1	4		0		3371	935					240
Greentiane11000001000 <t< td=""><td></td><td>NC7-perilla</td><td>25</td><td></td><td>0</td><td></td><td>0</td><td></td><td>86</td><td></td><td></td><td></td><td></td><td></td><td>1</td></t<>		NC7-perilla	25		0		0		86						1
NGT quant         (3)         (1)         (2)         (2)         (1)         (2)         (1)         (2)         (1)         (2)         (1)         (2)         (1)         (2)         (		NC7-portulaca	11		0		0		10		0				
Optimize         0/7         1         0         1         1         0.05         1         0        <		NC7-quinoa	424		12		0		1195						203
Criandia Criandia1430001236171410017Criandia Criandia9316333336171401100011Criandia Criandia9316333336171401100011Criandia Criandia931633361714011100011Criandia Criandia93193100001314100011Criandia Criandia93100000000111100011Criandia Criandia931000000000000000Criandia Criandia93100000000000000Criandia Criandia93100000000000000Criandia Criandia931000000000000000Criandia Criandia93100000000000000000Criandia <b< td=""><td></td><td>NC7-setaria</td><td>1077</td><td>1</td><td>10</td><td></td><td>T</td><td>1</td><td>4045</td><td></td><td></td><td></td><td></td><td></td><td>309</td></b<>		NC7-setaria	1077	1	10		T	1	4045						309
Crunkla, India         117         0		NC7-spinach	413		0		12		8008		1	0			49
Toth Contraction (Total)9161731090160138901200122Contraction Contraction91 </td <td></td> <td>NC7-umbels</td> <td>1197</td> <td></td> <td>0</td> <td></td> <td>1</td> <td>1</td> <td>6177</td> <td>1147</td> <td>Н</td> <td>0</td> <td></td> <td></td> <td>342</td>		NC7-umbels	1197		0		1	1	6177	1147	Н	0			342
		Total:			787		10407		80312	8990	12		0	1722	2809
Grammentika71800 <t< td=""><td>Carstens</td><td>NC7-medicinals</td><td>116</td><td></td><td>0</td><td></td><td>0</td><td>0</td><td>11956</td><td></td><td>8</td><td></td><td></td><td></td><td>815</td></t<>	Carstens	NC7-medicinals	116		0		0	0	11956		8				815
		NC7-ornamentals	719		0		0		152		20	1 m			153
Total3732410355000350010131		NC7-woody.landscape	1888		150		507		4566	813	40	0	0		1550
		Total:	3578		150		507		16674	1	68	0	0	1241	2518
	Marek	NC7-asters	432		0		0		8	1	1	0	0	0	
		NC7-brassica	2009		3794		23617		40507	-	0	0	0		922
		NC7-brassica.pvp	9		9	-	0		0		0				
Creations/py Creations/py10001100000Creations/py Creations29900000000000Creations/py Creations299000000000000Creations2960000000000000Creations296000000000000Creations296000000000000Creations/dam1660000000000000Creations/dam2960000000000000Creations/dam2960000000000000Creations/dam29600000000000000Creations/dam2960000000000000Creations/dam296000000000000Creations/dam<		NC7-crucifers	1267		574		6347		7311		0				42
Cricopten63000042027000013Cricoptent28400001387461387356000013Crianx cults28360000138746143822850000232Crianx cults28360001387461438218560000232Crianx cults283600001387461438218560000232Crianx cults283600001387461438218560001337Crianx cults28360000138746138761387000233Crianx cults283613230334323036233623362336233623362336233623362336233613371337Crianx cults23361332336 <td></td> <td>NC7-crucifers.pvp</td> <td>1</td> <td></td> <td></td> <td></td> <td>10</td> <td></td> <td>10</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		NC7-crucifers.pvp	1				10		10						
		NC7-cuphea	639				0		4260						ŝ
Criativals         254         0         <		NC7-euphorbia	209						0						
$C_{canculars}$ $2.0$ $0$ $0$ $1.8$ $0$ $0$ $0.8$ $0$ $0$ $0.8$ $0$ $0$ $0.8$ $0$ $0$ $0.8$ $0$		NC/-HAX	1007						1111						
		NC7 and and to	141				7991		200	15	1			26	6FG
Transmension monotosimal166000024011313070000Transmension Transmelle18600000000000Transmelle28600000138700000111Transmelle286000000000000Transmelle2860000000000000Transmelle286281200000000000Transmelle2863118471489957922004759029312900000Cransmelle26331184714899577220047580233233000000Cransmelle26331111911591151173213941334213421320000000Cransmelle263311119115111391320021342738211661332700000Cransmelle20041332004213427282116613327000000Cransmelle20350		NC7 cun culte SAM	886				U		U	TOT				24	20
		NC7-sun wilds ann	1665				245	5	40118	13(	0	0		9	120
		NC7-sun.wilds.per	886		0		430		13850		1	0			329
Total:12621243683433203655562129157291120011171 $C7-conkin5691022000$		NC7-sun.wilds.sp	12		0		0		0		0	0			
CT-corn kin1012000<		Total:			4368		32036		212915	7291	12		0	1111	286
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Cr-chicory       279       0       0       0       257       0       257         Cr-chicory       279       0       1377       43       0       0       257         Cr-chicory       328       5       1       1       1       0       0       257       0       0       0       257         Cr-chermisneue       3208       5       1       1       0       0       0       1285       1377       49       0       0       0       257         Cr-cuentisulds       3208       0       0       0       12855       317       249       0       0       0       75         Cr-cuentisuids       320       0       0       0       12855       317       249       0       0       0       75         Cr-cuentiswids       320       0       0       0       0       1587       227       0       0       0       75         Cr-cuentiswids       371       1360       1297       227       0       0       0       0       150         Cr-cuentiswids       373       0       0       267       22       5695       970       21 <td></td> <td></td> <td></td> <td></td> <td>9715</td> <td></td> <td>11005</td> <td>242</td> <td>272621</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>872</td>					9715		11005	242	272621						872
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Cr-denome         1473         37         470         20         3         1         19471         1360         20         0 <td></td> <td>NC7-cucums.winds</td> <td>078</td> <td></td> <td></td> <td></td> <td>6</td> <td></td> <td>100</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>168</td>		NC7-cucums.winds	078				6		100						168
C7-ceimun         104         1         0         0         0         635         98         0         0         13           C7-parsnips         73         0         0         0         0         0         153         71         18         0         1         13           C7-parsnips         73         0         0         0         0         153         71         18         0         0         1         1           C7-parsnips         7823         43         619         93         5         3         69769         7637         273         0         0         0         2745         1		NC7-dancus NC7-dancus	1473		470		4 00		17491						3150
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# **Appendix Figure 1**



# **Appendix Figure 2**

