# NCRPIS

AMES, IOWA

North Central Regional Plant Introduction Station

# 2018 NC7 Annual Report





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OWA STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

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#### NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION NC-7 ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2018

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

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

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

#### A. <u>Administrative Advisor</u>

\*J. Colletti, Interim Dean, Iowa State University, CALS

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

\*C. Gardner, USDA-ARS, Iowa

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

Voting members:

E. Sacks	7. Missouri	S. Flint-Garcia
L. Hoagland	8. Nebraska	D. Santra
T. Lübberstedt	9. N. Dakota	B. Johnson
M. Stamm	10. Ohio	P. Jourdan
A. Iezzoni	11. S. Dakota	M. Caffe-Treml
A. Lorenz	12. Wisconsin	W. Tracy
	E. Sacks L. Hoagland T. Lübberstedt M. Stamm A. Iezzoni A. Lorenz	E. Sacks7. MissouriL. Hoagland8. NebraskaT. Lübberstedt9. N. DakotaM. Stamm10. OhioA. Iezzoni11. S. DakotaA. Lorenz12. Wisconsin

#### Non-voting participants:

13. California-Davis	R. Karban	30. Missouri	S. Flint Garcia
14. Connecticut	M. Brand	31. Missouri	S. Jose
15. Delaware	R. Wisser	32. Nebraska	C. Urea
16. Hawaii	G. Presting	33. New Jersey	S. Handel
17. Illinois	J. Juvick	34. New York	J. Doyle
18. Illinois	G. Kling	35. New York	M. Gore
19. Illinois	S. Korban	36. New York	P. Griffiths
20. Illinois	D. Lee	37. New York	A. Hastings
21. Indiana	J. Janick	38. New York	M. Smith
22. Iowa	K. Lamkey	39. Oregon	A. Liston
23. Kansas	A. Fritz	40. South Dakota	L. Xu
24. Kansas	W. Schapaugh	41. Wisconsin	H. Kaeppler
25. Kansas	M. Jugulam	42. Wisconsin	S. Kaeppler
26. Kentucky	T. Phillips	43. Wisconsin	N. de Leon
27. Michigan	R. Grumet	44. Texas	D. Baltensperger
28. Michigan	J. Hancock	45. Texas	N. Subramanian
29. Mississippi	S. Popescu		

D.	<u>U.S. Department of Agriculture</u> (*Voting members)	
	1. ARS National Program Staff, Plant Germplasm	*P. Bretting
	2. ARS Plant Exchange Office	*G. Kinard
	3. ARS Area Director, Midwest Area	*J.L. Willett
	4. Cooperative State Research, Education and Extension Service	A. Thro
	5. National Center for Agric. Util. Research	*T. Isbell
	6. National Center for Genetic Resources Preservation	*S. Greene

### E. North Central Regional Plant Introduction Station, Ames, Iowa

See organizational chart, Figure 1 in the Appendix.

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

#### Personnel changes (June, 2018 – June, 2019):

#### <u>Departures</u>:

- Nikolas Ouellette, USDA-ARS Agri. Research Science Tech (Horticulture), July 2018
- Joyce Lok, ORISE staff, GEM Project Support, December 2018

#### Promotions:

- Jesse Perrett, USDA-ARS IT Specialist, Security Systems and Customer Support (grade)
- Candice Gardner, USDA-ARS Supr. Plant Biologist (grade)

#### New Hires:

- Jeffrey Schwartz, USDA-ARS Agri. Research Science Tech (Oilseeds), December 2018
- Anna Testen, USDA-ARS Plant Pathologist, February 2019
- Vivian Bernau, USDA-ARS Maize Geneticist/Curator, March 2019
- Elizabeth Melton, ORISE staff, Seed Storage Support

Transitions: None

#### Vacant USDA-ARS Positions:

- Agri Research Science Tech (Horticulture)
- IT Specialist (new, developer)
- Term Cat 3 Agronomist (Germplasm Enhancement of Maize GEM)
- Biol. Science Tech (Seed Storage)
- Germplasm Program Assistant

#### Vacant ISU Positions: None

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 FTE (full-time equivalent) part-time temporary positions in FY 2018, primarily via the Research Support Agreement with Iowa State University. The ORISE and other temporary positions support curatorial activities including regeneration, seed processing, viability testing, farm and facilities operations, IT support, and the GEM Project. Students were interviewed and selected by ISU Program Manager Fred Engstrom. Marci Bushman, PIRU Program Support Assistant, managed the administrative aspects of all student hires, with support and guidance from Admin. Officer Candace Weuve and Program Support Assistant Orlando Guzman.

#### **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 96% 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. ISU wage increases will be 2% in 2019, and this figure climbs to 97% if an additional 1% increase is granted in 2020, barring personnel changes. This limits professional meeting travel, technical training, and temporary student hiring with ISU resources.

FY2018 USDA-ARS funding was essentially the same as final FY2014 funding, minus a one percent assessment for 'Big Data' and smaller assessments for Digitop and SAS licenses. The PI CRIS was funded at \$2.38M (net to location) and the GEM CRIS at \$1.32M. Student hiring for summer 2018 was challenging, despite raising our starting wage for ISU students by almost \$2/hour to \$12/hour, and we were not quite able to fulfill the need for 25 summer FTE, but much better than in 2017. We attribute this to both the requirement for all agriculture students to complete internships, and the growing disparity in what we can offer for wages versus other hiring opportunities. ISU Program Manager Fred Engstrom advertised positions more widely across ISU colleges and excellent students were employed; several came from a molecular biology orientation and were looking for a hands-on experience with plants.

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 since 2014, a great deal of time and attention has been paid to recruitment and hiring activities. We feel very fortunate to have filled three ARS positions, plant pathologist, a second maize geneticist/curator, and an oilseeds technician with excellent candidates. The current four vacancies will be recruited as authorizations occur.

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

Seed storage space is becoming limiting and needs to be addressed within the next three to four years. A request to ARS leadership for support for a 2500 sq ft -20°C cold storage building to support essentially doubling the longevity of viability of many of our taxa was added to the Agency Construction Plan. 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*), a whitefly parasite (*Encarsia formosa*), and an insect new to the arsenal, the rove beetle (Staphylinidae, *Dalotia*) to help control thrips and other insects.

Please see the Information Management section of this report for details on upgrades that continue to enhance the NCRPIS' information technology infrastructure, and the Farm Support Team section for updates on maintenance and equipment. Most notable, two climate-controlled rooms were developed to provide additional vernalization environment space for brassica germplasm. Implementation of virtual servers using solid state drive systems continues to progress, and server backup capacity duplicated offsite.

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

(Part IV. summarizes the accomplishments and progress for calendar year 2018, presented in greater detail in the individual staff reports in the document.)

**Technical Exchange:** An exchange relationship continues to expand between the NCRPIS maize genebank and the CIMMYT maize genebank in Mexico. In addition to regeneration of highland tropical maize at CIMMYT's Tolucca site, new curatorial and database personnel will spend time with Ames staff in 2019, and maize accession information resources are being compared and augmented by both groups.

#### Acquisition and Documentation Highlights:

In 2018, collection development continued with the acquisition of 293 new accessions (Appendix Table 1). This compares with 250 accessions in 2017, 786 new accessions in 2016, 229 in 2015, 766 in 2014; 192 in 2013, 470 in 2012, and 485 in 2011. Details are provided in the individual curators' report sections.

The U.S. is now a partner to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Implementation by the NPGS is still under development as several Departments are involved. 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, pestand 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 2018, 1,245 accessions were grown for regeneration and 1,204 harvested (Appendix Table 2). This contrasts with 1601 accessions were grown for regeneration and 1210 harvested in 2017; 1033 accessions grown for regeneration and 1067 harvested in 2016; 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.

A 4,000 row tropical maize winter nursery planted in fall 2017 near Puerto Vallarta, Mexico was deemed successful with high quality seed return in 2018; positive results with this nursery provider was a high priority as this was our first experience with procurement of international nursery support. About 1100 accessions were made available to the public. Maize germplasm from Thailand was grown under quarantine permit in a winter greenhouse on the Iowa State University campus.

Accessions backed up at the National Laboratory for Genetic Resource Preservation (NLGRP) in Ft. Collins in 2018 numbered 795, compared to 595 in 2017, 428 in 2016; 431 in 2015; 1,231 in 2014; 781 in 2013; 799 in 2012; 792 in 2011; and 2,388 in 2010.

The overall percent of NCRPIS collection holdings backed up at the NLGRP remains at 80%. This ranges from 10-12% for maize crop wild relatives to 100% for several other taxa (Appendix Table 2). About 300 accessions were also sent to Ft. Collins for inclusion in the annual NPGS deposit to the Svalbard Global Seed Vault. 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 (S. McClintic-Friddle) and from Bejo Seeds (R. Maxwell)). USDA-ARS in Salinas, California (B. Mou) supported seed increase of domesticated spinach, and USDA-ARS in St. Croix supported increase of 21 tropical maize inbreds and populations.

USDA-ARS staff of Mayaguez, PR (R. Goenaga) and the St. Croix quarantine nursery staff supported regeneration of tropical maize accessions. 3<sup>rd</sup> Millennium Genetics in Puerto Rico returned an increase of lowland tropical maize populations from Central and South American from the Goodman racial collection. Raleigh ARS GEM Project Coordinator Matt Krakowsky provided increases of nine GEM lines and the Ames GEM team regenerated 12 GEM lines.

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

#### **Distribution:**

Approximately 48% of the 2018 germplasm distributions were to international and 52% to domestic requestors. Distributions set another new record (Appendix Table 3).

Year	# Items	<b># Unique Accessions</b>	<b># Orders</b>	<b>#</b> Requestors
2018	61,124	23,229	1,414	1,000
2017	55,474	22,801	1,410	1,019
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), continue to heavily target vegetable an ornamental germplasm, more than one-half of all orders to NC7 are cancelled nonresearch requests; other NPGS sites are also heavily targeted. 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. GRIN-Global's user friendly order module also helps individuals to select more, and more diverse germplasm from a number of NPGS sites at once.

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 2018	% of Total Collections	% of 2018 Distributions	% of 2017 Distributions	% of 2014 Distributions
Brenner	9,272	17	13	23	14
Carstens†	3,772	7	1	2	<1
Marek	12,775	23	27	35	16
Millard	21,097	39	43	22	35
Reitsma	7,858	14	16	18	28
Totals	54,774	100	100	100	100

<sup>†</sup>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 and pest 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. There was unusually high demand in 2018 for almost all major crop species. Germplasm requests continue to be driven by publication of information from genomic (genotyping by sequencing) and phenotypic analyses projects, some of which are supported with SCRI (Specialty Crop Research Initiative), AFRI, and NSF funding.

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

With the enhancements made to the 'Attachment Wizard' that works in conjunction with GRIN-Global, image loading has resumed. A large volume of accessionassociated images and other types of documents is being attached to accessions, orders, and inventories.

In 2018, the NCRPIS utilized more than 12,100 accession items internally for germination, seed increase, observation, evaluation and characterization for a wide array of descriptor information, and for viability testing (Appendix Tables 2, 4). More than 22,400 observation data points were loaded to the GRIN-Global database, (https://www.ars.grin.gov/npgs/) and more than 4,000 digital images associated with accessions. Other uses include pathogen testing to meet international distribution requirements and back up and herbarium preparation.

#### 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, implemented in 2015. This has been the sole primary focus of NCRPIS developer Pete Cyr since 2008, and a major focus of two other NCRPIS staff members, Mark Millard (system analyst) and Lisa Burke (beta testing, training) with substantial time invested by additional personnel. The Database Management Unit (DBMU) in Beltsville, is responsible for hosting and maintaining the system, developing the public interface, GRIN Taxonomy, and changes to the system's Middle (business) Tier and administration. Periodic video training conferences continue to be offered by DBMU personnel (contract documentation specialist Marty Reisinger) for NPGS site personnel participation, as they have been for the past five years, and other training as requested.

Software development efforts continue to center on the development and deployment of the successor to the GRIN system, GRIN-Global (GG), 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 is used to submit and address enhancement requests, prioritize development, assign work to developers, and to securely share new software applications between GG adopters to extend the system's functions and features.

Fourteen national or international genebanks have now implemented GRIN-Global for genebank use, and many of these have live public interfaces. Another 27 are in the process of evaluating and/or implementing the system, truly evidence of global adoption of this valuable resource.

For almost four years, 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. An international AdCom has been formulated with participation by key personnel from the Crop Trust, the US NPGS, CIMMYT, and CIP, and confers monthly. 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).

One focus of our current ARS Program cycle is to develop inter-operability between GG and other key information providers' portals, examples including MaizeGDB, Gramene, LIS (Legume Information System), or GOBii.

Please see the IT section for technical details of NCRPIS support activities. We owe IT Specialist Jesse Perrett, Kurt Kabriel, and Fred Engstrom sincere thanks for implementing the NCRPIS intercom communications system and the Monet environmental monitoring system. The Monet system provides continuous remote monitoring capability for greenhouses, cold storage areas, dryers, and other areas where extreme changes could impact processes or seed quality.

#### Germplasm's Viability and Health:

Viability testing was conducted on 8% of the collection accession in 2018; 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. Construction of a -18C cold storage building will provide for much longer period of viability for many of our taxa. This would bring significant cost savings over the long term, as most of the collection's seeds lose

viability long before inventory supply is depleted. Less frequent regeneration need would enable more rapid progress in making the collection fully available.

Pathology ASRT Dr. Narinder Pal, ISU Pathologist C. Block and new ARS Pathologist Anna Testen are collaborating to develop methods to eliminate the bacterial fruit blotch pathogen, *Acidovorax avenae*, from *Cucumis melo* seed.

Field inspections were made for all crops, the disease identified of most significance in 2018 was black rot of brassica. All cucurbit seedlings were screened routinely for presence of Squash Mosaic Virus via ELISA; Outcomes are detailed in the pathology section of this report.

In 2018, we continued testing for adventitious presence (AP) of genetically engineered organisms (GEO) in maize germplasm accessions new to the NCRPIS and sampled newly produced seedlots.

#### **Insect management:**

The Entomology staff provided six insect pollinator species to control pollinate 952 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.

#### **Enhancement:**

The Germplasm Enhancement of Maize Project (GEM) works with 61 active 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 of importance to human health and nutrition. International collaborators are screening GEM germplasm for late wilt, tar spot, maize rough dwarf virus, corn stunt, and others.

The Ames and Raleigh, NC GEM Projects and public collaborators have released 325 lines from 2001-2018 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 has released 204 doubled-haploid (DH) maize lines in partnership with the ISU Doubled

Haploid Facility. The next set of DH lines from the allelic diversity project will be released in January 2020. These lines have one-quarter exotic, three-quarters temperate background. In 2017 and 2018, the GEM Technical Steering Group's private sector members tested 47 GEM lines from the Ames and Raleigh, NC programs internally on their own proprietary testers. These data enabled calculation of the first general and specific combining ability estimates for GEM germplasm on company tester lines, important for breeders and researchers to gain insights into effective use of these genetic technologies.

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. The sunflower project has also used photoperiod control effectively to induce flowering in certain wild sunflower accessions. Photoperiod-control environment capacity on the order of one to three acres would be very useful in maintaining and providing unique genetic resources.

GEM field days are held every September and are well attended by scientists, breeders and graduate students. The field days offer a unique opportunity for more molecularfocused 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 2018. 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. ARS leadership has been requested to help secure resources for a -20°C cold storage building in order to extend longevity of seed viability. 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 address development of comprehensive, genetically diverse collections to meet research and development needs. More emphasis has been requested for advanced breeding materials, doubled haploid germplasm, mapping populations, single mother trees, and ephemeral genetic resources derived from NSF, AFRI, or SCRI-funded research.

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.

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.

Implementation of new, optical and spectroscopic-based technologies are in process and we hope they will enable us to better understand seed properties and improve the quality of our seed inventories.

Software development efforts continue to center on the development and deployment of the successor to the GRIN system, GRIN-Global (GG), 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 is used to submit and address enhancement requests, prioritize development, assign work to developers, and to securely share new software applications between GG adopters to 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 mission 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.

• At the 2018 NC7 RTAC meeting, NC7 Academic Advisor Joe Colletti (ISU Agricultural Experiment Station Interim), stressed the value of the NC-7 RTAC participants' information exchanges and the importance of demonstrating impact and public return on investment in germplasm research and utilization and the critical partnerships between NIFA, ARS, NASS and the universities for accomplishing mission. A thoughtful discussion on the impacts of tariffs on Iowa Agriculture, uncertainties associated with the agricultural economy, downstream impacts on university enrollment, and the challenges to crop diversification in Midwestern agriculture were of great interest.

Some of the NC-7 RTAC's specific suggestions and contributions from their 2018 Annual Meeting in Ames, IA include the following: (from the meeting minutes):

- Concurrence of the review and approval of the 2018/2019 NC-7 Hatch Project budget by the NCR AES Directors.
- 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 committee expresses its gratitude to President Wendy Wintersteen, who hosted the August 14 evening dinner and guest speaker.
- The committee recognizes and thanks Candice Gardner and the NCRPIS staff for their efforts and hospitality in organizing and hosting the NC7 RTAC meeting.
- Sincere thanks to ISU faculty guest speakers Baskar Ganapathasubramanian and Arti Singh for their excellent, informative presentations.

#### 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 2018, 64 applications for hourly employment were received and reviewed. There were approximately 50 interviews, resulting in 26 new and 32 returning hourly employees hired. Currently there are 25.2 (FTE) Biological Science Aides working at the NCRPIS.

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

- Fred Engstrom, (Program Manager II) Joined the staff July 1, 2016.
- 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.

#### 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. Installed infrastructure to support the Metrix and QSorter optical seed sorting instruments. Worked with QualySense on modification to support research applications.
- 2. Construction of two climate-controlled rooms utilized for vernalization of brassicas. Grow lights with timers were also installed.

- 3. Continued administered use biological controls for greenhouse pest control.
- 4. Installed pallet racking in Entomology building to maximize space utilization and improve pollinator syrup distribution system.
- 5. Installed additional intercom system components for improved communications during weather or other emergencies.
- 6. Arranged for repair of compressors, HVAC equipment in multiple areas.
- 7. Replaced various light fixtures with energy efficient LED lights in offices and other work areas as needed.
- 8. Planted cover crop on Amaranth field between cage frames for curator. This simplifies weekly field maintenance and allows access to the accessions after rainfall whereas tilled alley ways would be impassible.
- 9. Fall seeded Oilseeds project field with grass mix for improved soil conditions due to crew traffic. Results from this experiment will be reviewed for future plans.
- 10. Installed additional Monnit temperature and humidity sensors in facilities to track trends and alert us to abnormal conditions.

#### **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. John Deere 6130R tractor with autosteer was purchased and utilized this year for precision field work. This addition to the fleet improved field layout on most of the fields this year allowing for less manhours measuring plots by crews.
- 2. A six-foot rotary tiller allows for tilling a complete "cage" footprint for weed control or seed bed preparation.
- 3. Hyraulic dump trailer for hauling materials on station.
- 4. Greenhouse kit for a 40' x 100' 'cool' winter greenhouse.
- 5. Acquired two "people movers" to assist with larger field tours of the Plant Introduction Station.
- 6. A 'Metrix' analytic lab optical color sorting instrument from VMEK, Virgina, USA.
- 7. A 'QSorter' optical seed sorting instrument from QualySense, Switzerland that incorporates vision and NIRS.

#### **Tours**:

During 2018 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 annual updates for existing staff. Four NCRPIS staff members participate in the ARS ISU Campus Safety Committee.

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

Jesse Perrett served as the first-line of support for NCRPIS during 2018. Jesse is supervised by Pete Cyr who is dedicated to the GRIN-Global project. Jesse supervised Kurt Kabriel, ORISE intern in assisting with IT related tasks. The following list outlines the progress made by the IT team during 2018 at NCRPIS.

#### **Equipment:**

As of December 2018, the NCRPIS had 60 desktop and 35 laptop/tablet workstations installed for use by permanent staff members and part-time temporary student help. All station computers are equipped with solid state drives, have at least eight gigabytes of memory, and quad core processors. The centralized functions required by the station were supported by 15 physical servers and around 20 active virtual servers including those used for file storage, intranet, backups, and access security systems and monitoring. New field tablet devices with barcode scanners were issued to crews for data collection and pollinator tool usage.

A new WatchGuard Firebox M570 firewall was installed and configured in order to provide enhanced security as well as increased network performance in line with the 10-gigabit server network infrastructure. Each server rack is protected by a battery backup. In addition, a station generator system will 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 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 2019 to enhance storage performance of existing servers at minimal cost.

Installed eight new security system cameras to better monitor property entrances and outside activities. The storage capacity of the surveillance system was increased from four terabytes to 28 terabytes in order to accommodate the eight new cameras and to also increase the number of days of video recording retention.

Installed a new station-wide wireless Visiplex intercom system. The system includes 31 intercom speakers and allows all major areas (both inside and outside) to hear manual and recorded intercom messages. Messages for fire, tornado, and active shooter scenarios were pre-recorded and set up to broadcast over the intercom.

A new Monnit wireless environment monitoring system was installed and configured. The system has 78 sensors currently on-line for measuring temperature, humidity, light, and other sensors in greenhouses, buildings, growth chambers, server rooms, cold rooms, and dryer rooms. The system provides capabilities to review historical data, create event and other advanced notifications via text or email, and on-line access of all features and real-time sensor status from outside of the station network for afterhours monitoring of environmental conditions.

Installed and configured a new Lumigrow wireless light management system, allowing remote wireless control of 51 Lumigrow lights in the station's onsite

greenhouses. Color and intensity can be configured for all 51 lights remotely. Also installed light sensors on lights to allow for automatic configuration based on existing sunlight levels. This allows the system to compensate for natural fluctuations in sunlight intensity and automatically keep desired light levels while saving electricity and lowering greenhouse temperatures.

A new 802.11AC wireless system based on Cisco 3800 series access points (AP's) was installed. Four new AP's were installed and configured to enhance wireless performance and availability around the station.

The Microsoft SharePoint server was upgraded to SharePoint 2016. This upgrade included the installation and configuration of two new servers including the SharePoint 2016 server and a new SQL Server 2017 Standard server. The new setup should provide enhanced reliability and capability while increasing performance.

A new file server was installed and configured providing enhanced file transfer performance. A complete set of backup hardware was purchased and configured to cope with any hardware failures. The new backup hardware will provide the capability to restore backups and perform disaster recover dress rehearsals.

The server network infrastructure was upgraded to 10 gigabit speeds enabling increased bandwidth for backups and/or server to server file transfer operations.

A new offsite backup server was installed and configured in the National Laboratory for Atmosphere and the Environment building on campus. A wired direct connection was set up through an on campus VLAN allowing for backups to be transferred to an offsite facility automatically on a regular basis.

In 2018, the Lenel door access system was managed using the Epacs system. A LincPass Light Activation Station was used to facilitate the LincPass Activator role. This allows for card activation, credential updates, and LincPass PIN resets to be done locally. Jesse also served as a backup LincPass Activator for the NCAH (National Center for Animal Health) when their equipment was down, providing LincPass services to more than 40 people in the Ames area.

Staff printed over 4000 field-ready wooden stakes. Issues with print quality and print consistency were fixed to ensure minimal misprints.

#### Software:

All workstations at NCRPIS use Windows 10. Microsoft Office 2013, Microsoft Office 365, Adobe Acrobat Professional DC, Adobe Creative Suite, Pulse Secure, Activclient, Java, BigFix, and the GRIN-Global Curator Tool were installed on systems as 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 2018, 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 ensure that these workstations remain vulnerability free.

In 2018, 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.

#### **Documentation:**

Weather station history data was provided via SharePoint to allow users to download current and past weather data including calculated GDU and CHU (heat unit) data. The station uses SharePoint Server 2016 Intranet site for advanced document management and retention. Umbraco website management tool was used to configure the NCRPIS public webpage on USDA's website and for posting IT support videos and training documents, and information about farm operation, safety, and health to the NCRPIS intranet website.

#### Plans for 2019:

- Update documentation for IT systems and services.
- Upgrade station camera system with 2 new cameras.
- Upgrade/extend Monnit system to provide wireless monitoring to campus greenhouses.
- Continue to upgrade servers to Microsoft Server 2019
- Implement monthly windows updates via Microsoft WSUS.
- 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:**

GRIN-Global was the product of a joint partnership between the USDA-ARS NPGS, the Global Crop Diversity Trust and Bioversity International to develop a new genebank information management system that it could be deployed on any size computer with a minimum amount of effort and cost. Currently implemented by 14 national or international genebanks and in the process of evaluation/adoption by 27 other genebank entities, GRIN-Global was designed to support five different languages, can be configured to support four database systems, and can be installed on a single desktop computer or a network.



The development of the GRIN-Global curator desktop suite of applications (which includes the Curator Tool, Search Tool, various Wizards, and reports/labels) continued throughout 2018 in direct support of NCRPIS daily curatorial operations. New enhanced versions of the Curator Tool were developed, tested, and security scanned at NCRPIS before being released to the U.S. NPGS and to international GRIN-Global partners.

Some of the enhancements to the Curator Tool include upgrading the application to run natively on 64-bit processors and to use the new more secure .NET 4.6.2 Framework, enhancing the Viability Wizard interface to more rapidly and efficiently process viability data, enhancing the Attachment Wizard interface for easier management of attachment collections, enhancing the Curator Tool main interface to make data-view tab management easier, enhancing the Order Wizard to support processing the new features in the Public Website shopping cart, enhancing the speed of the Curator Tool suite of applications by compressing HTTP communications between the client and the server and caching data locally whenever possible.

Advisory Committee: The GRIN-Global Ad Com is comprised of genebank site users from NPGS sites for the purpose of communicating user development needs to development team members, collectively setting development priorities, and testing/approving software products to ensure performance meets user needs. The Ad Com meets biweekly and is chaired by Lisa Burke, NCRPIS Agronomist (IT) and Seed Storage Manager. In addition to Ms. Burke, other NCRPIS participants include Pete Cyr, Mark Millard, and Candice Gardner.

#### C. Information Management-Germplasm Collections (S. Estrada)

#### Acquisition:

The North Central Regional Plant Introduction Station (NCRPIS) acquired 355 (612 inventory lots) new accessions in 2018. Of these new accessions, 136 were received from within the National Plant Germplasm System (NPGS) through exploration and transfer (131 Seeds of Success and 126 NLGRP). Details of specific acquisitions are found in the curators' sections of this report.

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, IPR considerations, and any additional pertinent information provided by the donor. An excel workbook was developed to streamline the assembly of passport data and aid in loading the data to the GRIN-Global database.

#### Maintenance:

Curatorial assistance was provided by processing requests for taxonomic reidentifications and nominations of accessions to the inactive file. In total, 31 accessions received taxonomic re-identifications and two were inactivated.

Additionally, 417 accessions were assigned PI numbers. 375 of which were *Helianthus annuus* Canadian pre-breeding lines that were received in 2016 and 2017.

The NCRPIS continues to work on a project to digitize all paper documentation related to accession provenance, management, and performance. In total, 6,628 documents were uploaded to the GRIN database. This included 91 new documents filed in 2018 as well as 6,537 accession cards from the card catalog. The card catalog contains valuable passport information and inventory grow-outs for accessions that were received prior to GRIN-Global implementation.

Content of the Accession Passport Information cabinet and all remaining card catalog card contents were completely digitized in 2018. All new documentation, including passport files are being digitally maintained. We recorded important identifying information (Accession, Received Date, etc.) from the documents in Excel file format. The Excel files will enable us to rename files en-mass to conform to document naming conventions that more easily support future upload to the GRIN-Global database.

#### D. Order processing (S. Estrada)

The GRIN-Global public website has improved accessibility to germplasm information and the ability to search for desired crop characteristics. This year, the order processing team continued to refine the use of GRIN-Global order actions, attachments, and local order number in conjunction with Excel workbook templates to monitor order progress, streamline processing, and inform internal and external cooperators of order status. Order actions allowed both NCRPIS teams (curatorial personnel, seed storage, pathology) and other NPGS facilities (APHIS, GRIN Global feedback) to more easily monitor a germplasm order as it progresses through the pipeline towards fulfillment. Documentation related to orders is attached directly to the corresponding GRIN order via the Order Wizard's attachment tab, thus accessible to internal NPGS users. External users may also add attachments (usually an import permit, shipping instructions, or Excel file request list) through their public website order history. These processing improvements are exceptionally useful for communication and management of additional documentation that is required for international germplasm distribution.

During 2018, 2454 orders for NCRPIS collection germplasm were entered into GRIN-Global. Of these, 2027 entered the order processing system via the GRIN-Global Public Website. We continue to see a high proportion (30-40%) of non-research, non-educational (NRR) orders being submitted for consideration. 2,460 orders containing 113,815 items were processed in 2018. A detailed summary of NCRPIS distribution activity is summarized in the table below which illustrates use for internal purposes, and in Appendix Table 3. Over 60,600 items were distributed, 6,000 items more than in 2017.

	2010 - Detail Summary of Next 15 germphasm distributions										
				Grand Total					Shipped		
	Order Type	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order
_	Distribution	1,540	73%	69,689	61%	45	1,396	99%	60,554	84%	43
rna	Non-research, non-educational	576	27%	31,980	28%	56	7	0%	30	0%	4
Exte	Repatriation	3	0%	20	0%	7	3	0%	20	0%	7
۳	Total	2,119	100%	101,689	89%	48	1,406	100%	60,604	84%	43
	Backup	34	10%	1,397	1%	41	33	10%	1,292	2%	39
	Germination	178	52%	5,455	5%	31	177	52%	5,437	8%	31
	Herbarium/reidentification	5	1%	11	0%	2	5	1%	11	0%	2
rnal	Observation/evaluation	21	6%	672	1%	32	21	6%	672	1%	32
nte	Phytosanitary Testing	50	15%	3,440	3%	69	49	14%	3,254	4%	66
_	Replenishment/regrow	51	15%	1,145	1%	22	51	15%	1,145	2%	22
	Transfer	2	1%	6	0%	3	2	1%	6	0%	3
	Total	341	100%	12,126	11%	36	338	100%	11,817	16%	35
	Grand Total	2,460	100%	113,815	100%	46	1,744	100%	72,421	100%	42

#### 2018 - Detail summary of NCRPIS germplasm distributions

#### **Shipped orders:**

2,119 external orders were processed. Of these, 1406 (66%) were shipped and 713 orders cancelled. External orders were cancelled for a variety of reasons including: 569 (81%) were NRR, 75 were unable to secure an import permit, and 57 for other reasons such as mistakes, duplication, NCRPIS was unable to satisfy phytosanitary restrictions, or lack of response activity from the requestor for a year or more.

Domestic orders accounted for 75% of all distributed orders but only half of the distributed items (Appendix Table 3). Maize was the most popular crop in the United States. International orders were more balanced but heavily focused on maize and oilseeds curatorial groups.

Curatorial Group									
	Orde	rs	Order	ltems	Avg. Items per Order				
Curatorial Group	United States	International	United States	International	United States	International			
Amaranth	188	86	4,769	3,333	25	39			
Horticulture	135	8	579	29	4	4			
Maize	537	135	14,923	10,897	28	81			
Oilseeds	181	109	6,316	9,999	35	92			
Vegetables	120	45	4,827	4,932	40	110			
Grand Total	1,041	1,041 365		29,190	30	80			

#### 2018 - NCRPIS External Germplasm Distribution Summary by Curatorial Group

Order distributions were also summarized by curatorial group, as seen in the pie charts below. Horticulture and Oilseeds saw high demand for a few crop maintenance groups while Amaranth, Maize, and Vegetable requests were more balanced across crop groups.



International orders accounted for 25% of those shipped, but contained half of the distributed items. Approximately 1/3 of all international orders were shipped directly from the NCRPIS. The remaining 2/3 of requests were transferred to APHIS in Beltsville for phytosanitary certificate issue prior to export.

For a more detailed view of orders, distributed external orders are visualized in the context of destination.



Each country has unique restrictions regarding the importation of plant material. Therefore, a considerable amount of effort and documentation is required to process these international requests. We adapted the local order number field to distinguish shipping destination as well as serve as a 'quick reference' for documentation and additional quality assurance needed prior to dispatch of an order.



The order processing team was busy this year with many large requests for international germplasm distribution. Seventy five percent of all international orders required issue of a phytosanitary certificate prior to export and 18% of international distributions were shipped with an import permit. Import permit restrictions vary in complexity. There was an increase from 2017 in the number of orders which required additional pathology testing and other supporting documentation (see the pathology section of this report for activity details).

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

The seed storage area was staffed by one full-time, permanent federal employee (Lisa Burke), a three-quarter time temporary employee and three part-time student employees during 2018. We stored 2200 inventory lots, including 443 original seed lots. Of the increase lots, 1100 were produced in Ames and 426 were produced outside of Ames. Across all stored inventory seed lots, we sampled and reviewed seed quantity of 3432 lots, and any discrepancies with GRIN information were corrected in the GRIN database. Six hundred and thirty-eight samples were prepared and transferred to the -20C freezer for long-term storage.

We filled 1442 seed orders in 2018, including those for distribution, observation, germination, transfer and backup. NCRPIS distributed 64607 packets to meet distribution and observation requests. There were 1035 lots sent to the National Laboratory for Genetic Resources Preservation (NLGRP) for backup, involving both accessions new to NLGRP and additional seed quantities for previously deposited accessions. Thirteen inventory lots were transferred to other NPGS genebanks. In 2018, 405 samples were prepared and sent to the NLGRP in Ft. Collins for inclusion in the annual NPGS shipment to the Svalbard Global Trust Vault.

With the aid of our student workers, we prepacked 41058 packets from 3322 inventory lots. Prepacking increases efficiency of seed storage operations by speeding up seed order filling and also help keep the on-hand inventories more accurate. 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.

In 2018, scanning of original samples was postponed until the vacant seed storage technician position can be filled.

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

Lisa Burke and Lisa Pfiffner participated in the Second International Seed Longevity meeting held in Fort Collins, CO and presented a poster entitled: *The "Germination Cliff" and the implications on management of seed regenerations: Maize accessions maintained as populations at the NCRPIS.* 

Lisa Burke continued as the station's CPR/AED/First Aid instructor. She provided two-year First Aid certification for 28 NCRPIS student workers and two-year CPR/AED/First Aid certification for eight staff members. Each session was entered into the National Safety Council database and certificates of completion provided for each participant. Cooperative efforts with campus staff to improve the CPR/AED/First Aid training continues.

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

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

In 2018, the germination lab completed germination or Tetrazolium (TZ) testing on 143 orders containing 4,461 accessions.

Type of Order	# Orders	# Accessions
Regeneration/Original/Other	92	2111
Maintenance	45	2298
TZ	6	52

Progress was made in maintenance testing of the following crops, 100 Amaranthus inventory lots tested, 310 Panicum lots, 157 Daucus, 611 Helianthus lots, 245 Spinacia lots and 1935 Zea mays lots. An additional 504 inventory lots were TZ tested after standard germination testing was completed, which aids in differentiating dormant from dead seed. By testing additional ungerminated seeds following the initial germination test, the information verifies whether seed is viable or non-viable, giving a complete look at the state of the inventory lot.

Germination protocols are based on AOSA protocols and from the Handbook of Seed Technology for Genebanks. If a taxon is not found in either of these references, then protocols are researched and devised for that genus/species.

Curators of *Daucus pusillus*, *Euphorbia lagascae* and wild *Chenopodium spp*. requested that improved germination protocols be identified or developed for these species. Experiments were conducted, and research is ongoing.

Participated in the AOSA-SCST Annual Meeting in Raleigh, NC.

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

#### Controlled Insect Pollination Service Program (S. Hanlin, K. Judson) A.

## Summary of Pollinators supplied to 2018 regeneration cages

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Number of Unique ACCESSIONS per curator							
	Honeybee	Bombus	Osmia	ALC	Flies	TOTAL	
Amaranth/Misc. Umbels	12	0	1	12	18	43	
Horticulture	27	10	9	38	31	115	
Medicinals	4	11	0	4	6	25	
Oilseeds	90	6	81	177	111	465	
Vegetables	113	17	0	112	62	304	
OVERALL	246	44	91	343	228	952	
Number of TOTAL CAGE	/HIVES per	curator					
	Honeybee	Bombus	Osmia	ALC	Flies	TOTAL	
Amaranth/Misc. Umbels	14	0	1	13	18	46	
Horticulture	29	10	9	39	31	118	
Medicinals	8	12	0	4	6	30	
Oilseeds	100	7	81	181	111	480	
Vegetables	134	17	0	122	62	335	
OVERALL	285	46	91	359	228	1009	



#### **Progress:**

Caged pollination:

Bee pollinators (minus the alfalfa leafcutting bee) were supplied a single time to 422 cages for controlled pollination of 381 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 246 accessions in the field.



Crop Group	Total # of Accessions	# of Genera	# Accessions/Genera
Misc. Umbels	12	3	10 Torilis, 1 Coronilla, 1 Petroselinum
Horticulture	27	6	11 Spiraea, 8 Cornus, 3 Diervilla, 2 Caragana, 2 Euonymus, 1 Viburnum
Medicinals	4	2	3 Hypericum, 1 Monarda
Oilseeds	90	1	90 Helianthus
Vegetables	113	5	33 Cucubita, 33 Daucus, 28 Cucumis, 18 Cichorium, 1 Pastinaca
Total	246	17	

2018 Honeybee	Pollinator	<b>Deliveries</b> to	Regeneration	Cages
2010 Honeybee	1 Unnator	Denveries to	negeneration	Jugus

Overwintering success: 65% of the 32 three story parent colonies left outside, 81% of the 22 two story parent colonies and 33% of the 65 double-story nucleus colonies stored in the indoor wintering facility survived. These percentages are lower when compared to 2016/2017 percentages of 80%, 77% and 48%. It was observed in the double nucleus hives that 48% of the losses occurred by mid-January with an additional 27% occurring in early February.

All three-story hives were left outside during the winter 2018/2019 at two locations and were wrapped with thirty-pound roofing paper. All parent colonies stored inside were removed from the room starting on March 8, and all outside colonies unwrapped on March 2. The nucleus hives were removed from the over-wintering room on March 8. In the winter of 2018/2019, we placed in the overwintering room 30 two story parent colonies, 10 three story colonies and 78 double story nucleus hives. We left outside and wrapped 30 three story parent colonies at three locations (colony totals 8, 10, 12).

We purchased 60 five frame nucleus hives from three local suppliers and 50 Italian queens from a California supplier to supplement over-winter losses and to supply spring nucs used for cage pollinations. The queens arrived by USPS in early May, the nucs were picked up and put into full colony equipment in mid - May to early June, and allowed to increase in population until late June before producing nucs from them. The hives were given three feedings of high fructose corn syrup (HFCS) and two pollen patty treatments during the buildup period. The caged queens were placed in nucleus boxes with two frames of brood and a single frame of honey and adhering bees. For 20 of the nucleus hives purchased, it was decided to try Carniolan queens, however these

colonies increased bee numbers more slowly than the Italian queens. It was decided not to use this sub-species of queens in the future because of the lower number of nuc hives produced from them.

In early May we selected queens from eight resilient, over-wintered parent colonies and set them up as "cell builder colonies" for queen production during the summer 2018. During the summer, however three of the hives were switched because of their temperament during grafting. We did our first graft in late May, knowing that with the first graft we generally have only approximately 50% success, we could use the purchased queens to supply most of the nucs we were going to make at this time. Our average for the months' grafting was 19 queen cells per week, with nucleus hives produced until the end of July. In 2018, because nucleus hives were purchased locally (and not as packages from Texas), we had no issues of aggressive bees and all hives survived the summer. Of the purchased queens, only three were lost, even though some were held in queen banks for almost three weeks. All nucs which were not used in cages for pollination were fed HFCS, an additional super was placed below and treated for mites to prepare them for over-wintering.

All hives were fed syrup into early December, but most of the colonies and nucs went into winter lighter than in 2017 and will need to be fed sometime in February/March to assist with survival. The mortality rate prior to placing hives into the over-wintering room was 6% for the parent colonies and 45% for the nucleus hives.



Mite counts were made using the powdered sugar roll method in mid-June on 50% of the total colonies and double story nucs. Mite numbers were found to be between 0 to 23 mites per 100 bees. Most hives showed between 4 to 10 mites per sample, which is equal to the documented economic injury levels (EIL) of 5 mites per 100 bees. In August and September all colonies and nucleus hives were treated with Formic Acid (Mite Away Quick Strip®). A sugar roll sample was taken in October after the treatments were removed. Even though mite populations were lower, most hives went into winter with counts of 1 to 3 mites per 100 bees. In communicating with other area beekeepers, most individuals found mites in "after treatment" sampling but were not able to give a second treatment because of cool weather conditions. (See ARS Photo by Scott Bauer)

Starting in March through early April 2018, 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 October to early November, all hives were fed six feedings, but because the medication is no longer available, all feedings were non-medicated. During the summer neither European Foul Brood (EFB) nor American Foul Brood (AFB) were observed, however in August two treatments of Terra-Pro were given to possibly increase bee/brood production and prevent brood diseases from establishing.

As with the past three years, for wax moth control during the summer all stored supers with "cleaned" frames were stacked at right angles to each other to prevent adult moth migration in the equipment room. Starting in June through October, 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" was stored in the overwintering room at a temperature of  $60^{\circ}$  F. In late summer it was observed that the air conditioning unit had quit, the temperature rose to around  $80^{\circ}$  F, and wax moth was found in several supers of frames.

In 2018, we removed the 1,050-gallon polypropylene tank and replaced it with three 275-gallon gravity fed bulk tanks. We continued to use the 30-gallon poly "mixing" tank for filling feed containers. To prevent crystallization of the HFCS during the winter, we placed insulated blankets over the two tanks containing syrup and a single tank was left uncovered. We began using syrup on a weekly basis starting in February with no issues in syrup flow. It was thought that because the tanks were on racking close to the ceiling and the room's heating unit, that crystallization would not be a problem and it was not observed in any of the tanks of syrup. On May16, 852 gallons of HFCS were purchased for supplemental feeding during the summer and into the spring of 2019. We continue to use five-gallon buckets in the spring and fall for refilling feed containers in the field to reduce container damage and syrup waste.

As in 2018, hive registration with the Iowa Department of Agriculture and Land Stewardship (IDALS) was done using "Fieldwatch". Fieldwatch allows you to register yards by plotting them directly onto Google maps. Unlike 2017, the locations only had to be confirmed in the system that they would continue to be used in 2018 fin order to register them. The IDALS registry assists pesticide applicators in locating bee-yards and in obtaining contact information of appropriate beekeepers prior to spraying.

#### Bombus pollination:

"mini-research" Twenty-six colonies of *Bombus* impatiens were purchased from a commercial supplier and used to pollinate 46 field cages with 44 accessions. In 2018, we continued to use the gueen-less "mini-research" hive for pollination. For some cages loose worker bees had to be retrieved and released when the hive was switched to a different location because the bees would not go back into the hive. While in storage, we would place



sugar-soaked cotton wicks into holding containers for loose bees to feed on prior to release. 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 in 2018, because of the amount of vegetation, a single *Bombus* hive was combined with a honey bee nuc for more complete pollination in each cage.

		6	
Crop Group	Total # of Accessions	# of Genera	# Accessions/Genera
Horticulture	10	3	4 Diervilla, 3 Staphylea, 3 Cornus
Medicinals	11	2	10 Monarda, 1 Agastache
Oilseeds	6	1	6 Helianthus
Vegetables	17	1	17 Cucurbita
Total	44	7	

2018 Bombus Pollinator Deliveries to Regeneration Cages



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 the stand. In some cages, the plastic container was replaced with two full honey bee supers taped together and a telescoping lid placed over one end. For these shelters, no water bottles were needed because the weight of the boxes prevents them from blowing over in the wind.

The protective shelter, bottles and hive are placed on a full-size honey bee hive body and telescoping lid for a stand. The stand prevents the tub and hive from getting flooded in fields where standing water can occur.

#### Osmia cornifrons/O. lignaria pollination:

Osmia sp. were used to pollinate a total of 81 field cages and 10 greenhouse cages with 91 accessions.



2018 Osmu deer officiator Deriveries to Regeneration Cages						
Crop Group	# of Cages	Total # of Accessions	# of Genera	# Accessions/ Genera		
Misc. Umbels	1	1	1	1 Coronille		
Horticulture	9	9	3	5 Aronia, 3 Staphylea, 1 Spirea		
Oilseeds	81	81	3	74 Brassica, 6 Thlaspi, 1 Isatis		
Total	91	91	7			

#### 2018 Osmia Bee Pollinator Deliveries to Regeneration Cages

In the 2017 growing season, we obtained an increase of ca. 408 *Osmia* pupae (20.5 domiciles at 20 bees/domicile) which could be used for pollination and increase during the 2018 pollination season. We purchased an additional 3200 commercial cells in the spring of 2018. As in 2017, the pupae were shipped as loose cells prior to domicile placement in the field, 20 pupae (10 males and 10 females) were placed into specimen cups and were transferred into the domicile just before hanging it in the cage. For the greenhouse cages, six total pupae (3 male and 3 female) were placed into each cage. We also purchased an attractant to possible increase our *Osmia* "increase" numbers at our orchard locations and to help keep the bees we were releasing "close to home".

*Osmia* pupae were used to fill 109 two-inch domiciles. The two-inch domiciles were divided in the following manner, 91 were used in pollination cages and 19 were used at a single "increase" site.

In the fall of 2018, we collected ca. 260 pupae (13 domiciles at 20 bees) which will be used in the spring of 2019. Additional pupae will be ordered in the spring of 2019 to assure enough pollinators for the spring cages and for placing at "increase" sites.

#### Alfalfa leafcutting bee (ALC) Megachile rotundata:



ALC bees were purchased as larvae in leaf cells from a single supplier for use in 2018, arriving in Ames, IA on February 24. 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 March 2018 through early November 2018. The 2018 pollinations were all completed using the 2018 cocoons for emergence of adults. Because in 2017 we purchased only 15 gallons of pupae rather than the 18 gallons and

had used all cocoons by mid-October, we purchased 17 gallons for the 2018 season and as of December 2018 had nine tubs of pupae to use into the 2019 season.

Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period
Misc. Umbels	47	13	2	12	3	March – Oct.
Horticulture	135	39	6	38	8	March – Sept.
Medicinals	13	4	1	4	1	May – June
Oilseeds	982	181	4	177	5	Dec. (17) – Nov.
Vegetables	982	122	6	112	4	March – Oct.
Total	1177	359	19	343	21	Dec. (17) – Nov.

2018 Alfalfa Leafcutter Pollinator Deliveries to Regeneration Cages

In 2018, 1177 total ALC deliveries were made to a total of twelve fields and three greenhouses with 359 cages containing 343 accessions. A total of nine greenhouse cages were still receiving pollination at the transition from 2018 into 2019.

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 March through June 2018, greenhouse cages were supplied weekly with bees in the spring and summer. The 2018 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 last field cages supplied through early-November.

In 2018 we received Canadian sourced cells, which have fewer parasites and parasitoids than found in U.S. cells. Because the Thermo-Scientific® environmental growth chamber (EGC) was being used by several projects from February until early-June, we relocated the emerging bees to a Precision® incubator. By early June

through late December, all bee emergence was relocated back to the EGC. During both relocations, there was no observable decline in the emergence of adult bees available for pollination, however, the storage space available for screen trays of pupa was reduced for most of the season.

From September to early November, ALC were placed into cages of *Helianthus sp*, *Diervilla* sp, *Spirea* sp and several field vegetable cages. Under normal conditions ALC are not the major pollinator of some of these accessions nor are they used this late in the season in field cages. Because we had no fall greenhouse pollinations but continued to emerge a low number of bees, we chose to place them into field cages rather than discard 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 late December 2017 through early November 2018 for greenhouse and field pollinations. From early June through August, twenty-six orders of 10,000 house fly pupae were purchased and from December 2017 through September 2018, 241 cups of blue bottle fly pupae were purchased. In November and December, two orders of three cups of blue bottle pupae were



purchased for late season greenhouse pollinations that will continue into 2019. In 2018, 1234 fly deliveries were made to nine fields and three greenhouses with 231 cages containing 228 accessions representing 22 genera.

Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period
Misc. Umbels	113	18	3	18	4	March – Oct.
Horticulture	105	33	5	32	8	March – Sept.
Medicinals	13	4	1	4	1	May (11) – July
Oilseeds	465	113	3	112	5	Dec. (17) – Nov
Vegetables	538	63	3	62	4	Feb. – Oct.
Total	1234	231	15	228	22	Dec. (17) – Nov.

2018 Fly Pollinator Deliveries to Regeneration Cages

An average of four greenhouse cages and an average of 21 field cages received flies weekly from December 2017 through early November 2018. Three greenhouse cages of *Thlaspi sp.* and a single cage of *Isatis sp.* began getting blue bottle fly pupae in mid-December 2017 and transitioned into the 2018 season. We had a cage of *Crambe* sp. and two cages of *Daucus* sp. that were started in late November and will be transitioned into the 2019 season.

Because blue bottle flies work better at cooler temperatures and more cage requests were for the cooler greenhouse, only blue bottle flies were distributed weekly during the winter, spring and fall. During the summer, both blue bottle flies and houseflies were distributed weekly to greenhouse and field cages for pollination. Adult flies are re-supplied weekly to cages to ensure continued pollinator presence. Most cages which have fly pollinators introduced also have bee pollinators present to assure flower pollination based on promotion of insect competition. During the summer, if there was an excess of fly pupae available, flies were introduced to some accessions which lack favorable flowers for fly pollinators such as sunflower or melons. This decision was made the curators and the entomology staff to fully utilize the fly pupae.

#### **Tests:**

#### Feeding sugar in the winter:

In the winter of 2018, to improve hive survival but reduce the amount of excess HFCS that needed to be cleaned from the floors after hive removal, we tested feeding table sugar to the bees. Starting in early January, each hive was fed a cup of granulated sugar placed on a sheet of newspaper just above the cluster. During the first two feedings using this method, the newspaper/sugar was sprayed with water to produce a small amount of syrup. The hives were checked weekly and extra sugar was added if the original amount was reduced. By late January, it was observed that the parent colonies were able to benefit from this feeding method, but the double story nucleus hives did not show interest in using the sugar. A possible answer is that the nucs were not strong enough to break a loose cluster and feed or that they did not produce enough moisture to liquify the solid sugar. So, starting in February through March the nucleus hives were fed HFCS. Feeding table sugar worked well to maintain colony survival and made a minimal mess that needed to be cleaned up in the spring. For the nucs, it is felt that if the bees were forced to feed only on the granulated sugar that their mortality level would have been higher compared to 2017's.

#### Branding equipment:

As a method for assisting in identifying the station bee equipment and a deterrent for equipment being stolen, all woodware would be branded with an identifying stamp displaying that the hives are property of USDA, ARS and providing contact information. In the winter of 2017/2018 approximately 90% of all supers, both nucs and full, and approximately 95% of the frames were branded. All newly built frames were also branded. Prior to using the brand, a PPE (personal protective equipment) and JSA (job safety analysis) were written and submitted for approval. It is unclear if the branding of equipment prevented any theft from occurring, as prior to 2016 no equipment was stolen. We will continue to brand other woodware in the future as a precaution.

#### $2^{nd}$ year testing tablets with new actions and methods:

Prior to the start of the 2018 pollination season, curatorial staff which use pollinators, IT staff and the entomology staff met for a QandA so that all questions and issues could be addressed. Curators were encouraged to make "test" pollination requests and work with their tablets to get comfortable making requests. Because of this "workshop" the summer issues and problems were minimal, and most pollination requests were received with very few changes needed by the entomology staff. There were no major changes to the "action codes" or the "insect codes" prior to the 2018

season. It was decided by all participants that the present pollination system was adequate for the future and that a "workshop" in the spring of 2019 prior to the pollination season would be useful.

#### Safety:

#### Chemical Inventory:

In Mid-November 2018, C. Hopkins updated the Entomology chemical inventory and sent it to ISU and the USDA.

#### **Defensive Driving:**

Because of the amount of time that the bee crew is off site and the number of cumulative miles during the summer, annual driving training is needed to refresh good driving habits. Defensive driving courses were taken on AgLearn by both full-time entomology crew to fill this requirement. S. Hanlin on February 28 completed "Defensive Driving Fundamentals" and on March 6 "Distracted Driving". K. Judson on March 1 completed "Defensive Driving".

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

In early March, K. Grooms (nurse at ISU Occupational Medicine) was contacted for discussion and approval of the Epi-pen website and training materials on "the signs of anaphylactic shock and the correct use of Epi-pens". S. Hanlin provided the link to the Epi-pen website and "training completion document" to all permanent station staff to be completed at the end of March. On April 10, K. Grooms was asked to order four replacement pens and all PI staff "training documents" were filed at ISU Occ. Med. On April 18, K. Judson took expired Epi-pens to the ISU pharmacy and exchanged them for new pens. The Epi-pens are available at four NCRPIS locations for use in prevention of anaphylactic shock caused by bee stings or other allergic reactions.

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

On April 30, S. Hanlin presented a power point presentation at the DMACC (Des Moines Area Community College) Boone campus for "Earth Day". The presentation consisted of an introduction about the NCRPIS and then went into the different pollinators used in our program and why. The final portion of the presentation was about a selection of native pollinators which had become extinct or were endangered and what factors has caused their reduction. The students had the opportunity to look at live and drawered specimens and sample honey sticks during the Q and A. The presentation was given to approximately 30 students and faculty.

On April 30 and May 1, S. Hanlin, K. Judson and L. Burke spoke to two groups of Vocational Agriculture/FFA groups. The group was introduced to the variety of pollinators used at the station and then most of the 20-minute talk was focused on honey bees including disease problems and control, queen rearing and how to get started in beekeeping including where to obtain equipment and what the costs may be. Both groups consisted of approximately 20 students and staff and each was given a honey stick during the QandA session at the end.

On May 17, K. Judson, K. Dameron and S. Hanlin talked to approximately 120 sixth grade students about honey bees and other pollinators in six half hour presentations at the Squirrel Hollow Outdoor Classroom in Jefferson, IA. The students were told

about the makeup of the hive, what each of the castes of honey bees do, how bees produce honey and wax, the equipment used in beekeeping including trying them on and possible benefits and issues associated with honey bees.

On October 23, K. Dameron and S. Hanlin went to Gilmore City Grade School in Gilmore City, IA and gave six half hour presentations to groups of students ranging from kindergarten to sixth grade. The students were told about the various pollinators used at the station and shown pinned and live specimens and given facts about each insect. The students had the opportunity to try on and use some bee keeping equipment. The makeup of the hive including the castes of honey bees and how they make honey and wax was discussed. The students were also shown how honey is extracted and given a honey stick to eat or take home. Each session ended with a Q&A session.

#### Plans for 2018:

#### Feeding sugar in the winter:

In 2018 we tried feeding granulated sugar to both the colonies and nucs as a late winter source of nourishment for inside over-wintered hives and also as a cleaner feeding method than HFCS. We found that the colonies did use the granulated sugar, but the nucleus hives did not and had to be fed HFCS. For 2019 we will test the feeding of a mix of HFCS and granulated sugar to nucs in the form of a patty or more solid liquid feed which will be placed on wax paper. For the colonies, we will feed a purchased "winter patty" which consists of pollen, assorted nutrients and HFCS. The "winter patties" were used in 2017/2018 for winter feeding and were consumed by the colonies, but not by the nucs in late winter. We also plan to put the "winter patties" on outside colonies as soon as the weather allows us to remove the lids/inner covers and not chill the cluster.

#### Branding equipment:

In the winter of 2017/2018, we branded most of the supers and frames as a theft preventative. We will continue to brand any missed supers or frames, but also will begin to brand bottom boards, inner covers and lids. We will also brand any new frames which are built in 2018/2019 season. After two winters of branding, we should have most of the equipment which goes to the field marked with the "Property of USDA; ARS" for a deterrent for theft in the future.

#### Additional Osmia supplier:

In late December, S. Hanlin contacted a new *Osmia* bee supplier. Because the price was lower than the present supplier and these bees could be used to supplement our bee stocks, a small quantity of pupae will be purchased and tested both in cages and at "increase" sites to determine if the bees are comparable to the present supplier's product. The bees from the new supplier will be compared based on emergence percentages, summer build-up (increase) and health. All observations will be done visually and will be based on spot checks of cages and increase sites.
# B. <u>Plant Pathology (N. Pal)</u>

#### Personnel changes:

Dr. Anna Testen, Plant Pathologist joined NCRPIS in February 2019.

#### **Research:**

<u>Real-time PCR to specifically detect *Pantoea stewartii* subsp. *stewartii* from corn <u>seeds</u>:</u>

Current seed health screening methods for the Stewart's wilt pathogen of maize, Pantoea stewartii subsp. stewartii rely on enzyme-linked immunosorbent assays (ELISA). However, these assays are non-specific and detect the non-pathogenic Pantoea stewartii subsp. indologenes. A manuscript titled "A real-time PCR differentiating Pantoea stewartii subsp. stewartii from P. stewartii subsp. indologenes in corn seed" was drafted and submitted to *Plant Disease*. The manuscript was and will be published in the May issue of *Plant Disease* accepted (https://doi.org/10.1094/PDIS-06-18-0936-RE). In this study a real-time PCR method was developed to detect P. stewartii subsp. stewartii directly from a seed wash and distinguish it from the closely related, but non-pathogenic, P. stewartii subsp. indologenes. Use of the real-time PCR methods described in this article would avoid false positive results due to non-specificity in ELISA testing for Pantoea stewartii subsp. stewartii and help reduce unnecessary restrictions on international movement of corn seed.

#### Screening for resistance to anthracnose in American Sycamore:

Anthracnose of American sycamore (*Platanus occidentalis*) is caused by the fungus, *Apiognomonia veneta* and is a key disease of this native tree. Identifying genetic resistance to this disease would help preserve sycamores in the landscape and a project was undertaken to screen sycamore seedlings for disease resistance. The fungal pathogen was isolated from symptomatic leaves and twigs of American sycamore trees located in Iowa. Conidial suspensions were used to inoculate a replicated trial of seedlings grown from the open-pollinated seed of *P. occidentalis* and *P. occidentalis* var. glabrata mother trees, collected from Iowa and Texas respectively. The screening experiment was conducted in a shade house where six-week-old seedlings were sprayed with conidial suspension during the first week of May. Veinal necrosis, characteristic of anthracnose (Fig. 1 A-C). was observed on the leaves 16 days following inoculation. Disease progression stopped in June because of environmental conditions (dry and hot), and we could not draw any conclusions regarding differences in disease incidence or disease severity.

### Bacterial Fruit Blotch (BFB) grow out assay for *Cucumis melo* accessions:

Bacterial Fruit Blotch (BFB) caused by, *Acidovorax citrulli* (Aac) is an economically important disease of cucurbits. The pathogen is seedborne and infected seed is considered to be the most important cause of disease outbreaks. To determine if the seed lots of seven *Cucumis melo* accessions (PI 126126 17ncax01, PI 136181 17ncax01, PI 164855 17ncax01, PI 169309 17ncax01, PI 174142 17ncax01, PI 182950 17ncax01, PI 321005 17ncax01) are infected or not, 200 seeds from each of these accessions were screened by a greenhouse grow-out assay (Fig. 1D-E). Seeds were planted in plastic trays filled with potting mix. The trays were watered and placed inside turkey bags

with the mouth of the bag tucked underneath the tray to create hot and humid conditions conducive for disease expression. Of the seven accessions, seeds of only one accession (PI 136181 17ncax01) were found to be BFB-contaminated. Characteristic water-soaked lesions and necrosis was observed on the cotyledons of infected seedlings (Fig. 1F). Seedlings were further confirmed to be BFB positive by enzyme linked immunosorbent assay (ELISA). To test for the localization of *Acidovorax citrulli* in the infected seedlings, ELISA was performed on the cotyledon, hypocotyl and root tissues individually after thoroughly washing the hypocotyl and root under distilled water to remove any surface contamination. All the three tissues (cotyledon, hypocotyl and root) tested strong positive by BFB ELISA thereby suggesting the systemic presence of *Acidovorax citrulli* in the naturally-infected melon seedlings.

#### Seed treatments for BFB contaminated seed lots of *Cucumis melo* accessions:

Seed lots of *Cucumis melo* accessions transferred to NCRPIS from Griffin, Georgia were found to be BFB contaminated by previous greenhouse grow-out assays or field plantings. Our research objective was to test seed treatments to eradicate the pathogen, Acidovorax citrulli, from naturally-contaminated seed lots so that they can be grown for seed increase to obtain clean seed for distribution to the requestors. Two published seed treatments: a streptomycin soak for 16 hours [Sowell and Schaad (1979) Plant Disease Reporter 63(6): 437-441] and a dry heat treatment at 85°C for 72 hours [Kubota et al. (2012) J. Phytopathol. 160: 364-368] were evaluated on naturallycontaminated seed lots of 16 C. melo subsp. melo accessions. Seed treatments were performed on 100-200 seeds from each accession according to the published methods and treated seeds planted in the greenhouse. Seedlings (cotyledon tissue) were tested by BFB ELISA prior to transplantation in the field. Several accessions (5/16 and 4/16 accessions among the streptomycin and dry heat treatment groups respectively) had tested ELISA positive. Prominent disease symptoms (Fig. 1G) were observed on some accessions in the field indicating that neither of the two published methods was completely effective.

We tried a new approach which was a combination of physical (dry heat) and chemical (streptomycin) treatment. One hundred seeds from naturally-contaminated seed lots of 13 *C. melo* accessions were subjected to dry heat at 85°C for 72 hours, slow rehydration at room temperature for 72 hours followed by soak in streptomycin solution (1 mg/ml) for 72 hours.

Seeds were planted in the greenhouse and 200 ppm of streptomycin solution further applied as a spray (five times at about two-week intervals) and soil drench (two times 47-60 days after germination).

Though the treatment had some effect on germination and development, and cotyledon samples from 3 out of 13 accessions had tested positive by BFB ELISA, none of the true leaf samples collected from the 13 accessions were ELISA positive and no obvious disease symptoms were observed on any plant until fruits were harvested and plants discarded. Seeds obtained from these plants will be tested for BFB contamination by a grow out assay.



**Figure 1.** A, B, C) Veinal necrosis on Sycamore seedlings following inoculation with conidial suspension of the anthracnose fungus, Apiognomonia veneta. D) Greenhouse grow-out assay for BFB screening of melon accessions. E, F) Water-soaked lesions and necrosis on BFB infected seedlings of accession PI 136181 17ncax01. G) BFB symptoms on leaves of melon accession (PI 126079 91ncab01) in the field.

#### **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.

#### Maize:

During July and August, we made field inspections of 212 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, 616 GEM plots were inspected for the same group of diseases. In terms of disease incidence, the dominant diseases were gray leaf spot (Cercospora zeae-maydis), common rust (Puccinia sorghi), northern corn leaf blight (Exserohilum turcicum), physoderma brown spot (Physoderma maydis), eyespot (Kabatiella zeae), and anthracnose leaf blight (Colletotrichum graminicola) (Fig. 2 A-F). Southern rust (*Puccinia polysora*) was observed on few accessions during July. Common smut (Ustilago maydis) was present in several plots. Anthracnose leaf blight was observed in almost every maize seed increase plot during July but plants were not severely infected (lesions restricted to lower leaves only) except for the accession PI 193438 18ncai01, which was severely infected and plants showed anthracnose top dieback also. None of the diseases of phytosanitary concern: Stewart's wilt, Goss's wilt, head smut, crazy top or other downy mildews, and viral diseases were observed.

#### Oilseeds:

Field inspections were carried out during the months of June and July for sunflower, flax and *Brassica* accessions. A total of 194 sunflower accessions were inspected

multiple times during the early growing season in the month of June for the presence of downy mildew (*Plasmopara halstedii*) or apical Chlorosis (*Pseudomonas syringae* pv. *tagetis*). No downy mildew (the main phytosanitary issue) or apical chlorosis was observed in any plot.

Among the 14 flax (*Linum usitatissimum*) accessions inspected during the months of June and July, Cercospora leaf spot was observed in all plots.

Black rot (*Xanthomonas campestris* pv. *campestris*), a disease of phytosanitary importance was observed on 31 of the 75 *Brassica* accessions. Other than black rot, Cercospora leaf spot was present on some accessions.

#### <u>Vegetables</u>:

Routine disease testing for squash mosaic virus was conducted on all cucurbit seedlings prior to transplanting; annual testing has been done since 1993. Fifty-nine accessions and 2,572 plants were sampled and tested by ELISA. Test results are summarized in Table 1. One *Cucurbita pepo* accession PI 442299 80 ncpo01 was found to have SqMV-infected plants. 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 plantings. SqMV ELISA test results were uploaded to GRIN database.

Species	# of Accs tested	# of Accs with infected plants	# of plants tested	# of SqMV infected plants
Cucumis spp. (melo, sativus)	29	0	1656	0
Cucurbita pepo	30	1	916	28
Total	59	1	2572	28

Table 1: Squash mosaic virus testing results for 2018

Multiple disease inspections of the vegetable collection seed increases were conducted from June through early September. A total of 147 cages that included 43 Cucumis spp., 33 Cucurbita pepo, 1 Pastinaca sativa, 18 Cichorium intybus, and 52 Daucus carota were inspected. Aster yellows was present in several D. carota accessions. Infected plants were removed from the cages to prevent spread of the aster vellows phytoplasma by leafhoppers. Cercospora leaf blight (Cercospora carotae) (Fig. 2H) was observed on several (8/52) carrot accessions. Two carrot accessions showed symptoms of bacterial blight (Xanthomonas campestris py. carotae) (Fig. 2I and J). The parsnip accession PI 652111 18ncai01 had yellow flecking on leaves and poor seed set. Anthracnose (Colletotrichum orbiculare) (Fig. 2G) was observed on Cucumis spp. (C. sativus and C. melo). Lesions were present on leaves, stem and fruits. Cercospora leaf spot (Cercospora citrullina) was present on some C. sativus, C. melo and C. pepo accessions. Because of frequent rains during 2018 growing season, Cercospora leaf spot and anthracnose diseases were severe and plants had to be sprayed with protectant and systemic fungicides: Bravo 720 (chlorothalonil; group M5 fungicide) and Quadris (azoxystrobin; group 11 fungicide) for disease management.

Amaranthus, Chenopodium, Panicum, Setaria and Miscellaneous Apiaceae and Poaceae:

Disease inspections were conducted in the field and greenhouse. Other than *Pyricularia* leaf blast on some *Setaria italica* accessions (Fig. 2K) and minor leaf spots (*Phomopsis amaranthicola*) on *Amaranthus* accessions, no other unusual disease problems were observed in the field. The fungus *Pyricularia* has been isolated from the symptomatic leaves and species identification will be done by fungal ITS sequencing. In the greenhouse plantings, 98 accessions were inspected during September-October. No disease problems were observed.

A total of 3,632 observations from the 2018 field and greenhouse plantings were uploaded to the GRIN database.



**Figure 2.** Images of disease symptoms on corn (A-F), cucurbits (G), carrots (H-J) and foxtail millet (K) from field scouting. A) Physoderma brown spot. B) Anthracnose top dieback. C) Anthracnose leaf blight. D) Northern corn leaf blight. E) Gray leaf spot. F) Eye spot. G) Anthracnose. H) Cercospora leaf blight. I) and J) Bacterial blight. K). Pyricularia leaf blast.

### Seed Health Testing/Seed Treatment:

We carry out a seed health testing and seed treatment program to support international seed shipments. A total of 1,670 maize accessions were tested for seedborne pathogens including: Stewart's wilt (1401 accessions), Maize chlorotic mottle virus (143 accessions) and Wheat streak mosaic virus (126 accessions). Eight *Daucus carota* accessions were tested for *Xanthomonas hortorum* pv. *carotae* by seed wash dilution-plating and PCR. A total of 457 laboratory tests for different fungal pathogens (*Plasmopara halstedii*, *Puccinia helianthi*, *Sclerotinia sclerotiorum*, *Septoria helianthi*, and *Verticillium* sp.) were performed for 159 *Helianthus* accessions.

Laboratory seed health testing records (2,135 results) were uploaded to the GRIN database.

Phytosanitary documentation, i.e. freedom from specific pathogens, was provided to support seed shipments for 148 international seed orders.

## **Publication:**

N. Pal, C. Gardner and C.C. Block. 2019. A real-time PCR differentiating *Pantoea stewartii* subsp. *stewartii* from *P. stewartii* subsp. *indologenes* in corn seed. Plant Disease. https://doi.org/10.1094/PDIS-06-18-0936-RE.

## Manuscript in progress:

N. Pal and C.C. Block. 2019. Stewart's wilt of corn – a review of testing methods (For submission to Plant Health Progress).

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:

We acquired 15 accessions: 1 Amaranthus, 5 Chenopodium, 1 Dalea, 1 Dysphania, 1 Echinochloa, 1 Eryngium, 2 Melilotus, and 3 Setaria.



PI 689082 Melilotus albus was collected under palm trees at a Pinellas County beach in Florida.

A two-year plant exploration led by David Brenner in the Tampa, Florida area, funded by a USDA-ARS Plant Exploration grant, resulted in 12 new accessions in 2017. A second visit to the same area in June 2018 resulted in 10 additional accessions. The *Chenopodium* accessions (Ames 34323, PI 689074, PI 689075, PI 689076, PI 689077) add germplasm with sub-tropical winter adaptation as compared to our temperate summer adapted germplasm holdings. *Amaranthus blitum* (Ames 34322), *Dysphania anthelmintica* (PI 689078), *Echinochloa walteri* (PI 689080), two *Melilotus albus* (PI 689081 and PI 689082), and *Setaria corrugata* (PI 689085) were collected on the 2018 exploration. Two accessions of taxonomically ambiguous varieties of *Setaria viridis* were also collected. They are the red bristled (PI 689681) and green bristled (PI 689682) forms of giant-sized weedy foxtail, growing as tall as giant foxtail, *Setaria faberi* but with small seeds which are diagnostic for *Setaria viridis*. They are a common, widespread field weed, and may be intermediate with cultivated *Setaria italica*, but are oddly under-studied, previously not in the collection, and a topic for new research.

Jeff Carstens collected an *Eryngium leavenworthii* (Ames 34172) near Winfield, Kansas which has ornamental potential for its bright blue flowers, and new *Dalea purpurea* accession (Ames 34340) near Guthrie Center, Iowa. They are both beautiful species suited to landscaping and cut-flower use.



David Brenner collecting *Chenopodium berlandirei* (PI 689076) at Fort De Soto county park in Pinellas County, Florida. This plant had mature seeds on June 28, 2018, so it is wintergrowing and springmaturing unlike the fall-maturing examples of the same species at higher latitudes.

### Maintenance:

#### Haldrup LT-20 thresher:

The station purchased a Haldrup LT-20 thresher to improve seed cleaning efficiency. It successfully processed foxtail millet seeds and we anticipate improved efficiency with other crops in 2019.

### Cover crop:

An experimental cover crop of Quick-2-Thick blend, and other miscellaneous grasses was planted by Fred Engstrom and Brian Buzzell in our field between the pollination cages and in the millet observation-field alleys. The cover crop planting was mowed during the growing season and was very successful for improving the footing, reducing compaction, and reducing erosion. In late 2018 Brian made a similar new seeding of combined 'Fawn' and 'Kentucky 31' tall fescue to emerge in spring 2019 and replenish the grass cover.

### Beneficial rove beetles:

We used beneficial rove beetles *Dalotia coriaria* (=*Atheta coriaria*) to control western flower thrips in our campus greenhouse. The ISU Agronomy Department's

Greenhouse Manager, Aaron Brand, was already using them successfully in other greenhouse areas. The rove beetles were released in late summer 2018, and no thrips were observed in the 2,500 square-foot protected area during the fall or early winter 2019.

#### <u>Glyceria striata:</u>

We had an innovative break-through in methods to regenerate an accession of *Glyceria striata* (Ames 29899). We have grown it for years with only weak nonsynchronized flowering. In 2018 we forced the plants to flower in the campus greenhouse with very long 22-hour days and were able to successfully harvest seeds. Starting on April 26, 2018. When our 17 plants were 20 months old, they were exposed to the long days until the final harvest on June 15, 2018. A similar species, *Glyceria notata* (PI 380865), did not flower in the same long-day environment.

#### Miscellaneous Apiaceae:

We had small 2018 field-cage year increasing 15 cages of *Torilis*. Since the Apiaceae seeds are aging in storage, we anticipate years of larger field-cage increases starting with *Coriandrum* in 2019.

#### <u>Spinacia</u>:

Seeds of 14 accessions of domesticated spinach were sent to Beiquan Mou of the USDA-ARS in Salinas, California for regeneration. We anticipate receiving the harvests in 2019 and sending more seeds for planting and 2020 harvests. Two wild spinach accessions were regenerated in our Ames greenhouses, and one more was planted for harvesting in 2019. In 2018, one cultivated spinach accession was increased in a field-cage.

### Characterization/evaluation/taxonomy:

The GRIN database was enhanced with useful updates listed below in the "Updating GRIN 2018" table. Stacey Estrada was very helpful with the pollination code data loading. The entries will be useful for selecting accessions for seed orders and processing seed orders.

Count	Name	Description
		Taxonomic changes were made in eight genera. Seven of the
17	Taxonomic Re-IDs	changes were in <i>Chenopodium</i> .
50	DI numbono	Permanent "PI" number identifiers were assigned to
96	r1 numbers	accessions with temporary Ames numbers.
		Links to research publications loaded onto cited accessions
		in GRIN. The citations included: Stetter, M.G., T. Muller,
193	Citations	and K.J. Schmid. 2017. Genomic and phenotypic evidence
		for an incomplete domestication of South American grain
		amaranth, linked to 117 accessions.
		Narinder Pal loaded her 2017/2018 pathology inspection
227	Pathology notes	notes into GRIN to support rapid identification of order
337		items that meet phytosanitary requirements or require
		additional testing.
		Older pathology inspection notes by Charlie Block were
1,586	Pathology notes	loaded in GRIN by David Brenner, to be readily available
		for approving order items that require inspections.
		Observations were loaded into GRIN on traits such as
6,102	Observations	flower color, male sterility, and millet adaptation for seed
,		maturity in Ames, IA, for public user access.
		Pollination control codes for seeds lots dating back to
		the 1980s were loaded from the site-inventory part of
24.202		GRIN into the inventory part of GRIN by David Brenner
24,392	Pollination codes	with help by Stacey Estrada. In the Site-inventory they
		were part of a legacy system, and now in inventory, they
		conform to the modern GRIN schema.

#### Updating GRIN 2018

### Grasses:

Purpletop tridens, or greasegrass (*Tridens flavus*), and Sand Reed Grass (*Calamovilfa longifolia*) are commercialized. To my surprise many vendors are offering seeds for what were until recently obscure wild species with "potential". *Tridens* is a late flowering bunch grass used in native species conservation plantings in the mid-western and eastern United States. Germplasm from our collection of 74 accessions was distributed on 16 orders in the last ten years. *Calamovilfa*, with eleven accessions, is a strongly rhizomatous native grass that is adapted to colonize sandy areas and was distributed in 11 orders in the last ten years. I do not know if our germplasm is involved in the parentage of the commercial offerings.

### Amaranthus:

There is a continuing taxonomic controversy involving some (and probably most) of our 112 Amaranthus caudatus accessions from India. On a molecular basis they cluster with another species, Amaranthus hypochondriacus and not South American Amaranthus caudatus. A new amaranth morphological type was created to document these accessions in the GRIN descriptors and coded as CAUIND (A. caudatus from India). This characterization is intended to help researchers select vetted accessions for further study. In early 2019 CAUIND was populated with 20 greenhouse-observed accessions including 16 new observation from 2018.

### Chenopodium:

Seven *Chenopodium* taxonomic re-identifications in 2018 were possible with expert consultation with: Thomas M. Davis of The University of New Hampshire, Eric Jellen of Brigham Young University, and Nuri Benet Pierce of San Diego State University.

### Millets:

We grew 69 accessions of foxtail millet (*Setaria italica*) in 2018. During a routine test of seed viability 20 years since the last test, Lisa Pfiffner found that viability had generally declined by about 10%, but some more than 20%. Foxtail millet seed lots for distribution are generally between 90% and 100% viable, but viability was in the 70-80% range, and we suspected from field plantings that poor vigor was causing poor establishment. Backup planting-seeds for 55 of these regenerations were sent from the NLGRP in Fort Collins, CO. Previous curators deposited backup samples there for exactly this purpose 25 to 30 years ago. The backup seeds germinated well, and greenhouse-grown seeds increases were harvested in late 2018 for storing in 2019. This second cycle of seed regeneration, 34 years after the first cycle, was considerably easier because we now use greenhouse methods as compared to field-transplanting used in the first cycle. A similar viability test of proso millet (*Panicum miliaceum*) indicated no decline in their viability.

Dr. Dave Stoltenberg, a weed scientist at the University of Wisconsin, was asked about donating herbicide resistant germplasm of weedy proso millet (*Panicum miliaceum*) and replied that he has not observed herbicide resistance in weedy proso. It is valuable to have this state-of-the-art information, since I have been asked for herbicide resistant weedy proso millet, which I'm not aware exists.

We evaluated 124 accessions of *Setaria*, 64 of *Panicum*, and one of *Echinochloa* in a millet field planted on May 18, 2019. Data were collected on adaptation for seed maturity and other field traits. Most of these accessions are adapted in Iowa, 180 (98%) reached seed maturity before a freeze on October 20. The four that did not reach maturity are from Tamil Nadu, India and Zimbabwe.



PI 614817 foxtail millet with a green long-bristled off-type head that may be the result of spontaneous crossing with weeds in the previous generation.

PI 180878	Shortest foxtail millet, 55 cm tall, with many small heads, collected by Jack Harlan in Turkey
PI 649318 'White Wonder'	Well adapted, and as learned at the millet conference, the standard foxtail millet variety in Colorado
PI 433387 'Taichung No. 6'	Widest foxtail millet leaves, 46 cm wide
PI 365848 C.P.I. 46977 PI 476398 'Irtykoe-201'	Earliest proso millets, matured grain on July 18, 61days after planting
PI 204918	Most of the heads on this foxtail millet are forked at the tip.

## Notable accessions in the 2018 Millet Observation Field

## Crop Germplasm Committee reports:

A written progress report was prepared for the New Crops, Crop Germplasm Committee.

## Service:

David served as the Crop Science Society of America, 2018 Genetic Resources (Division C08) Chair. The tasks in 2018 included choosing symposium topics, inviting speakers, scheduling secessions, and communicating with the Genetic Resources Section by email. Sessions in 2018 emphasized crop wild relatives, and landscape genomics. As Past Chair in 2019, David will organize a committee of people to select the outstanding genetic resources paper published in Crop Science, in 2018, and chair the committee for selecting the invited speaker for the 2019 Calvin Sperling lecture.

# **Outreach and Presentations:**

David was an invited Plenary speaker on the topic Grain Amaranth Developments at the start of the Primer Congreso Mundial del Amaranto in Cholula, Puebla Mexico, October 10 to 12, 2018. He also spoke at the 3<sup>rd</sup> International Millet Symposium, August 8 to 12, 2018 in Fort Collins, CO., entitled: Millet Genetic Resources from the North Central Regional Plant Introduction Station, Ames, Iowa.

David organized and lead a field trip, 3.5-mile hike, at the Soldiers Delight Natural Environment Area as part of the American Society of Agronomy meeting in Baltimore, MD. The area is a serpentine barren with large grasslands dominated by grass species that are also common in Iowa prairies, but include local endemic species such as an endemic chickweed that tolerates the thin and poor soil in the barrens. Historically the barrens were mined for chromium ore, and the old mine shafts are still present.



The male fertile amaranth stem above has open anthers on long filaments, the male sterile amaranth below has characteristic short filaments and greenish shrunken anthers.

#### Plans for 2019:

A cytoplasmic male sterile amaranth line (PI 686465) was developed by David at the NCRPIS and was made publicly available in early 2018. A publication was accepted by the Journal of Crop Registrations for publication in early 2019.

David, with Harold E. Brockelman, and Karen A. Williams wrote a book chapter on North American wild relatives of grain crops for 2019 publication in S.L. Greene et al. (eds.), North American Crop Wild Relatives, vol 2.

David plans to release a dwarf plant breeding line of *Amaranthus cruentus* (DB 2008910) via Iowa State University.

We will have an insect pollinated cage-field with 60 to 70 cages mostly to replace *Coriandrum* seed lots with reduced viability.

### Publications about our germplasm:

Andini, R. 2018. Breeding amaranth for enhanced nutritional values; emphasizing on protein content. In: Singh, S., R. Singh and N. Thakur, Antioxidant Properties of Vegetable Crops, Jaya Publishing House, Delhi, India. Pages 251–276.

Andini, R. 2018. Natural polyploidy in amaranths (*Amaranthus* spp.). AIP Conference Proceedings 2002, 020053. https://doi.org/10.1063/1.5050149.

**Brenner, D.M**. 2018. Grain amaranth developments: Optical sorting, geosmin flavor, and male sterility. In: Espitia-Rangel, E., D. Escobedo-Lopez, A. Meza-Hernandez, and P. Rivas-Valencia. 2018. De Mexico para el mundo; Memorias del primer congreso mundial del amaranto. October 10–12, 2018. Choula, Puebla, Mexico. Pages 441–442.

Chaluvadi, S. and Bennetzen J. 2018. Species-associated differences in the belowground microbiomes of wild and domesticated *Setaria*. Frontiers in Plant Science. 9:1183. doi:10.3389/fpls.2018.01183. Desai, J. S., E. Slabaugh, D.J. Liebelt, J.D. Fredenberg, B. N. Gray, J.S.V. Krishna, O. Wilkins, and C.J. Doherty. 2018. Neural net classification combined with movement analysis to evaluate *Setaria viridis* as a model system for time of day of anther appearance. Frontiers in Plant Science. 9:1585. doi:10.3389/fpls.2018.01585.

Esan, Y.O., O. Omoba, and V.N. Enujiugha. 2018. Biochemical and nutritional compositions of two accessions of *Amaranthus cruentus* seed flour. American Journal of Food Science and Technology. 6:145–150. doi: 10.12691/ajfst-6-4-3.

Hinojosa, L., J.A. González, F.H. Barrios-Masias, F. Fuentes, and K.M. Murphy. 2018. Quinoa Abiotic Stress Responses: A Review. Plants. 7:106. doi:10.3390/plants7040106.

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Luo, K., M.Z.Z. Jahufer, H. Zhao, R. Zhang, F. Wu, Z. Yan, J.Zhang, and Y.Wang. 2018. Genetic improvement of key agronomic traits in *Melilotus albus*. Crop Science. 58:285–294. doi:10.2135/cropsci2017.08.0495.

Murphy, B.P., P.J. Tranel. 2018. Identification and validation of Amaranthus speciesspecific SNPs within the ITS region: Applications in quantitative species identification. Crop Science. 58:304–311. doi:10.2135/cropsci2017.06.0359.

Myers, R. 2018. Amaranth: An Ancient grain and exceptionally nutritious food. Harvest Road Publishing, Columbia, MO. https://www.amazon.com/Amaranth-Ancient-Grain-Exceptionally-Nutritious-ebook/dp/B07FZFNFF7.

(This wonderful, popular book about the amaranth crop is very supportive to us in the NPGS.)

Odonkor, S., S. Choi, D. Chakraborty, L. Martinez-Bello, X. Wang, B. A. Bahri, M.I. Tenaillon, O. Panaud, K.M. Devos. 2018. QTL mapping combined with comparative analyses identified candidate genes for reduced shattering in *Setaria italica*. Frontiers in Plant Science. 9:918. doi:10.3389/fpls.2018.00918.

Smith, J.D., F.F. Dinssa, R.S. Anderson, F-C Su, and R. Srinivasan. 2018. Identification of major insect pests of *Amaranthus* spp. and germplasm screening for insect resistance in Tanzania. International Journal of Tropical Insect Science. 38:261–273. https://doi.org/10.1017/S1742758418000115.

Thapa, R., and M. Blair. 2018. Morphological assessment of cultivated and wildamaranthspeciesdiversity.Agronomy.8:272.http://dx.doi.org/10.3390/agronomy8110272.

Viljoen, E., D.A. Odeny, M.P.A. Coetzee, D.K. Berger, and D.J.G. Rees. 2018. Application of chloroplast phylogenomics to resolve species relationships within plant

genus *Amaranthus*. Journal of Molecular Evolution 86:216–239. https://doi.org/10.1007/s00239-018-9837-9.

Van Eck, J. 2018. The status of *Setaria viridis* transformation: agrobacteriummediated to floral dip. Frontiers in Plant Science. 9:652. doi:10.3389/fpls.2018.00652.

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Yang, J., S. Thames, N. B. Best, H. Jiang, P. Huang, B. P. Dilkes, A. L. Eveland. 2018. Brassinosteroids modulate meristem fate and differentiation of unique inflorescence morphology in *Setaria viridis*. The Plant Cell. 30:48–66. doi: 10.1105/tpc.17.00816.

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

The Horticulture project currently holds 3,772 accessions representing 186 genera (Table 1.0). Significant NC7-medicinal collections include: Actaea (44), Agastache (74), Echinacea (193), Calendula (83), Hypericum (224), Monarda (134), Prunella (53), and *Tanacetum* (53). Significant NC7-ornamentals collections include: Alcea (34), Malva (53), Phacelia (52), Potentilla (106), Sphaeralcea (90), and Thalictrum (52). Significant NC7-woody landscape collections include: Aronia (100), Betula (158), Cornus (201), Euonymus (59), Fraxinus (473), Gymnocladus (89), Rhus (80), Salix (53), Spiraea, (95), Staphylea (44), and Ulmus (44). Jeff Carstens serves as curator. Nickolis Ouellette departed from the horticulture technician position in July 2018 and the position is currently vacant. Targeted acquisitions were focused on collecting the newly described (2011) Monarda luteola (10) in Texas and Arkansas; collection of Sapindus saponaria (5) in Kansas and Oklahoma for the WLPGR; acquisition of Drymocallis arguta (8) via donations from the University of Northern Iowa Tallgrass Prairie Center (2) and the Chicago Botanic Garden Dixon National Tallgrass Prairie Seed Bank (3), targeted collections by Carstens (1) and the Iowa Department of Natural Resources (2). The first NPGS accession of diploid Ulmus americana (Ames 34187) and an accession of *Gymnocladus* (Ames 34173) from the extreme western edge of the species native range in Kansas were collected during the 2018 NCRPIS Sapindus collection trip. Ames 34173 also represents a new county record for the state of Kansas. The following species were deposited into the NPGS for the first time: Dirca palustris, Cunila origanoides and Salix serissima.

Management group	Genera	Accessions
NC7-medicinals	35	1053
NC7-ornamentals	54	738
NC7-woody landscape	97	1981
Total	186	3772

Table 1. Active accessions maintained in the NC7 horticulture collections (medicinals, ornamentals, and woody landscape) as of December 31, 2018

### Acquisitions:

During 2018, we acquired a total of 69 accessions including 38 medicinal, 8 herbaceous ornamental, and 23 woody landscape accessions to the horticulture collections. An additional 15 accessions were collected through the horticulture project and transferred to other NPGS genebanks. Significant contributions included *Sapindus* (5), *Cucurbita* (3) and *Baptisia* (2).

Collection trips were completed by Jeff Carstens, Andy Schmitz, Nick Ouellette and Cole Hopkins in Oklahoma and Kansas sampling *Sapindus* (5); and Carstens and Jared Trent sampling *Monarda luteola* (10) in Texas and Arkansas.

A significant contribution from other cooperators included *Betula fargesii* (1) from Michael Dosmann at the Arnold Arboretum, which is the first accession of this species deposited into the NPGS.

#### Maintenance:

#### Regeneration:

Existing plantings that mostly consisted of *Aronia*, *Cornus*, *Hypericum*, *Monarda*, *Spiraea* and *Staphylea* were harvested via controlled pollinations. A total of 44 accessions were harvested.

We attempted germination for future regeneration of 26 accessions focused on *Monarda* and *Spiraea* accessions.

A total of 25 accessions were transplanted to the field mostly focused on *Aronia*, *Echinacea*, *Euonymus*, *Monarda* and *Spiraea* accessions.

An accession of *Salix glauca* that was harvested in 2004 was pulled for regeneration and only six seedlings germinated. For a taxa that has historically been termed recalcitrant, this provides insight on seed longevity in -18C, especially if propagules are handled promptly.

#### Availability and Backup:

Currently, approximately 72% of the medicinals, 72% of the herbaceous ornamentals, and 53% of the woody landscape accessions are available.

Currently, approximately 76% of the medicinals, 77% of the ornamentals, and 46% of the woody landscape accessions are backed up at the National Center for Genetic Resources Preservation in Ft. Collins, Colorado.

The horticulture crew assumed the management of pollination screen repair on November 2018. The team utilizes a commercial sewing machine to repair torn seams and broken zippers. A total of circa 55 screens were repaired in 2018.

#### Viability Testing:

A total of 145 seed viability assessments were made for the horticulture collections including maintenance (24), increase (25) and original (96) seed samples. New in 2018, seaweed agar was used to help establish taxa (e.g. *Salix*) that are extremely small and difficult to handle. This new method resulted in approximately a 25% increase in survivability of newly germinated, seedling transplants.

### **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 143 external orders to 116 requestors totaling 662 items from 426 accessions. We cancelled 264 orders from 186 requestors representing 2,360 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. A total of 27 accessions of *Aronia* were rooted at NCRPIS and provided to the University of Illinois for a study to evaluate commercial potential and also provide elite lines for breeding. Leaf tissue of 51 accessions as of *Fraxius quadrangulata* was provided to the Arnold Arboretum for a genetic characterization study.

Таха	Most distributed (greatest to least)
Medicinals	Origanum
	Monarda
	Echinacea
	Agastache
	Actaea
Ornamentals	Potentilla
	Sphaeralcea
	Phacelia
	Malva
	Lythrum
Woody landscape	Fraxinus
	Aronia
	Rhus
	Ulmus
	Xanthoceras

Table 2. Taxa most distributed from the NC7 horticulture program in 2018

		No. of	No. of	No. of Items	No. of Accs				
Crop	Year	Orders	Recipients	Distributed	Distributed				
Medicinals*	2014	21	18	133	87				
	2015	39	39	218	174				
	2016	36	33	99	99				
	2017	53	44	387	233				
	2018	44	36	218	169				
	Average	39	34	211	152				
Ornamentals*	2014	41	40	186	160				
	2015	21	20	78	74				
	2016	33	30	72	61				
	2017	27	26	174	160				
	2018	46	40	117	93				
	Average	34	31	125	110				
Woody Landscape	2014	73	58	230	139				
	2015	95	66	335	191				
	2016	97	69	302	168				
	2017	71	56	367	146				
	2018	82	68	327	164				
	Average	84	63	312	162				

Table 3. External domestic and foreign germplasm distributions for theNCRPIS horticulture program during 2018

\* During 2015, all NC7-mints accessions were reassigned to either the NC7-medicinals or NC7ornamentals collections.

#### Characterization/taxonomy:

During 2018, five horticulture accessions (*Agastache, Alnus, Baptisia, Echinacea* (2)), were renamed based on morphological characteristics and/or ploidy analysis. No PI numbers were assigned.

#### **Evaluation:**

In 2018, observation data collected included capitulum diameters of *Monarda*, fruit diameters of *Aronia*, seed diameters of *Gymnocladus*, seed lengths and widths of *Fraxinus*, plant heights and widths of *Salix* and Omernik Level III Ecoregion classifications, totaling 172 observations loaded to GRIN.

A common garden study/evaluation plot of select  $Gymnocladus \ dioicus$  accessions was established in 2017. This evaluation plot (ongoing) includes 52 wild collected accessions from across the species native range with typically three mother trees from each accession, replicated five times totaling 720 trees. The main goal is to identify superior accessions of *G. dioicus* germplasm. *G. dioicus* has recently become one of the more popular, widely-planted, urban street trees.

We continue to capture observation data on nine *Betula nigra* accessions (145 trees), a common garden study hoping to identify elite lines for the Midwest. Data captured to date includes chlorophyll concentration, caliper, and tree height.

In 2018, we shipped samples of Quercus (5) and Salix (2) to the NLGRP in order to better understand optimum storage regimes by testing moisture and temperature variables.

A total of 533 items were attached in GRIN as either images (446) or as documents (87) including publications, collection trip reports, viability cards, permits and/or passport data.

#### **Enhancement:**

A second generation grow out of *Quercus prinoides* (Ames 23752) established in 2016 totaling approximately 150 seedlings continues to be maintained. Seedlings will be screened for mildew resistance and superior growth habit. A hybrid of *Quercus prinoides* x *Quercus macrocarpa* was identified, which is not known in the nursery trade.

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

In 2018, the horticulture project distributed 160 plants of five accessions to thirteen sites for long-term evaluation. An additional 36 plants were shipped to four sites. Accessions distributed included *Carpinus caroliniana*, *Carya glabra*, *Juglans microcarpa* and *Nyssa sylvatica*.

### Posters, Presentations, and Seminars:

In 2018, Jeff Carstens hosted a tour of the NCRPIS and gave a presentation on viability testing to the Iowa State University Horticulture (Plant Propagation) class to approximately 40 students and also the Iowa State University Agroforestry class to approximately 10 students. Carstens gave a presentation on *Gymnocladus* to the Woody Plant Cultivars Class (Horticulture 341) at Iowa State University and to the Missouri Department of Conservation Natural Heritage Program Annual Plant Workshop. Carstens co-authored with Andy Schmitz (The Brenton Arboretum) an article on *Gymnocladus* in the Arnoldia (Vol. 76, No. 1, pp. 2-16) and also a poster presented at the American Public Gardens Association for the Excellence in Plant Collections Management Symposium.

### **Conclusions and Plans for 2019:**

The 2018 growing season was generally productive in terms of overall regeneration of the horticulture collections. Progress continued in the acquisition and curation of *Monarda* germplasm. Future acquisitions will focus on sampling of western *Fraxinus* species, *Monarda* spp., Midwest *Aronia* and collaboration with Kevin Conrad of the National Arboretum to sample *Cladrastis kentukea*.

### Curation:

For 2019, we will attempt to obtain seed increases from 20 medicinal and ornamental accessions and from approximately 20 NC7-woody landscape accessions. Significant time will likely be spent reviewing plant inventories and removal of successfully increased accessions. A number of accessions could be assigned PI numbers and paperwork completed for those needing inactivation.

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

## **Personnel:**

The NCRPIS was given permission to hire a second maize curator at the end of FY'18 classified at the GS11-14 level. The position was advertised, candidate resumes were reviewed, and candidate interviews occurred in November. At the end of December 2018, the process to finalize hiring a candidate was well underway. This development has been in the works for a long time. The maize curatorial tech team is fully staffed with the hiring of Dr. Vivian Bernau, a new maize geneticist/curator in March 2019. 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. In addition to regular collection maintenance duties, David continues to develop, refine, and maintain computer applications specific to the maize project. Brady North, NCRPIS maize tech, travelled to our tropical maize contractor, Semillas y Agroproductos Moreno Retis S. de P. R. de R. L. located in Nayarit, Mexico near Puerto Vallarta in January and documented the status of the nursery and exchanged information on management techniques we use for unimproved landrace accessions by Ames staff. Brady has continued his regular interactions with the CIMMYT staff by visiting a CIMMYT location.

## **Research Progress:**

Pedigrees have been extracted from the Plant Variety Protection (PVP) certificates for all available expired PVPs. One of the bits of data in high demand by the maize user community is the pedigree of the expired PVP accessions. The data is available online in the PVP certificate, but it can be tedious to extract from those certificates and comparisons are difficult. Below is a screenshot of the data entry presentation in GRIN-Global. Currently, the description is now presented on the public website (the far-right column). This field is searched when doing a general GRIN wide query. In future versions of the public interface parents will be presented. This data will be maintained as PVPs expire.

Accession	Female Accession	External Female Accession	Male Accession	External Male Accession	Pedigree Description
PI 591840	PI 601404	LH60	PI 600955	LH51	LH60 x LH51
PI 601466		(Mo17 X H99)		LH53	(Mo17 X H99) X LH53
PI 601494		(Mo17 X LH18)		LH53	(Mo17 X LH18) X LH53
PI 601317		(Mo17 X H99)		LH53	(Mo17 X H99) X LH53
PI 596306	PI 539920	LH160		LH59	LH160 X LH59
PI 593037		(LH85 X LH61)	PI 601416	LH61	(LH85 X LH61) X LH82
PI 564543	PI 601317	LH57	PI 601170	LH82	LH57 X LH82

After much trial and error, balancing time of acquisition and the highest resolution practical, the maize project set the image resolution on ear scans at 600 dpi. The scanners will do 1200 dpi, but on the large format scanners (OpticPro A320) being used, this can take 15 minutes or more per scan. Previously the scans were done at 400 dpi. Average archival tiff files are now 100-200 MB in size. Resulting medium compressed tiffs are 1-3 MB in size for web posting. Previously tiffs were 20-40 MB and web jpgs .25-.5 MB.

The GRIN-Global Curator Tool Attachment (Image) Wizard was put to good use in 2018. Over 4,000 images on over 800 accessions were loaded to GRIN in 2018. This increased the maize images on GRIN from 8,500 to over 12,500 images.

It was learned that another taxa of *Zea* can be regenerated using nodal cuttings. The maize crew had to resort to using this technique in the spring of 2018 after an accession of *Zea mays subsp. huehuetenangensis* failed to be induced to flower during the winter of 2017-2018 except for one plant. Most of the plants were growing into the greenhouse roof. When it became warm enough to move the plants outside, nodal cuttings of 0.5 to 1.0 meter in length were taken high up in the canopy of tiller tops and placed in five-gallon buckets of water. After a week or so, secondary roots will emerge from above the cut node or on previously formed secondary root buds. This technique has been used on *Zea nicaraguensis* and *Zea diploperennis*.

#### Acquisition:

We received 33 new accessions in 2018. These included 27 expired PVPs, four inbreds and two populations.

#### **Regeneration:**

A large nursery funded by ARS was harvested in Mexico and a second nursery was planted in 2018. It represents the first time the NCRPIS has contracted for tropical maize regenerations in a foreign country. Four thousand rows were planted in the fall of 2017 to regenerate 250 mid-altitude to lowland accessions from all over the tropics by Semillas y Agroproductos Moreno Retis S. de P. R. de R. L., Nayarit, Mexico. The biggest challenge presented by this material resulted from wind events that blew down many accessions. They were able to manually stand some accessions upright as we do here in Ames. Several goosenecked on their own. By the time Brady visited much of the nursery was manageable for pollinations. A few higher altitude accessions as well as many non-Mexican races were included to gauge adaptability. It is clear certain things cannot be grown there. The quality of the resulting ears from most of the nursery was excellent. Ears were shipped back for imaging and description.



Figure 1

Quarantine maize was grown in a NCRPIS winter greenhouse for the first time.



Figure 2 Quarantine Greenhouse

We received 14 inbreds and two populations under APHIS quarantine permit from Dr. Sansern Jampatong at Kasetsart University, Thailand. Pathologist Dr. Charles

Block at Iowa State University oversees the growout. These can only be increased in an inspected greenhouse room that meets quarantine requirements during the winter months in Ames. St. Croix's APHIS permit does not allow for growing maize from SE Asia. Our campus greenhouse presents some challenges, but some seed was obtained from most accessions. An unexplained situation occurred where a population KS23(S)C5 pictured below (right)was successful, but an accession representing an earlier cycle of selection KS23(S)C3 growing directly next to it failed. Below is an image of the successful population increase. There were the same number of plants germinating for both accessions, but as time progressed plants slowly died on KS23(S)C3. Pollen viability of these varieties is quite poor in the winter greenhouse. Seed of the successful accession increases was immediately passed on to the GEM team for shade house short-day growing.

At the end of November 2018, those that were unsuccessful in the first quarantine nursery were planted for the winter of 2018-19 season. Eight inbreds and one population were tried again. Several of the eight inbreds had a handful of seeds from the first increase but were grown again to confirm that enough seed was obtained before destruction of the original seed which is required by the quarantine permit.



Figure 3 Greenhouse Grown Suwan Population

The NCRPIS is trying yet another location to grow highland Andean maize. CIMMYT is growing 25 accessions from Peru at their Toluca location under USDA contract. On the right is an image of PI 572029 (Apurimac 29) representing the race Morocho collected at 3200 meters growing in Toluca. All accessions had or were flowering at the time of this image in August 2018.



Figure 4 PI 572029 (Apurimac 29)

A summary of the 2018 Zea program increases follows and includes the highlights mentioned above. The Zea program had 352 regeneration attempts in 2018. (1.7% of the collection). This compares with 338 regeneration attempts in 2017 (1.6% of the collection), 442 regeneration attempts in 2016 (2.1% of the collection), and 555 (2.7%) in 2015.

1. Regeneration numbers were maintained in 2018. Temporary help hiring for Ames during the summer pollination period continues to be difficult to obtain but improved over 2017. Twenty percent of the planned nursery was not planted to accommodate the deficit. These resources were channeled into tropical nurseries.

The Ames summer nursery was maintained at 172 accessions in 2018. This compares to 120 in 2017, 267 in 2016, 259 in 2015 (2,128 vs. 1,726 vs. 3,110 vs. 2,674 25-foot rows). The nursery was composed of 52 expired or soon to be expired PVPs, 30 BGEM inbreds, 11 other inbreds, nine GEM populations, and 17 other populations. Nursery size was reduced due to the uncertainty of the budget until late in the spring and then difficulty hiring temporary labor in the summer.

Four planting dates occurred on May 7, May 14, May 18, and May 25, 2018 to spread out the pollination season and to allow time for nursery size to be determined as hiring was completed. An early June planting was planned but cancelled. After an irrigation line was laid in early summer, no irrigation was required. After an easy peak, the pace rapidly relaxed as later maturities were well distributed through the latter half of the season. Harvest followed the same non-pressured cycle assisted by a long fall that we have experienced the last few years. Excessive rainy days after mid harvest did cause more ear rot deterioration than in recent years and a rush to get the last rows in before a heavy freeze. This summer's regeneration is still rated as above average.

No Stewart's wilt was observed in any increase plots in 2018, as in every year between 2010-2018. ELISA testing is still necessary on Ames increase lots to meet phytosanitary requirements because the state cannot be declared Stewart's wilt free.

- 2. Eight of the Thailand accessions passing quarantine were grown by the GEM team in their new short-day induction shade house during the summer of 2018. They make breeding crosses with the pollen and self-pollinate the stand for increase satisfying the needs of both programs. Despite being planted late, yield was good.
- 3. Four GEM lines were regenerated by the Ames GEM team in 2018 for the maize collection.
- 4. Five GEMs were regenerated by the Raleigh GEM team in 2018 for the maize collection.
- 5. Ames greenhouse increases consisted of six population accessions planted at the end of 2017 and harvested in 2018. Populations consisting of one *Coix*, two *Tripsacum*, and one *Zea mays subsp. huehuetenangensis* (teosinte) were planted in the fall of 2017 for a spring 2018 harvest. The teosinte is the only accession of this subspecies in the collection. As mentioned above, this teosinte failed to flower and was taken outside until fall when it was brought into the greenhouse and flowered during the fall of 2018.
- 6. Quarantine maize was grown in a NCRPIS winter greenhouse for the first time during the winter of 2017-18 (See above). We received 14 inbreds and 2 populations under APHIS quarantine permit from Dr. Sansern Jampatong at Kasetsart University, Thailand. Pathologist Dr. Charles Block at Iowa State University oversees the growout. These can only be increased in an inspected greenhouse room that meets quarantine requirements during the winter months in Ames. St. Croix's APHIS permit does not allow for growing maize from SE Asia. Success was mixed during the first growout. We succeeded with one population and 10 inbred lines. Six of these inbreds were grown again starting in the fall of 2018 for the 2018-19 season. The permit only allows for winter increases.
- 7. A large nursery funded by ARS was received from Mexico in 2018. This is the first time the NCRPIS has contracted directly for tropical maize regenerations in a foreign country. Four thousand rows were planted in the fall to regenerate 250 mid-altitude to lowland accessions from all over the tropics by Semillas y Agroproductos Moreno Retis S. de P. R. de R. L., Nayarit, Mexico (Moreno Retis).
- 8. A smaller tropical nursery consisting of 125 accessions was sent to Moreno Retis in Nayarit Mexico in the fall of 2018 for early 2019 harvest. Accessions were chosen based on successful increases from the above nursery.
- 9. St. Croix grew 21 accessions in 2018. They are currently growing tropical inbreds and populations. At present, APHIS will not issue a permit to grow quarantine maize in the field, only in a greenhouse. Dr. Goenaga is working with others in ARS to try to remedy this situation.
- 10. As mentioned above under highlights, **CIMMYT grew 25 Peruvian highland accessions** in Toluca, Mexico under USDA-ARS contract.

### Maintenance:

There were 20,996 accessions of *Zea* held at the NCRPIS as of December 31, 2018. This compares with 20,952 in 2017, 20,819 in 2016, and 20,744 in 2015. GEMs and expiring PVPs made up most of the additions. The maize curator maintains an additional 101 accessions from the *Coix* and *Tripsacum* genera.

There were 14,915 There were available Zea accessions held at the end of 2018 (71% of the total). 14,718 available Zea accessions held at the end of 2017 (70% of the total). This compares with 14,718, at the end of 2017, 14,376 (69%) in 2016, and 14,144 (68%) in 2015. 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 past in kind regeneration assistance of Monsanto, DuPont Pioneer, and GEM programs at North Carolina and Iowa. Company consolidations have put a damper on in-kind support recently, but after a period of stability it is hoped that more in-kind support can be arranged. Having enough funding to contract tropical nurseries is also critical to future progress.

Yearly Accession Availability								
Year	<b>Total Accessions</b>	Available Accessions	% Available	New Accessions				
2009	20,166	12,239	65.70%	105				
2010	20,347	13,338	65.60%	178				
2011	20,540	13,572	66.10%	180				
2012	20,579	13,753	66.80%	39				
2013	20,624	13,757	66.70%	39				
2014	20,694	13,876	67.70%	98				
2015	20,744	14,144	68.20%	53				
2016	20,819	14,144	69.10%	110				
2017	20,952	14,718	70.25%	105				
2018	20,996	14,915	71.04%	33				

The table above indicates that Zea accession availability continues to improve though the collection continues to grow and accessions become unavailable. Efforts in recent years have been focused on increasing inbreds and expired PVPs to meet demand.



Figure 5. Maize Collection Holdings and Availability Statistics, December 31, 2018

There were 1,888 accessions tested for viability in 2018 (9% of the collection); 1,177 maize accessions tested in 2017 (6% of the collection). Most accessions are tested on a 10-year plan. Recently we have found some elite inbred accessions that appear to deteriorate faster and will be put on a 5-year plan after regeneration. In 2018 we began doing indicator tests of two reps of 10 seeds on older lots of unavailable accessions with few seeds or accessions that have not been germinated in over 10 years. The idea is to find those accessions that have the poorest viability to determine regeneration priorities and overplanting needs. A benefit of this particular Mexican provider for tropical regenerations is that they can overplant population. Some past contracted nursery providers and in-kind growers could not overplant and thin because of the type of mechanical planters they use. We plan to continue doing a statistically sound full germination test of 4 reps of 50 seeds on new increases. It is hoped that the indicator tests will help us catch up on the 10-year cycle.

Two hundred-two accessions were backed up at the NLGRP in Fort Collins in 2018. This compares with 97 in 2017, 56 in 2016, and 101 in 2015. The percent of the collection backed up held at 73%. Currently, NLGRP needs are not the highest priority for regenerating, but are considered. Since there is a large backlog of regenerations needed, viability of the Ames inventory and availability are the more important priority setting factors. At storage of a new increase, NLGRP holdings are reviewed for each accession and seed is sent to NLGRP if their backup is considered substandard.

All GEM and BGEM accessions with temporary Ames numbers in the collection were assigned permanent PI numbers in 2017. Since the SOP for GEM is that all GEM releases are moved to the collection after two years, it is planned that at transfer there

will be a PI number on the accession for the first distributions from the maize collection. In 2018 the new GEM accessions entering the collection received PI numbers immediately

## **Distribution:**

Orders for all accessions that are maintained by the maize curator including those of the genera *Tripsacum* and *Coix* increased **107%** in **2018** compared to a 7.2% increase in 2017, a 15.7% increase in 2016, a decrease of 12.4% in 2015. A good share of the increase was due to four orders over 1,000 accessions. Insect and pathogen resistant screening and phenotyping/genotyping were reasons given. 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. Targeted requests for fewer accessions continue to comprise the bulk of the orders. Handling this number of packets would be very difficult without GRIN-Global and the hard work of Ms. Stacey Estrada, Ms. Lisa Burke and their teams in order processing and seed storage.

Annual Distribution Data									
Year	Total Packets	Foreign Packets	Total Accs	Foreign Accs	Orders	Foreign Orders	Requestors	Foreign Requestors	
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	
2017	12,541	2,095	4,137	1,042	655	99	451	73	
2018	25,993	10,846	8,833	4,834	671	131	456	107	
Averages 2014-18	13,773	3,608	4,056	1,848	630	100	482	87	

Orders for expired PVPs were sent to 177 requestors (39% of all *Zea* requestors). Expired PVPs made up some portion of 44% of all Zea orders shipped.

### **Characterization:**

There were 16,173 data points on 1,241 accessions loaded into GRIN in 2018. This compares with 11,324 data points on 1,279 accessions in 2017, 9,715 data points on 1,718 accessions loaded in 2016 and 11,791 data points on 346 accession in 2015. We imaged 565 accessions in 2018 compared with 499 accessions in 2017, 527 accessions in 2016 and 420 in 2015. Image loading to GRIN included 4,070 images on 812 accessions in 2018. Currently there are 12,799 images on 5,868 accession in GRIN.

# **Evaluation:**

Two disease-screening nurseries were distributed in 2018. Dr. Marc Mancl screened 106 accessions for fusarium resistance and 124 accessions for head smut resistance at Woodland California. Many thanks are extended to DuPont/Pioneer Hi-Bred (now Corteva Agriscience) for this long-term, continuing contribution. ARS pathology technician Narinder Pal observed all nurseries in Ames for disease occurrence during the growing season for phytosanitary purposes.

#### Plans for 2019:

The biggest change for 2019 will be bringing onboard a second maize curator. It is expected that much progress will be made in accession documentation, deactivation, and permanent number assignment.

In 2019 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 to Ames for distribution and management.

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

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 2019. GRIN-Global should greatly assist in this PI assignment project.

We will continue acquiring germplasm from public collections.

We will augment the collection of images currently on GRIN of 5,000 accessions with images of additional accessions in 2019. The GRIN-Global image loader (Attachment Wizard) will be used.

We will be converting the last of old maize increase slide images to digital format in 2019.

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

### **Project management:**

Curator Dr. Laura Marek was assisted by full time Agronomy Department staff, Grace Welke, ISU Agronomy Assistant Scientist III. John Reinhardt, ISU Farm Equipment Operator works with the NCRPIS Oilseed Project November through March. The project is also supported by a team of hourly student workers. The ARS Agricultural Research Technician position attached to the project, vacant since late March 2016 was refilled in December 2018 with the hiring of Mr. Jeff Schwartz. Mr. Schwartz has prior experience in the seed industry. As a result of the staffing shortage during 2018, regenerations were scaled back and simplified, decreasing the number of genera attempted and resulting in more uniformity in regeneration protocols and other management concerns.

#### Acquisitions:

The oilseed project received 63 new accessions in 2018.

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

Fifty-six cultivated *H. annuus* pre-breeding lines were received from Dr. Gerald Seiler, USDA botanist, Sunflower and Plant Biology Unit, Fargo, ND. The lines were developed by Dr. C.C. Jan when he was the cytogeneticist in the Fargo group. Dr. Jan retired in January 2017. The majority of the lines (54) have introgressions from 21 different wild annual (5) or perennial (49) *Helianthus* species. Two of the lines are selections from cultivated *H. annuus* crosses.

Six wild perennial *H. maximiliani* sunflower accessions, which filled geographic gaps in the species representation in North Dakota and Minnesota, were received from Dr. Jarrad Prasifka, USDA Sunflower and Plant Biology Unit, Fargo, ND.

One wild annual sunflower, *H. exilis*, collected east of Healdsberg, CA was received from Dr. Tom Gulya, retired USDA plant pathologist.

#### **Collection Maintenance:**

General statistics about availability and management of the collections are presented in Appendix Tables 1 and 2. Selected details for oil seed accessions increased during 2018 are noted below.

#### Helianthus, Ames regenerations:

Cultivated *H. annuus* accessions are 70% available for distribution. The collection includes two specialized subsets, an association mapping population of 288 lines, UGA-SAM1, and a set of 360 pre-breeding lines developed in Canada, GB\_UBC. These 648 accessions are only available with special curator permission until regeneration lots from the 2018 growing season are processed and stored. We expect 95% of the subset lines to become freely available during 2019 which will restore cultivated *H. annuus* collection availability to 90-95%. We manage regenerations to ensure that core collection accessions and other accession subsets of interest to specific stakeholder groups are available.

In 2018, 234 cultivated *H. annuus* accessions were regenerated in the field including 184 of the Canadian pre-breeding lines. The pre-breeding lines were planted in two, side-by-side 20 ft rows (148) with heads bagged or in screened cages (26) to control pollination. An additional 50 cultivated accessions were caged and screened including 10 UGA-SAM1 accessions. Seed was harvested from all of the cultivated sunflower plots and processing is under way.

Wild annual *Helianthus* accessions are 96% available and wild perennial accessions are 84% available. Two new wild annual and three new wild perennial sunflower regenerations were started in Ames in 2018. One previously established perennial plot from 2017 was re-caged. Seeds were harvested from all populations and processing is underway.

Typically, two or three cultivated sunflower accessions requiring long seasons and/or short days to flower are increased in an NCRPIS greenhouse during the winter. In winter 2017-2018, we grew accessions of wild species for the first time, one annual and one perennial. The annual accession did very well; the perennial species did not

flower. For winter of 2018-2019 we are growing one accession of the same wild annual species that did not do well in 2017-2018 and one cultivated accession. One accession of the wild annual species H. *debilis ssp. debilis* is maintained clonally in the greenhouse and distributed as vegetative clones.

#### Miscellaneous asters:

The miscellaneous asters are 35% available. No miscellaneous asters were grown at the NCRPIS in 2018. Four asters were sent to our alternate grow-out site, see section below.

Helianthus and Miscellaneous asters, Parlier alternate grow-out site regenerations:

We continue to partner with National Arid Lands Plant Genetic Resources Unit (NALPGRU), Parlier, CA to regenerate wild taxa requiring a longer growing season than is 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 cages purchased by the NCRPIS and can grow up to 40 NCRPIS oilseed accessions per year. We germinate seeds in Ames and ship live seedlings to Parlier in late March and early April. Parlier staff transplant the seedlings and manage plant growth. Following our Ames protocol, plots are caged before flowering, pollinator insects are introduced (Ames funds the pollinator services), and seed heads are harvested as they mature. Harvested material is shipped to Ames for threshing and processing.

In 2018, seedlings for 34 wild sunflower accessions and four miscellaneous asters were shipped to Parlier. Heads were harvested from one of the miscellaneous asters (*Centrapalus pauciflorus* ssp. *mutomoensis*) and from all of the wild sunflower accessions although two of the perennials did not yield much seed and are being maintained into 2019. Plants for two accessions of *Centrapalus pauciflorus* ssp *australis* did not start to flower until December and the plants froze before they produced mature seed. Plants of one accession of *Linzia glabra* did not produce seed, but the plants were not killed by frost and are being maintained into 2019. Seeds did not germinate for three accessions: one each wild perennial sunflower, wild annual sunflower, and wild miscellaneous aster. These accessions will be inactivated.



**Figure 1.** Regeneration cage, Parlier, CA, *Helianthus radula*, Ames 33055. *H. radula* is native to the longleaf pine wiregrass ecosystem in the far southern U.S. and does not flower in Ames. This species does not produce ray flowers; occasional plants in some populations produce rudimentary rays as shown in this image.

The Parlier staff record basic field data (transplant, flowering and harvest dates and takes some images) but they do not have the resources to record standard descriptor data such as ray and disc flower color, plant height, and branching characteristics nor to take all images. Phenotypic information is a valuable component associated with each accession and it is important to capture the observation data. In September 2018, Ms. Welke and I traveled to Parlier to record descriptor information and to take images.

We have an excellent partnership with the NALPGRU staff, ensuring successful regenerations of many taxa. We are most grateful for the dedicated efforts of Dr. Claire Heinitz, Curator, and Mr. Jerry Serimian, Agricultural Research technician, and their staff.

#### Brassicaceae regenerations:

Brassicaceae accessions are 83% available. Availability is lower than in past years because 2016 and 2017 viability maintenance testing of distribution lots of *Brassica* accessions determined a larger than expected decrease in viability (some lots fell by as much as 80% in the six or 10 year interval since prior testing) resulting in 205 accessions of *B. napus* and *B. rapa* changing from status "Available" to "Low germination". This is unfortunate because the average seed quantity on hand for these accessions is almost 100,000.

The majority of the 205 accessions with low germination distribution lots are *B. napus* (96%). Approximately 80% of the NCRPIS *B. napus* collection is of winter flowering

type, meaning that regeneration requires a vernalization period to induce flowering. If accessions are winter hardy in Ames, vernalization can take place in the field after an early fall planting. However, only 20% of the 40 winter flowering type accessions we have attempted to vernalize in the field (9% of the winter flowering type *B. napus* collection) have survived the winter to flower and produce seed in the following spring. Therefore, we start winter type *Brassica* accessions in the greenhouse and vernalize them in a NCRPIS vernalization space. In December 2017, we started 75 winter type *Brassica* accessions which were kept in the greenhouse until late February 2018 and then moved to vernalize in the NCRPIS "cave". The vernalized plants were transplanted to the field in early May by which time a number of accessions were already bolting. Due to weather delays, the plots could not be caged for controlled pollination for about two weeks requiring that flowers be cut from many plots. Possibly due to contact with soil resulting from the trimming and splash up from rain, about 20% of the plots appeared to suffer from black rot or other stem rot with the result that we could not harvest seeds from 17 of the plots.

NCRPIS Greenhouse #2 (GH-2) is managed in the winter to provide conditions that approximate a Mediterranean climate allowing us to regenerate Brassicaceae accessions native to that region, and to grow 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 focused on making all accessions of this species available. *T. arvense* flowers very early in the season in Iowa and is present in all NCRPIS farm fields as a weed. To obtain reasonable regenerations and to ensure the genetic integrity of each accession, we increase *T. arvense* in GH-2. We grew *T. arvense* (10), *Crambe laevigata* (1), and *Isatis boissieriana* (1) in the 2017-2018 winter greenhouse. As soon as the 2018-produced seeds are stored, all viable accessions of *T. arvense* will be available for distribution. In fall 2018, we started *Camelina* spp (6), *Lepidium sativum* (2) and *T. arvense* (2) in FGH-2 for late spring 2019 harvest. Two *Camelina* accessions donated without specific epithet were also started for the purpose of taxonomic clarification.

#### *Linum* regenerations:

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 NCRPIS viability lab has determined that seed viability has started to decline for some distribution lots and maintenance germination efforts have been increased. Based on the data, we are regenerating 10 to 15 accessions of cultivated flax every year, with priority given to accessions with distribution lots having viability scores less than 50% with the long-term goal that all distribution lots have viability scores of at least 70%. In 2018, 14 cultivated flax accessions whose distribution lots ranged from 53 to 59% viability were successfully regenerated. Maintenance germination tests in 2018 identified seven cultivated flax accessions with distribution lot viability scores of less than 50% and 20 had viability scores between 50% and 70%. These accessions will be the focus of 2019 regenerations efforts. About 1000 flax accessions are scheduled for maintenance testing in 2019 to inform future regeneration. No wild flax accessions were attempted in 2018.

## *Cuphea* regenerations:

No *Cuphea* regenerations were attempted in 2018. 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 agronomic development efforts by members of the National *Cuphea* Consortium. Thirteen accessions of *Cuphea* are maintained as clones in the greenhouse and distributed as vegetative cuttings. Over all, the *Cuphea* collection is 80% available.

## Euphorbia regenerations:

The Euphorbia collection is 49% available. No Euphorbia accessions were grown in 2018. The taxon within this genus of greatest interest for seed oil production is *E. lagascae* and accessions of this species are 89% available. *E. lagascae* grows reasonably well during an average Iowa growing season; we were growing five to six accessions annually with a goal of having all accessions available within five years. Euphorbia seed pods shatter as they mature making this genus more difficult to manage than other NCRPIS oilseeds; therefore, we did not grow Euphorbia lagascae in 2018. Six Euphorbia accessions are maintained as clones in the greenhouse and distributed as vegetative cuttings.

## **Distributions:**

General statistics about oil seed distributions are presented in Appendix Table 3. A summary of the distributions separated by international versus domestic distributions is presented in Table 1.

					-				
	2018		international	international	international	domestic	domestic	domestic	total
crop	orders	packets	orders	packets	requestors	orders	packets	requesters	requesters
Helianthus	130	11006	58	7730	44	72	3276	57	101
Brassicaceae	145	5530	55	2633	46	90	2897	68	120
Linum	30	387	7	68	6	22	319	17	23
Cuphea	8	17	0	0	0	8	17	7	7
Euphorbia	9	27	2	5	2	7	22	7	9
misc Asters	23	57	6	13	6	17	44	15	22

# Table 1: Oilseed Crops 2018 Distributions

# <u>Helianthus</u>:

Roughly 49% of the total *Helianthus* items distributed in 2018 were sent to seed companies or other commercial entities, 90% of which were sent to international destinations, reflecting the current locations of the majority of commercial sunflower breeding programs. About 22% of the total distributed items were sent for UGA-SAM1 and GB\_UBC pre-breeding line evaluations (2369 packets) focused on drought, salt, and flooding tolerance in sunflower as well as disease resistance. The remaining 25% of the 2018 sunflower distributions went to programs associated with public institutions, primarily universities. Seventy-five percent of the distributed packets were cultivated *H. annuus*, 15% were wild annual sunflowers including wild *H. annuus* and 10% were wild perennial sunflowers.

#### Brassicaceae:

The genus *Brassica* accounted for 70% of the 2018 distributions (3889 packets, 1617 unique accessions). The Brassicaceae genus with the second largest number of packets distributed was *Eruca*: 769 packets, 14% of the total. The largest individual distribution, 1555 packets encompassing more than 90% of the available accessions in the genus *Brassica*, was sent to Europe for disease screening and genetic research. The two next largest orders contained 1260 items (23% of the 2018 distributions) and were sent to U. S. research programs investigating plant responses to metal toxicity. The diversity present in the Brassicaceae collection (262 taxa in 21 genera) supports a wide range of research purposes.

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

Forty-seven percent of the distributed flax packets were cultivated flax accessions. As declared by the requesters, half of the orders, representing 85% of the packets, were sent to support genetic studies and varietal development.

### Cuphea:

The majority (13 accessions, 76%) of the 2018 *Cuphea* distributions were sent for botanical taxonomic investigations.

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

*Euphorbia* accessions were distributed in 2018 for a range of research purposes including both ornamental and agronomic varietal development and chemical analysis.

### Miscellaneous asters:

Seventy percent of the miscellaneous asters distributed in 2018 were part of larger requests involving other NCRPIS projects. Stated research purposes included botanic and taxonomic investigations and anthropological research (35%), genetic studies and varietal development (30%), and chemical studies (17%).

### **Research Activities:**

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

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

<u>SAM evaluations/Pre-breeding lines</u>: We cooperate with the NSF and Genome Canada projects which involve field and greenhouse evaluations using the UGA-SAM1 association mapping population and the GB\_UBC developed pre-breeding lines. The projects are managed by Dr. John Burke and Dr. Lisa Donovan, UGA, USA and Dr. Loren Rieseberg, UBC, Canada. We provided seeds for evaluations in drought environments in southern California and Uganda, a salt environment in central Canada and a nutrient stress environment in Israel as well as greenhouse and laboratory based studies at UGA and UBC. Additional international cooperators are located in Chile, India and Argentina. <u>Pre-breeding Lines</u>: Loren Rieseberg's lab at the University of British Columbia developed the GB\_UBC pre-breeding lines with support from The Global Crop Diversity Trust. The Crop Trust also provided funding for an international evaluation effort of these lines, "Evaluation of Sunflower Pre-Bred Lines for Stress Resistance and Associated Trade-off with Yield", managed by the University of British Columbia group with partners in Chile, Argentina, Africa, India, Israel and Ames. We contribute to the partnership by providing seeds for the international locations and recording descriptor data during the regeneration process. Funding was received in 2017 for two years; a no-cost extension will be requested to spend any remaining funds.

Brassicaceae:

Brassica rapa flowering type evaluation:



**Figure 2.** Brassica rapa accessions growing for assessment of winter or spring flowering type. Seedlings are not vernalized and those which do not bolt are presumed winter types

We are in the process of evaluating the 544 accession *B. rapa* collection for flowering type (winter or spring). Knowing whether or not vernalization is required to obtain flowering (winter type) allows more efficient management of regeneration efforts. Spring flowering types can be direct seeded in the field in the spring, a much less resource intensive process than growing seedlings in the greenhouse, transferring to and maintaining plants in a vernalization location, followed by transplanting to the field. In 2017-2018, we evaluated a set of 80 accessions of which 80% were spring type (flowered fully without vernalization). In November 2018 we started another set of 80 accessions (Figure 2). Observation priority was determined based on viability data; lowest viability accessions were evaluated first.

<u>Brassica napus</u> winter survival of winter flowering types: In fall 2018, 15 Brassica napus and two *B. rapa* winter type accessions previously determined to have some winter hardiness in Ames (successful vernalization and regeneration by overwintering in the field) were direct seeded in a common garden plot to confirm their winter hardiness. There is general interest in more winter hardy Brassica both to improve the yield for canola production and to improve the performance of Brassica used as a winter cover crop.

# **Professional Activities:**

Meetings and Presentations:

<u>January</u>: I attended the Plant and Animal Genome XXIV Conference in San Diego and the one-day satellite meeting of the Genome Canada and NSF sunflower group. I made a short presentation about regenerations, seed stocks and seed order processes for the UGA-SAM1 association mapping population which both groups are using for trait evaluations. In addition, I participated in a meeting with the Crop Trust's sunflower GB\_UBC pre-bred lines evaluation group.

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

June: I attended the NPGS PGOC meeting at the Carver Center in Beltsville, MD.

<u>August</u>: I participated in the NCRPIS RTAC meeting in Ames, IA and made a short presentation about the status of the Oilseed Project.

<u>September</u>: I attended the AAIC meeting in London, Ontario, Canada and provided a report to the New Crops Crop Germplasm Committee on oilseed crops of interest held at the NCRPIS: Brassicaceae, *Euphorbia*, *Cuphea* and the miscellaneous asters genus *Centrapalus* ssp.

<u>November</u>: I attended the CSSA and ASA annual meeting in Baltimore, MD. As past chairperson of the C08 (plant genetic resources) Division of the Crop Science Society of America, I was responsible for organizing the Calvin Sperling Lectureship as well as leading the committee responsible for selecting the C08 outstanding plant genetic resources paper published in Crop Science in 2017.

Training:

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

### **Publications:**

Peer reviewed journal articles:

Mathew F, Olson T, Marek L, Gulya T, and Markell S. 2018. Identification of sunflower (*Helianthus annuus*) accessions resistant to *Diaporthe helianthi and Diaporthe gulyae*. Plant Health Progress 2018. 19:97-102. doi.org/10.1094/PHP-10-17-0060-RS.
Okello P, Petrovic K. Kontz B, Ali S, Marek L, Mathew F. 2018. Root rot caused by species of *Fusarium* on *Brassica carinata* in South Dakota. Plant Health Progress 2018. 19:188-192. doi.org/10.1094/PHP-04-15-0012-RS.

#### Book chapters:

Marek LF. 2018. "Practicalities of Collecting Wild Plants in North America: Insights from the United States". In: "North American Crop Wild Relatives, Volume 1. Conservation Strategies" SL Greene, Williams KA, Khoury CK, Kantar MB, and Marek LF, editors. Cham Switzerland, Springer, pp 229-244.

#### Posters:

Marek LF and Welke G. 2018. Winter survival in a subset of *Brassica napus* and *Brassica rapa* PIs in Ames, IA. Crop Science Society Meeting, Baltimore, MD November 4-7.

Marek LF. 2018. Sunflower: A North American native crop and its wild relatives. Crop Science Society Meeting, Baltimore, MD November 7.

#### **Active Grants:**

#### <u>Crop trust</u>:

In 2017 the Oilseeds project received a \$25,000 grant from the Global Crop Diversity Trust administered through the University of British Columbia to support regenerations of the Canadian developed GB\_UBC sunflower pre-bred lines received in 2016. The grant also provides partial support of my travel to the project's annual meeting at the Plant and Animal Genome meeting in San Diego, CA in January.

#### Collecting:

I serve as the PI for a USDA Plant Exchange Office funded Plant Exploration Grant for David Brenner to collect wild relatives of amaranth, millets, bedding plants, corn and quinoa in Florida

#### **Service Activities:**

#### Journal peer review:

I served as a peer reviewer for submissions to Industrial Crops and Products (2) and the Journal of Plant Registrations (1).

#### Book editor:

I served as one of five editors for a two-volume book about crop wild relatives being published by Springer. The first volume, North American Crop Wild Relatives, Volume 1. Conservation Strategies, was published in December 2018.

#### Plant Germplasm Operating Committee (PGOC):

I serve as a member of the PGOC GIS and Geo-referencing Subcommittee and the Molecular Subcommittee.

#### NCRPIS Committee:

I severed on the search committee for the Oilseeds Agricultural Research Technician.

## CSSA:

As immediate past chair of the C08 division (Plant Genetic Resources) of the Crop Science Society of America, my responsibilities for 2018 were to organize the Calvin Sperling Lectureship and to lead the committee responsible for selecting the Outstanding Paper(s). There were 43 papers in the award pool, a higher than average number due to the June 2017 publication of a special issue featuring Crop Wild Relatives, and three papers were selected as a result.

CSSA along with ASA and SSSA, featured Crop Wild Relatives for one of their weeklong blogs in September 2018. I provided the sunflower entry for the blog.

## 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", but are also summarized specifically for the Vegetable Project in the table below.

										Percent	
Site Crop		Number			Percent	PI	Ames	NSL		Backed	
(Maintenance	Number	Accs	Number	Percent	Avail Last	Numbered	Numbered	Numbered		up	
Policy)	Accs	Acquired	Available	Available	Year	Accs	Accs	Accs	Backup	NCG &	Svalbard
NC7-chicory	284	5	244	86	76	230	28	26	258	91	114
NC7-cucumis.cucs	1398	10	1327	95	95	1230	143	25	1331	95	985
NC7-cucumis.melo	3216	2	1964	61	61	2906	279	27	2597	81	551
NC7-cucumis.wilds	318	0	203	64	63	245	73	0	208	68	51
NC7-cucurbita	979	0	728	74	75	884	90	5	824	84	298
NC7-daucus	1484	0	1223	82	80	968	486	30	1322	89	940
NC7-ocimum	106	0	99	93	93	93	13	0	100	94	76
NC7-parsnips	73	0	58	79	77	52	19	2	58	68	33
Totals	7858	17	5846	74	74	6608	1131	115	6698	84	3048

## Acquisition:

Five retired *Cichorium endivia* cultivars were donated to the NPGS by Nunhems Netherlands, BV. Ten *Cucumis sativus* and two *Cucumis melo* cultivars from NLGRP were received for incorporation into the NPGS collection due to interest generated from work by members of the Cucurbit Coordinated Agricultural Project (CucCAP). The NLGRP also shared seeds of an expired *Ocimum basilicum* PVP, and seeds of a PI-numbered *Daucus muricatus* accession that had never been included in the NCRPIS collection at Ames.

## Maintenance:

Data for vegetable crop regenerations attempted and number of accessions harvested in 2018 are summarized in the appendices in "Table 2: NCRPIS Accessions (Accs) Germinated, Regenerated, Made Available, Backed Up." Eighteen accessions of *Cichorium* were planted for regeneration in 2018 due to low viability and/or seed quantity. The harvests have been threshed and are awaiting blowing and hand picking. Preliminary assessment of seed quantities appears good, quality be determined viability testing is completed.

Cucumis increases primarily focused on newly received accessions (two C. melo, 10 C. sativus) from the NLGRP, and also greenhouse and field regenerations of 34 unavailable wild Cucumis species accessions. One of four C. melo accessions failed to germinate, one produced an insufficient quantity of seed in a greenhouse cage, and two regenerated in field cages produced large quantities of fruit/seed and will be made available for distribution after viability testing in April 2019. All eleven C. sativus accessions regenerated produced good seed quantities and will be available for distribution after viability testing. Greenhouse regenerations were attempted on seventeen wild Cucumis species. Three accessions failed to germinate, and vines of two accessions that failed to set fruit in the greenhouse were trimmed and transplanted to summer field cages resulting in successful increases. The remaining twelve accessions produced fruit in the greenhouse cages, seven had sufficient seed quantities to make available for distribution and for backup at NLGRP. A 2019 greenhouse regeneration will be attempted on the accessions which failed to germinate or did not produce sufficient seed quantities in 2018.

*Cucurbita pepo* field regenerations focused on accessions with low seed quantities or distribution lots 20+ years old. Twenty-eight of 35 accessions were successfully regenerated in field cages or greenhouses, one failed to germinate, two had no fruit at harvest (though insect pollinators were introduced when male and female flowers were present), two had low fruit/seed production, and two need additional increase for backup at NLGRP. One accession with low fruit production had positive ELISA for squash mosaic virus (SqMV) prior to transplanting to the field cage. Many of these seedlings were destroyed which contributed to the low fruit production. We will again attempt to regenerate this accession in 2019 using a dry-heat treatment on the parent seed lot to eradicate the virus prior to planting. The two accessions regenerated via hand pollination in the NCRPIS greenhouses were late-maturing accessions that could not be grown in the field All 2018 increase lots will be inventoried and stored after viability testing in April 2019.

NCRPIS *Daucus* regeneration efforts focused primarily on Ames-numbered wild, annual species (from Morocco, Spain, Tunisia) and on old PI-numbered accessions having lower seed quantities. Fifty-seven annual accessions were planted, and seeds were harvested from 53 accessions. Two accessions failed to germinate, and one had an insufficient population, another died in the field cage due to wet conditions. Processing the 2018 harvests is still ongoing. In addition to the Ames, IA *Daucus* 2018 regenerations, we received seed increases of six accessions from Sage McClintick-Friddle, Seminis Vegetable Seeds (Monsanto), Idaho and six accessions from Rob Maxwell, Bejo Seeds, Idaho.

The one *Pastinaca* accession regenerated in 2018 was a population of plants resulting from seeds that germinated after the 2017 vernalization treatment. The non-bolted plants were dug from the field cage, held in the greenhouse over the winter,

vernalized, and transplanted to a field cage in 2018. The resulting 2018 increase was to be bulked with the 2017 increase, but most plants in the population were slow to bolt and flower resulting in little seed set before frost. The regeneration was not successful.

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 the future 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. Backup samples of 124 accessions from the Vegetable Project were sent to NLGRP in 2018, including five "retired" *Cichorium endivia* cultivars donated to the NPGS by Nunhems, The Netherlands. 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).

### **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 2018, 9788 items (packets) involving 4871 accessions were distributed to fulfill 169 orders (124 domestic, 45 foreign) equaling 130 recipients. Vegetable research requests received in 2018 specified objective topics as disease evaluations, breeding for specific traits and disease resistances, genetic and molecular studies, and diversity assessment for biotic and abiotic stress tolerance. The set of 691 *Daucus* accessions was distributed four times in 2018 for Dr. Philipp Simon's (USDA-ARS) Carrot SCRI (Specialty Crops Research Initiative), and 3673 cucurbit accessions were distributed to an international seed company. Requests for approximately 1700 items were cancelled for international orders because we were not able to satisfy import requirements and the requestors were not able to secure waivers for the additional declarations on the import permits.

Non-Research Requests (NRR), i.e., home gardener requests, continue to make up a significant portion of the Vegetable Project requests with over 6400 items not being distributed.

#### **Germinations:**

In 2018, 328 vegetable inventory lots were tested for viability (Appendix Table 2), with the majority of the testing attributed to germinations of new increase lots and initial viabilities on original seed lots for the new *Daucus* germplasm collected in Spain 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 2018 will be loaded to GRIN-Global. Images

are taken to document plant, leaf, flower, fruit, or root characteristics. These data and images will be made available via GRIN-Global.

Taxonomic identities are reviewed and confirmed as each accession is regenerated. The 2018 taxonomic changes included nine accessions reidentified to two *Cucumis melo*, four *C. metuliferus*, and three *C. zambinaus* accessions previously identified as *Cucumis spp*.

### **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 of Cucurbita has been conducted since 1994 and Cucumis since 1999. 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* and *Cucurbita* germplasm to some countries. The Vegetable Project is working with Dr. Narinder Pal (NCRPIS Pathology Project), Dr. Charles Block (Iowa State University, Seed Science Center), and new ARS plant pathologist Dr. Anna Testen, 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 section of this NCRPIS annual report for more details on our field and greenhouse experiments using streptomycin and dry heat seed treatments in 2018.

David Spooner (USDA, ARS, Madison, WI) is wrapping up work on the taxonomic revision of the genus *Daucus* and allied species as he prepares to retire. There are still pending publications as well as labeling and disposition of herbarium specimens collected during the project. The specimens will be deposited in the University of Wisconsin herbarium in Madison.

The *Daucus* SCRI Project (initiated in 2017) lead by Dr. Philipp Simon (USDA-ARS) has a goal to use 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. Thus far, the ~700 accession set of cultivated wild germplasm, has been distributed as nine separate orders to participants in the SCRI. The set is being evaluated in Wisconsin and California fields for flowering time, stand establishment, top size, nematode and *Alternaria* resistance, flavor, root nutritional pigments, cavity spot, and root appearance (shape, external and internal color). Images of typical roots are also being collected.

The SCRI and the Crop Trust projects are developing breeding pools by intercrossing plants with superior performance for traits mentioned above. They are also genotyping phenotyped plants for both projects and developing a database.

The CucCAP Project (started in 2016) 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). It was reported that all genotype by sequencing (GBS) has been completed for the NPGS cucumber, melon and watermelon collections during the third annual meeting of the CucCAP in Raleigh, NC April 4-5, 2018. The GBS of the NPGS Cucurbita collections will be completed within a month. Core collections of  $\sim 300$  PIs and a mix of  $\sim 100$ breeding lines, cultivars, and heirloom varieties will be established to best capture the genetic variability as determined by the GBS. These cores will be grown for resequencing and self-pollination to create enhanced lines but will not be made available through the NPGS. Phenotypic characterization of the cores is also planned but may have to be pursued under CucCAP II. The project's website, https://cuccap.org/, posts a list of publications resulting from the research, and provides access to cucurbit genomics tools and databases via the Cucurbit Genomics Database website. All phenotypic 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.

#### **Publications/Posters:**

No new publications at this time.

#### Plans for 2019:

#### **<u>Regenerations</u>**:

The 2019 vegetable regenerations will include approximately 70 *Daucus* (annual, biennial field and greenhouse increases), and as yet an undetermined number of cucurbits in the summer field cages. Regenerations of wild *Cucumis* species and hard-to-handle *Cucumis* will continue in the greenhouse as time, space, and other resources permit.

The Vegetable Project will continue to collaborate with the Plant Pathology project researching new regeneration protocols for the cucurbit collections to produce pathogen free seed lots, primarily regarding the *Acidovorax citrulli* pathogen in the *Cucumis melo* collection.

### Characterization:

Image loading to GRIN "Classic" was suspended in 2013 in preparation for the launch of the new GRIN-Global database. With the development of the new Inventory Attachment Wizard for GRIN-Global, the Vegetable Project is able to load the backlog of images during the summer of 2019 including: images acquired as part of the regeneration process from 2013 to the present, images from the 2013 and 2017 *Daucus* observation plantings, 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 for 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 (GBS) 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 with the completion of the projects.

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

## Administration and Leadership Activities:

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. New five-year project plans for the NC-7 Project and both of the ARS CRIS projects were approved in 2017.

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 serves on the advisory group for the Daucus SCRI Project. She served as leader of the review team for the USDA-ARS Soybean Genebank Review in Urbana, IL in March 2018, and also on a selection panel for a new soybean Cat 4 geneticist/curator for the Urbana soybean genebank. About 10% of her time was devoted to assisting GRIN-Global System development team members, 15% to the GEM Project, 5% to activities related to implementation of the new optical sorting technology, and about 70% to genebank issues and writing in the past year.

## 2019 Plans:

With the purchase of the QSorter from the Swiss company, QualySense with USDA-ARS Midwest Area and HQ support, we have a unique instrument capable of capturing 3D images and NIR spectra from seeds, and sorting seeds based on calibrations developed for specific traits or size/color parameters. QualySense has enabled the instrument research-read and we will now focus on collecting image and NIRS data in preparation for algorithm development activity. The VMEK Metrix is a fast, visual optical sorter than has enabled us to optimize maize seedlots that will result in much better emergence and remove contaminants from a variety of crop seeds. We will be experimenting with a variety of other crop seeds to determine if we can eliminate much manual seed picking prior to storage.

As a result of a two-year collaboration with CIMMYT and INIFAP co-authors, a chapter on the status of the wild relatives of maize was published (cited below).

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

## **Publications:**

Cruz-Cardenas, C.I., Cortes-Cruz, M., Gardner, C.A., and Costitch, D.E. 2018. Wild Relatives of Maize. IN: North American Crop Wild Relatives, Volume 2: Important Species. Editors: S. Greene, K. Williams, C. Khoury, M.B. Kantar, and L. Marek. Springer International Publishing. doi: 10.1007/978-3-319-97121-6

Vanous, A., Gardner, C.A., Blanco, M., Martin-Schwarze, A., Lipka, A.E., Flint Garcia, S.A., Bohn, M., Edwards, J.W., Lübberstedt, T. 2018. Association mapping of flowering and height traits in Germplasm Enhancement of Maize doubled haploid (GEM-DH) lines. The Plant Genome. doi:10.3835/plantgenome2017.09.0083.



Year 2018	Table 1.		NCRPIS Ac	cossions (A	ees) Acquir	od Availah	lo
01/01/2018 to	0 12/31/2018		NUMP 15 AC	cessions (A	ces), Acqui	eu, Avanab	ie
CURATOR	GENUS_CROP	Number Accs	Number Accs Acquired	Percent Acquired	Number Available	Percent Available	Percent Avail Last Year
Brenner	NC7-amaranth	3334	1	0%	3230	97%	96%
	NC7-celosia	59	0	0%	38	64%	64%
	NC7-echinochloa	311	1	0%	278	89%	89%
	NC7-grasses	138	0	0%	82	59%	65%
	NC7-legumes	299	1	0%	159	53%	53%
	NC7-melilotus	1006	2	0%	892	89%	89%
	NC7-panicum	936	0	0%	906	97%	97%
	NC7-perilla	25	0	0%	22	88%	88%
	NC7-portulaca	12	0	0%	9	75%	75%
	NC7-quinoa	438	6	1%	292	67%	70%
	NC7-setaria	1111	3	0%	1006	91%	92%
	NC7-spinach	413	0	0%	353	85%	95%
	NC7-umbels	1190	1	0%	755	63%	63%
	Total:	9272	15	0%	8022	87%	87%
Carstens	NC7-medicinals	1053	53	5%	760	72%	71%
	NC7-ornamentals	738	17	2%	537	73%	73%
	NC7-woody landscape	1981	36	2%	1062	54%	52%
	Total:	3772	106	3%	2359	63%	61%
Marok	NC7-asters	447	15	3%	155	35%	33%
Matek	NC7 brossies	9019	10	0%	1697	910/s	03%
	NC7 brassica pup	2013	1	0%	1007	0470	95%
	NC7 amugifors	1999	19	10/	1150	070	0%
	NC7 emusifere pup	1202	15	170	1150	90%	90%
	NC7 suppos	629	0	0%	510	800/	0% 80%
	NC7 cupherbic	910	0	0%	102	400%	400%
	NC7 flox	210	1	0%	102	49%	100%
	NC7 flox wilds	160	12	070 80/	192	77%	80%
	NC7 sup cults	2210	56	070 20/	120	76%	70%
	NC7 sup culta SAM	2319	50	270	1700	1070	19%
	NC7 sun wilds ann	1677	19	10/	1616	170 060/	0%
	NC7-sun wilds por	1077	12	170	759	9070	90%
	NC7 sun wilds on	097	9	170	100	0470	00/
	Total	19775	190	10/	10682	Q 40/	070 860/
Millord	NG7 som hin	12773	120	1 70	10003	04%	00%
Millard	NC7-corn.kin	101	0	0%	1	1%	9%
	NC7-maize.gems	316	0	0%	301	95%	77%
	NC7-maize.inb	2622	4	0%	2174	83%	85%
	NC7-maize.pop	17129	2	0%	11904	69%	68%
	NC7-maize.pvp	490	27	6%	462	94%	95%
	NC7-maize.wilds	439	0	0%	74	17%	18%
	NC7-zea.totals	20996	33	0%	14915	71%	70%
	Total:	21097	33	0%	14922	71%	70%
Reitsma	NC7-chicory	284	5	2%	244	86%	77%
	NC7-cucumis.cucs	1398	10	1%	1329	95%	95%
	NC7-cucumis.melo	3216	2	0%	1970	61%	62%
	NC7-cucumis.wilds	318	0	0%	204	64%	64%
	NC7-cucurbita	979	0	0%	738	75%	75%
	NC7-daucus	1484	1	0%	1228	83%	80%
	NC7-ocimum	106	1	1%	99	93%	93%
	NC7-parsnips	73	0	0%	58	79%	77%
	Total:	7858	19	0%	5870	75%	74%
NCRPIS To	tal:	54774	293	1%	41856	76%	76%

Year 2018	Table 2.				NCRI	PIS Accessic	ons (Accs) Ge	rminated, Re	generated,	Made Availa	ble, Backed	1 Up			
CURATOR	GENUS CROP	Number Accs	Number Accs Germed	Percent Accs Germed	Number Attempted Regen	Number Harvested Regen	Number Perm Perennial	Number Perennial Harvested (Vegetative)	Number Accs Growing	Number Accs Made Available	Number Accs Backed Up at NLGRP for YR	Number Accs Backed Up at Svalbard for YR	Number Accs Backed Up at Other Locations for YR	Total Number Accs Backed Up	Percent Accs Backed Up
Brenner	NC7-amaranth	3334	226	7%	69	28	0	0	0	110	96	0	0	3260	98%
	NC7-celosia	59	9	10%	0	0	0	0	0	0	0	0	0	42	71%
	NC7-echinochloa	311	14	5%	2	2	0	0	0	2	2	0	0	275	88%
	NC7-grasses	138	27	20%	8	1	0	0	0	0	0	0	0	06	65%
	NC7-legumes	299	0	%0	0	3	0	0	0	0	ũ	0	0	217	73%
	NC7-melilotus	1006	10	1%	0	2	0	0	0	1	2	0	0	929	92%
	NC7-panicum	936	305	33%	20	4	0	0	0	0	0	0	0	916	98%
	NC7-perilla	25	90 G	32%	4	4	0	00	0	0	0	0	0 0	24	96% 00%
	NC7 minos	120	7 0	706	00	97					0 9			294	740%
	NC7-setaria	1111	120	11%	208	17	0		0		0	0	0	972	87%
	NC7-spinach	413	0	0%0	16		0	0	0	0	0	0	0	399	97%
	NC7-umbels	1190	22	2%	24	24	0	0	0	19	14	0	0	780	66%
	Total:	9272	748	8%	430	185	0	0	0	140	125	0	0	8238	89%
Carstens	NC7-medicinals	1053	81	8%	6	48	0	0	0	61	39	0	0	794	75%
	NC7-ornamentals	738	5	1%	0	33	0	0	0	6	5	0	0	564	76%
	NC7-woody landscape	1981	40	2%	18	58	20	9	0	99	40	0	0	884	45%
	Total:	3772	126	3%	27	109	20	9	0	136	84	0	0	2242	59%
Marek	NC7-asters	447	ũ	1%	0	0	0	0	0	12	1	0	0	168	38%
	NC7-brassica	2013	111	6%	0	57	0	0	0	23	19	0	0	1984	99% - 2007
	NC7-brassica.pvp	1909	000	0%0 907	0 [	0				0				91147	000%
	NC7-crucifers num	1 2071	000	%0	1T	OT				01				1411	100%
	NC7-cuphea	638	0	0%0	0	0	0	0	0	0	0	0	0	583	91%
	NC7-euphorbia	210	0	%0	0	0	0	0	0	0	0	0	0	66	47%
	NC7-flax	2834	214	8%	14	14	0	0	0	14	14	0	0	2832	100%
	NC7-flax.wilds	160	2	1%	0	0	0	0	0	6	0	0	0	130	81%
	NC7-sun.cults	2319	558	24%	222	209	0	0	0	34	30	0	0	1837	79%
	NC7 and milds and	202	90	707 0/6T	IO	י מ				0	0 10			1500	0.402
	NC7-sun.wilds.per	897	57	6%9	0	-	0			27	14	0	0	748	83%
	NC7-sun.wilds.sp	2	0	%0	0	0	0	0	0	0	0	0	0	0	%0
	Total:	12775	1139	%6	257	301	0	0	1	171	110	0	0	11115	87%
Millard	NC7-corn.kin	101	15	15%	1	2	0	0	0	0	0	0	0	12	12%
	NC7-maize.gems	316	77	24%	10	14	0	0	0	64	151	0	0	218	%69
	NC7-maize.inb	2622	484	18%	110	106	0	0	0	29	2	143	0	1570	60%
	NC7-maize.pop	17129	1243	%1	187	287	0	0	0	322	88	0	0	13157	77%
	NC7-maize.pvp	490	69	14%	44	45	0	0	0	51	31	157	0	468	96%
	NC7-maize.wilds	439	0	%0	0	[	0	0	0	0	0	0	0	44	10%
	NU7-zea.totals	20996	1873	9%	351	453	0	0	0	466	272	300	0	15457	74%
Roitema	NC7-chicom	18017	19	18%	18	18				51 51	414 71	000		976 976	87%
Metrema	NC7-cilicory	1 308	10	20% 10/0	11	11				10	10			1334	01/0
	NC7-cucumis melo	3916	15	0%0	66	11				9	TF C			5604	81%
	NC7-cucumis wilds	318	2 m	1%	15	19				6	- 0			2002	65%
	NC7-cucurbita	979	13	1%	31	34	0	0	0	14	6	0	0	823	84%
	NC7-daucus	1484	157	11%	75	60	0	0	0	58	72	0	0	1321	89%
	NC7-ocimum	106	ũ	5%	0	0	0	0	0	4	2	0	0	100	94%
	NC7-parsnips	73	19	26%	7	1	0	0	0	13	6	0	0	58	79%
III DIG GOA	TOURI	0001	309	4%	RIT	104	00			0.61	100	000	2	5400 5600	0/02
NCRPIS TG	otal:	54774	4210	8%	1245	1204	20	9	-	1109	617	300	0	43758	80%

Year 2018	Table 3.		Exter	nal NCRPIS	Distributio	ns - Includes ho	th DI (resear	ch and educa	tion). RP (Ren	atriation). OB	(Observation)	and NR (hom	ie garden) ord	er tynes
01/01/2018 t	0 12/31/2018		5	C I Domo	Lin District			Ponoion D	The second second		Touris I		L Foundation D:	atui huti au a
CURATOR	GENIIS 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	NC7-amaranth	3334	919	594	68	49	698	505	19	17	1617	868	87	66
	NC7-celosia	59	10	10	9	9	ŝ	2	5	2	13	11	8	8
	NC7-echinochloa	311	31	22	10	6	33	27	5	5	64	37	15	14
	NC7-grasses	138	9	9	4	4	5	5	2	2	11	10	9	9
	NC7-legumes	299	13	12	δ I	0	0	1 00	0	000	18	12	12	12
	NC7-melilotus	1006	28	27	2	9	x I	7	21	21	36	34	6	x ;
	NC7-panicum	936	76	72	9	5	367	256	6	6	443	278	15	14
	NC7-perilla	25	36	21	9	0	9	9	0	1	42	21		9
	NC7-portulaca	12	6	2 2	× 9	80 00	0	0000	0	000	6	5	× 1	× 5
	NC7-quinoa	438	360	1/2	43	33	1911	269	31	77	1001	162	14	19
	NC7-setaria	1111	398	284	12	24	349 556	204	12	II	147	416 204	39	30
	NC7-imbels	1190	329	971	33	2.8	65	55	0 9	9 9	394	303	68	34
	Total:	9272	4974	1893	190	140	3286	1775	85	74	8260	2680	275	214
Carstens	NC7-medicinals	1053	202	160	41	33	16	16	00 0	0	218	169	44	36
	NC7-ornamentals	738	111	92	41	35	9	9	5	5	117	93	46	40
	NC7-woody landscape	1981	320	158	78	65	7	7	4	ŝ	327	164	82	68
	Total:	3772	633	410	135	109	29	29	œ	7	662	426	143	116
Marek	NC7-asters	447	43	40	17	15	10	6	က	co	53	47	20	18
	NC7-brassica.pvp	9	0	0	0	0	0	0	0	0	0	0	0	0
	NC7-brassica	2013	2210	1165	57	40	1805	1583	29	25	4015	1633	86	65
	NC7-crucifers	1282	735	459	43	37	724	361	24	21	1459	510	67	58
	NC7-crucifers.pvp	1	0	0	0	0	0	0	0	0	0	0	0	0
	NC7-cuphea	638	17	17	xo -	2	0 1	0 1	0 0	0 0	17	17	x0 «	2
	NC7-euphorbia	210	9	9	4	ro 9	0	5	21	21 -	11	6	9	5
	NC7-flax	2834	112	107	11	10	- ,	- 0		- 0	113	108	12	11
	NC7-flax.wilds	160	178	97	13	10	13	6	4	ro ;	191	102	17	13
	NC7-sun.cults	2319	2145	1137	45	34	4784	2141	45	39	6929	2159	60	73
	NC7-sun.cults.SAM	682	348	282	7 00	2 00	1.60	Lar 282	4	4	949	2020	0 1	0
	NC7-sun wilds ner	268	930	262	20 96	93	831	635	0	L CT	0201	674	100	30
	NC7-sun.wilds.sp	2	0	0	0	0	0	0	0	. 0	0	0	0	0
	Total:	12775	6438	3808	186	139	9983	5789	108	89	16421	6419	294	228
Millard	NC7-corn.kin	101	18	8	12	12	1	1	1	1	19	8	13	13
	NC7-maize.gems	316	316	165	45	35	431	301	15	13	747	302	60	48
	NC7-maize.inb	2622	4319	1266	275	204	3830	2193	80	73	8149	2221	355	277
	NC7-maize.pop	17129	6299	4848	165	142	2192	1803	42	38	8491	5761	207	180
	NC7-maize.pvp	490	3919	462	228	125	4176	462	64	52	8095	462	292	177
	NC7-maize.wilds	439	275	62	46	42	217	74	16	15	492	19	62	57
	NUT-zea.totals	20996	15128	6820	534	345	10846	4833	130	901	25974	8825	664	451
Roitemo	NC7 abioout	16012	15140	130	040 7	349	10547 915	4034 913	151	10/	20993	5533 918	b 1/9	490 0
NILLON DATE	NC7-cucumis.cucs	1398	817	722	22	17	1768	1087	7 E	1 0.	2585	1275		26
	NC7-cucumis.melo	3216	685	538	37	31	2195	1998	20	17	2880	2020	57	48
	NC7-cucumis.wilds	318	46	41	8	8	214	204	õ	5	260	204	13	13
	NC7-cucurbita	979	163	124	33	29	48	48	8	8	211	163	41	37
	NC7-daucus	1484	2644	784	26	18	328	305	11	11	2972	878	37	29
	NC7-ocimum	106	341	66	22	20	156	98	4	4	497	66	26	24
	NC7-parsnips	73	8	L	101	00	1090	8 1000	- 1	1	0766	14	4	4
MCD DIG TO	10val.	1000	0005	15921	1055	40 108	4004	1066	950	000	0018 01197	T 1 0 <del>1</del>	P171	1000
NUKEIS IG	)tal:	94/14	14026	10304	TUDD	001	11067	10330	909	707	47TT0	67797	1414	TUUT

Year 2018	Table 4.				~	NCRPIS Acce	essions (Accs) (	bservations	(Obs) in GRIN	I, Images in	1 GRIN			
1 01/7/10/10	0102/16/21													
CURATOR	GENUS_CROP	Number Accs in Collection	Number of Accs Obs Trials	Number of Obs in GRIN for Year	Number of Accs with 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 Imaged	Number of Accs with Images in GRIN for Year	Number of Images in GRIN for Year	Number Acc Images in GRIN (all years)	Number of Images in GRIN (all years)
Brenner	NC7-amaranth	3334	7	5891	446	31	31	54820	3328	0	0	0	741	1166
	NC7-celosia	59	0	0	0	0	0	164	56	0	0	0	16	39
	NC7-echinochloa	311	1	0	0	9	2	1168	306	0	0	0	64	130
	NC7-grasses	138	0	0	0	0	0	290	113	0	0	0	21	23
	NC7-legumes	299	0	0	0	0	0	547	244	0	0	0	29	41
	NC7-melilotus	1006	0	0	0	0	0	7235	966	0	0	0	190	244
	NC7-panicum	936	99	0	0	629	206	4230	935	0	0	0	125	240
	NC7-perilla	25	0	0	0	0	0	86	25	0	0	0	10	17
	NC7-portulaca	12	0	0	0	0	0	10	4	0	0	0	2	6
	NC7-quinoa	438	0	196	96	0	0	1457	375	0	0	0	149	203
	NC7-setaria	1111	133	1	1	40	12	4668	1078	0	0	0	156	309
	NC7-spinach	413	0	0	1	29	5.	8039	403	0	0	0	17	49
	NC7-umbels	1190	0	0	0	1	1	6145	1146	0	77	4	200	343
	Total:	9272	207	0609 0	544	736	257	88859	6006	0	2	4	1720	2810
Carstens	NC7-medicinals	1053	0	20 2	10	0	0	11976	457	0	137	290	509	1105
	NC7-ornamentals	738	0	0	0	0	0	152	101	0 0	45	67	141	220
	NC7-woody landscape	1981		143	58	1.07	41	4891	845	0 0	128	419	8/3	2694
,	Total:	3772	0	163	68	707	41	17019	1403	0	310	776	1523	4019
Marek	NC7-asters	447	0	0	0	0	0	8	1	0	0	0	0	0
	NC7-brassica	2013	89	0	0	0	0	40507	1996	0 0	0	0	332	922
	NC7-brassica.pvp	9	0 -	0	0	0	0	0	0	0 0		0	0	0
	NC7 ownoifows with	12021				000	0	116/	1				670	0
	NC7 ambao	1 698						101	1 979				12	0
	NC7-eupliea	910						4200	017				0 0	04
	NC7-flax	2834			0	0	0	1717	285			0	1	
	NC7-flax.wilds	160	0	0	0	0	0	852	82	0		0	2	2
	NC7-sun.cults	2319	2	0	0	0	0	104316	1826	0	1	4	254	- 666
	NC7-sun.cults.SAM	289	0	0	0	0	0	0	0	0	0	0	0	0
	NC7-sun.wilds.ann	1677	0	0	0	0	0	40118	1307	0	0	0	64	120
	NC7-sun.wilds.per	897	0	0	0	0	0	13850	630	0	0	0	124	329
	NC7-sun.wilds.sp	2	0	0	0	0	0	0	0	0	0	0	0	0
	Total:	12775	92	0	0	386	79	212949	7292	0	1	4	1119	2872
Millard	NC7-corn.kin	101	0	0	0	0	0	0	0	1	0	0	7	7
	NC7-maize.gems	316	33	876	57	1115	49	8475	350	55	30	187	133	599
	NC7-maize.inb	2622	35	3842	255	1291	269	84486	2492	82	19	60	601	1173
	NC7-maize.pop	17129	119	9119	503	7230	845	186053	13326	381	500	1687	4635	8151
	NC7-maize.pvp	490	94	2336	426	1688	011	18681	490	47	263	2136	385	2754
	NC7-maize.wilds	439	0	0	0	0	0 1970	331	200	0 202	010	0	101	10700
	NULI-zea.totats	20330	182	16173	1941	11324	12/3	291942	16658	200	819 819	4070	1000	19790
Reitsma	NC7-chicory	284	0	0	0	0	0	4700	279	4	0	0	257	913
	NC7-cucumis.cucs	1398	0	0	0	0	0	26149	1377	11	0	0	920	1231
	NC7-cucumis.melo	3216	25	0	0	0	0	12286	3196	16	4	12	653	1086
	NC7-cucumis.wilds	318	0	0	0	0	0	680	286	12	0	0	75	110
	NC7-cucurbita	979	ŝ	0	0	0	0	5667	970	34	0	0	150	326
	NC7-daucus	1484	0	0	0	0	0	19505	1361	49	0	0	681	3153
	NC7-ocimum	106	0	0	0	0	0	635	98	0	0	0	13	17
	NC7-parsnips	73	0	0	0	0	0	153	17	0	0	0	1 0770	1 0000
	T OTAL:	0021	202	n	0	0	<b>N</b>	01.1.69	0001	120	4	12	2/00	1650
NURPIS TC	otal:	54774	613	22426	1853	13153	1656	686544	42200	692	1129	4866	12980	29337

# **Appendix Figure 1**

