

NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION







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NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION NC7 ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2022

I. **PROJECT TITLE:**

NC7 "Conservation, Management, Enhancement and Utilization of Plant Genetic Resources"

II. COOPERATING AGENCIES AND PRINCIPAL LEADERS (current):

A. <u>Administrative Advisor</u>

* C. Lawrence-Dill, Associate Dean, Iowa State University, CALS

B. <u>Regional Coordinator</u>

*D. Peters, USDA-ARS, Iowa

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

Voting members:

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

Non-voting participants:

or o			
13. California-Davis	E. Forrestel	28. Michigan	A. Thompson
14. California-Davis	R. Karban	29. Missouri	S. Flint-Garcia
15. Connecticut	M. Brand	30. New Jersey	S. Handel
16. Hawaii	G. Presting	31. New York	J. Doyle
17. Illinois	J. Juvick	32. New York	M. Gore
18. Illinois	G. Kling	33. New York	M. Smith
19. Illinois	D. Lee	34. Oregon	A. Liston
20. Indiana	D. Wang	35. Oklahoma	E. LoPresti
21. Iowa	K. Lamkey	36. South Dakota	L. Xu
22. Kansas	A. Fritz	37. South Carolina	W. Park
23. Kansas	M. Jugulam	38. Texas	D. Baltensperger
24. Kansas	R. Lollato	39. Texas	N. Subramanian
25. Kansas	W. Schapaugh	40. Wisconsin	H. Kaeppler
26. Kansas	M. Jugulam	41. Wisconsin	S. Kaeppler
27. Michigan	R. Grumet	42. Wisconsin	N. de Leon

1. ARS National Program Staff, Plant Germplasm	*P. Bretting
2. ARS Plant Exchange Office	*G. Kinard
3. ARS Area Director, Midwest Area	*A. Pantoja
4. Cooperative State Research, Education and Extension Service	
5. National Center for Agric. Util. Research	*S. Cermack
6. National Institute of Food and Agriculture	*A. Stapleton
7. National Laboratory for Genetic Resources Preservation	*S. Greene

D. 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 (January 2022 – December 2022) Now Hiros:

<u>New Hires</u>:

• Skye Bradley, USDA-ARS Agri. Science Research Tech. (GEM), November 2022

Vacant USDA-ARS Positions:

- Agri. Science Research Tech (Plant Pathology)
- Agri. Science Research Tech (Entomology)
- Secretary Office Automation

Vacant ISU Positions:

• ISU Agri. Specialist II (Maize Curation)

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

Management of Federal and ISU Student Temporary Employees:

USDA-ARS resources were to provide for 19 student full-time equivalent (FTE) parttime temporary positions in FY 2022, primarily via the Research Support Agreement with Iowa State University (ISU). However, due to pandemic-associated restrictions, field operations were scaled back and only 16 student FTE were hired due to continuing pandemic restrictions. The temporary positions support curatorial activities including regeneration, seed processing, viability testing, farm and facilities operations, IT support, and the Germplasm Enhancement of Maize (GEM) Project. Students were interviewed and selected by ISU Assistant Director of Research Administration, Fred Engstrom. Marci Bushman, Plant Introduction Research Unit (PIRU) Program Support Assistant, managed the administrative aspects of all student hires, with support and guidance from Administrative Officer Candice Weuve and Administrative Support Assistant, Orlando Guzman.

Budget:

The Agricultural Experiment Stations (AES) of the North Central Region (NCR) authorized a 9.5% increase in their Hatch funds annual support which becomes effective in 2023. The Hatch fund budget had stood at \$522,980 for the past 20 years. These funds support the salaries of eight ISU staff members, their professional travel, and some supply expenses. In addition, the ISU Agricultural Experiment Station provides support valued at over \$609,000 annually which supports infrastructure, administration, and benefits for current NCRPIS-ISU staff members and retirees.

We are grateful Hatch funding resources will be increased. The authorized increase will ensure continued stability, which is critical to NCRPIS operations, now and in the future. Prior to the increase, about 95% of Hatch NC7 funds were devoted to the wages and salaries of the eight permanent ISU employees. This will be reduced to 87% which will provide essential reserves needed to accommodate future ISU wage increases without further limiting professional advancement, professional meeting travel, technical training, and temporary student hiring.

Fiscal year (FY) 2022 USDA-ARS funding of the PI CRIS remained essentially the same as final FY2014 funding (2.38M net to location), minus a one percent assessment for big data and smaller assessments for Digitop and SAS licenses. The GEM CRIS project received the same funding as the final FY2021 (1.56M net to location) amount, minus similar IT related assessments as the Plant Introduction (PI) CRIS. Student hiring for summer 2022 was challenging. The requirement for all agriculture students to complete internships and the growing disparity in what we can offer for wages versus other hiring opportunities continue to be our major challenges. Continuing pandemic constraints made recruiting difficult. Fred Engstrom advertised positions more widely across ISU colleges and excellent students were employed from diverse academic backgrounds.

Like many other research units, our ability to cover all aspects of our mission is challenged. 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.

Construction and Facilities:

Seed storage space is limited and needs to be addressed within the next two to three years. The 2018 request to ARS leadership for support to construct a 2,250 sq ft -20°C cold storage building which could essentially double the longevity of viability of many of our taxa was added to the Agency Construction Plan, and funds provided in FY2020 were utilized for design. A contractor was hired to work with station staff on design and location considerations. Construction plans cannot progress without Congressional appropriation for the facilities. In general, space is extremely tight for all personnel and functions. Addition of this building would enable dividing the collection inventories appropriately between 4° C and -20° C, greatly extending longevity of viability for many of our taxa.

The Information Management section of this report provides details on upgrades that continue to enhance the NCRPIS' information technology infrastructure, and the Farm Support Team section provides details for updates on maintenance and equipment.

IV. PROGRESS IN GERMPLASM AND INFORMATION MANAGEMENT, RESEARCH, AND EDUCATION (D. PETERS):

(Part IV. summarizes the accomplishments and progress for calendar year 2022, presented in greater detail in the individual staff reports in the document.)

Acquisition and Documentation Highlights:

Collection development continued with the acquisition of 336 new accessions (Appendix Table 1). Details are provided in the individual curators' report sections. A historical perspective to provide a comparison of acquisitions over the past nine years is provided below.

Year	# New Accessions	Year	# New Accessions	Year	# New Accessions
2022	336	2019	437	2016	786
2021	255	2018	293	2015	229
2020	262	2017	250	2014	766

The U.S. is now a partner to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Implementation by the National Plant Germplasm System (NPGS) is still under development as several Departments are involved. International collection continues to be challenging as countries adopt variations of the Standard Material Transfer Agreement (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 essential 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. Despite these challenges, excellent quantities of seed provided by collectors of many new accessions have made a significant proportion available and immediately distributable.

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

Regeneration and Maintenance Highlights:

Regeneration was attempted for 1,475 accessions. Of these, 949 were harvested (Appendix Table 2); efforts are described in the curators' report sections. Comparisons with previous regeneration attempts are provided in the table below. Differences may reflect challenges due to pandemic constraints and limited labor availability. Overall collection availability held steady at 80%, despite 10.5% growth in collection size since 2006. The increased focus on inactivating accessions which were received with low viability and could not be maintained, and resolution of duplication has also impacted collection availability.

	# Accessions		# Accessions]		# Accessions
Year	Regenerated	Year	Regenerated		Year	Regenerated
2022	949	2019	1,562		2016	1,033
2021	837	2018	1,245		2015	1,637
2020	915	2017	1,601		2014	1,230

Assistance in regeneration was provided by USDA-ARS staff of Parlier, CA for increase of wild Helianthus taxa. Daucus regeneration efforts were supported through seed increases from Seminis Vegetable Seeds (L. Maupin) and from Bejo Seeds (R. Maxwell). USDA-ARS in Salinas, California (B. Mou) supported seed increase of domesticated spinach. Spinach regenerations were also supported by cooperative efforts between the USDA-ARS and Sakata Seed America, Inc. in Salinas, CA.

USDA-ARS staff at the St. Croix quarantine nursery staff supported regeneration of 41 tropical maize inbred lines and populations. Raleigh ARS GEM Project Coordinator Matt Krakowsky provided increases of GEM lines and the Ames GEM team regenerated and provided GEM lines.

Accessions backed up at the National Laboratory for Genetic Resource Preservation (NLGRP) in Ft. Collins in 2022 numbered 1,343, with an overall collection backup average of 84%. There is wide variation for percent backup across the various crop collections, from 11% (teosinte, a maize ancestor) to 100% for flax (Appendix Table 2). Variation may be due to lack of appropriate environmental conditions to support the growth and reproductive requirements for some taxa, lack of methods to induce and synchronize flowering for some, and/or insufficient representation of male/female individuals, among other factors. Seed of 848 accessions was prepared and shipped to Ft. Collins for inclusion in the NPGS deposit to the Svalbard Global Seed Vault.

Year	# Accessions Backed Up	Year	# Accessions Backed Up	Year	# Accessions Backed Up
2022	1,343	2019	623	2016	428
2021	975	2018	795	2015	431
2020	1,454	2017	595	2014	1,231

Distributions:

Approximately 35% and 65% of the 2022 germplasm distributions were sent to international and domestic requestors, respectively. This proportion is closer to the average historical balance of 45% international and 55% domestic distribution than has been experienced the past two years. Distributions continued to reflect high demand in 2022 (Appendix Table 3). The 2020-2022 timeframe reflects 22% lower distributions than the previous three years.

Year	# Items	# Unique Accessions	# Orders	# Requestors
2022	43,978	20,894	1,054	771
2021	41,690	21,132	1,140	847
2020	46,627	21,089	1,153	836
2019	54,232	22,271	1,296	902
2018	61,124	22,229	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

Research demand for our plant genetic resource collections continues to be high; requests for diversity and relationship analyses, disease and pest resistance, biofuel, and health and nutrition contribute to these increases, as well as for basic research applications such as photoperiod response, and an array of performance traits. Germplasm requests continue to be driven by publication of information from genomic (genotyping by sequencing) and phenotypic analyses projects. Some of these studies are supported with Specialty Crop Research Initiative (SCRI), Agriculture and Food Research Initiative (AFRI), or National Science Foundation (NSF) funding.

The relative numbers of distributions vary from year to year, but generally correlate well with the proportional makeup of the collections and vary from year to year.

Demand for maize is usually greater than any other crops in our collection. Demand for maize and oilseed species remained strong and requests for vegetable crop accessions returned to historic average levels.

Curator		% of Total Collections	% of 2022 Distributions	% of 2021 Distributions	% of 2014 Distributions
Brenner	9,538	17	18	17	14
Carstens	4,052	7	1	2	<1
Marek	12,967	24	23	28	16
Millard/Bernau	20,161	37	43	45	35
Reitsma	7,986	15	15	8	28
Totals	54,704	100	100	100	100

Evaluation and Characterization:

Enhancements made to the 'Attachment Wizard' that work in conjunction with GRIN-Global, image organizing, and loading continue to progress rapidly. A large volume of accession-associated images and other types of documents are being attached to accessions, orders, and inventories. In 2022 image loading dropped to pre-pandemic levels with 2,406 images loaded. Loading of observation data also substantially decreased, with 15,278 observations associated with 873 accessions. For the NC7 collections, 42,705 accessions have one or more trait observation data points available via the GRIN-Global database, (https://www.ars.grin.gov/npgs/) and more than 27,378 have at least one associated image.

Information technology and telecommunications:

The NCRPIS staff continued to provide expertise and leadership for the development of GRIN-Global (GG), the successor to the Germplasm Resources Information Network (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 (Advisory Committee Chair, beta testing, training) with substantial time invested by additional personnel. The Database Management Unit (DBMU) in Beltsville, MD is responsible for hosting and maintaining the database and the system, developing the public interface, GRIN Taxonomy, 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 training, as for the past five years, and other training as requested.

Software development continues to center on the development and deployment of user tools that improve curatorial workflows, user experience, applications for data capture and transfer, enabling increased availability of accession-associated information to the public. 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. Currently 29 national or international genebanks have implemented the GRIN-Global system for genebank use, and many of these have live public interfaces. Another 16 genebanks are in the process of evaluating and/or implementing the system, true evidence of global adoption of this valuable resource.

For almost seven 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. An international AdCom was formulated with participation by key personnel from the Crop Trust, the U.S. NPGS, CIMMYT, and CIP, and confers monthly. A process was developed for international development products to be checked into branches of the Git vault (maintained by the Trust at CIMMYT) and then vetted.

One focus of the current ARS Program cycle is to develop inter-operability between GG and other key information providers' portals, examples including Maize Genetics Database (MaizeGDB), Gramene, LIS (Legume Information System), or GOBii. Pete Cyr developed and implemented the Breeding Application Programing Interface (BrAPI) specification using a RESTFUL webservice interface and integrated it with the GRIN-Global middle tier. It is being tested by genomic database personnel on a test server residing in Ames.

Please see the IT section for technical details of NCRPIS support activities and GRIN-Global development progress.

Germplasm's Viability and Health:

In 2022, about 11% of the collection was tested for viability during periodic maintenance (5,891 accessions). Documentation of collection quality necessitated an increase of resources devoted to this effort. A concerted effort is being made to ensure all seed lots 10 years or older have current germination information. Our storage conditions (4°C, 25-35% relative humidity) are very good, and efforts devoted to seed cleaning ensure storage of very clean seed lots, which is important for longevity of viability. Construction of an additional -20°C cold storage unit would provide a much longer period of viability for many of our taxa. Long-term cost savings would be significant, as most of the collection's seeds lose viability long before the inventory supply is depleted. Less frequent regeneration would enable more rapid progress in making the collection fully available.

ARS Pathologist Dr. Colleen Warfield has provided each curatorial team with guides and protocols for improved field and greenhouse practices to support healthy plant and propagule production. Collaborations continue for development of methods to eliminate the bacterial fruit blotch (BFB) pathogen, Acidovorax citrulli, from Cucumis melo seed.

Field inspections were made for all crops focusing on diseases of phytosanitary concern. All cucurbit seedlings were screened routinely for the presence of Squash mosaic virus via enzyme-linked immunosorbent assay (ELISA) prior to transplanting; outcomes are detailed in the pathology section of this report.

We continued to test, using a commercial laboratory vendor, for adventitious presence (AP) of genetically engineered organisms (GEO) in maize germplasm accessions new to the NCRPIS and newly produced seedlots.

Insect management:

The Entomology staff provided six insect pollinator species to control pollinate 805 accessions. Honeybees continued to be the primary pollinator used in the NCRPIS regeneration program, followed by the Alfalfa leafcutter bee (ALC).

Detailed observations and interpretative information regarding field pollinator research activities can be found in the extensive entomology section of this annual report describing the 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 the project, limited sources of such information, and relevance to the broader germplasm conservation world. Feedback and suggestions on experimental approaches are welcomed.

Enhancement:

The GEM Project works with 69 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 338 lines from 2001-2022 representing more than 60 maize races. An important goal is the 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 Spring 2023. These lines have one-quarter exotic, three-quarters temperate background.

Photoperiod sensitive tropical maize often does not flower until September in Ames. GEM and maize curatorial teams have continued to collaboratively develop an effective method for photoperiod control in the field. 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 traditionally held every September and are attended by scientists, breeders, and graduate students. The field days offer a unique opportunity for more molecular-focused researchers to understand the diversity of materials available for research, and the activities that support germplasm development. Easing pandemic related restrictions for visitors at the NCRPIS allowed field day plans to move forward in 2022 with approximately 40 visitors attending.

Outreach and Scholarship:

Normally our staff hosts more than 400 visitors per year and participates in a wide range of outreach activities involving students from grade K to postgraduate level and outreach events to civic and other organizations about germplasm conservation and management, and the work done at the NCRPIS. Easing pandemic related restrictions allowed gradual resumption of site tours 2022. The number of tours was limited, and participant numbers were kept small. Iowa State University (ISU) hosted the National Association of Plant Breeders (NAPB) 2022 annual meeting in August. The NCRPIS participated in the event field tour program with approximately 240 guests visiting the field demonstration plots. Scientific and technical staff members continue to publish scholarly journal articles and make presentations at scientific meetings.

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 our efforts to improve conservation methods to utilize the resources available more efficiently to us, and to develop labor and resource saving technologies. We continue to evaluate activities that can be reasonably reduced without sacrificing collection health and quality, and to improve efficiency.

Continued emphasis will be placed on communicating with research stakeholders to address development of comprehensive, genetically diverse collections to meet research and development needs. Stakeholders have requested increased emphasis 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 based on geographic information system (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.

High quality phenotypic characterization information is essential to enable welltargeted use of the collections, especially given the increasing constraints of limited research and conservation resources. Availability of plant genetic resources (PGR) significantly impacts research applications, including taxonomy.

Software development efforts will continue to center on the development and deployment of GG resources, and on information management tools that facilitate information transfer from various providers and integrate the information in useful

ways for researchers. 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. 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 for research being conducted at NCR institutions was not requested of the Regional Technical Advisory Committee (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 use. Requests have become increasingly more 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 per 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 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-Global 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.

The 2022 NC7 RTAC meeting was held using a hybrid format (in person and virtual) and hosted by the NCRPIS and NC7 RTAC member Thomas Lubberstedt. Minutes of this meeting can be found on the National Information Management and Support System (NIMSS) website.

Some of the NC7 RTAC's specific suggestions and contributions include the following: (from the meeting minutes):

- The NC7-RTAC committee is grateful for support from AES Directors and most appreciative for additional Hatch funding to the NCRPIS to meet current and future staffing and seed request needs more adequately.
- The NC7-RTAC committee recognize and thank Host David Peters, Chairman Professor Lubberstedt, Academic Advisor Carolyn Lawrence-Dill of ISU, and colleagues who contributed.
- The NC7-RTAC committee extends sincere thanks to Carolyn Lawrence-Dill, Administrative Advisor and Associate Dean ISU regarding dedication, experience, insights, and guidance in formulating the current 5-year NCRPIS project and ongoing support.
- The NC7-RTAC committee encourages funding for the -20° C seed storage room, as this will greatly increase germplasm efficiency by reducing the frequency of seed-regeneration, which is a costly and labor-intensive process.

VI. SUPPORT TEAM REPORTS:

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

The farm support team supervised and coordinated daily operations at the NCRPIS, including management of all facilities, fields, and greenhouse space. This included all pesticide applications in the field and campus greenhouses, response to maintenance requests, and the selection, coordination, and scheduling for the student labor force.

The team coordinated and completed facility construction and upgrades along with safety inspections. The COVID-19 pandemic related restrictions significantly changed our operation over the last three years. The challenges forced us to find alternatives to the traditional methods we have become accustomed to in our operation.

Labor:

During 2022, 38 applications for hourly employment were received and reviewed. There were approximately 24 interviews, resulting in 13 new and 18 returning hourly employees hired.

NCRPIS Farm Crew Personnel:

- Fred Engstrom, (Assistant Director of Research Administration) joined the staff July 1, 2016.
- Brian Buzzell (ISU Farm Equipment 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 multiple projects which enhanced the efficiency and safety of the station operations. These projects ranged from improving storage areas in several buildings, to organizing supplies, to purging of obsolete equipment and supplies. Routine maintenance of equipment and emergency repair need continuously change based on field and laboratory activities, and the Facilities team is tasked with those repairs. This keeps the team busy providing support to all the curatorial and support teams affiliated with the PI Station.

Purchasing:

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

Tours:

Tours were conducted on site in 2022 but were still limited due to COVID-19 restrictions.

Staff Training:

Tractor and Utility Vehicle Safety, Hazard Communication, and Worker Protection Standard training sessions were conducted for the new staff and student employees as well as annual updates for existing staff. The maintenance crew was involved in the rewriting of Job Hazard Analyses (JHA's) for the site and assisted with training.

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

Jesse Perrett served as the first line of support for NCRPIS during 2022. Jesse is supervised by Pete Cyr who is dedicated to the GRIN-Global project. The following list

outlines the progress made by the Information Technology (IT) team during 2022 at NCRPIS.

Equipment:

As of December 2022, the NCRPIS had 25 desktop and 60 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 6 physical servers and roughly 20 active virtual servers including those used for file storage, intranet, backups, and access security systems and monitoring.

A firewall was maintained 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.

Twenty laptop and desktop computers were deployed with all required equipment to set up users for teleworking. All users were ensured remote access to needed files, email systems, and networking capabilities for remote work.

The station continues to utilize the Monnit wireless environmental monitoring system with over 90 sensors placed in various locations around the station. This allows for real time monitoring of temperature and humidity for plant material and valuable equipment throughout the station.

The IT support team responded to numerous Client Experience Center (CEC) data calls for converting IT systems to a new centrally managed CEC implementation.

All site computers were compliant with department installations of Tanium for patch and software deployment and update monitoring as well as reporting to ARS IT specialists.

The GG label programs were updated with enhancements and fixes as needed. Work was coordinated with germplasm management staff to configure label printing for simplicity and functionality.

Multiple SharePoint lists are used at the station for tracking purchasing requests, maintenance requests, farm spray records, and other issues. The lists allow multiple users to add and monitor requests for new supplies and requests simultaneously.

Security system cameras are installed around the station to monitor property entrances and outside activities. Two security cameras needed replacement this year.

Software:

All workstations at NCRPIS use Windows 10. Microsoft Office 365, Adobe Acrobat Professional DC, Adobe Creative Suite, Cisco Anyconnect VPN, ActivClient, Tanium, Microsoft Windows Defender and the GRIN-Global Curator Tool were installed on systems as necessary. Laptops and tablets were encrypted by bit-locker.

PDQ Inventory and PDQ Deploy were also used for deployment and monitoring of non-Windows software packages such as the GRIN Global curator tool.

Documentation:

Training and documentation were provided for various station processes. New SharePoint lists for JHA documentation were implemented and collaboration with the Safety Team a new system for managing JHA training for employees was implemented.

Weather station history data was provided via SharePoint to allow users to download current and past weather data including calculated growing degree unit (GDU) and crop heat unit (CHU) data. The station uses a 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 (internal use).

Plans for 2023:

- Upgrade government smart phones to Intune system.
- Research and implement Multi-Factor Authentication for all users as well as whole drive encryption for data at rest wherever possible.
- Continue to update documentation for IT systems and services.
- Continue to replace user desktop systems with laptops and extended warranties and docks to prepare for the CEC implementation.
- Continue to replace NCRPIS workstations on an as needed basis (targeting a 3-5 year lifespan for daily use workstations).

GRIN-Global:

The GG database management system is the product of a partnership between the USDA-ARS NPGS, the Global Crop Diversity Trust and Bioversity International to develop a new genebank information management system that can be deployed on any size computer with a minimum amount of effort and cost. The GG system is currently implemented by 29 international genebanks and is being evaluated for adoption by additional 16 other genebank entities. GG is designed to support an unlimited number of languages (seven languages are currently installed) and has the capacity to store the genebank data in one of the four relational database engines (SQL Server, Oracle, MySQL, or PostgreSQL). The complete GG system can be installed on a stand-alone desktop computer or in a network server/client configuration.

The USDA-ARS GG development team is located primarily in the PIRU at Ames, Iowa and in the DBMU at Beltsville, Maryland. Pete Cyr is responsible for the Curator Tool (CT), Search Tool and development of associated wizards and forms. CT v1.23.1.26 is in beta now and will soon be released for production use at NPGS and international genebanks. In FY2022 Mr. Cyr developed 6 new versions of the CT Software Suite for testing and distribution. Included in the 6 CT versions released to the public domain are 79 enhancements and 4 bug fixes primarily in the new Attachment Wizard and the new Reports Manager interface in the CT. In addition to the CT development work, Mr. Cyr has continued to develop and implement updates to the GG implementation of the BrAPI specification in an effort to address requests from plant model organism database systems like MaizeGDB and Soybase. New versions of the BrAPI endpoints are hosted at a NCRPIS server in Ames and developers for MaizeGDB, SoyBase, PeanutBase, and Legume Information System are testing these enhancements. Mark Millard serves as the business analyst, and Lisa Burke serves as chair of the GG AdCom.

DBMU personnel at the National Germplasm Research Laboratory (NGRL) in Beltsville are responsible for the administration of the GG database, the Middle Tier security features, and the public website, <u>https://npgsweb.arsgrin.gov/gringlobal/search.aspx.</u> The public website 2.3.3.0 is currently under development. The NGRL botanist responsible for GRIN taxonomy works closely with DBMU developers.

Plans for 2023:

- Design, develop and test a RESTful interoperability application programing interface (API) for enabling mobile applications to insert and/or update data in the GG from the field.
- Enhance CT to support SQL Server Reports in addition to Crystal Reports.
- Continue enhancing the CT installation process to further minimize the installation tasks that require administrator permission to perform in an effort to enable end users to update reports without system administrator intervention.
- Enhance the CT to present a user-friendly interface for managing dataview tabs, Crystal Report updates, and Wizard updates gracefully.
- Design, develop, and test prototype mobile applications for field data collection.

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

Acquisition:

The NCRPIS acquired 397 new accessions in 2022. Of these new accessions, 50 were received from within the NPGS through exploration and transfer. Number assignments by curatorial project included: 6 for Pseudocereals, 79 for Horticulture, 91 for Oilseeds, 8 for Vegetables, and 180 new maize accessions- including 7 accessions from the GEM Project, and 45 newly expired Plant Variety Protection (PVP) lines. An additional 32 accessions were incorporated into the NPGS by NCRPIS personnel and then transferred to the appropriate priority site. Details of specific acquisitions are found in the curators' sections of this report.

As new accessions are recorded in the GG database, as much passport information as possible is included. Typical passport information would include a source history, cooperator records, collection-site descriptions and geographic coordinates for wild collections, pedigree, secondary identifiers, intellectual property rights (IPR) considerations, and any additional pertinent information provided by the donor. An excel workbook streamlines the assembly of passport data and aids in loading the data to the GG database.

Maintenance:

Curatorial assistance was provided by processing requests for taxonomic reidentifications and nominations of accessions to the inactive file. In total, 20 accessions received taxonomic re-identifications and 86 accessions were inactivated. 11 accessions were inactivated due to failure to germinate/not viable, and 80 accessions were inactivated due to duplication.

Additionally, 145 accessions were assigned PI numbers, 5 wild Sunflower accessions, 102 for David Brenner which included 45 spinach, 31 Umbels (mostly parsley), 13 Amaranth, and 13 Chenopodium and 41 maize accessions from various collections.

The NCRPIS continues to work on a project to digitize all paper documentation related to accession provenance, management, and performance. In total, 339 new passport files were uploaded to the GRIN database in 2022. We were unable to digitize additional legacy archival documentation this year due to staffing shortages caused by the COVID-19 pandemic.

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 masse to conform to document naming conventions that more easily support future upload to the GG database.

D. Order processing (S. Estrada)

The GRIN-Global public website has continued to improve accessibility to germplasm information and the ability to search for desired crop characteristics. The order processing team continued to refine the use of GG order actions, attachments, and local order numbers in conjunction with Excel workbook templates to monitor order progress, streamline processing, and inform internal and external cooperators of order status. Order actions allowed NCRPIS teams (curatorial personnel, seed storage, pathology) and other NPGS personnel (i.e., APHIS, GG feedback) as well as our external PW users to monitor a germplasm order more easily as it progresses through the pipeline towards fulfillment. This year, new order actions related to the phytosanitary inspections were added to better inform users about the process and scheduling between APHIS inspectors and NPGS personnel. These new actions will also improve long-term monitoring in the number of requests that require a phytosanitary certificate. Supporting documentation related to orders is attached directly to the corresponding GRIN order via the Attachment Wizard, thus accessible to internal NPGS users. The attachment wizard allows for uploading files to multiple orders en masse which simplifies uploading documents. External users may also add attachments (usually an import permit, shipping instructions, or Excel file request list) through the web order view of 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 2022, 1,542 orders were entered into GG. A total of 1,615 orders containing 66,930 items were completed. Of these, 1,043 entered the order processing system via the GG Public Website (PW). This year, the NCRPIS only processed 35 requests, (2%) of non-research, non-educational (NRR) orders submitted for consideration. This dramatic drop from 40-60% of all processed requests is a result of the introduction of the NPGS-wide NRR web order processing wizard in late 2021. In addition, standard email rejection letters and correspondence is now managed at the NPGS-wide level. A detailed summary of NCRPIS distribution activity is summarized in the table below which illustrates various internal use purposes, and in Appendix Table 3. External cooperators were shipped 43,978 items through 1,054 requests (812 domestic). Order processing capacities and operations have remained functional yet diminished since the COVID-19 pandemic began in 2020. We continue to annually distribute approximately 200 less requests and 5,000 – 20,000 fewer items than the annual processing volume seen since our requestors could submit orders directly through the GG PW (Nov 2015).

			2	022 - 11	CIAI 15	Jermh		Suibut	lions 50	iiiiiai	1
			0	Grand Tota	ıl		Distributed				
		Orders	Orders (%)	Order Items	ltems (%)	Avg. Items per Order	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order
Distribution		1,198	95%	54,204	81%	45	1,037	98%	43,508	80%	42
Non-research, non-education	al	35	3%	506	1%	14					
Observation / evaluation		22	2%	736	1%	33	14	1%	467	1%	33
Repatriation		3	0%	3	0%	1	3	0%	3	0%	1
	Total	1,258	100%	55,449	83%	44	1,054	100%	43,978	81%	42
Backup		64	18%	2,514	4%	39	60	18%	2,458	5%	41
Germination		214	60%	6,469	10%	30	201	61%	5,998	11%	30
Phytosanitary Testing Replenishment/regrow		22	6%	1,298	2%	59	19	6%	1,007	2%	53
Replenishment/regrow		54	15%	1,197	2%	22	47	14%	1,087	2%	23
Transfer		3	1%	3	0%	1	3	1%	3	0%	1
	Total	357	100%	11,481	17%	32	330	100%	10,553	19%	32
Gra	nd Total	1,615	100%	66,930	100%	41	1,384	100%	54,531	100%	39

2022 - NCRPIS Germplasm Distributions Summary

Shipped orders:

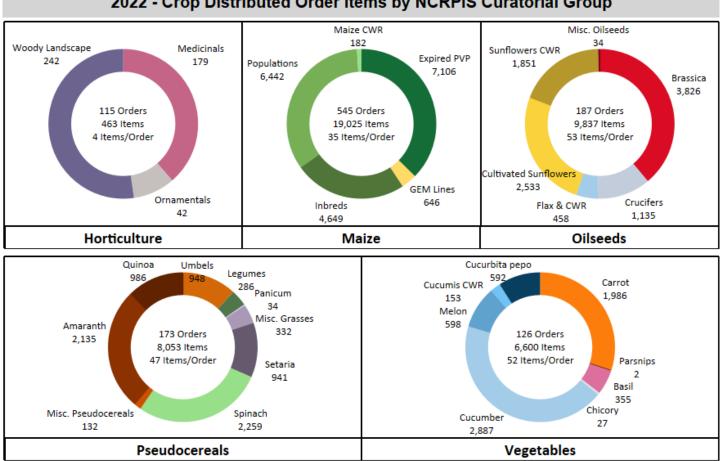
A total of 1,258 external orders were processed. Of these, 1,054 (84%) were shipped and only 116 orders were cancelled. External orders were cancelled for a variety of reasons including: 33 (28%) were NRR, 53 requestors were unable to secure an import permit, and 30 for other reasons such as mistakes, 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 77% of all externally distributed orders and 65% of the distributed items (Appendix Table 3), indicating U.S. requestors received fewer items per order on average. Maize was the most highly distributed crop both within the U.S. and internationally.

	Orders		Order It	tems	Avg. Items per Order		
Curatorial Team	U.S.A.	Int'l	U.S.A.	Int'i	U.S.A.	Int'i	
Horticulture	104	11	368	95	4	9	
Maize	451	94	13,942	5,083	31	54	
Oilseeds	118	69	5,212	4,625	44	67	
Pseudocereals	120	53	4,588	3,465	38	65	
Vegetables	84	42	4,580	2,020	55	48	
Grand Total	812	242	28,690	15,288	35	63	

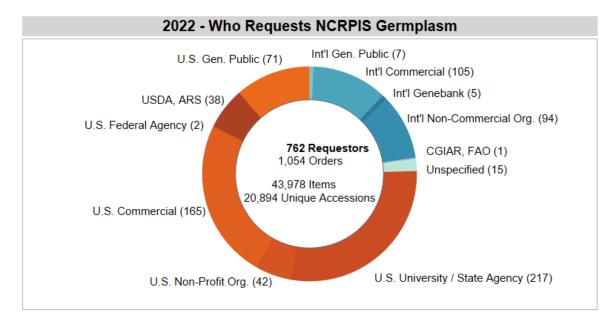
2022 - NCRPIS External Germplasm Distribution Summary by Curatorial Group

Order distributions were also summarized by curatorial group, as seen in the pie charts below. Vegetable and Horticulture curatorial groups saw high demand for select crop maintenance groups while Oilseeds, Maize, and Pseudocereals requests were more balanced across crop groups. The 10 largest orders, ca. 700 - 2,000 items, were for maize lepidoptera bioassays of maize landrace populations and inbreds, genome-wide association studies (GWAS) of Cucumis and maize inbreds, pathology or insect resistance screening, and also for varietal development of Cucumis, sunflower, millets. We received 10 requests for the Cucumis melo International Cucurbit Powdery Mildew Initiative (ICPMI) differential set (22 accessions) that was available for the first time beginning in January of 2022. See specific curator's sections for detailed information.



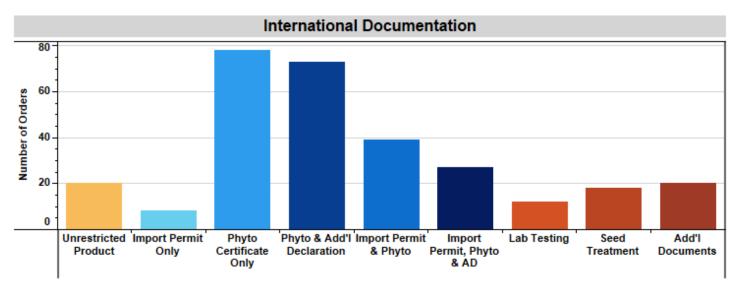
2022 - Crop Distributed Order Items by NCRPIS Curatorial Group

For a more detailed view of orders, distributed external orders are visualized in the context of destination and requestor affiliation. We continue to see a large number of requests from domestic and international universities and commercial companies.



International orders accounted for 23% of those shipped and for 35% of all distributed items. Approximately 70% of international requests were transferred to the NPGS' National Plant Germplasm Inspection Station in Maryland for consolidation with other NPGS site's orders and centralized APHIS phytosanitary inspection prior to export. The European Union (EU) now requires a phytosanitary certificate for all plant and plant part shipments so most international requests shipped directly from NCRPIS were sent to Canada or Mexico in accordance with the United States-Mexico-Canada Agreement (USMCA).

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. Our local order number field distinguishes shipping destinations as well as serving as a 'quick reference' for documentation and additional quality assurance needed prior to dispatch of an order. New order actions were added to GG to improve monitoring of orders that require phytosanitary inspection. Altogether, order actions now allow for phytosanitary certificates, additional declaration statements, seed treatment, quality testing, and import permits to be easily queried and better documented.



Complexity of international shipments increases from left to right. Orders with no phytosanitary certificates are the least complex while orders with many additional conditions, testing, and/or additional supporting documentation increase processing times. The red bars at the far right represent additional processes or steps that are necessary to satisfy the terms of import.

Import restrictions vary in complexity depending on the crop and destination country. Eighty-one percent of all international orders required at least the issue of a phytosanitary certificate prior to export. An import permit was associated with 29% of international distributions.

EU regulations were updated in 2022 and shipments now must meet stricter testing criteria for detecting *Pantoea stewartia* subsp. *stewartia*, the causal agent of Stewart's Wilt (detection of 0.5% level of infestation with a 99% confidence interval). As a result, maize curation, pathology, and seed storage personnel worked together to devise and implement a new strategic approach to ensure shipments comply with these stricter

terms of import. These changes resulted in delayed processing times of up to nine months for shipments of maize to EU requestors.

The Cucumis melo ICPMI powdery mildew differential set introduced new challenges to international shipments because the set was not grown inside the U.S. Therefore, shipments require a re-export phytosanitary certificate and extensive communication with the importing country to determine the specific terms of import for the germplasm prior to export.

E. <u>Seed Storage (L. Burke, A. Sonner)</u>

The seed storage area was staffed by two full-time, permanent federal employees (Lisa Burke and Ashley Sonner), one part-time student employee, and one Limited Appointment (LA) employee during 2022.

The seed storage team placed 1,015 seed inventory lots into storage including 339 original lots, which are the first samples received for an accession. Of the increase lots, 498 were produced in Ames and 137 were produced at external locations outside of Ames. Across all stored inventory, seed lot quantities were verified for 2,918 lots. Any discrepancies with information in the GG database were corrected. Original and reference samples of 507 lots were prepared and placed at -20°C for long-term storage.

In 2022, 1,165 seed orders were filled. These included requests for distribution, observation, germination, transfer, or backup. The NCRPIS distributed 43,579 packets to fulfill distribution and observation requests. There were 1,465 seed lots sent to the NLGRP for backup, including accessions new to the NLGRP as well as additional seed quantities for previously deposited accessions. Eight hundred forty-eight accessions were packaged for shipment to Svalbard for long-term storage.

With the assistance of student workers, 35,422 packets from 3,363 inventory lots were prepacked. Prepacking increases efficiency of seed storage operations by speeding up order fulfillment and also helps 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 2022, 143 accessions received PI numbers and 533 inventory samples were relabeled and moved to their chronologically correct locations.

Lisa Burke continued to participate in the development of GG. She served on the GG AdCom as chairperson and chaired 19 meetings in 2022. During the meetings, progress on curator tool and public web site enhancements were discussed, priorities were established, and the functionality of new software products verified. The focus this past year was to concentrate on developing the GG public website's ability to satisfy ordering parameters for vegetatively propagated germplasm in the NPGS clonal repositories.

Lisa Burke continued as the station's CPR/AED/First Aid instructor. She provided CPR/AED/First Aid certification training to 16 NCRPIS student workers and 8 staff members. Due to COVID-19 pandemic restrictions, these classes were conducted online with skills certification completed in a socially distanced setup. Each session was entered into the National Safety Council database and certificates of completion provided for each participant.

Ashley Sonner continued to image original seed samples in 2022. Original samples of 601 accessions were scanned. Images were uploaded to GG utilizing the attachment wizard. In addition to imaging original seed, Ashley assisted the Vegetable Crew in relabeling and loading over 600 images onto GRIN (Table 1). Ashley is working with Lisa Pfiffner and the International Seed Morphology Association (ISMA) to develop fact sheets for seed identification. This past year Lisa and Ashley focused their efforts on Helianthus ciliaris and Cucurbita pepo with the goal of submitting these fact sheets for review in early summer 2023.

Curatorial Group	Original samples imaged
Maize	0
Oilseeds	203
Horticulture	72
Pseudocereals	265
Vegetable	61

Table 1. Images uploaded to GRIN in 2022

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 2022, the germination lab completed germination or Tetrazolium (TZ) testing on 190 orders. TZ testing is a biochemical method to determine seed viability and predict the probability of seed germination.

Type of Order	Number of Orders
Regeneration	52
Maintenance	92
Original	12
Re-germ	21
TZ	13
Total	190

Table 1. Number of orders received by type

		Number of accessions tested			
Curator	Inv. maintenance policy	Maintenance	Increase	Original	
Brenner	NC7-pseudocereal.amaranth	378	33	0	
	NC7-pseudocereal.celosia	8	1	0	
	NC7-grass.echinochloa	20	0	0	
	NC7-grass.misc	0	3	0	
	NC7-legume.misc	24	0	0	
	NC7-legume.melilotus	57	0	0	
	NC7-grass.panicum	267	0	0	
	NC7-pseudocereal.perilla	4	2	0	
	NC7-pseudocereal.portulaca	0	1	0	
	NC7-pseudocereal.quinoa	0	69	6	
	NC7-grass.setaria	5	0	0	
	NC7-spinach	0	0	0	
	NC7-umbels	42	7	0	
Carstens	NC7-medicinals	117	13	11	
	NC7-ornamentals	3	2	2	
	NC7-woody.landscape	35	3	17	
Marek	NC7-asters	53	0	3	
	NC7-brassica	10	10	5	
	NC7-crucifers	1	7	55	
	NC7-cuphea	25	0	0	
	NC7-euphorbia	37	0	0	
	NC7-flax	0	45	0	
	NC7-flax.wilds	0	8	0	
	NC7-sun.cults	546	39	7	
	NC7-sun.wilds.ann	14	29	1	
	NC7-sun.wilds.per	3	19	0	
Millard	NC7-maize.gems.inb	0	0	0	
	NC7-maize.inb	596	10	90	
	NC7-maize.inb.stndrd	2	0	0	
	NC7-maize.pvp.clear	82	99	2	
	NC7-maize.check	5	15	0	
	NC7-maize.gems.pop	67	1	0	
	NC7-maize.pop	1,353	64	38	
	NC7-maize.pop.GalinA	11	2	0	
	NC7-maize.pop.GoodR	135	2	0	
	NC7-maize.pop.GoodR2	16	1	0	
	NC7-maize.pop.GoodR3	11	2	0	
	NC7-maize.pop.Gtrop	748	10	0	
	NC7-maize.pop.GtropA	118	15	0	
	NC7-maize.teosinte	54	1	0	
	NC7-maize.coix&tripsacum	0	0	0	

Table 2. Number of accessions tested for germination/viability and order type in 2022

Reitsma	NC7-chicory	26	46	0
	NC7-cucumis.cucs	12	24	0
	NC7-cucumis.melo	0	4	21
	NC7-cucumis.wilds	14	3	0
	NC7-cucurbita	12	6	2
	NC7-daucus	146	71	0
	NC7-ocimum	11	0	0
	NC7-parsnips	7	0	0
Total		5,075	667	260

The backlog in maintenance testing was eliminated for the majority of crops in 2022.

Several Association of Official Seed Analysts (AOSA) webinars and virtual meetings were attended as continuing education to maintain certification to stay in good standing and remain a voting member of the AOSA. Lisa Pfiffner and Ashley Sonner began working on a project for AOSA to develop a digital reference for seed identification of the species on the AOSA/SCST exam list. The goal of the project is to have reliable resources for individuals who are studying to become certified seed analysts. Ashley is providing images of seeds and is jointly developing seed identification sheets for several species with Lisa.

VII. CURATORIAL AND SCIENTIFIC TEAM REPORTS:

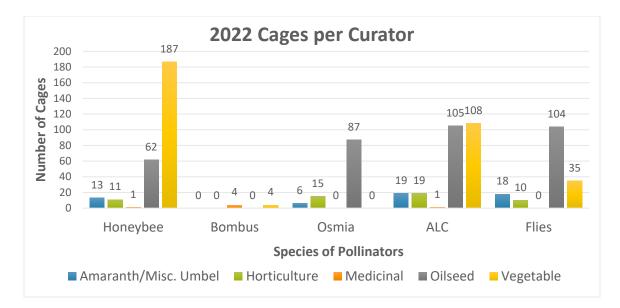
A. <u>Controlled Insect Pollination Service Program (S. Hanlin)</u>

Overview:

A total 805 insect pollinators were supplied throughout the pollination season. The table and graph below display the total number of insect pollinators provided in 2022 minus honeybee "health" replacements and "two nuc" *Cucurbita* cages. This total does not also include the multiple doses of alfalfa leafcutting bee (ALC) or flies that were added on a weekly basis, only the initial request. Pollinators were supplied a single time to 765 accessions in 2022.

Crop Group	Honeybee	Bombus	Osmia	ALC	Flies
Amaranth/Misc. Umbel	13	0	6	19	18
Horticulture	11	0	15	19	10
Medicinal	1	4	0	1	0
Oilseed	62	0	87	105	104
Vegetable	187	4	0	108	35
Total	274	4	108	252	167

2022 Cage Totals per Curator per Pollinator



ALC and flies are distributed multiple times to the same cage over the pollination season. Except for several weeks during the summer, every cage needing either pollinator gets at least a single dose of ALC and flies on a weekly basis. ALC and flies were used on similar plant types. In the summer both Blue Bottle Flies and Common House Flies were introduced together for all requests. But, during the Spring and Fall, only Blue Bottle Flies were used in cage pollinations.

Osmia are used in both the greenhouses and field cages and work best in early spring or in the "cooler greenhouse" when temperatures are between $50-70^{\circ}$ F ($10-21^{\circ}$ C). The average nesting season for Osmia is between 6-12 weeks. At the end of the pollination season, Omsia domiciles are collected and individual pupae are counted and used the following year.

Bombus colonies are used throughout the summer on plants with larger flowers. Because bumble bees are a more efficient pollinator, they need to be in cages for a shorter time period and a single *Bombus* colony can be used in numerous cages throughout the summer.

Due to better proficiency in managing honey bees, they are the most used pollinator at the station. We can use a nuclues hive (nuc) in multiple cages throughout the pollination season, but in general a nuc is left in a cage until pollination is completed. Feeding of high fructose corn syrup (HFCS) is required on a weekly basis and a pollen patty mix every other week to promote continual brood production.

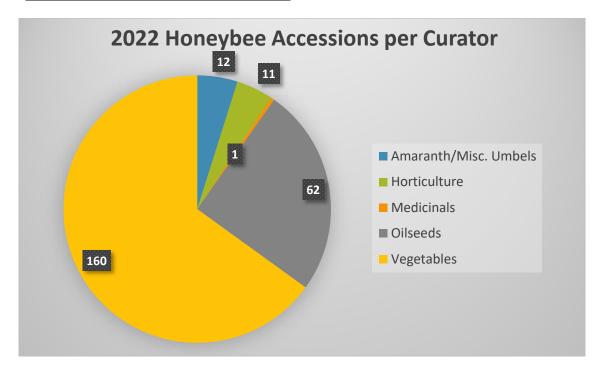
Health requests were made by the entomology crew or other curators throughout the summer in *Bombus* and honey bee colonies. Nucs failed for multiple reasons: queen failure, weakened hive, age, damage. Once notified or observed, the nucleus hive was replaced with another to maintain pollination in that cage. There were 65 insect health requests during the pollination season. The graphs and charts do not include duplicate plantings, multiple nucs per cage or accessions which were relocated from greenhouse to field.

Honeybee Pollination:

Honeybees were used to pollinate 274 cages in the field including duplicate accessions. In the following table only single honeybee requests of single accessions are recorded.

Crop Group	Total # of Accessions	# of Genera	# of Accessions/Genera	
Misc. Umbels	12	3	10 Dalea; 1 Coriandrum; 1 Melilotus	
Horticulture	11	5	5 Spiraea; 3 Coleus; 1 Physocarpus; 1 Salix; 1 Viburnum	
Medicinals	1	1	1 Agastache	
Oilseeds	62	1	62 Helianthus	
Vegetables	160	5	45 Cucumis; 32 Cucurbita; 32 Daucus 27 Cichorium; 24 Ocimum	
Total	246	15		

2022 Honeybee Pollinator Deliveries to Regeneration Cages



Overwintering:

During the winter of 2021/2022, a total of 24 three-story colonies were left outside at three locations and were wrapped with forty-pound roofing paper. Six three-story parent colonies were located at the NCRPIS in the caged in area used for queen rearing during the summer. An additional six three-story colonies were located at a northern off-site location and an additional twelve colonies were placed at an eastern location. Forty-eight two-story parent colonies, seven three-story colonies and 138 double-story



nucleus hives were placed into the overwintering room. It was observed that 17% of the double-story nucleus hives were lost prior to putting them into the indoor facility and 9% of the parent colonies were lost prior in the fall of 2021. However, none of the outside colonies were lost prior to wrapping them. All parent colonies stored inside were removed from the room starting on March 11, and all outside colonies unwrapped on March 2. The nucleus hives were removed from the over-wintering room on March 11. In the winter of 2021/2022, 16% of the wrapped three-story colonies left outside survived, 71% of the inside colonies survived and 44% of the double-story nucleus colonies stored indoor survived. While the hives were in the over-wintering room, they were fed a syrup/sugar patty combination in February and March to improve their survival in the spring.

In the Fall, all nucleus hives which were not used in cages for pollination were fed HFCS, an additional superstructure (box) was added, and the bees treated for mites to prepare them for over-wintering. For winter preparation, all hives were fed HFCS into mid-November prior to being placed into the overwintering room or wrapped. Feeding will begin February/March of 2023 to assist with survival of hives into the spring. In the fall of 2022/2023, we placed a total of 58 double nucleus hives and 3 single nucs, 51 two-story colonies and 25 three-story colonies into the over-wintering facility. A total of 20 three-story hives were wrapped at three sites at NCRPIS with forty-pound roofing paper.



Hives were sampled in mid-August prior to applying mite treatment. In mid-August, all colonies and double nucleus hives were given two treatments of Thymol (Apiguard[®]) to prevent any varroa mite build up over the winter. Hives were not sampled treatment confirm after to effectiveness of control. During the summer neither European Foul Brood (EFB) nor American Foul Brood (AFB) were observed.

Nucs and packages:

In the spring of 2022, 40 five-frame nucleus hives were purchased from a local supplier, 20 three-pound packages were purchased from an additional local beekeeper and 50 Italian queens from a California supplier. These were used to supplement overwintering losses and to supply spring nucs used for cage pollinations. The queens arrived by USPS in early May, the packages were picked up and put into full sized equipment in mid-April and the nucs were placed into full sized equipment prior to pick up in June. The colonies were given four feedings of HFCS and two pollen patty treatments during the buildup period to promote queen laying and brood production. The purchased queens were placed into nucleus boxes with two frames of brood and a single frame of honey with adhering bees. Only three of the purchased queens did not survive to be put into "spring" nuc production.

Queen-rearing:

In late May we selected six resilient, over-wintered parent colonies and set them up as "cell builder colonies" for queen production during the summer 2022. The first queen grafts were done on June 1 and the first two grafts only produced approximately 19 cells per week. However, later in the summer the queen production improved to a weekly average of 32 cells. The last graft was done in mid-August. As in the last three years, because packages/nucleus hives were purchased locally, we had no issues of aggressive bees.



Feeding:

Starting in March through early June, all parent colonies and nucleus hives were given a sugar patty and pollen patty and then eight feedings of HFCS. In October to mid-November, all hives were fed weekly. In 2022, we were again able to mix Fumagilin – B® medication used to treat dysentery (Nosema) with the syrup, In the fall, all hives received an initial two feedings of unmedicated syrup, they then received two feedings of medicated syrup and then one to two additional unmedicated feedings.

We continued to use the "bulk tank system" with the 30-gallon poly "mixing" tank for filling feed containers. To prevent crystallization, insulated blankets were used to cover two tanks containing syrup and a programable tank heater was placed in the third at 93° F. On May 25th, 825 gallons of HFCS was purchased for supplemental feeding during the summer and into the spring of 2023. Use of 5-gallon buckets was continued in the spring and fall for refilling feed containers in the field to reduce container damage and syrup waste.

We continued in 2022 with the "winter/spring" feeding system of using granulated sugar and HFCS to increase hive survival. A mixture of HFCS and granulated sugar was applied to all overwintering nucleus hives and colonies inside the overwintering room starting in February. In March, outside colonies which were unwrapped and confirmed alive, were fed a "candy board" (mix of sugar, HFCS and vinegar) which was placed on the top box as a spring supplement feed. With both methods of feeding, it was found that hive survival increased, and the hive sustained itself until they could be fed HFCS.

Wax moth:

As in past years for wax moth control during the summer, all stored supers with "cleaned" frames were stacked at right angles to each other and placed in the "cooler" equipment room to prevent adult moth migration. Starting in June through September, the lights in the equipment room were left on during working hours (8 hours; 5 days) to deter an establishment of moths. All equipment removed from the field as "dead hives" was placed in the over-wintering room, to prevent a fall outbreak of wax moth in the equipment room. However, in the late summer we did find supers containing wax moth larvae, so the frames were either discarded if they had excessive damage or placed on strong colonies of bees to control the immature moths.



Bee yard registry:

As in 2021, hive registration with the Iowa Department of Agriculture and Land Stewardship (IDALS) was done using "Field-Watch". Field-Watch allows registration of yards by plotting the apiary locations directly onto Google maps. For 2022, registration consisted of confirming all 2021 registered locations which would be used during the summer. The IDALS registry assists pesticide applicators in locating beeyards and in obtaining contact information of appropriate beekeepers prior to spraying.

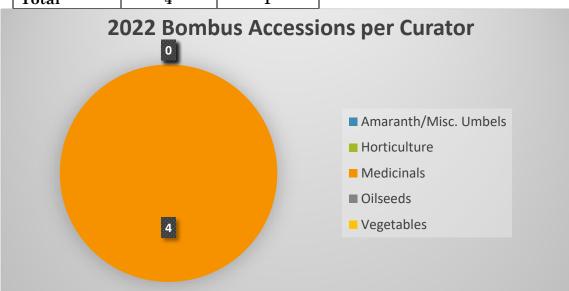
Bombus pollination:

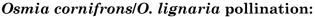
Four "mini research" colonies of *Bombus impatiens* were purchased from a commercial supplier and used for pollination at the station. Bombus was used to pollinate 4 field cages containing *Monarda* sp. After removal from requested cages, Bombus hives were placed in *Cucurbita* or *Helianthus* cages and were used in combination with honeybees for more efficient pollination. 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. While in holding, hives were stored in a 73° F (23° C) rearing room.



	Total # of		
Crop Group	Accessions	# of Genera	# of Accessions/Genera
Misc. Umbels			
Horticulture			
Medicinals	4	1	4 Monarda
Oilseeds			
Vegetables			
Total	4	1	

2022 Bombus Pollinator Deliveries to Regeneration Accessions





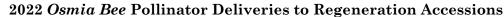


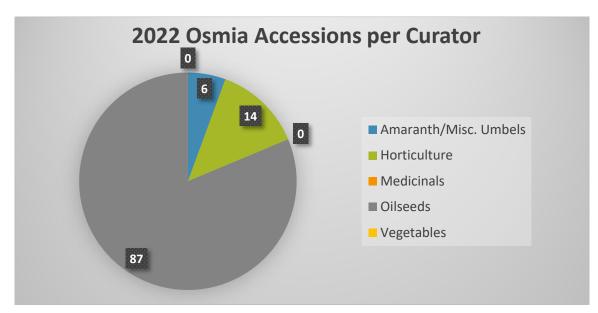
Osmia sp. were used to pollinate a total of 108 cages containing 107 accessions in five fields and two greenhouses. Osmia pupae arrived in March from two previously used suppliers based in Idaho and Washington state. A total of 3,500 pupae were shipped (2,000 Idaho and 1,500 Washington). For "early" greenhouse pollinations, the pupal cocoons were allowed to hatch or were opened prior to placing them into the cages. In the spring of 2022, 108 Osmia domiciles were hung in greenhouse and field cages per request. An additional 50 domiciles were placed at a single "increase" site in hopes of collecting additional bees for the 2023 season.

In November of 2022, Osmia tubes were sorted and cut open to retrieve any cocoons. No viable bees

were found in the tubes and pupae would need to be ordered for pollination use in 2023. It is hypothesized due to the cold, dry spring that either tubes could not be lined with mud, or the larva got chilled. An additional 2,000 Osmia were ordered from a prior commercial supplier for delivery in early March of 2023.

Crop Group	Total # of Accessions	# of Genera	# of Accessions/Genera
Misc. Umbels	6	3	4 Melilotus, 1 Caucalis, 1 Corandrum
Horticulture	14	5	7 Aronia, 3 Spiraea, 2 Salix, 1 Physocarpus, 1 Viburnum
Medicinals			
Oilseeds	87	6	80 Brassica, 2 Camelina, 2 Lepidium, 1 Eruca, 1 Erysimum, 1 Thlaspi
Vegetables			
Total	107	14	





Alfalfa leafcutting bee (ALC) Megachile rotundata:

ALC bees were purchased as pupae in leaf cells from a single supplier for use throughout the 2022 pollination season for the pollination of a variety of crops. Arriving on March 17th, the bee cells were held in Precision® incubators at 39° F (4° C) until requested for pollination after which they were placed into an Environmental Growth Chamber (EGC) at 86° F (30° C) and bee emergence boxes. The cocoons were from a Canadian source, which means they are less infested with parasitoids than found in U.S. cells. Bees were available weekly throughout the year for use in plant regeneration cages in the field and greenhouses from January 2022 into 2023.

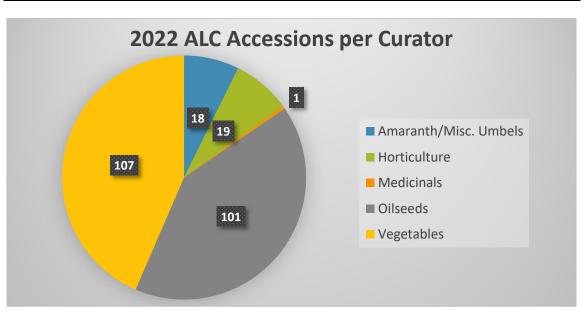


The 2022 pollinations were began using the remaining pupal tubes from the 2021 cocoons and finishing the season with the 2022 cocoons. There was a period in late

February and early March, prior to the arrival of the 2022 cocoons, in which bees were unavailable for greenhouse cage pollination and were not used until late March. In 2022, 2,286 total ALC deliveries were made to a total of eleven fields and four greenhouses with 252 cages containing 246 accessions and 24 genera.

	# of	# of	# of	# of	
Crop Group	Deliveries	Accessions	Locations	Genera	Time Period
Misc. Umbels	113	18	3	4	Feb - Sept
Horticulture	101	19	5	6	April - Oct
Medicinals	11	1	1	1	July - Sept
Oilseeds	948	101	3	9	Jan - Oct
Vegetables	1,113	107	4	4	May - Oct
Total	2,286	246	16	24	Jan - Oct

2022 Alfalfa Leafcutter	Pollinator Deliveries to	Regeneration Accessions
		negeneration necessions



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 summer months. However, at the station ALC are used outside of the normal time frame. From January 2022 through June, greenhouse cages were supplied weekly with bees and field cages started in early April and the number of weekly active cages increased through mid-August and then declined with the last field cages supplied through late-October.



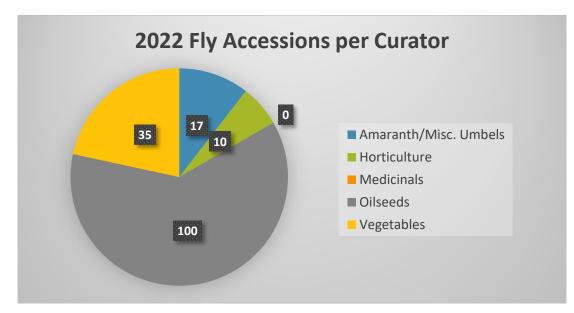
From late August to mid-October, ALC were placed into cages of *Helianthus sp, Linum sp., Coleus sp., Agastache sp* and several field vegetables cages. Adult bees are resupplied weekly to cages to ensure continued pollinator presence. Under normal conditions ALC are not the major pollinator of some of these accessions nor are they used this late in the season in field cages. Because we had two fall greenhouse cages, we continued to emerge a low number of bees for pollination into 2023.

Flies (Blue Bottle Flies and Houseflies):

Fly pupae of *Calliphoridae* and *Musca domestica* were purchased from two suppliers and incubated for weekly use from late January 2022 through mid-October 2022 for greenhouse and field pollinations. From June through August, 12 orders of 20,000 house fly pupae were purchased and from mid-January 2022 through early September, 248 cups of blue bottle pupae were purchased. In 2022, 1,545 fly deliveries were made to seven fields and three greenhouses with 167 cages holding 162 accessions of 16 genera.

	# of	# of	# of	# of	
Crop Group	Deliveries	Accessions	Locations	Genera	Time Period
Misc. Umbels	116	17	3	4	Feb - Sept
Horticulture	23	10	3	3	April - June
Medicinals	-	-	-	-	-
Oilseeds	1,012	100	4	8	Jan - Oct
Vegetables	394	35	1	1	May - Sept
Total	1,545	162	11	16	Jan - Oct

2022 Fly Pollinator Deliveries to Regeneration Accessions



From January 2022 through early June, greenhouse cages were supplied weekly with flies. The 2022 field requests for flies started in early April and the number of weekly active cages increased through mid- August and then declined with the last field cages supplied through late-October. 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 house flies 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 had fly pollinators introduced also had other pollinators present to assure flower pollination based on promotion of insect competition. If there was an excess of fly pupae available during the summer, flies were introduced to some accessions which lack favorable flower formation for fly pollinators such as *Brassica* sp, sunflower or melons. This decision was made by the curators and the entomology staff to fully utilize the fly pupae and prevent wasting of pupae.

Branding equipment:

As in the past years, all new woodware (frames and other hive parts) were branded with an identifying stamp displaying hives as the property of USDA, ARS and providing contact information. The goal was to assist in identifying the station's bee equipment and serve as a theft deterrent.



Safety:

Defensive Driving:

Because of the amount of time the bee crew spends off-site, and the number of cumulative miles driven during the summer, annual driving training was completed on AgLearn by each full-time entomology crew member as a refresher on good driving habits and prevent possible auto accidents.

Epi-pens:

In April 2022, new Epi-pens were purchased. However, with the recommendations of ISU Occupational Medicine, the four expired pens were retained as "emergency pens" as long as the active materials did not appear cloudy within the pens. The distribution and use of the new pens was based on the stipulation that in 2022 all station personal would complete the Epi-pen training in the spring. In late-March, K. Grooms (nurse at ISU Occupational Medicine) was contacted about required training and it was decided because of no PowerPoint presentation by ISU Environmental Health and Safety (EH&S) on anaphylactic shock and Epi-pen use, the Epi-pen website training would continue to be used. However, in 2023, the recommended training would be based off the PowerPoint created in the ISU Toth Bee Lab. In mid-April all staff had completed the Epi-pen site training and filled out forms which were filed with ISU Occupational Medicine and pens were distributed to all station locations for access in case of anaphylactic shock.

B. <u>Plant Pathology (C. Warfield)</u>

Phytosanitary Inspections for Seed Increase Crops:

The plant pathology team conducts field and greenhouse inspections to support the issuance of phytosanitary certificates necessary for international movement of seed. Importing countries often require seed to be free of seedborne plant pathogens or to be harvested from plants inspected during active growth. In addition, the plant pathology team provides support for curators and their crews in addressing plant

health concerns and providing disease diagnostics and management recommendations.

Maize:

During August, field inspections of 373 maize seed increase plots were conducted 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 and southern rust, common smut, head smut, sorghum downy mildew and wheat streak mosaic virus (WSMV). Similarly, 692 GEM plots were inspected for the same group of diseases. The disease pressure was very low throughout the growing season. The most prevalent diseases in terms of incidence of infected plants in the maize curation plots were common smut (Ustilago maydis) observed on ears, stalks, and/or tassels, gray leaf spot (Cercospora zeae-maydis), northern corn leaf blight (*Exserohilum turcicum*), and common rust (*Puccinia sorghi*). No diseases of phytosanitary concern were observed in the maize curation plots. Disease incidence in the GEM plots was extremely low. A few instances of common smut, gray leaf spot, maize leaf spot (Cochliobolus carbonum), northern corn leaf blight, and common rust were observed. Crazy top (Sclerophthora macrospora) was observed in the GEM plots located in the North Field for the first time in many years and was most prevalent in the border rows/plot edges.

Oilseeds:

Two field phytosanitary inspections, the first on 26 June or 1 July and the second on 27 July, were carried out for 78 sunflower seed increase plots. Plots were inspected for downy mildew (*Plasmopara halstedii*), Septoria leaf spot, Phoma black stem, Sunflower apical chlorosis, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Alternaria*, viruses, and phytoplasmas. No downy mildew or sunflower apical chlorosis (the main phytosanitary issues) were present. Cultivated sunflowers in a couple cages in the North Field either severely declined or died. The pattern of damage was consistent with phytotoxicity. While the leaf and stem symptoms alone suggested a biotic cause, no fungi or bacteria were isolated from the leaf or stem tissue. Dr. Charles Block was consulted, and his field observations and laboratory isolations also failed to identify a cause. The cause of these symptoms remains a mystery. Herbicide toxicity could not be ruled out and remains the most likely cause.

Flax (*Linum* sp.) increase plots (63 accessions) were inspected on 23 June and 27 July. No disease symptoms were observed. Significant lodging was observed throughout the plots because of flooding earlier in the season.

Two phytosanitary inspections were conducted in June, and July for all 84 *Brassica sp.* seed increases. Field observations were made for any disease symptoms including those associated with Xanthomonas black rot, powdery mildew, black leg, white rust, downy mildew, Cercospora leaf spot or phytoplasmas. Lodging and lower leaf senescence were prevalent throughout the plots attributed to flooding earlier in the seasons. No black rot or other diseases of phytosanitary concern were observed.

Cucurbits/Vegetables/Herbs:

Routine disease testing for squash mosaic virus was conducted on all cucurbit seedlings prior to transplanting, as has been done since 1993. Of the 32 accessions tested by ELISA in 2022, two accessions had Squash Mosaic Virus (SqMV) positive seedlings. While all seedlings were tested prior to transplant in the field, viral symptoms were noted in a field cage and confirmed as SqMV for one of the accessions which had SqMV positive seedlings. Mechanical transmission of this virus is known to occur, and it is likely the virus was spread to other seedlings during sampling. In the future, seedling goings into the field cages from an accession with SqMV positive seedlings should be re-sampled and tested again 2 or 3 weeks after transplant into the field.

Two disease inspections of the cucurbit cages took place during July. A very low incidence of powdery mildew was present on a few accessions. Timely fungicide applications limited further development. No diseases of phytosanitary concern were observed.

No diseases were observed for the 26 *Ocimum* and 36 *Daucus* accessions inspected. A high incidence of stem fasciation was noted in two *Cichorium endivia* plots and one *Cichorium intybus* plot. Based on previous PCR-based assay results, the fasciation is attributed to genetics/physiology rather than a biotic cause. No diseases of phytosanitary concern were found.

Amaranthus, Celosia, Chenopodium, Echinocloa, Erygnium, Foeniculum, Melilotus, Setaria, Petroselinum, and Miscellaneous Apiaceae and Poaceae:

Greenhouse inspections were conducted in spring and fall. No diseases of phytosanitary concern were noted among the 138 accessions scouted in the spring or the 122 accessions scouted in the fall.

Seed Health Testing:

Maize seed health testing was the primary testing activity with many international orders requiring testing for *Pantoea stewartii* (Stewart's wilt), *Clavibacter michiganensis* subsp. *nebraskensis* (Goss's Wilt), Maize chlorotic mottle virus (MCMV), and WSMV. The majority of seed health assays performed use ELISA as the primary detection method. Due to an EU phytosanitary requirement change in 2022, any maize samples shipped to the EU must now conform to new minimum requirements regarding the percentage of seed that must be tested to meet a detection standard of 0.5% level of infestation with a 99% level of confidence. For maize accessions with high demand the entire lot is being tested. For items with anticipated demand, sublots of 1,000 seed are being tested which allows for 9 seed orders to be distributed from the sublot. For those items with limited demand, 10% of each shipping sample is tested. This has resulted in a substantial increase in maize seed health testing for Stewart's Wilt, as few existing lots in storage meet this requirement.

To meet import requirements of various countries, the pathology team provides written additional declarations (AD) to support phytosanitary certification for seed lots shipped internationally. In most cases import permit requirements can be satisfied through field inspections, seed health testing or fungicide seed treatments. When the import conditions cannot be met, the pathologist prepares letters to assist seed requesters in obtaining waivers from regulatory officials in their respective countries. Approximately 63 seed orders required an AD and 12 orders required a waiver letter in 2022.

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>

Acquisitions:

Six accessions were acquired in 2022: 3 Amaranthus, 2 quinoa-related, and 1 Dalea.

The *Amaranthus* accessions (Ames 35903 to Ames 35905) were donated by Dr. Mitch McGrath of the USDA-ARS at Michigan State University. Both accessions are plant breeding selections for amaranth grain production.

The two quinoa related accessions (PI 701481 & PI 701482) were both collected by David Brenner. PI 701481 is a *Chenopodium album* from northern Minnesota, and PI 701482 is a *Chenopodium berlandieri* collected in the wild on a sand bar of the Des Moines River in Boone County, Iowa. In the Midwest *C. berlandieri* grows in sand along rivers. Now that the habitat is understood, a substantial gap in the collection for this crop wild relative of quinoa can be filled.

The *Dalea villosa* accession (Ames 35918) was donated by William Watson who coordinated with Jeff Carstens. This species is state listed as endangered in Iowa, and this accession is from a prairie remanent in Black Hawk County.

Collection Maintenance:

Dalea (prairie clovers):

Ten *Dalea* accessions were grown for caged seed increase in the field. Eight of them are perennials scheduled for removal after seeds are harvested in 2023. One annual accession PI 231728 was direct seeded in the field on May 13, 2022, after the viability declined and had a successful seed increase harvested on October 21, 2022. We anticipate years of regenerating *Dalea* since there is a substantial backlog of 34 accessions needing regeneration.

Panicum (proso and little millet):

The seed viability team performed a scheduled viability test of some of our oldest proso millet seed lots. Most proso seed lots from 1959 are doing very well with viability over 90%. However, 155 old seed lots with viability below 90% should be replaced in the next ten years. Fortunately, the *Panicum* millets are suited for efficient greenhouse regeneration methods, and sufficient space is available. Fresh



Samuel Flomo harvesting seeds.

replacement seeds are being produced for the oldest three seed lots from 1951, with viability between 75% and 85%. Replacement seed lots were grown for 15 old seed *Panicum* lots in 2022, and a similar number of regenerations are intended for 2023. For the millets replacing old seed lots is a priority, but not urgent since the seeds have good tolerance for long-term storage.

Petroselinum (parsley):

Parsley seed viability has fallen, with 116 accessions (62% of the parsley collection) having less than 70 percent viability. Many of the failing seed lots are 50 years old. Replacing old seed lots will be laborious as parsley has a two-year biennial life cycle requiring the use of field cages in the second year. This new parsley regeneration cycle was started with a July 2022 planting of 38 accessions using seeds from NLGRP backup storage in Fort Collins, CO.

Spinacia (spinach):

Spinach regeneration cooperator, Beiquan Mou of the USDA-ARS in Salinas, California regenerated 15 accessions of spinach in 2022 and shipped the seed lots to NCRPIS in early 2023. He planted 14 accessions in 2022 for harvesting in early 2023. A wild spinach accession was regenerated in Iowa with a seed harvest in early 2022, and in the fall, a second wild spinach was planted and one cultivated spinach for harvesting in early 2023. Plans to store 54 spinach seed lots from 2021 and 2022 in 2023 are in progress. This is 13% of the 414-accession spinach collection. Substantial effort and commitment from the Salinas group driving our spinach germplasm maintenance was greatly appreciated. In addition to increasing seeds Beiquan Mou takes notes on the presence of rare dioecious plants which are posted publicly in the GRIN-Global spinach descriptors.

Dr. Sandra Branham of Clemson University donated 738 images of our spinach accessions growing in her field. In 2022 these images were loaded in GRIN. PI 647865 is an example of a spinach accession with Branham's images.

<u>Chenopodium</u>:

Many wild species accessions of *Chenopodium* are being grown after the entire genus became a research priority for improving *Chenopodium quinoa*. Thirty-eight seed lots of *Chenopodium* were harvested in 2022, and a similar number will be grown in 2023.

Miscellaneous maintenance progress:

The 2022 field plantings included 151 rows for observation comprised of: 48 rows of amaranth, 78 rows of millets, 3 rows of sweet clover, and one row each of fennel, and perilla. These field plantings were to observe adaptation to the Iowa climate, height, and to image of accessions grown in the field. Seventeen of these rows were part of a substantial station-wide demonstration planting for the NAPB meeting in August 2022. Thirteen seed increase plantings mostly of the *Dalea* described above were also grown.



PI 700072 *Chenopodium album* seeds with a distinctive seed coat that develops only near where the seeds are attached to the mother plant. This image was taken by David Brenner with a microscope purchased in 2021.

The GRIN-Global database was enhanced with updates listed below: Undating GRIN-Global 2022

Updating GRIN-Global 2022									
Count	Name	Description							
12	Taxonomic Re-IDs	Taxonomic changes were made in seven genera. Three of the changes were in <i>Amaranthus</i> .							
16	Duplicate accessions merged	These are amaranths from Peru that were accessioned twice, once in 1984 and again in 1990.							
52	Increased Seed lots stored	Most of these were grown for regeneration in 2021. They include 33 (63%) <i>Amaranthus</i> and smaller numbers of accessions from three other groups.							
102	PI numbers	Permanent PI number identifiers were assigned to accessions with temporary Ames numbers. They include 23 parsley, 45 spinach accessions and a mix of other genera.							
386	Observations	Observations were loaded into GRIN-Global.							
423	Citations	Links to research publications were loaded onto cited accessions in GRIN-Global.							
1,934	Images loaded	Including 1,315 (68%) loaded by Samuel Flomo, 489 (25%) magnified seed images taken and loaded by Ashley Sonner, 106 <i>Chenopodium</i> images donated by Eric Jellen of BYU, and 547 spinach images donated by Sandra Branham of Clemson University.							

Stereo microscope:

A stereo microscope purchased in 2021 with a digital camera was augmented with an electronic focusing feature that is integrated with z-stacking for improved imaging. The microscope is scheduled to get enhanced lighting installed in 2023. This

microscope is suited for taking images of small seeds that are about 1 mm long. Jesse Perrett is helping with augmenting the microscope and Ashley Sonner uses the microscope for GRIN-loaded images of small seeds.

<u>Amaranthus</u>:

<u>Amaranth pre-breeding</u>: Two *Amaranthus hypochondriacus* breeding lines were released via ISU Office of Innovation Commercialization: DB 2003878 and DB 2003883 (Brenner, 2022b). They are from plant breeding with PI 584523 which is the source of their superior non-lodging stem.



DB 2003883 the two rows on the left and DB 2003883 the two rows on the right were released by David Brenner via Iowa State University. They are about 1 meter tall and lodging resistant.

Work with male sterile amaranths that can cross-pollinate 100% and make hybrid seeds is underway. This is already done commercially to get hybrid vigor in many crops including sunflowers and onions. In 2022 male sterile versions of seven agronomically successful accessions were used as females and crossed to standard male fertile accessions with the intention of finding useful combinations. Observations on these hybrids will be made in 2023.

Millets:

Dr. Asheesh K. Sing of the ISU Agronomy Department initiated a millet breeding program. In this first year of his program a large field planting of NPGS millet germplasm was grown for observation. The millet genera under study include: *Echinochloa, Eleusine*, and *Setaria*.



The new Iowa State University millet breeding group led by Dr. Asheesh K. Sing with *Echinochloa* millet during a visit by David Brenner in August 2022.

Outreach and Presentations:

David Brenner presented two talks, one on male sterility in amaranths for an Amaranth Institute virtual meeting (Brenner, 2022a) and a second at the American Society of Agronomy (ASA) meeting in Baltimore on domesticated white seeded grain *Chenopodium* accession from India similar to quinoa (Brenner & Jellen 2022). David also was a presenter in a tour of demonstration plots during the NAPB conference in August 2022.

Service:

Brenner led a field trip at the ASA meeting in Baltimore on November 6, 2022: It was a foray to historic Fort McHenry.

https://scisoc.confex.com/scisoc/2022am/meetingapp.cgi/Session/23863

David Brenner was the presenter in 4 new videos posted in GRIN U (Douglass and Brenner 2023a,b,c,d).

Plans for 2023:

Spinach disease resistance ratings:

In February 2023 Dr. Aniong Shi of The University of Arkansas provided spinach disease resistance data for *Pythium*, *Stemphylium* leaf spot, and white rust. These data are intended for loading into GRIN but, descriptors will need to be written and installed before the data can be loaded.

Images in GRIN descriptors:

A new GRIN feature allows loading images of traits such as amaranth stem and leaf colors, in the publicly available crop descriptors. Some of these images have already been taken will be loaded to illustrate how traits are observed.

Publications about our germplasm:

Baturaygil A, Schmid K. 2022. Characterization of flowering time in genebank accessions of grain amaranths and their wild relatives reveals signatures of domestication and local adaptation. Agronomy. 12:505. https://doi.org/10.3390/agronomy12020505

Bhattarai G, Shi A, Mou B, Correll J. 2022. Resequencing worldwide spinach germplasm identifies downy mildew field tolerance QTLs and genomic prediction tools. Horticulture Research. 9:uhac205. <u>https://doi.org/10.1093/hr/uhac205</u>

Bhattarai G, Olaoye D, Mou B, Correll JC, Shi A. 2022. Mapping and selection of downy mildew resistance in spinach cv. whale by low coverage whole genome sequencing. Frontiers in Plant Science. 13:1012923. https://doi.org/10.3389/fpls.2022.1012923

Brenner DM. 2022a. Amaranth male sterility. Online conference presentation. Amaranth Institute, Tennessee State University. April 11–12. <u>http://amaranthinstitute.org</u>

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D. <u>Horticulture (J. Carstens, A. Sherwood)</u>

The Horticulture project currently holds 4,052 accessions representing 194 genera (Table 1). Significant NC7-medicinal collections include: Actaea (44), Agastache (101), Echinacea (196), Calendula (85), Hypericum (222), Monarda (173), Prunella (54), and Tanacetum (53). Significant NC7-ornamentals collections include: Alcea (34), Malva (54), Phacelia (53), Potentilla (127), Sphaeralcea (90), and Thalictrum (52). Significant NC7-woody landscape collections include: Aronia (112), Betula (170), Cornus (209), Euonymus (60), Fraxinus (515), Gymnocladus (90), Rhus (101), Salix (69), Spiraea, (106), Staphylea (45), and Ulmus (44).

(metremuls, or numericals, and woody fundscupe) as of December 91, 2022									
Management group	Genera	Accessions							
NC7-medicinals	35	1,157							
NC7-ornamentals	57	783							
NC7-woody landscape	102	2,112							
Total	194	4,052							

Table 1. Active accessions maintained in the NC7 horticulture collections (medicinals, ornamentals, and woody landscape) as of December 31, 2022

During 2022, 80 accessions were added to the collection, including 28 medicinal, 6 herbaceous ornamental, and 46 woody landscape accessions to the horticulture collections. An additional 20 acquisitions (*Acer* (1), *Cannabis* (1), *Chilopsis* (1), *Chionanthus* (1), *Crataegus* (1), *Fragaria* (6); *Glycyrrhiza* (1); *Hydrangea* (1); *Liatris* (7) were also acquired through NCRPIS horticulture team collection activities (7) or through other collaborators (13) and transferred to other NPGS repositories for curation.

Targeted acquisitions were focused on collecting *Fraxinus* (9) in Arizona/New Mexico and*Monarda lindheimeri* (11) funded by the USDA-ARS Plant Exchange Office. A <u>NCRPIS Collection Trip Report (*Monarda lindheimeri*) and <u>Germplasm Exploration</u> <u>Report – Arizona and New Mexico (*Fraxinus*)</u> was published to GRIN-Global for each accession acquired during these explorations. Additional trips were made to collect *Fraxinus nigra* (6) in Wisconsin and *Morus rubra* (3) in Iowa.</u>

The following acquisitions merit recognition:

• *Crataegus wootoonia* (<u>WLP 2705</u>) was collected in New Mexico and represents one of the first *ex situ* collections of the species for the NPGS. It is listed as a U.S. Fish and Wildlife Service Species of Concern and is endemic to New Mexico.

- *Betula murrayana* (<u>Ames 35970</u>) was collected in Michigan and donated by Michael Dosmann of the Arnold Arboretum. This species is only known to occur in two sites in North America.
- *Hypericum swinkianum* (<u>Ames 35971</u>): a recently (2016) described taxon known to exist in limited distribution in the eastern portions of the Midwest. This is the first accession of this species to be deposited into the NPGS.
- Quercus prinoides (Ames 35936): this rhizomatous, densely suckering accession was collected in southern Iowa and represents the only accession of Q. prinoides in the NPGS with this growth habit.

Maintenance:

Regeneration:

Existing plantings consisting mostly of *Aronia, Agastache, Monarda*, and *Spiraea* were increased via controlled pollination and resulting seed was harvested. A total of 31 accessions were increased as seed. A total of 17 *Salix* accessions were regenerated clonally by shoot cuttings. Table 2 (Number Harvested Regen) also includes the number of original seed samples harvested (57 accessions) to keep records of harvest dates in nature.

Germinations were attempted for future regeneration on 34 accessions focused on *Coleus, Heuchera, Salix, Sorbaria, Monarda,* and *Physocarpus.*

A total of 64 accessions were transplanted to the field mostly focused on *Aronia*, *Coleus, Salix, Symphoricarpos*, and *Monarda*.

Availability and Backup:

Approximately 70% of the medicinals, 71% of the herbaceous ornamentals, and 54% of the woody landscape accessions are currently available.

Approximately 75% of the medicinals, 78% of the ornamentals, and 47% of the woody landscape accessions are currently backed up at the NLGRP in Ft. Collins, Colorado.

Viability Testing:

A total of 220 seed viability assessments were made for the horticulture collections including increase (5%), maintenance (84%) and original (11%) inventories. Maintenance tests were performed on available *Hypericum* accessions (91). A total of 63 samples of *Calendula* were tested with 35% (22 samples) scoring below 50% viable.

Research conducted on *Salix* determined seed viability can be maintained at room temperature in sealed packets for approximately three weeks. After three weeks, viability drops significantly with viability of *S. humilis, S. discolor, and S. candida* reaching nearly 0% after six weeks (Figure 1). This information will be useful for handling future acquisitions. Further research will be undertaken to evaluate the impact on seedling vigor following a decline in seed quality.

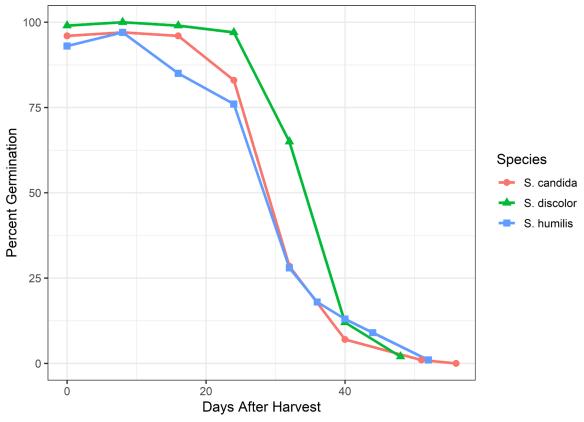


Figure 1. Viability of select Salix species following storage at room temperature

Distribution:

Distribution numbers for the horticulture collections are summarized in Tables 2 and 3, below, and Appendix Table 3. For the combined horticulture program, 115 external orders were distributed to 95 requestors totaling 463 items from 310 accessions. In addition to the 115 distribution orders, 30 orders totaling 267 items were requested by 17 recipients and represented herbarium specimens sampled by NCRPIS or found in nature. Orders in 2022 were requested for a variety of uses including breeding, additions to botanical gardens/arboretums, disease resistance screening, seed testing for the Association for Official Seed Analysts (AOSA) exam, anthropological research, and restoration projects requiring known provenanced germplasm. Notable requests include those involving genetic analysis of *Fraxinus* and *Morus rubra*.

Таха	Most distributed (greatest to least)
Medicinals	Echinacea
	Agastache
	Calendula
	Tanacetum
	Monarda
Ornamentals	Phacelia
	Potentilla
	Anemone
	Althaea
	Malva
Woody Landscape	Fraxinus
	Morus rubra
	Salix
	Aronia
	Viburnum

Table 2. Frequency of distribution by taxa sent in 2022

Table 3. External domestic and international germplasm distributions 2018-2022

Сгор	Year	No. of Orders	No. of Recipients	No. of Items Distributed	
Medicinals	2018	44	36	218	169
	2019	5 3	47	404	293
	2020	44	42	267	196
	2021	36	36	338	269
	2022	30	30	179	168
	Average	42	39	288	221
Ornamentals	2018	46	40	117	93
	2019	22	21	63	55
	2020	30	28	108	100
	2021	21	21	83	67
	2022	19	19	42	42
	Average	28	26	87	75
Woody Landscape	2018	82	68	327	164
	2019	72	58	206	154
	2020	59	48	202	138
	2021	78	61	247	144
	2022	78	61	242	100
	Average	74	59	249	142

Characterization/taxonomy:

Eight horticulture accessions *Amelanchier* (1), *Forestiera* (4), *Fraxinus* (1); *Quercus* (1); and *Teucrium* (1) were re-identified based on updated taxonomy and/or morphological characteristics. No PI numbers were assigned.

Evaluation:

A common garden study/evaluation plot of select *Gymnocladus dioicus* (Kentucky coffeetree) accessions was established in 2017. This evaluation plot 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.

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

A common garden study/evaluation plot of select *Hydrangea quercifolia* accessions (150 specimens) was established in 2020. This evaluation plot includes 15 wild-collected accessions from across the species native range represented as 10 specimens per accession, planted in a completely randomized design. The main goal is to identify accessions of *H. quercifolia* with superior qualities including fall color, growth habit, floral display, and cold hardiness.

A total of 1,707 records were attached in GRIN-Global as either images (1,499) or as documents (208) including publications, collection trip reports, viability cards, permits, field maps, passport data, and USDA Natural Resources Conservation Service (NRCS) release documents.

A total of 1,226 observations were entered in GRIN-Global for MONARDA and ULMUS. The *Ulmus* data was extracted from historical publications, which were also added to GRIN as citations.

Enhancement:

An interspecific cross between *Agastache foeniculum* x *Agastache scrophularifolia* was acquired in 2020 as <u>Ames 35585</u> and prompted efforts to manually recreate this cross. In 2021, *A. foeniculum* (<u>Ames 35586</u>) originating from southeastern Montana and *A. scrophularifolia* (<u>Ames 35234</u>) originating from southeastern Minnesota were crossed via isolated hand pollinations resulting in four individual specimens with confirmed intermediate morphological characters. Plans are to write a description of the hybrid, designate a type specimen to be deposited at the US National Arboretum Herbarium, and determine whether a name for this nothospecies is warranted.

Coordination of the NC-7 Regional Ornamental Trials:

The Horticulture project distributed three accessions in 2022: *Betula lenta* (Ames 31054 and Ames 34218) and *Populus grandidentata* (Ames 33965) to a total of 13 sites for a total of 35 items. Select accessions of *Magnolia acuminata* (1), *Betula lenta* var. *uber* (2) are currently being grown for future distribution.

Posters, Presentations, and Seminars:

Activities involving conferences and tours were highly restricted or cancelled in 2022 due to COVID-19 pandemic protocols.

Conclusion and Plan for 2023:

Similar to 2021, the 2022 growing season was a very dry season at NCRPIS. Approximately 150 more hours were spent on watering compared to an average year.

E. <u>Maize Curation (V. Bernau, M. Millard, S. Armintrout)</u>

Project Management:

Curators Mark Millard and Dr. Vivian Bernau are assisted by a full time USDA-ARS Agriculture Science Research Technician, Samantha Armintrout.

Acquisitions:

In 2022, 159 new accessions were acquired. These included seven GEM lines, 46 PVPs expiring in 2023, 66 inbreds donated by Illinois Foundation Seed Inc., and 31 accessions submitted to the Journal of Plant Registrations.

Outreach:

More than 100 NCRPIS maize ears were sent to the United States Botanic Gardens (USBG) in 2021 for a new temporary exhibit, "Cultivate: Growing food in a changing world" (Figure 1). This exhibit opened in July 2022 and will be on display until December 2023. The ear display will be sent to NCRPIS at the end of the exhibit.



Figure 1. Maize diversity display at the US Botanic Garden

Maintenance:

There were 20,120 accessions of *Zea* in the active collection at the NCRPIS as of January 1, 2023. The maize curators maintain an additional 41 accessions from the *Coix* and *Tripsacum* genera for a total collection of 20,161 accessions (Figure 2).

There were 15,339 available Zea accessions held at the end of 2022 (76.12% of the total). This represents a 1.5% reduction in availability, due largely to several years with smaller nurseries as well as the identification of distribution lots with low viability identified through increased testing. Low viability dictated making 146 accessions unavailable. A total of 172 new distribution lots were made available in 2022. Progress would not be possible without in-kind regeneration assistance. Sherry Flint-Garcia, Jim Holland, Wenwei Xu, Kristin Mercer, and the GEM programs in North Carolina, and Iowa are appreciatively acknowledged.

Accession Backups:

A total of 945 accessions were backed up at the NLGRP in Fort Collins in 2022. This compares with 868 in 2020 and 383 in 2021. There are now 16,555 accessions (82% of the total) with backup samples at NLGRP. 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. Backup samples of 226 accessions were also sent to the Global Seed Vault at Svalbard.

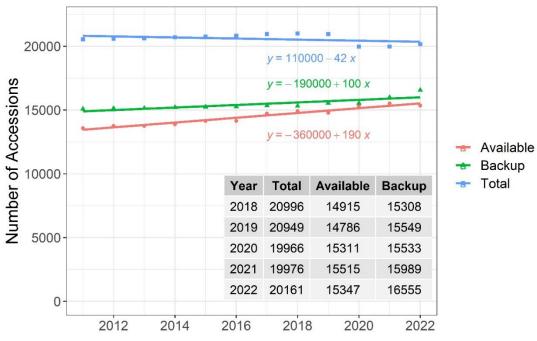


Figure 2. Collection size, availability, and backup status over the last 10 years.

<u>Regenerations</u>:

Regenerations were attempted on a total of 650 accessions in 2022, compared to 667 in 2021. As of December 2022, 290 harvests had been made during the calendar year.

No Stewart's wilt was observed in any increase plots in 2022, as in every year between 2010 and 2022. ELISA testing is still necessary on Ames increase lots to meet foreign phytosanitary requirements because the state cannot be declared Stewart's wilt free.

As of April 2022, the EU updated the additional information needed to satisfy the additional declaration requirements for the presence of Stewart's wilt. In short, testing must be conducted according to International Standards for Phytosanitary Measures (ISPM) 31 standards and satisfy the 99% confidence interval and the 0.5% detection rate. Moving forward, all new lots will be tested at the higher level prior to storing. The distribution lots of high demand accessions are being re-tested to meet this higher standard, and other items requested will be tested on the shipment level to satisfy the requirement.

Greenhouse increases included 24 inbred accessions and two landraces planted in January 2022. One accession of *Z. perennis* (Ames 21890 2021ncab01 SD) was carried over from the previous year for a second harvest. The second harvest of Ames 21890 was moderately successful.

Two additional accessions of teosinte were planted in May 2022 (*Z. mays* subsp. *mexicana*, PI 331779 and *Z. mays* subsp. *huehuetenango*, PI 343233). PI 331779 began flowering at the end of September 2022, and PI 343233 began flowering at the end of November 2022. Harvests of both accessions were quite poor, partly due to greenhouse spider mite infestations.

In total, two wild accessions were made available in 2022, in addition to two in 2021. With the regeneration of more wild maize, feedback from stakeholders on standard traits that should be measured is being pursued.

A tropical nursery consisting of 112 mid-altitudes to lowland accessions was sent to a custom winter nursery provider near Puerto Vallarta, Mexico in the fall of 2021 and harvested in early 2022. Seed was received on the ear in March 2022. The quality of the increase was above average with 45% of increases having greater than 100 ears, 35% 50-100 ears, and 20% less than 50 ears. It appears germination was the biggest factor in the smaller harvests. Because opportunities to grow good quality seed from U.S. southwestern landraces in Ames are infrequent, a number were tried in this nursery without success. Accessions from higher altitudes often show more ear rot at this location. Accessions from Central America (Costa Rica, Honduras, and Panama) were especially successful at this location as were several Brazilian accessions with apparent race Tusón influence. There were some very good Mexican Tuxpeño increases also.

In previous years we were unable to obtain a re-export phytosanitary certificate necessary to send seed grown in Mexico back to Mexico for planting. Following more conversations with APHIS and our state phytosanitary certifier, we were able to get the necessary phytosanitary certificate. However, the shipment was rejected by SENASICA (National Agro-Alimentary Health, Safety and Quality Service in Mexico) upon arrival in Mexico because it lacked a copy of the original export phytosanitary certificate, a document which did not exist at the time of import from Mexico. A resolution to this issue is being explored. This nursery was set up to assist in regenerating accessions that would grow best in the tropics. Seed of several thousand accessions grown in Peru, Mexico, and Colombia in the 1986-1995 exist in our collection. APHIS has suggested proceeding with shipping a small nursery, as they are unable to address the issue with SENASICA until there is a shipment in consideration.

St. Croix grew 15 accessions in the 2021-2022 winter cycle and 17 accessions in the 2022 summer cycle. Seed was received on the ear in February 2022 and October 2022, respectively. 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.

In-kind regenerations were initiated in 2022 by Jim Holland (USDA-ARS, six accessions), Sherry Flint Garcia (USDA-ARS, nine temperate populations), Bill Tracy (University of Wisconsin-Madison, one temperate population) and Kristin Mercer (Ohio State University, two accessions of wild maize). In most cases these have been successfully harvested and will be transported to Ames in early 2023.

Viability Testing:

There were 3,557 accessions tested for viability in 2022 (18% of the collection). A less resource-consuming viability testing plan was implemented in 2018 using two replications of 10 seeds followed by standard testing (four replications of 50 seeds) for accessions with low viability. This testing plan continues to be used and has provided better information for curators as they choose planting quantities for the increase nurseries.

The current distribution lots of 24% of the available accessions (and 28% of the whole collection) were grown in 1990 or earlier; with further maintenance viability testing more accessions are expected to be made unavailable (Figure 3).

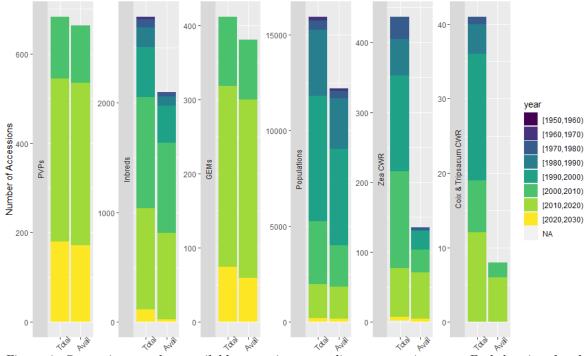


Figure 3. Comparison total to available accessions according to accession type. Each bar is colored according to the approximate age of the current or last distribution lots.

Characterization and Evaluation:

There are now images uploaded to GRIN for 82% of accessions in the maize collection. Just 66 images on 29 accessions were loaded to GRIN in 2022, compared to 31,377 images on 14,235 accessions loaded in 2021. This increased the maize images on GRIN to 42,944 images on 16,613 accessions. Major efforts on increasing the availability of images on GG have been made over the last three years.

There were 11,549 data points on 492 accessions loaded to GRIN in 2022. This compares with 36,697 data points on 6,200 accessions loaded to GRIN in 2020 and 4,735 data points on 350 accessions loaded to GRIN in 2021. In 2022 365 accessions were imaged compared to 1,701 accessions in 2020, and 400 accessions in 2021. Field regenerations have been mostly limited to high priority inbreds and PVPs, which do not generate as many data points as populations.

Germplasm Management:

In 2022, 29 maize accessions were inactivated. Inactivation of an accession occurs when it has been determined by the curator of the Crop Germplasm Committee (CGC) that the accession cannot or should not be maintained as part of the active inventory of the NCRPIS. Accessions can also be inactivated if they are duplicates of an earlier received accession—this was the case for 25 of the maize accessions inactivated.

PI numbers were assigned to 40 maize accessions in 2022. Preparations for PI number assignment include proofing and updating of all passport data, determining that there are no duplicate accessions, verifying accession viability, and determining whether any Material Transfer Agreement (MTA) or IPR restrictions apply to the material.

Curator Vivian Bernau traveled to Wooster, Ohio in October 2022 at the invitation of David Francis to review maize germplasm holdings at the Ohio State University (OSU). The cooler in one storage room (holding primarily breeding populations and landraces) failed several years ago. The cooler in a second room (holding primarily inbreds) remained functional but lacked humidity control. Inbred lines stored in sealed plastic jugs were prioritized for rescue. Material brought back to NCRPIS has not yet been inventoried or tested for viability. It was determined some recombinant inbred lines (RILs) from the program were sent to Maize Genetic Stock Center (MGSC), and some inbreds have been incorporated into the collection of the USDA-ARS Corn Virus breeding program in Wooster.

Dr. Bernau also traveled to Columbia, Missouri in December 2022 to assist Sherry Flint-Garcia with reviewing University of Missouri germplasm for donation to NCRPIS. Flint-Garcia conducted preliminary germination tests indicating material grown in the 1950s is still viable.

Distribution:

Overall, order items filled from the maize collection items increased by 1% in 2022 relative to 2021 (Figure 4). The number of total and foreign orders, recipients, and accessions ordered fell slightly relative to 2021. The number of foreign orders fell by 28%--likely due to the increased stringency on Stewart's wilt testing for shipping to the EU. Foreign and total requests for *Coix*, *Tripsacum* and wild *Zea* fell relative to

2021. There were no foreign requests for *Coix* or *Tripsacum* filled. PVPs were included in 50% of all *Zea* orders and 63% of foreign *Zea* orders.

Almost all orders are now entered by requestors in GG. Expired PVP-lines continue to be a major maize distribution category followed by NAM (Nested Association Mapping) 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 GG and the hard work of Stacey Estrada, Lisa Burke and their teams in order processing and seed storage.

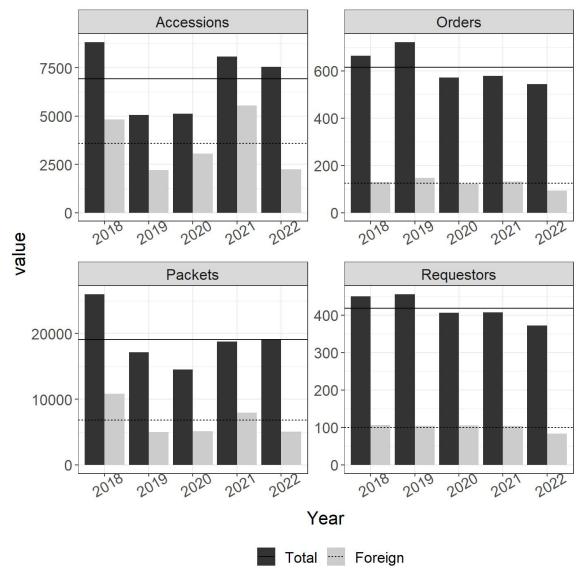


Figure 4. Distribution statistics over the last five years for the maize collection. Total distributions are represented by the black bars with the average across five years represented by the black lines. Foreign distributions are represented by grey bars and the dotted lines.

Plans for 2023:

Management of regeneration nursery and seed processing will be a priority again in 2023. The highest priority is processing PVP accessions prior to their expiration dates so they can be shipped to stakeholders and included in breeding nurseries.

Transfer of tropical maize seed lots from NLGRP for incorporation into the maize collection will be resumed. In October 2019, an initial batch of about 1,200 tropical maize seed lots were transferred from NLGRP, but processing was disrupted by COVID-19 restrictions. From this transfer 431 accessions have been made available for distribution; 81 others have been used as parent lots for tropical increase nurseries. Processing includes: 1) adding an accession and/or inventory to the GG database, 2) incorporating passport data (typically from CIMMYT's germplasm database), 3) collecting images of the seed, 4) confirming identity with other sources, 5) checking viability, and 6) packaging a critical backup sample to be sent back to NLGRP. The transfer of seed lots with >2,500 seeds are being prioritized as part of this long-term project.

National Storage Laboratory (NSL) and Ames numbered accessions will continue to be reviewed, and PI numbers assigned. Over 1,037 Ames-numbered and NSLnumbered accessions available for distribution need to be reviewed and considered for permanent PI numbers in 2023. Accessions that have not been successfully propagated will also continue to be reviewed for inactivation.

Germplasm will continue to be acquired from public collections. Material acquired from Ohio and Missouri will be inventoried and evaluated for viability.

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

Project Management:

Curator Dr. Laura Marek (ISU Research Scientist III) was assisted by Grace Welke, ISU Research Scientist III, and Jeff Schwartz, USDA-ARS Agricultural Research Technician. John Reinhardt, ISU Farm Equipment Operator, worked with the NCRPIS Oilseed Project through March. The Oilseeds Project was also supported by a team of hourly student workers. Due to pandemic effects on scaled-back operations, oilseed regenerations were 30% lower compared to 2019 and focused on fewer taxa and those with straightforward regeneration requirements.

Acquisitions:

The oilseed project received 47 new accessions in 2022: 25 *Camelina macrocarpa* and 10 *Thlaspi arvense* collected by Jordan Brock primarily in the western US; three *Eupatorium perfoliatum* collected in Iowa and donated by Laura Walter; one *Helianthus petiolaris* ssp. *petiolaris* collected by Andrew Hopkins in Kansas; two wild *H. annuus*, one collected in Arizona by the Borderlands Restoration Network and one collected in Iowa by Laura Marek; and six cultivated *H. annuus* inbred lines developed by Lili Qi.

Maintenance:

General statistics about availability and management of the collections are presented in Appendix Tables 1 and 2. Selected details for oilseed accessions increased during 2022 are noted below.

Helianthus, Ames regenerations:

Cultivated *Helianthus annuus* accessions are 93% available for distribution. Regenerations were managed to ensure core collection accessions and accession subsets of interest to specific stakeholder groups remain available. In 2022, 40 cultivated *H. annuus* accessions were attempted for regeneration. One accession succumbed to disease mid-summer and all plants were removed. Seed heads were harvested from the remaining 39 plots which were either grown in screened cages with added pollinators or grown in rows with hand pollination and bagged seed heads. These techniques are illustrated in Figure 1.

Wild annual *Helianthus* accessions are 95% available and wild perennial accessions have 87% availability. Twenty-eight wild annual sunflowers were attempted for regeneration in the field in Ames in 2022 and seeds were harvested from 27 plots. Seedlings of one accession were not hardy and did not survive transplant to the field. Seven perennial accessions were attempted for regeneration in 2022 and five new plots were established. Six perennial plots were maintained from 2021 and seeds were harvested from five of the plots. Six accessions did not flower in time to produce mature seed before the first freeze.

Typically, several oilseed accessions requiring long seasons or short days to flower are increased in a NCRPIS greenhouse during the winter. However, no sunflowers were started in the greenhouse for the winter 2021-2022 seasons; all available space was needed for winter flowering *Brassica* regenerations. One accession of the wild annual species *H. debilis* ssp. *debilis* developed for ornamental uses and dune restoration is maintained clonally in the greenhouse and distributed as vegetative clones.



Figure 1: Illustration of the three techniques used for field regeneration of sunflowers at the NCRPIS. From left to right: large (10x10x20 ft) screened cage, small (7x7x20 ft) screened cage, rows with bagged heads. Cultivated sunflowers are grown using all three methods while wild sunflowers are only grown in cages. The smaller sized cage is also used for Brassicaceae and Flax regenerations.

<u>Miscellaneous asters; Ames regenerations</u>:

The miscellaneous asters are 34% available. No regeneration attempts were made during 2022 for the Ames location.

<u>Parlier alternate grow-out site regenerations for *Helianthus* and Miscellaneous asters:</u>

A continuing partnership with the National Arid Lands Plant Genetic Resources Unit (NALPGRU) in Parlier, CA allowed for regeneration of wild taxa requiring a longer growing season than can be reliably obtained in Ames, as well as species poorly adapted to midwestern humidity and heavy soils. The Parlier location utilized pollination cages purchased by NCRPIS and can grow up to 40 NCRPIS oilseed accessions per year. Seeds were germinated in Ames and live seedlings were shipped to Parlier in late March and early April. Parlier staff transplanted the seedlings and grew the plants to maturity. Following the protocol used in Ames, plots were caged prior to flowering; insect pollinators were introduced (honeybee services were also supported with Ames resources); and seed heads were harvested as they matured. Harvested material was shipped to Ames for threshing and processing.

In 2022, seedlings of 39 wild sunflower accessions were shipped. Harvested material was returned for all accessions. Final harvests were made for *Encelia californica*, a population of miscellaneous aster, sent in 2020.

The Parlier staff recorded basic field observations (transplant, flowering, and harvest dates) as well as captured images. However, resources were not available to image all accessions or to record standard descriptor data. Ray and disc flower color, plant height, and branching characteristics, which are valuable phenotypic components used to assess species identity, should ideally be captured. Dr. Marek traveled to Parlier in September 2022 to record data and examine plants for species confirmation.

An excellent partnership with the NALPGRU staff ensured successful regenerations of many wild taxa. The dedicated efforts of Dr. Claire Heinitz and Mr. Juan "John" Jimenez agricultural research technician were gratefully acknowledged.

Brassicaceae regenerations:

Brassicaceae accessions are 90% available.

Brassica accessions identified with low viability continued to be prioritized for regeneration. Maintenance viability testing started in 2016 and completed in 2020 revealed a decrease in viability. Viability of some seed lots decreased by as much as 80% during the six- or 10-year interval since the prior testing with 460 lots at 50% or lower viability and an additional 388 falling between 50 and 70% viability during this interval. Regeneration of 368 of the accessions with low viability was completed between 2018-2022 starting with those accessions with the lowest viability. Seed for regenerations was obtained from the Fort Collins germplasm back-up repository.

Of the distribution lots with low germination, most accessions were B. napus (83%) and most were winter flowering types requiring a vernalization period to induce flowering. In past trials in Ames, most winter flowering accessions did not survive overwintering in the field; therefore, winter flowering types are started in the

greenhouse and flowering is induced in a NCRPIS vernalization space. In December 2021, 75 *Brassica* accessions which were either winter flowering types or had unknown vernalization requirements were sown; 73 accessions germinated. Accessions were maintained in the greenhouse until mid-February 2022 and then moved to vernalization rooms except for two accessions which flowered without vernalization and were caged and maintained in the greenhouse. Vernalized plants were transplanted into the field during April. Thirteen spring flowering type accessions were started in April 2022 and the 12 which germinated were transplanted to the field after the transplanting of the vernalized winter flowering type plants was completed. All 85 field plots were harvested and seed processed.



Figure 2. Illustration of plot preparation prior to transplanting (left) and successfully transplanted and flowering Brassica napus PI 469754 (right).

NCRPIS Greenhouse 2 is managed in the winter to provide conditions that approximate a Mediterranean climate allowing regeneration of Brassicaceae accessions native to that region, and to grow other Brassicaceae taxa which flower very early in the growing season before field preparations can be reliably started in the spring.

In fall 2021, we started 12 accessions, three of which did not germinate. Plants of *Berteroa incana* (two accessions), *Camelina laxa*, *Camelina sativa*, *Eruca vesicaria* ssp. *sativa*, *Lepidium sativum* (two accessions) and *Thlaspi arvense* grew well and were harvested in 2022. One accession of *Erysimum gomez-campo*, started in fall 2020, did not flower winter of 2021 and was moved to the field. In fall 2021, plants were dug from the field and kept in a vernalization room for three weeks and returned to the greenhouse in early winter where they began to flower and ultimately set seed. Similar to the *Erysimum gomez-campo* accession started in 2020, an accession of *Erysimum witmannii* started in fall 2021, did not flower in the greenhouse in winter 2022 nor after transfer to the field and plants were put into a vernalization room. Based on previous experience with these two *Erysimum Brassica* spp. are handled, may allow *Erysimum* accessions to flower in the greenhouse in one season.

In fall 2022, 14 accessions were started in Greenhouse 2, one of which did not germinate. Plants of *Camelina sativa*, *Eruca vesicaria* ssp. *sativa*, *Thlaspi arvense* (10 accessions), and *Noccaea perfoliata* will be ready to harvest in 2023.

Linum regenerations:

Cultivated flax accessions are 99% available; wild flax accessions are 76% available. Ninety-seven percent of the 2,834 accession cultivated flax collection was transferred to Ames in 1998/1999 and were of uniform seed age. Due to declining viability, 45 accessions of cultivated flax are being regenerated each year, including 2022, with priority given to accessions with lowest viability. The long-term goal for all distribution lots is 80% or higher viability. Seven new wild flax accessions were started in 2022, five of which did not produce seeds. Two plots of accessions, started in 2021 and over-wintered, flowered and successfully produced seeds.

Cuphea regenerations:

No *Cuphea* field regenerations were established in 2022. Seeds are available for 96% of the accessions of seven species (*Cuphea calophylla*, *C. carthegenensis*, *C/lanceolata*, *C. lutea*, *C. tolucana*, *C. viscosissima*, *C. wrightii*) and for the *Cuphea* hybrid accessions that were part of agronomic development efforts of the now inactive National *Cuphea* Consortium. Thirteen accessions of *Cuphea* are maintained as clones in the greenhouse and distributed as vegetative cuttings. Overall, the *Cuphea* collection is 80% available.

Euphorbia regenerations:

The *Euphorbia* collection (210 accessions) is 49% available. The taxon of interest for seed oil production within this genus is *Euphorbia lagascae* and its accessions are 93% available. No *E. lagascae* accessions were attempted in 2022. Six *Euphorbia* accessions are maintained as clones in the greenhouse and distributed as vegetative cuttings.

Distributions:

One hundred seventy-nine unique orders containing 9,522 oilseed packets were shipped in 2022. General statistics about oilseed distributions are presented in Appendix Table 3. A summary of the distributions separated by international and domestic distributions is presented in Table 1. below. Although the NCRPIS was able to fill and ship most seed orders during and after the pandemic, international shipments were halted for months during 2020, a backlog of inspections for issuance of phytosanitary certificates still exists at the national office. Oilseed orders shipped in 2022 were less than 2021 and roughly 20% fewer items were distributed.

	(orders may contain items from more than one crop category)													
	shipped		international	international	international	domestic	domestic	domestic	total					
Сгор	orders 2022	packets	orders	packets	requestors	orders	packets	requestors	requestors					
Brassicaceae	83	4652	34	2342	32	49	2310	30	62					
Cuphea	3	20	0	0	0	3	20	3	3					
Euphorbia	0	0	0	0	0	0	0	0	0					
Helianthus	85	4374	28	1986	22	57	2388	42	64					
Linum	8	462	2	277	2	6	185	6	8					
misc asters	6	14	3	5	3	3	9	3	6					

Table 1. 2022 Distribution Summary(orders may contain items from more than one crop category)

<u>Helianthus</u>:

Roughly 34% of the total Helianthus items distributed in 2022 were sent to seed companies or other commercial entities at international destinations; 7% of the total distributions went to domestic commercial entities, reflecting the current locations of most of the commercial sunflower breeding programs; 47% of the total distributions were sent to public institutions, primarily universities and national institutes, at domestic locations. One research program at the Danforth Plant Sciences Center received 20% of the sunflower distributions in 2022.

Sunflower packets were sent to support breeding programs (55%), a broad range of physiological based assessments (25%), and pathology research (17%), with smaller numbers for botanical or taxonomic investigations, and anthropological research. Fifty-eight percent of the total distributed packets were of cultivated *H. annuus* accessions, 42% were wild sunflower species including wild *H. annuus*, a progenitor of the cultivated crop.

Brassicaceae:

The genus *Brassica* accounted for 74% of the 2022 Brassicaceae distributions (3,444 total packets) followed by *Camelina* with 315 packets sent. The diversity present in the Brassicaceae collection (262 taxa across 21 genera) supports a very wide range of research purposes including genetic studies, pathology research related to disease resistance, varietal development and other breeding related discovery, flowering control research, ploidy level investigations and glycosylate analyses.

Linum:

Sixty-seven percent of the flax packets distributed in 2022 were cultivated flax accessions and the most common stated use was for breeding (four different programs). Wild accessions were sent to a researcher investigating seed mucilage and to a program developing new perennial oilseed crops.

<u>Cuphea</u>:

Cuphea accessions were distributed in 2022 to two different research programs. One expressed the intent to study seed oil profiles and the other stated evaluation as a research purpose.

Euphorbia:

No Euphorbia accessions were distributed in 2022.

Miscellaneous asters:

Miscellaneous aster accessions were distributed in 2022 for breeding programs, for seed reference and identification, and for genomic sequencing directed toward understanding aspects of C4 photosynthesis.

Research Activities:

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

<u>Helianthus</u>:

Observations: landrace collection from Spain: In 1990, the NCRPIS received a collection of 94 Spanish landrace lines from CIDA Cordoba. Some of these accessions are not available due to low inventory quantities. To facilitate future regenerations, in spring 2022 we planted all 94 accessions in a common plot with one 15' row for each line to obtain Ames specific information about flowering time, branching characteristics and plant height. The plants grew well and 92% flowered by September 6th, suggesting most would produce seeds during years with a first frost no earlier than the mid-October average. Thirty accessions, however, were taller than 9.5 ft and could not be regenerated in our largest cages. Half of those accessions had plants taller than 11.5 feet meaning bagging heads and hand pollinating using ladders would also be problematic. Two accessions had plants taller than 14 ft. In fall 2022 we planted seeds of six of the tallest growing accessions in pots in the greenhouse to test the idea that longer nights might trigger flowering before the plants would get so tall, without having to use hormone applications which have been problematic in the past. The plants did not flower before year's end but neither did they grow impossibly tall, and they remain in the greenhouse.

Brassicaceae:

Brassica rapa flowering type evaluation: Evaluation of the 675 accession NCRPIS *Brassica rapa* collection for flowering type (winter or spring) is currently in process. Knowing whether vernalization is required to induce 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. The evaluation process involves starting seeds in the late fall and growing 12 plants per accession in NCRPIS Greenhouse 1 through late winter, discarding accessions as all plants bolt and flower. Accessions are scored as winter flowering type if no plants flower during the scoring period. In 2021-2022, 100 accessions were evaluated of which 80% were spring type. In early December 2021, a set of 125 accessions was started. Observation priority was determined based on viability data; lowest viability accessions were evaluated first so those accessions could be incorporated into regeneration activities. After the 2022-2023 set are scored, roughly 150 accessions will remain to be screened. Scoring is final for 420 accessions and of those, 75% are spring type, whereas the *B. napus* collection is about 80% winter type. About 55 accessions need to be re-tested.

Publications:

Lee JS, Jahani M, Huang K, Mandel JR, Marek LF, Burke JM, Langlade NB, Owens GL, Rieseberg LH. 2022. Expression complementation of gene presence/absence polymorphisms in hybrids contributes importantly to heterosis in sunflower. J. Adv. Res. 42:83-98.

Service Activities:

Tours:

Oilseeds planted and maintained 15 plots of 31 accessions in the NCRPIS 2022 demonstration garden in place for the tour by the NAPB annual meeting in Ames.

Journal peer review:

Dr. Marek served as a peer reviewer for one submission to Oilseeds and Fats, Crop and Lipids.

PGOC:

Dr. Marek served as a member of the PGOC GIS and Geo-referencing Subcommittee and the Molecular Marker Subcommittee.

AOSCA:

Dr. Marek served as a member of the Sunflower Variety Review Board. In 2022 the committee reviewed 41 applications, seven of which she was the lead.

USDA NIFA:

Dr. Marek was a reviewer for a NIFA grants panel meeting for two days in August.

G. <u>Vegetables (K. Reitsma, B. Chapman, 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, Acquired, Available, but are also summarized specifically for the Vegetable Project in the table below.

The Vegetable project is led by Curator Kathy Reitsma and assisted by Brandyn Chapman (full-time ISU Agricultural Specialist II) and Cole Hopkins (half-time ISU Agricultural Specialist I, shared with the Farm Management team). We are assisted by 3 to 6 ISU students part-time during the year equaling approximately 3 FTE.

									Total			T . 1
					Percent				Number	Percent	Accns	Total
Site Crop		Number			Avail	PI	Ames	NSL	Backed	Backed	Sent to	Backed
(Maintenance	Number	Accs	Number	Percent	Last	Number	Number	Number	Up at	Up at	Svalbard	Up at
Policy)	Accs	Acquired	Available	Available	Year	Accs	Accs	Accs	NLGRP	NLGRP	for YR	Svalbard
NC7-chicory	285	0	258	91	89	231	28	26	262	92	11	190
NC7-cucumis.cucs	1401	0	1335	95	95	1230	143	28	1335	95	27	1125
NC7-cucumis.melo	3252	2	1982	61	59	2908	283	35	2632	81	0	1054
NC7-cucumis.wilds	318	0	223	70	69	245	73	0	226	71	0	73
NC7-cucurbita	984	3	734	75	73	888	90	6	839	85	78	376
NC7-daucus	1565	1	1298	83	82	968	567	30	1370	88	0	703
NC7-ocimum	108	2	100	93	94	95	13	0	102	94	0	76
NC7-parsnips	73	0	58	79	79	52	19	2	58	79	0	33
Totals	7986	8	5988	74	74	6617	1216	127	6824	85	116	3630

Acquisitions:

Eight new vegetable cultivars/breeding lines were acquired in 2022. An old *Cucurbita pepo* ornamental gourd variety 'Bicolor Spoon' was received from the NLGRP for incorporation into the active collection in Ames. The seven other new accessions were

expired PVPs received from the NLGRP: two accessions each of *Cucumis melo* ('XLT-86', 'M98'), *Cucurbita pepo* ('Bush Delicata', 'Little October') and *Ocimum* ('Pesto', 'Sanremo') and one accession of *Daucus* ('BetaKing').

Maintenance:

Data for vegetable crop regenerations attempted and number of accessions harvested in 2022 are summarized in the appendices in Table 2: NCRPIS Accessions Germinated, Regenerated, Made Available, Backed Up.

<u>Regenerations</u>:

The NCRPIS Vegetable Project regeneration program remained scaled back in 2022 due to restricted budget, personnel availability, and COVID-19 pandemic related restrictions.

<u>Cichorium</u>: Regenerations were attempted on 35 Cichorium (6 C. endivia, 29 C. intybus) accessions having low seed viability. Thirty-one accessions were harvested. Four accessions had poor germination and were not carried through for field cage increases. One C. intybus accession had a small population of plants in a field cage but the seeds harvested were of very poor quality and were discarded. Expired PVP Cichorium endivia PI 544067 ('Priscilla'), bolted in late September so plants were dug from the field cage, transplanted to pots in the greenhouse for continued regeneration over the winter, but failed to produce viable seeds. Twenty-one accessions produced inadequate seed quantities due to poor plant populations or irregular bolting and flowering. These accessions will be regenerated again in 2023 with resulting seed increases to be bulked with the 2022 increase lots to replace existing distribution lots with declining seed viability.

<u>Cucumis sativus</u>: Availability of the cucumber collection is at 95% with 67 accessions unavailable for distribution. The unavailable accessions include four accessions that will be inactivated (exhausted seed supply, unable to maintain), and the remaining 63 accessions will require regeneration in the greenhouse to accommodate the need for a longer growing season, short day-length, or the application of growth regulators to address male-female flower ratios. Forty-seven cucumber accessions were planted for field cage regeneration in 2022 and focused primarily on accessions having old distribution lots. Successful seed increases were secured on 37 accessions, 29 of which produced significant seed quantities between 10,000 and 43,000 seeds. Regenerations were not successful on ten accessions - three of which failed germinate, and seven which flowered in late July and early August, producing few fruits/seeds before frost. Alternate parent seed lots will be used in 2023 to increase the three accessions that failed to germinate, and the late flowering accessions will have to be regenerated in the greenhouses in the future.

<u>Cucumis melo</u>: No melon accessions were regenerated in 2022.

<u>*Cucumis spp.*</u>: Two perennial wild melon accessions were started in the greenhouse in December of 2019 and transplanted to field cages in 2020, 2021, and 2022 for continued regeneration because the plants produced few fruits/seeds each year. The *C. heptadactylus* and *C. hirsutus* accessions have dioecious (separate male and female) plants which produced small (3 cm x 6 cm) fruit. Though the vines grew well in the field cages, only a few plants in the population were female and produced few flowers and fruits. The 2022 harvest of the *C. heptadactylus* appears to have produced a sufficient quantity of seeds allowing the accession to be made available for distribution when bulked with the seed increases from 2020 and 2021. The 2022 increase of the C. hirsutus was not sufficient, so plants were dug from the field cage at the end of the season, potted, and maintained in the greenhouse over the winter to be again transplanted to a field cage in 2023 for continued regeneration. Unlike other cucurbits, seeds of *C. heptadactylus* appear to lose viability rather quickly over a ten year period in the 4° C seed storage room. Storing seeds of these two perennial species in the -20° C freezer may improve long-term seed viability and availability.

<u>Cucurbita</u>: Thirty-one accessions of pumpkin/squash were regenerated in 2022. One accession failed to germinate. Plants of another accession were destroyed due to the high incidence of seed transmitted SqMV from the parent seed lot, a subsample of which will undergo dry-heat treatment in an attempt to inactivate the virus and hopefully get a clean seed regeneration in 2023. Six accessions produced female flowers late in the season resulting in zero to few fruits at harvest in mid-October. These accessions will require greenhouse regeneration where short daylength and/or a longer growing season can be provided. Harvests on the remaining accessions were generally very successful producing significant quantities of fruits which were stored until seed extraction in December to allow for continued seed maturation.

In 2020, we switched from harvesting and storing the fruits in multiple rubber trugs/hard plastic bushel baskets per accession to using plastic-lined collapsible and stackable wire crates that are hauled to and from the field on a flat-rack. In most cases, one crate holds the entire harvest from one accession.



Twenty-four crates of squash/pumpkins stacked and stored until seed extraction.

Use of these crates increased the efficiency of the harvesting process and optimized the use of the limited storage space for the fruits. We currently have eight large (48" x 40" x 42.5") crates and twelve small (40" x 32" x 35") wheeled crates for use. Using

these crates saved personnel a lot of work as the forklift was used for most of the heavy lifting. The wheeled crates made transporting fruits to the processing area more convenient. The wire crates were lined with a heavy plastic bag to prevent possible contamination and keep the facility clean in case fruits began to decline during storage.

All seeds have been processed and should be made available for distribution after viability testing in April 2023.

Daucus: Regenerations focused on annual wild species of carrot for which regeneration protocols are still under development (there is little information in published literature to provide guidance). Seeds of 37 accessions were treated with hot water to eliminate pathogens on the seed coats and then planted into flats in the greenhouse in March. Plants were then transplanted to field cages in mid-May. Nine accessions had low germination resulting in smaller plant populations which may require a subsequent regeneration of the accessions to improve genetic representation (resulting 2022 and 2023 seed increases will be bulked for the distribution lots). Five accessions reported to be annuals had high percentages of plants that failed to bolt. These will be regenerated again in 2023 as mixed annual/biennial populations and receive a vernalization treatment to hopefully better synchronize bolting, flowering, and seed production. Several other accessions exhibited sporadic bolting and flowering with many plants flowering and setting seeds late in the growing season. These also may benefit from a short vernalization period to induce and synchronize flowering in future increases. Processing of the 2022 harvest has not yet been completed so the success of the regeneration effort cannot be reliably assessed at this time.

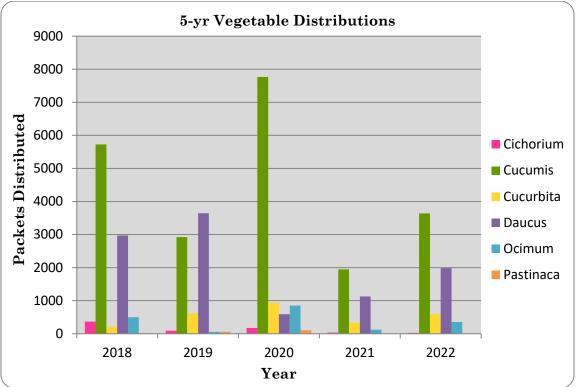
Seeds of thirty *Daucus* accessions were planted in November 2022 as biennials for regeneration in the 2023 summer field cages. Twenty-two accessions were from the *Daucus* crop wild relative (CWR) collections sponsored by the Millennium Seed Bank Project and the Royal Botanic Gardens (Kew). Little information is known about these CWR accessions, some of which appear to be of mixed annual and biennial life cycles. Bolting plants will be maintained as annuals and placed in isolation greenhouse cages for pollination with flies and Osmia (mason bees) to improve pollination. Biennial plants will be vernalized and transplanted to field cages in May 2023 for pollination with flies, alfalfa leaf cutting bees, and honeybees. Seed harvests from the annual and biennial portions of each accession will be bulked together prior to seed storage to conserve the mixed life-cycle characteristic of the accessions.

Seed increases of twelve accessions were received from two cooperators in 2022 - six from Laura Maupin, Seminis Vegetable Seeds (Bayer), Idaho and six from Rob Maxwell, Bejo Seeds, Idaho. The continued support of our cooperators' regenerations of cultivated, biennial *Daucus* germplasm allows the NCRPIS program to focus efforts on regenerating the more challenging wild, and unimproved accessions in the collection.

<u>Ocimum</u>: Twenty-six accessions of basil were planted for regeneration in 2022, one failed to germinate, and 25 were transplanted to field cages. Large quantities of seeds were harvested from six accessions, moderate quantities were harvested from eight

accessions and small quantities of seed were harvested from ten accessions. Four accessions of *Ocimum gratissimum* were late to flower and produced low quantities of seed. One accession was dug from the field and transplanted to the greenhouse where alfalfa leaf cutting bees were used to improve pollination. The remaining three *O. gratissimum* will likely require greenhouse regeneration in the near future.

<u>Germplasm Backup</u>: As NCRPIS accessions are regenerated, backup seed samples are sent to the NLGRP in Ft. Collins. Backup samples of 156 accessions from the Vegetable Project were sent to the NLGRP in 2022. Overall, 85% 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). The NCRPIS has also sent an additional 116 accessions (11 *Cichorium*, 27 *Cucumis sativus*, 78 *Cucurbita pepo*) for deposit in the Svalbard seed vault with a total of 3,630 vegetable accessions backed up at that facility.



Distributions:

Packet and accession distributions for research and education for the vegetable collections are summarized in the appendices in Table 3: External NCRPIS Distributions. In 2022, 6,600 items (packets) involving 3,475 accessions were distributed to fulfill 126 orders (84 domestic, 42 foreign) for 106 unique recipients. The total number of vegetable crop distributions has rebounded after a decline caused in part to research activities being limited by COVID-19 restrictions and the completion of *Daucus carota* germplasm evaluations by the Carrot Specialty Crops Research Initiative (SCRI) and the cucurbit germplasm evaluations by the Cucurbit Coordinated Agricultural Project (CucCAP). Vegetable research requests received in

2022 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.

There were twelve international orders in 2022 for which an import permit and/or a waiver for additional declarations to meet import requirements was not received. This resulted in 1,637 cucurbit accessions not being distributed.

The ICPMI melon race differential host set was distributed eight times in 2022 (2 domestic orders, six international orders). The shipment of seeds of differential line **ICPMI 1 10** has not yet been received from The Netherlands where regeneration under quarantine conditions is taking place (the NCRPIS is responsible only for distribution of the seeds at 25-count per accession). Until the seed stock is received, the NPGS will distribute accession PI 414723 in its place to satisfy germplasm requests. The set was also viability tested and backed up at NLGRP.

The four-accession Collaboration for Plant Pathogen Strain Identification Host (CPPSIH) Differential Set for Melon Fusarium Wilt was distributed three times in 2022 (1 domestic, 2 international orders).

Germinations:

In 2022, 380 vegetable inventory lots were tested for viability (Appendix Table 2), with most of the testing attributed to maintenance germinations on *Cichorium* and *Daucus* distribution lots.

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*.

Taxonomic identities are reviewed and confirmed as each accession is regenerated. No taxonomic changes were submitted in 2022, but several are pending for *Cucumis* and *Daucus*.

Evaluation/Utilization:

We continue to screen all *Cucurbita* and *Cucumis* seedlings grown for regeneration for the presence of SqMV, using ELISA, before seedlings are transplanted to the field cages. All vegetable field and greenhouse plantings are visually inspected for disease during the growing season to ensure healthy seeds stocks for distribution. These assessments by the plant pathologist often enable us to address additional declarations accompanying import requirements for international germplasm requests.

The CucCAP1 Project which began in 2016 had 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 curators participated in the project providing information and guidance regarding the germplasm collections and the NPGS. For NCRPIS germplasm collections, CucCAP evaluations focused on disease resistance in *Cucumis sativus* (downy mildew, *Phytophthora*), *Cucumis melo* (powdery mildew, *Fusarium*, Cucumber yellow stunting disorder virus, Cucumber mosaic virus), and *Cucurbita pepo* (powdery mildew, *Phytophthora*, Papaya ring spot virus, Cucumber mosaic virus). All genotyping by sequencing (GBS) was completed for cucumber, melon, watermelon and *Cucurbita* genera and the data made publicly available via the CucCAP website (not all data has yet been published).

Pursuit of phenotypic characterization of the cores was planned as part of CucCAP2 which received four-year funding beginning in 2021. An aspect of the genomics tool development was to establish genomically-informed core diversity panels for the four primary cucurbit species in the U.S. (*Cucumis sativus, Cucumis melo, Citrullus lanatus, Cucurbita pepo*) after performing genotyping by sequencing (GBS) of the available cucurbit NPGS collections. Accessions selected for the core populations represent greater than 95 percent genetic diversity of each collection and have been or are in the process of being self-pollinated for several generations by collaborating seed companies. These cores have been or are being re-sequenced at 30-40x coverage to serve as a community resource for GWAS and other approaches to identify genes underlying important traits for breeding. The CucCAP2's next annual meeting is in April 2023. Discussion is planned with the NPGS crop curators and the respective station research leaders regarding how best to handle the distribution of seeds for the core populations.

The project's website, <u>https://cuccap.org</u>, posts a list of publications resulting from the research, and provides access to cucurbit genomics tools and databases via the Cucurbit Genomics Database website. 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:

Hernandez C, Labate J, Reitsma K, Fabrizio J, Bao K, Fei Z, Grumet R, Mazourek M. Characterization of the USDA *Cucurbita pepo, C. moschata, and C. maxima* germplasm collections. Frontiers in Plant Science, Sec. Functional and Applied Plant Genomics. Vol. 14-2023. <u>https://doi.org/10.3389/fpls.2023.1130814</u>

Plans for 2023:

Regenerations:

Vegetable Project regeneration plans for 2023 be similar to 2022. Student recruitment and retention continue to be a concern and may impact regeneration efforts.

Thirty accessions of *Daucus carota* were planted in the greenhouse in November 2022 and included accessions with biennial, mixed (annual-biennial), and unknown life cycles. Thirteen of the thirty accessions have annual plants present in their populations. These plants will be maintained in greenhouse isolation cages and insect pollinators will be introduced to effect pollination. The non-bolted plants of these populations will be vernalized and transplanted to field cages for pollination in late April or early May. In addition to the Ames, Iowa *Daucus* regenerations, six accessions each of cultivated biennial carrot accessions were sent to cooperators in August 2022 for seed increase in 2023.

Thirty *Cichorium* accessions having low viabilities or low seed quantities will be planted for regeneration – twenty of which did not produce an adequate quantity of seed during the 2022 season, or which had small number of plants that bolted in the 2022 regeneration population.

Approximately 20 *Ocimum* accessions with low viability, low seed quantity, or older distribution seed lots will be planted for field regeneration. Ten of the *Ocimum* regenerated in 2022 did not produce enough seeds or had low plant populations so will be grown again in 2023. The *O. gratissimum* accessions require a longer growing season and will be regenerated in the greenhouse in the fall.

Thirty *Cucurbita pepo* and approximately 50 *Cucumis* will be planted for field regeneration. One wild perennial species (*Cucumis hirsutus*) originally planted in 2019, did not produce enough fruits/seeds and was dug from the 2022 field cage for continued maintenance in the greenhouse over the winter. These plants will again be transplanted to a field cage in 2023. If an insufficient quantity of seeds results from the 2023 field planting, the roots will again be transplanted to pots in the greenhouse in the fall. This process will be repeated until we are able to secure a sufficient quantity of seed for backup at NLGRP and for distribution.

Characterization:

Many years of fruit characterization data on cucurbits need to be converted and loaded into GG as do many image files of seeds, plants, and fruits. Loading these images to GG is still a high priority, but this work will be delayed as we work through a shift in duties and responsibilities of project personnel and learn the process of image loading for GG. 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 anticipate receiving data on various disease resistance evaluations and phenotypic/molecular characterization on Daucus, Cucumis, and Cucurbita pepo resulting from NPGS funded evaluation proposals, the Carrot Specialty Crops Research Initiative, and CucCAP.

H. <u>Research Leader Activities (D. Peters)</u>

Administration and Leadership Activities:

The RL administers the five-year project plan objectives for the USDA-ARS Plant Introduction Research Unit's two CRIS Projects, Plant Introduction Research, and the GEM Project and contributes to the coordination and execution of activities which support those objectives. Dr. Peters also served as the Coordinator of the Hatchfunded Multistate NC7 Project. In 2022, COVID-19 pandemic associated precautions continued to add complexity for university and federal operations, but these restrictions eased considerably in quarter 4. Health and safety concerns were guided by both ISU and USDA-ARS. Practices included enhanced sanitation, safe distancing, wearing of masks when in proximity to others (inside buildings and in the field), and maximum telework. Field activities continued at a reduced level across all curatorial and GEM projects. Student hiring was reduced proportionately, and our stats indicate accession regeneration remained reduced when compared to pre-pandemic levels. Travel remained restricted to mission critical needs which limited the number and type of curation collection trips allowed. GEM was able to plant, manage, and harvest yield trial plots but phenotypic data collection for yield trials remained reduced. Scientific and curatorial personnel continued to meet with colleagues virtually through most of the year. The station was closed to visitors and tours through the first half of the year, but restrictions eased in the last half. Limited site visits and tours resumed in quarter 4. Similarly, participation in scientific meetings began to shift back to in- person attendance. Distribution of seed to U.S. requestors continued, and international requests requiring APHIS handling were approaching pre-pandemic schedules by quarter 4.

Fiscal 2022 appropriations were fully available in early quarter 3 of 2022 following several continuing resolutions. Delays in the release of funds created uncertainty for program planning and complicated completion of hiring and procurement and exacerbated administrative management overload throughout the system. Making timely decisions for work plans for many taxa that require germination and vernalization treatments in the winter is challenging under these circumstances.

Budget savings resulting from reduced field activities and associated labor costs were used to replace or repair aging curational regeneration and seed storage equipment. Through close collaboration with ISU and with the budgetary assistance of the USDA ARS MWA office, the cooling system for the main 4° C cold storage room was approved for replacement with work anticipated for completion by mid-2023. A lease exception was negotiated between ISU and ARS during the last half of 2022 to allow for use of ARS budget money to replace the failing cooling system of the main 4° C facility.

The five-year project plan period of Hatch Multistate Project NC7 of 10/01/2017 - 9/30/2022 ended and the newly approved project plan has taken effect. The new project plan will cover the period of 10/01/2022 - 9/30/2027.

The ARS CRIS projects located at the NCRPIS were developed in coordination with the ARS Office of National Programs in the last half of 2022 and submitted for full panel review. The updated project plans will take effect before the middle of 2023.

2023 Plans:

Review of the lease agreement between ISU and ARS immediately after the derecho storm in August 2020 revealed the lease needed to be reviewed and updated. The lease will be reviewed and updated in 2023 to ensure the lease properly reflects the current station activities, facility footprint, and maintenance responsibilities on the ISU experiment station site. Review of the lease will also allow for the potential addition of -20 ° C cold storage capacity and construction of an additional hoop greenhouse.

We hope to reengage use of the QSorter from the Swiss company, QualySense, purchased with USDA-ARS Midwest Area and headquarters support, captures 3D images and near infrared spectroscopy (NIR) spectra from seeds, and can sort seeds based on calibrations developed for specific traits or size/color parameters. The COVID-19 pandemic delayed progress in this area but collaboration with ISU Agronomy Department partners should move this initiative forward in 2023.

Pete Cyr will continue to focus on development of RESTFUL interface applications to enable ready extraction of GRIN-Global information that can be combined with information from other resource providers (such as genomic information resources) by researchers.

Focus will continue on recruiting to fill vacant PIRU and NCRPIS positions with outstanding individuals, facilitate smooth transitions, and assist graduate students in completion and publication of their work.

List of Acron	lyms
<u>Acronym</u>	Definition
AD	Additional Declarations
AdCom	Advisory Committee
AES	Agricultural Experiment Station
AFB	American foulbrood
AFRI	Agriculture and Food Research Initiative
ALC	Alfalfa Leafcutter Bees
AOSA	Association of Official Seed Analysts
AP	Adventitious Presence
APHIS	Animal and Plant Health Inspection Service
API	Application Programing Interface
ARS	Agricultural Research Service
ASA	American Society of Agronomy
AT	Administrator Tool
BFB	Bacterial Fruit Blotch
BLM	Bureau of Land Management
BrAPI	Breeding Application Programing Interface
CALS	College of Agriculture and Life Sciences
CEC	Customer Experience Center
CGC	Crop Germplasm Committee
CGIAR	Consultative Group for International Agricultural Research
CIMMYT	International Center for Maize and Wheat Improvement
CPPSIH	Collaboration for Plant Pathogen Strain Identification Host
CRIS	Current Research Information System
CT	Curator Tool
CucCAP	Cucurbit Coordinated Agricultural Project
CuGenDB	Cucurbit Genomics Database
CWR	Crop Wild Relative
DBMU	Database Management Unit
Dev Team	Development Team
DH	Doubled Haploid
EAB	Emerald Ash Borer
\mathbf{EFB}	European Foul Brood
EGC	Environmental Growth Chamber
EHS	Environmental Health and Safety
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FTE	Fulltime Equivalent
GBS	Genotyping by Sequencing
GDU	Growing Degree Units
GEM	Germplasm Enhancement of Maize
GEO	Genetically Engineered Organism
GG	GRIN-Global, Successor to legacy GRIN system
GHU	Growing Heat Units

GIS	Geographic Information System
GRIN	Germplasm Resources Information Network
GUI	Graphic User Interface
GWAS	Genome Wide Association Study
HFCS	High Fructose Corn Syrup
IBS	Identity by Sequence
ICPMI	International Cucurbit Powdery Mildew Initiative
IDALS	Iowa Department of Agriculture and Land Stewardship
IPR	Intellectual Property Rights
ISMA	International Seed Morphology Association
ISPM	International Standards for Phytosanitary Measures
ISU	Iowa State University
IT	Information Technology
ITPGRFA	International Treaty for Plant Genetic Resources for Food and Agriculture
JHA	Job Hazard Analysis
LA	Limited Appointment
LIS	Legume Information System
MaizeGDB	Maize Genetics Database
MCMV	Maize Chlorotic Mottle Virus
MGSC	Maize Genetic Stock Center
MS	Microsoft
MT	Middle Tier
MTA	Material Transfer Agreement
NA	National Arboretum
NALPGRU	National Arid Land Plant Genetic Resource Unit
NAM	Nested Association Mapping
NAPB	National Association of Plant Breeders
NC7	Hatch Multistate Project NC-007
NCR	North Central Region
NCRPIS	North Central Regional Plant Introduction Station
NGRL	National Germplasm Resources Lab
NIMSS	National Information Management Support System
NIR	Near Infrared Spectroscopy
NLGRP	National Lab for Genetic Resource Preservation
NP	National Program
NPGS	National Plant Germplasm System
NRR	Non Research Request
NSF	National Science Foundation
NSL	National Storage Laboratory
OPGC	Ornamental Plant Germplasm Center
OSU	Ohio State University
PGOC	Plant Germplasm Operating Committee
PGR	Plant Genetic Resources
PI	Plant Introduction
PIRU	Plant Introduction Research Unit

PVP	Plant Variety Protection
PW	Public Website
RIL	Recombinant Inbred Line
RTAC	Regional Technical Advisory Committee
SAM	Sunflower Association Mapping
SCRI	Specialty Crop Research Initiative
SCST	Society of Commercial Seed Technologists
SENASIC	National Agro-Alimentary Health, Safety, & Quality Service in Mexico
SMTA	Standard Material Transfer Agreement
SqMV	Squash Mosaic Virus
ΤZ	Tetrazolium
USBG	United States Botanical Gardens
USDA	United States Department of Agriculture
USFWS	U.S. Fish and Wildlife Service
USMCA	United States-Mexico-Canada Agreement
WSMV	Wheat Streak Mosaic Virus

Year 2022 Table 1 01/01/2022 to 12/31/			NCRPIS A	ccessions (A	.ccs), Acquir	red, Availab	le
CURATOR	GENUS_CROP	Number Accs	Number Accs Acquired	Percent Acquired	Number Available	Percent Available	Percent Avail Last Year
Brenner	NC7-grass.echinochloa	315	0	0.0%	285	90%	90%
	NC7-grass.misc	142	0	0.0%	84	59%	59%
	NC7-grass.panicum	936	0	0.0%	910	97%	97%
	NC7-grass.setaria	1117	0	0.0%	1044	93%	94%
	Subtotal Grasses:	2510	0	0.0%	2323	93%	93%
	NC7-legume.melilotus	1006	0	0.0%	868	86%	86%
	NC7-legume.misc	312	1	0.3%	166	53%	53%
	Subtotal Legumes:	1318	1	0.1%	1034	78%	79%
	NC7-pseudocereal.amaranth	3338	3	0.1%	3230	97%	97%
	NC7-pseudocereal.celosia	61	0	0.0%	39	64%	64%
	NC7-pseudocereal.perilla	25	0	0.0%	24	96%	96%
	NC7-pseudocereal.portulaca	13	0	0.0%	11	85%	77%
	NC7-pseudocereal.quinoa	664	2	0.3%	412	62%	61%
	Subtotal Pseudocereals:	4101	5	0.1%	3716	91%	90%
	NC7-spinach	414	0	0.0%	294	71%	76%
	NC7-umbels	1195	0	0.0%	824	69%	69%
	Brenner Total:	9538	6	0.1%	8191	86%	86%
Carstens	NC7-medicinals	1157	27	2.3%	805	70%	71%
curstons	NC7-ornamentals	783	6	0.8%	557	71%	71%
	NC7-woody.landscape	2112	38	1.8%	1120	53%	54%
	Carstens Total:	4052	71	1.8%	2482	61%	62%
Marek	NC7-asters	462	3	0.6%	160	35%	34%
	NC7-brassica	2024	5	0.0%	1837	91%	91%
	NC7-crucifers	1328	35	2.6%	1174	88%	87%
	NC7-cuphea	638	0	0.0%	507	79%	80%
	NC7-euphorbia	210	0	0.0%	105	50%	47%
	NC7-flax	2834	0	0.0%	2809	99%	99%
	NC7-flax.wilds	172	0	0.0%	130	76%	77%
	NC7-sun.cults	2686	45	1.7%	2514	94%	93%
	NC7-sun.wilds.ann						
		1708	2	0.1%	1629	95%	95%
	NC7-sun.wilds.per	903	0	0.0%	782	87%	87%
	NC7-sun.wilds.sp	2	0	0.0%	0	0%	0%
	Subtotal Wild Sunflower:	2613	2	0.1%	2411	92%	92%
D 0 M:11 1	Marek Total:	12967	92	0.7%	11647	90%	90%
Bernau & Millard	• •	41	0	0.0%	8	20%	20%
	NC7-maize.gems	405	7	1.7%	381	94%	99%
	NC7-maize.inb	2787	76	2.7%	2096	75%	80%
	NC7-maize.pop	15806	30	0.2%	12064	76%	78%
	NC7-maize.pvp	685	46	6.7%	663	97%	100%
	NC7-maize.teosinte	437	0	0.0%	135	31%	17%
	Subtotal Zea:	20120	159	0.8%	15339	76%	78%
	Bernau & Millard Total:	20161	159	0.8%	15347	76%	78%
Reitsma	NC7-chicory	285	0	0.0%	258	91%	89%
	NC7-cucumis.cucs	1401	0	0.0%	1335	95%	95%
	NC7-cucumis.melo	3252	2	0.1%	1982	61%	59%
	NC7-cucumis.wilds	318	0	0.0%	223	70%	69%
	NC7-cucurbita	984	3	0.3%	734	75%	73%
	NC7-daucus	1565	1	0.1%	1298	83%	82%
	NC7-ocimum	108	2	1.9%	100	93%	94%
	NC7-parsnips	73	0	0.0%	58	79%	79%
	Reitsma Total:	7986	8	0.1%	5988	75%	74%
NCRPIS Total:		54704	336	0.6%	43655	80%	80%

01/01/2022 to 12/31/2	•				NCR	PIS Accessio	ons (Accs) Ge	rminated, Reg	generated,	Made Availa	able, Back	ed 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-grass.echinochloa	315	20	6%	60	9	0	0			0	33	0	287	91%
	NC7-grass.misc	142			0		0	0	0	2	1	0			
	NC7-grass.panicum	936		29%	18										98%
	NC7-grass.setaria	1117			24					0	0				
	Subtotal Grasses:	2510		12%			0	0	0	2	1	41		2314	92%
	NC7-legume.melilotus	1006		6%				0	0	0	0				
	NC7-legume.misc	312		8%	3			-			1				72%
	Subtotal Legumes:	1318	81	6%		15					1				
	NC7-pseudocereal.amaranth	3338		12%		30		-	0						
	NC7-pseudocereal.celosia	61	9			4	0	0			1	0	1		
	NC7-pseudocereal.perilla	25									1	7			
	NC7-pseudocereal.portulaca	13		8%		1	0				1				
	NC7-pseudocereal.quinoa	664			91										
	Subtotal Pseudocereals:	4101		12%											
	NC7-spinach	414					0				0				
	NC7-umbels	1195									-				
	Brenner Total:	9538		10%	327	167	0	27							0.000
Constant		010000000000000000000000000000000000000	100.000	53500 CONTRACTOR (1997)	1000000	200.0708.		1						200700700700700700700	100000
Carstens	NC7-medicinals	1157			6							-			
	NC7-ornamentals	783													
	NC7-woody.landscape	2112													
	Carstens Total:	4052		4%		1		59			100.10				61%
Marek	NC7-asters	462													
	NC7-brassica	2024													
	NC7-crucifers	1328													
	NC7-cuphea	638													
	NC7-euphorbia	210		18%	0										48%
	NC7-flax	2834			45										
	NC7-flax.wilds	172													
	NC7-sun.cults	2686		22%											
	NC7-sun.wilds.ann	1708		3%											
	NC7-sun.wilds.per	903			29		0								
	NC7-sun.wilds.sp	2													
	Subtotal Wild Sunflower:	2613							. 0						
	Marek Total:	12967	859	7%		216									
Bernau & Millard	NC7-maize.coix&tripsacum	41													
	NC7-maize.gems	405			0										
	NC7-maize.inb	2787		25%	105			0	0 0						
	NC7-maize.pop	15806	2520	16%	49	136	0	0	0 0	85	839	48	0	13635	86%
	NC7-maize.pvp	685	210	31%	59	56	0	0	0 0	78	73	88	0	684	100%
	NC7-maize.teosinte	437	56	13%	437	3	0	0	0	2	3	0	0	49	11%
	Subtotal Zea:	20120	3557	18%	650	290	0	0	0	172	945	226	0	16538	82%
	Bernau & Millard Total:	20161	3557	18%	650	290	0	0	0	172	945	226	0	16555	
Reitsma	NC7-chicory	285			35	1									
	NC7-cucumis.cucs	1401	29		47		0						201		
	NC7-cucumis.melo	3252			0										
	NC7-cucumis.wilds	318		5%							1				
	NC7-cucurbita	984	18						-	-					
	NC7-daucus	1565		13%	59										
	NC7-ocimum	108		10%	26		0	-	0						
	NC7-parsnips	73													
	Reitsma Total:	7986	380			171	0								
NCRPIS Total:	atortonia i otan	54704	5891	11%		949		79							84%

Year 2022 Table 3 01/01/2022 to 12/31/2			1	External NC	CRPIS Distr	ibutions - Inc	ludes both D	l (research ai	nd education)	, RP (Repatri	ation), and OI	3 (Observatio	n) order type	s
			External Domestic Distributions				Foreign Distributions				External Domestic and Foreign Distributions			
CURATOR	GENUS_CROP	Number Accs in Collection	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients
Brenner	NC7-grass.echinochloa	315	317	261	5	4	8		5	5	325	262	10	9
	NC7-grass.misc	142	5	5			2	2	1	1		7	4	
	NC7-grass.panicum	936	15	15			19	18	6			33	11	
	NC7-grass.setaria	1117	838	793	26		103	99	8	7		857	34	
	Subtotal Grasses:	2510	1175	1074	31	25	132	127	13	12		1159	44	37
	NC7-legume.melilotus	1006	10	10	3	3	264	262	2	2	274	268	5	
	NC7-legume.misc	311	5	5		4	7	7	1	1		9	5	
	Subtotal Legumes:	1317	15	15	7	7	271	269	3			277	10	
	NC7-pseudocereal.amaranth	3335	325	279	28	24	1810	1224	20			1341	48	
	NC7-pseudocereal.celosia	61	37	33	8		31	30	2	2		33	10	10
	NC7-pseudocereal.perilla	25	25	21	3	3	16	16	1	1	41	24	4	4
	NC7-pseudocereal.portulaca	13	20	10	4	4	3	3	1	1	23	10	5	5
	NC7-pseudocereal.quinoa	662	279	216	31	23	707	306	13			345	44	
	Subtotal Pseudocereals:	4096	686	559	59	48	2567	1579	33	32		1753	92	
	NC7-spinach	414	2069	316	16	13	190	176	4	4	2259	316	20	17
	NC7-umbels	1195	643	238	17	16	305	285	3	3	948	343	20	19
	Brenner Total:	9532	4588	2202	<u>120</u>	<u>99</u>	3465	2436	53	<u>51</u>	8053	3848	173	150
Carstens	NC7-medicinals	1136	98	88	26	26	81	81	4	4	179	168	30	30
	NC7-ornamentals	781	32	32	13	13	10	10	6	6	42	42	19	19
	NC7-woody.landscape	2092	238	97	75	58	4	4	3	3	242	100	78	61
	Carstens Total:	4009	368	217	<u>104</u>	84	<u>95</u>	<u>95</u>	11	11	463	310	115	95
Marek	NC7-asters	462	9	9	3	3	5	4	3	3	14	12	6	6
	NC7-brassica	2024	2041	1407	32	26	1785	1256	21	21	3826	1531	53	47
	NC7-crucifers	1342	574	373	32	30	561	545	17	16	1135	796	49	46
	NC7-cuphea	638	20	20	3	2	0	0	0	0	20	20	3	2
	NC7-euphorbia	210	0	0	0	0	0	0	0	0	0	0	0	0
	NC7-flax	2834	40	40	3	3	267	261	2	2	307	287	5	5
	NC7-flax.wilds	172	145	133	3	3	6	6	1	1	151	133	4	
	NC7-sun.cults	2686	1682	1352	38	29	851	639	25	22	2533	1613	63	51
	NC7-sun.wilds.ann	1708	329	291	22		957	814	15			927	37	
	NC7-sun.wilds.per	903	372	280	23		193	178	6			403	29	
	NC7-sun.wilds.sp	2	0	0			0	0	0			0	0	
	Subtotal Wild Sunflower:	2613	701	571	37	33	1150	992	15			1330	52	
	Marek Total:	-	5212	3905	118		4625	3703	69			5722	187	
Bernau & Millard	NC7-maize.coix&tripsacum	41	7	5	6	6	0	0	0	0	7	5	6	6
	NC7-maize.gems	398	276	194	41	30	370	280	12	12	646	346	53	42
	NC7-maize.inb	2787	3522	1432	203	158	1127	685	52	51	4649	1602	255	209
	NC7-maize.pop	15806	5728	4599	134	105	714	649	32	31	6442	4855	166	136
	NC7-maize.pvp	675	4279	650	213	116	2827	596	59	49	7106	668	272	165
	NC7-maize.teosinte	437	130	56	39	33	45	36	6	6	175	63	45	39
	Subtotal Zea:	20103	13935	6931	446	286	5083	2246	94	83	19018	7534	540	369
	Bernau & Millard Total:	20144	13942	6936	451	289	5083	2246	94	83	19025	7539	545	372
Reitsma	NC7-chicory	285	9	9		2	18	18	2	2	27	27	4	
	NC7-cucumis.cucs	1401	1921	1335	19	17	966	761	18	18	2887	1335	37	35
	NC7-cucumis.melo	3250	162	123	18	18	436	375	16	15	598	450	34	33
	NC7-cucumis.wilds	318	7	7	3	3	146	135	6	6	153	140	9	9
	NC7-cucurbita	984	375	286	28	22	217	211	4	4	592	385	32	26
	NC7-daucus	1565	1977	1035	16	9	9	9	3	3	1986	1036	19	12
	NC7-ocimum	108	129	100	9	9	226	100	6	6	355	100	15	15
	NC7-parsnips	73	0	0	0	0	2	2	1	1	2	2	1	1
	Reitsma Total:	7984	4580	2895	84	65	2020	1611	42	41	6600	3475	126	106
NCRPIS Total:		54650	28690	16155	812		15288	10091	242			20894	1054	

	l				NCR	PIS Accessio	ons (Accs) O	bservations	(Obs) in GR	IN, Images	in GRIN			
01/01/2022 to 12/31/2 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	Obs In GRIN	Number of Accs with Obs In GRIN Last Year	Number of Obs in GRIN (all years)	Obs in GRIN (all years)	Number of Accs Imaged	Images in GRIN for Year	Number of Images in GRIN for Year	Number Acc With Images in GRIN (all years)	Number of Images in GRIN (all years)
Brenner	NC7-grass.echinochloa	315	57											
	NC7-grass.misc	142	0											
	NC7-grass.panicum	936	3				0					18	378	
	NC7-grass.setaria	1117	18										364	
	Subtotal Grasses:	2510	78		0		0					122	888	
	NC7-legume.melilotus	1006	3			~							226	
	NC7-legume.misc	312	0			d				16		29	91	
	Subtotal Legumes:	1318	3	0	0		0	8032				43	317	
	NC7-pseudocereal.amaranth	3338	14		112		19					177	1393	
	NC7-pseudocereal.celosia	61	0				0						27	
	NC7-pseudocereal.perilla	25	1	0							4			
	NC7-pseudocereal.portulaca	13	0				0					16	10	
	NC7-pseudocereal.quinoa	664	10		72					255			568	
	Subtotal Pseudocereals:	4101	25	382	184	37	21	58571		401	401	822	2023	4424
	NC7-spinach	414	0	0	0	0	0	8883	413	3	1	2	412	500
	NC7-umbels	1195	0	0	0	•	0	6141	1145			20	370	
	Brenner Total:	9538	106	382	184	37	21	92008	9098	592	530	1009	4010	7906
Carstens	NC7-medicinals	1154	0	697	36	0	0	12678	481	86	55	234	648	1698
	NC7-ornamentals	782	0	0	0	0	0	152	101	51	8	12	205	374
	NC7-woody.landscape	2112	3	2650	161	36	12	7615	894	188	128	818	1151	4208
	Carstens Total:	4048	3	3347	197	36	12	20445	1476	325	191	1064	2004	6280
Marek	NC7-asters	462	0	0	0	0	0	8	1	30	6	6	31	35
	NC7-brassica	2024	299	0	0	606	77	42352	1996	8	5	5	426	1189
	NC7-crucifers	1342	4	0	0	0	0	7325		78	42	46	422	
	NC7-cuphea	638	0	0	0	0	0	4260	278	0	0	0	13	34
	NC7-euphorbia	210	0	0	0	0	0	0	0	1	0	0	7	19
	NC7-flax	2834	0	0	0	0	0	1717		45		45	91	174
	NC7-flax.wilds	172	0	0	0	0	0	852				2	29	33
	NC7-sun.cults	2686	0	0	0		0	104316				45	482	
	NC7-sun.wilds.ann	1708	0		0		0					54	102	
	NC7-sun.wilds.per	903	0			-						4	169	
	NC7-sun.wilds.sp	2	0				0							
	Subtotal Wild Sunflower:	2613	0	0	0	-	0			107			346	
	Marek Total:	12981	303				77		1				1847	
Bernau & Millard	NC7-maize.coix&tripsacum	41	1	0	0		0		4	Contractor Contractor		0		
Dernau & Minaru	NC7-maize.gems	405	0		7		41			7		1	344	
	NC7-maize.inb	2787	9		14		14			65				
		15806	0		323		14			154		23	13938	
	NC7-maize.pop	685	2		87		132							
	NC7-maize.pvp NC7-maize.teosinte	437	0		61									123
	Subtotal Zea:	20120	11	11549	492		350			363			16613	42944
			11			-	-		1					-
Reitsma	Bernau & Millard Total:	20161 285	6		492		350			360	29	1	16624 263	
	NC7-chicory		6	0	0	0	0			-		-		
	NC7-cucumis.cucs	1401	0	0	0	0	0	26149				13	933	
	NC7-cucumis.melo	3252	0	0	0		0	12286				40	686	
	NC7-cucumis.wilds	318	0	0	0		0	680			0	0	75	
	NC7-cucurbita	984	6		0	0	0	5667				3	156	
	NC7-daucus	1565	14		0	0	0	19504				3	765	
	NC7-ocimum	108	8		0	0	0	635			0	0	14	
	NC7-parsnips	73	0	1000	0	5075)	0					0	1.27	77
	Reitsma Total:		34				-							
NCRPIS Total:		54,714	458	15,278	873	5,414	460	756,957	42,705	1,822	1,000	2,406	27,378	68,56

Appendix Figure 1

