USDA/ARS NC7 Annual Report January 1 - December 31, 2021

PLANT INTRODUCTION STATION



NCRPIS ANNUAL REPORT – 2021

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NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION NC7 ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2021

I. PROJECT TITLE:

NC7 "Plant Germplasm and Information Management and Utilization"

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

A. Administrative Advisor

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

В. **Regional Coordinator**

*D. Peters, USDA-ARS, Iowa

C. **State Experiment Stations Representatives**

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:

13. California-Davis	E. Forrestel	28. Michigan	J. Hancock
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 (June 2021 - May 2022)

<u>Departures</u>:

- Mike Peters, USDA-ARS Agri. Research Science Tech. (GEM), September 2021
- Rita Mueggenberg, USDA-ARS PFT Secretary Office Automation, January 2022
- Narinder Pal, USDA-ARS Agri. Research Science Tech. (Pathology), January 2022
- Kallie Judson, USDA-ARS Agri. Research Science Tech. (Entomology), March 2022

Promotions:

- Andrew Sherwood, USDA-ARS Agri. Research Science Tech. (Horticulture), (grade), June 2021
- Samantha Armintrout, USDA-ARS Agri. Research Science Tech. (Maize Curation), (grade), May 2022

New Hires:

- Brandyn Chapman, ISU Agri. Specialist II (Vegetable Curation), October 2021
- Tracie Hennen-Bierwagen, USDA-ARS Agri. Research Science Tech. (GEM), March 2022
- Dr. James McNellie, USDA-ARS Term Support Geneticist (GEM), March 2022

Transitions:

 Dr. Adam Vanous, previously USDA-ARS Term Support Geneticist at the PIRU accepted the role of PIRU GEM Coordinator, October 2021

Vacant USDA-ARS Positions:

- Agri. Research Science Tech (GEM)
- Agri. Research Science Tech (Plant Pathology)
- Agri. Research Science 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 21.68 student FTE (full-time equivalent) part-time temporary positions in FY 2021, primarily via the Research Support Agreement with Iowa State University. However, field operations were scaled back and only 13.25 student FTE were hired due to pandemic restrictions. These temporary positions support curatorial activities including regeneration, seed processing, viability testing, farm and facilities operations, IT support, and the GEM Project. Students were interviewed and selected by ISU Assistant Director of Research Administration, Fred Engstrom. Marci Bushman, PIRU Program Support Assistant, managed the administrative aspects of all student hires, with support and guidance from Admin. Officer Candice Weuve and Administrative Assistant Orlando Guzman.

Budget:

We appreciate the support of the Agricultural Experiment Stations of the North Central Region, which maintained their annual support of \$522,980. These funds supported the salaries of our eight ISU staff members, their professional travel, and some travel, supply, and maintenance expenses. In addition, Iowa State University's Agricultural Experiment Station provided annual support valued at over \$560,000 for site infrastructure and administration and provided benefits for current NCRPIS-ISU staff members and retirees.

We are grateful Hatch funding resources were maintained. Their continued stability is critical to NCRPIS operations, now and into the future. Currently, about 95% of Hatch NC7 funds are devoted to the wages and salaries of the eight permanent ISU employees. Future ISU wage increases without additional Hatch funding support will inevitably limit professional advancement, professional meeting travel, and technical training.

FY2021 USDA-ARS funding of the PI CRIS remained unchanged as it has each year since FY2014 (2.38M net to location), minus a one percent assessment for 'Big Data' and smaller assessments for Digitop and SAS licenses. The GEM CRIS funding matched the final FY2020 funding (1.56M net to location), minus similar IT related assessments as the PI CRIS.

Any future reductions in funding will force a reduction in student hiring and further hinder our ability to attract students who are critical for executing our gene bank's mission. Student hiring for summer 2021 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. ISU Assistant Director of Research Administration 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. Our personnel strive to perform all functions and serve the collections entrusted to us and our stakeholders to the best of our ability. A high turnover in permanent staff continued in 2021, requiring a great deal of time and attention spent on recruitment and hiring activities.

Construction and Facilities:

Seed storage space is limited and must be addressed within the next three to four years. A 2018 request to ARS leadership for a 2,250 sq ft -20°C cold storage building 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. However, construction plans cannot progress without Congressional appropriation for the facilities. In general, space is extremely tight for all personnel and functions. Addition of this cold storage building will enable dividing the collection inventories appropriately between 4°C and -20°C, greatly extending longevity and viability for many of our taxa.

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

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

(Part IV. summarizes the accomplishments and progress for calendar year 2021, presented in greater detail in the individual staff reports contained within this document.)

Acquisition and Documentation Highlights:

In 2021, collection development continued with the acquisition of 255 new accessions (Appendix Table 1). Details are provided in the individual curators' report sections. A historical perspective to provide comparison of acquisitions over the previous nine years is provided below.

Year	# New Accessions
2021	255
2020	262
2019	437

Year	# New Accessions
2018	293
2017	250
2016	786

Year	# New Accessions
2015	229
2014	766
2013	192

The U.S. has been a partner to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) since March 2017. However, implementation by the NPGS is still under development as several Departments are involved. International collection continues to be challenging as countries adopt variations of the Standard Material Transfer Agreement (SMTA) or other requirements the NPGS cannot accept. Of ongoing concern is the successful entry into the U.S. of germplasm collected from international explorations. It is critical clean, pest- and pathogen-free seed be shipped or hand-carried by collectors; sufficient time must be devoted to collection sample preparation and sufficient care post-collection are all essential for successful entry. Despite these challenges, excellent quantities of seed provided by collectors of many new accessions has enabled a significant proportion to be available and distributable immediately.

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

Regeneration and Maintenance Highlights:

Regeneration was attempted for 1,273 accessions. Of these, 837 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 hiring constraints. Overall collection availability is 80%, an increase of 1% over 2020, despite 8% growth in the collection size since 2006. Part of the increased availability was attributed to the inactivation of accessions which were received with low viability and could not be maintained or resolution of duplication.

Year	# Accessions Regenerated
2021	837
2020	915
2019	1,562

***	# Accessions
Year	Regenerated
2018	1,245
2017	1,601
2016	1,033

	# Accessions
Year	Regenerated
2015	1,637
2014	1,230
2013	1,148

Continued positive results from use of a contracted tropical maize winter nursery planted in fall 2021 near Puerto Vallarta, Mexico encourages us to continue to invest in these efforts to increase maize germplasm availability.

Assistance in regeneration was provided by USDA-ARS staff of Parlier, CA for increase of wild *Helianthus* taxa. *Daucus* regeneration efforts were supported by seed increases from Seminis Vegetable Seeds (L. Maupin) and from Bejo Seeds (R. Maxwell). USDA-ARS in Salinas, California (B. Mou) supported seed increase of domesticated spinach, and USDA-ARS in St. Croix supported increase of 17 tropical maize inbred lines and populations. 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 supported regeneration of tropical maize accessions. Raleigh ARS GEM Project Coordinator Matt Krakowsky provided increases of GEM lines and the Ames GEM team regenerated and provided GEM lines. The International Center for Maize and Wheat Improvement (CIMMYT) regenerated highland maize, which has very specific growing requirements, at their Tolucca, Mexico site.

Accessions backed up at the National Laboratory for Genetic Resource Preservation (NLGRP) in Ft. Collins in 2021 numbered 975, with an overall collection backup average of 83%. There is wide variation for percent backup across the various crop collections, from 10% (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, and/or insufficient representation of male/female individuals, among other factors. Seed of 1,713 accessions was prepared and shipped to Ft. Collins for inclusion in the NPGS deposit to the Svalbard Global Seed Vault.

Year	# Accessions Backed Up
2021	975
2020	1,454
2019	623

Year	# Accessions Backed Up
2018	795
2017	595
2016	428

Year	# Accessions Backed Up
2015	431
2014	1,231
2013	781

Distributions:

Approximately 55% and 45% of the 2021 germplasm distributions were sent to international and domestic requestors, respectively. This proportion is atypical compared to an average historical balance of 45% international and 55% domestic distribution. Distributions continued to reflect high demand in 2021 (Appendix Table

3). The 2019-2021 timeframe reflects 8% lower distributions than the previous three years.

Year	# Items	# Unique Accessions	# Orders	# Requestors
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	23,229	1,414	1,000
2017	55.474	22.801	1,410	1,019
2016	39,520	18,093	1,254	963
2015	34,188	14,279	1,186	945
2014	41,655	17,558	1,285	993
2013	40,409	17,788	1,523	1,204
2012	45.115	18,811	1,632	1,344
2011	38.402	18,634	1,501	1,180

Research demand for our plant genetic resource collections continues to be very high; requests for diversity and relationship analyses, disease and pest resistance, biofuel, and health and nutrition contribute 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 SCRI, AFRI, or NSF funding.

The relative numbers of distributions vary from year to year, but generally correlate well with the proportional makeup of the collections. Demand for maize is usually greater compared to other crops in our collection. Despite a reduction in demand in 2020, requests for maize surged in 2021 accounting for 45% of all distributions. Demand for oilseed species remained strong, but vegetable crops requests decreased to less than half of 2020 levels.

Curator		% of Total Collections	% of 2021 Distributions	% of 2020 Distributions	% of 2014 Distributions
Brenner	9,552	17	17	16	14
Carstens	3,990	7	2	1	<1
Marek	12,896	24	28	29	16
Millard/Bernau	19,976	37	45	31	35
Reitsma	7,977	15	8	23	28
Totals	54,391	100	100	100	100

Non-research requests (home gardeners) continue to heavily target vegetable and ornamental germplasm. More than one-half of all orders to NC7 are cancelled non-research requests and other NPGS sites are also heavily targeted. Home gardeners are redirected to other sources of commercially available materials. Although our resources cannot support maintaining and distributing the collections to home gardeners, we inform these requestors about plant genetic resource conservation and encourage interested individuals to save seeds, conserve them, and share germplasm and associated information. The proliferation of websites instructing non-research

requestors how to deceive curators at various germplasm sites in order to get free germplasm continues to be problematic. The careful efforts that go into each and every increase, characterization, imaging, processing, storage, viability testing, and distribution surely make these seeds among the most expensive to provide. GRIN-Global's user friendly order module also helps individuals to select more diverse germplasm from a number of NPGS sites at once. Software tools were developed by the DBMU (Database Management Unit, Beltsville) that enable rapid classification of NRR (non-research requestor) orders and reduce personnel processing time. This tool was put into operation in late 2021 and has provided an effective way to identify probable NRR orders and improved consistency of response to these requests.

Evaluation and Characterization:

With the enhancements made to the 'Attachment Wizard' that work in conjunction with GRIN-Global, image organizing, and loading has rapidly progressed. A large volume of accession-associated images and other types of documents are being attached to accessions, orders, and inventories. In 2021 image loading increased dramatically from 2020 levels with 33,776 images loaded (compared to more than 2,844 images in 2020). Loading of observation data also substantially increased, with 41,328 observations associated with 7,379 accessions (compared to more than 26,500 trait observations loaded/associated with 2,764 accessions in 2020). For the NC7 collections, 42,492 accessions have one or more trait observation data points available via the GRIN-Global database, (https://www.ars.grin.gov/npgs/) and more than 26,917 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 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 and 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 efforts continued 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, truly evidence of global adoption of this valuable resource.

For almost six years, the NPGS has utilized a GRIN-Global Advisory Committee (AdCom) as a forum for genebank personnel and developers to identify development needs, prioritize them, test, and approve software for release. The AdCom is chaired by NCRPIS staff member Lisa Burke and has been highly productive. An international AdCom 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 our current ARS Program cycle is to develop inter-operability between GG and other key information providers' portals; examples include MaizeGDB, Gramene, LIS (Legume Information System), and GOBii. Pete Cyr developed and implemented the 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 2021, about 7% of the collection was tested for viability during periodic maintenance (3,865 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 a -20°C cold storage building would provide for a much longer period of viability for many of our taxa. This could bring significant cost savings over the long term, as most of the collection's seeds lose viability long before inventory supply is depleted. Less frequent regeneration 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 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 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 748 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 Germplasm Enhancement of Maize Project (GEM) works with 68 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 334 lines from 2001-2021 representing more than 60 maize races. An important goal is development of a set of inbred lines representative of the diversity inherent to all of the races of maize. In addition to traditional introgression methods, the project has released 204 doubled-haploid (DH) maize lines in partnership with the ISU Doubled Haploid Facility. The next set of DH lines from the allelic diversity project will be released in Fall 2022. 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 well attended by scientists, breeders, and graduate students. The field days offer a unique opportunity for more molecular-focused researchers to understand the diversity of materials available for research, and the activities that support germplasm development.

Pandemic related restrictions for visitors at the NCRPIS forced the cancelation of the field day in 2021, for the second consecutive year.

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. These events simply were not possible during the 2021 pandemic year. 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. More emphasis has been requested for advanced breeding materials, doubled haploid germplasm, mapping populations, single mother trees, and ephemeral genetic resources derived from NSF, AFRI, or SCRI-funded research.

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

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

Software development efforts will continue to center on the development and deployment of GRIN-Global resources, and on information management tools that can 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 RTAC members this year. NC7 Region researchers typically account for nearly half of domestic plant germplasm distributions from the NCRPIS. Requests for germplasm continue to increase for research as well as non-research use. Requests become increasingly better targeted as the quantity and quality of information associated with the collection improves, thus sharing of findings resulting from use of NPGS germplasm, linked with the germplasm's identity and source, is critically important.

The linkage of the GEM Project, the maize curation project, and public and private collaborators throughout the U.S. facilitates the use of exotic maize germplasm by public and private sector maize researchers. This unique partnership offers great potential for diversifying the genetic base of U.S. maize production 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 Regional Technical Advisory Committee (RTAC) has provided valuable direction in the following areas:

- Requesting and suggesting organizational structure of information needed to
 determine project impact and provide accountability. This includes advice on
 useful formats for analyzing and evaluating the nature of distributions, whom
 they benefit, and how benefits are realized, which are essential for determining
 the impact and value of the project.
- Identifying needed improvements to the public GRIN interface.
- Providing input from their respective AES Directors to curators, genebank, and other administrators.
- Providing guidance to increase the NCRPIS program's relevance to NCR stakeholders.
- Providing technical expertise, particularly in the areas of diversity assessment and taxonomy.
- Providing added breadth in understanding issues at genebanks beyond the NCRPIS.

Understanding the challenges faced by public researchers partnering with other
public institutions' researchers, both governmental and non-governmental. This
has provided useful insights for ARS and NCR administrators to guide
programmatic decision-making, as well as operational guidance; this function is
key because of its direct impact on the public interest as well as the specific
research interests of more directly involved stakeholders.

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

The 2021 NC7 RTAC meeting was held virtually and hosted by the NCRPIS and NC7 RTAC member Aaron Lorenz. Minutes of this meeting can be found on the NIMSS website.

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

- The NC7-RTAC committee encourages Expt. Station Directors to provide additional funding to the NCRPIS to meet future staffing and seed request needs more adequately.
- The 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.
- The committee recognize and thank Host David Peters, Chairman Professor Lorenz, Academic Advisor Carolyn Lawrence-Dill of ISU, and colleagues who contributed.
- The committee extends sincere thanks to outgoing members Amy Iezzoni (MI) and Pablo Jourdan (OH) for their past dedication, insights, and numerous contributions to the committee.
- The committee extends sincere welcome to new committee members Rebecca Grumet (MI), Jonathan Fresnedo-Ramirez (OH) and Carolyn Lawrence-Dill, Administrative Advisor and Associate Dean Iowa State University.
- The committee extends its highest appreciation and admiration to Candice Gardner, outgoing Research Leader at the NCRPIS for 21 years of outstanding service in accomplishment, resourcefulness, and perseverance.

VI. SUPPORT TEAM REPORTS:

A. <u>Farm (F. Engstrom, B. Buzzell, C. Hopkins)</u>

We supervised and coordinated daily operations at the NCRPIS farm, including management of all facilities, fields, and greenhouse space. We conducted all pesticide applications in the field and campus greenhouses. We responded to maintenance requests from staff members at the farm and the campus location. We selected, coordinated, and scheduled the student labor force. We coordinated and completed facility construction and upgrades along with safety inspections. The COVID-19 pandemic significantly changed our operation. The challenges forced us to find alternatives to the traditional methods we have been accustomed in our operation. Increasing social distancing mandated fewer people in a given area while our labor needs are heavy.

The COVID-19 impact on staffing levels and lower labor availability placed the facility crew into a "survival" mode supporting the various projects. Restrictions on contractors accessing the facility due to pandemic guidelines kept us from initiating projects that would require external support. The following statement is the same from last year's report yet is just as relevant as it was originally.

Recent changes in field operations have allowed lower staffing levels for spring field preparations. In the past, all cage frames were removed from the field at the end of the season to allow fall tillage and crop rotation with a row crop. We have created "permanent" fields where the cage frames remain year-round decreasing the labor needed to remove, and then grid, measure, and reinstall the cages annually. This process also allows us to have turf in the alley ways which then allows access to the fields regardless of precipitation events. New equipment that fits physically in the cage is a key factor allowing this development. The key factors in choosing this operation were due to perceived soil compaction affecting accession growth and health due to equipment traffic patterns, erosion of exposed soil between cages for weed management, and access to cages after a rain event. One benefit that has been realized this year was the decreased labor effort which has been instrumental in a year of COVID-19.

Labor:

During 2021, 28 applications for hourly employment were received and reviewed. There were approximately 20 interviews, resulting in 10 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 I) joined the staff in September 2016, and assists the vegetable project half-time, and facility operations half-time.

Maintenance projects:

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

- Reseeded grass ways in multiple fields.
- Coordinated the repair of the Seed Storage roof which was severely damaged during the 2020 Derecho event.
- Replaced additional exterior building security lights with LED units.
- Arranged for repair of HVAC equipment in multiple areas.
- Replaced additional light fixtures with energy efficient LED lights in offices and other work areas as needed.

Purchasing:

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

Equipment Purchased:

• Autosteer guidance system was purchased for the John Deere 6430 tractor utilized for field layout, tillage and spraying operations.

Tours:

During 2021 there were fewer than 20 visitors due to COVID-19 restrictions. Pandemic restrictions did not allow any tour groups to visit the facility.

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.

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

Jesse Perrett served as the first line of support for NCRPIS during 2021. Jesse is supervised by Pete Cyr who is dedicated to the GRIN-Global project. The following list outlines the progress made by the IT team during 2021 at NCRPIS.

Equipment:

As of December 2021, the NCRPIS had 35 desktop and 50 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 batteries for the rack ups systems were replaced in 2021.

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.

Deployed 15 laptop and desktop computers with all required equipment to set up users for teleworking during the COVID-19 pandemic. Ensured all users had remote access to needed files, email systems, and networking capabilities for remote work.

Deployed Tanium clients on all computers for department management of updates and patches.

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

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

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

Updated GRIN-Global label programs with enhancements and fixes as needed. Worked 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 were replaced in 2021.

Staff printed over 5,000 field-ready wooden stakes. Issues with print quality and print consistency were resolved to ensure minimal misprints.

Software:

All workstations at NCRPIS use Windows 10. Microsoft Office 365, Adobe Acrobat Professional DC, Adobe Creative Suite, Pulse Secure, ActivClient, Java, Tanium, 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:

Updated station information system security documentation and disaster recovery plans. The new documentation includes information necessary to repair or reconfigure station IT systems in the event of a natural disaster or equipment failure.

Weather station history data was provided via SharePoint to allow users to download current and past weather data including calculated GDU and CHU (heat unit) data. The station uses 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 2021:

- Continue to update documentation for IT systems and services.
- Develop a strategy to implement new infrastructure for and convert existing workstations to CEC images with USDA.net connectivity.
- Continue to replace user desktop systems with laptops and extended warranties and docks to prepare for the Client Experience Center (CEC) implementation.
- Continue to replace NCRPIS workstations on an as needed basis (targeting a 3-5 year lifespan for daily use workstations).

GRIN-Global:

GRIN-Global 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 it can be deployed on any size computer with a minimum amount of effort and cost. The GRIN-Global system is currently implemented by 29 international genebanks and is being evaluated for adoption by additional 22 other genebank entities. GRIN-Global 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 GRIN-Global system can be installed on a stand-alone desktop computer or in a network server/client configuration.

The USDA-ARS GRIN-Global development team is located primarily in Ames, Iowa (PIRU) and in Beltsville, Maryland (DBMU, or Database Management Unit). Pete Cyr is responsible for the Curator Tool, Search Tool and development of associated wizards and forms. Curator Tool 1.22.4.19 is in beta now and will soon be released for production use at NPGS and international genebanks. In FY2021 Mr. Cyr developed 12 new versions of the Curator Tool Software Suite for testing and distribution. Included in the 12 Curator Tool versions released to the public domain are 32 enhancements and 11 bug fixes in various components like the Order Wizard, Cooperator Wizard, Viability Wizard, Curator Tool and the Search Tool. In addition to the Curator Tool development work, Mr. Cyr has recently developed and

implemented the upgrades to the GRIN-Global implementation of the BrAPI specification 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 GRIN-Global Advisory committee.

DBMU personnel at the NGRL in Beltsville are responsible for the administration of the GRIN-Global database, the Middle Tier and security features, and the public website (PW), https://npgsweb.ars-grin.gov/gringlobal/search.aspx. PW 2.2.2.0 is currently under development. The NGRL botanist responsible for GRIN taxonomy works closely with DBMU developers.

Plans for 2021:

- Enhance the BrAPI implementation currently being tested to include native GRIN-Global security so that the BrAPI interface can be used for inserting and updating data in the GRIN-Global database using mobile applications in the field.
- Enhance the Attachment Wizard to support all attachment tables in the GRIN-Global database.
- Enhance Curator Tool to support SQL Server Reports in addition to Crystal Reports.
- Enhance the Curator Tool to leverage modern connectivity technology (like gRPC) to replace the legacy SOAP XML communication technology currently being used.
- Enhance the Curator Tool installation process to minimize the need for administrator permissions steps required to make the Curator Tool work with recent Microsoft security changes.
- Enhance the Curator Tool to present a user-friendly interface for managing dataview tabs, Crystal Reports, and Wizards gracefully.

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

Acquisition:

The North Central Regional Plant Introduction Station (NCRPIS) acquired 285 new accessions in 2021. Of these new accessions, 50 were received from within the National Plant Germplasm System (NPGS) through exploration and transfer. Number assignments by curatorial project included: 38 for the Pseudocereals, 65 for Horticulture, 27 for Vegetables project-including 22 accessions from the international Cucumis powdery mildew differential (ICPMI) set, and 126 new maize accessions-including 10 accessions from the GEM project, 19 from the University of Hawaii, and 80 newly expired PVPs. Twenty-seven of these accessions across all curatorial teams were collected through the SOS project. Details of specific acquisitions are found in the curators' sections of this report.

As new accessions are recorded in the Germplasm Resources Information Network (GRIN-Global) database, we include as much passport information as possible. Typical passport information includes a source history, cooperator records, collection-

site descriptions and geographic coordinates for wild collections, pedigree, secondary identifiers, 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 GRIN-Global database.

Maintenance:

Curatorial assistance was provided by processing requests for taxonomic reidentifications and nominations of accessions to the inactive file. In total, 17 accessions received taxonomic re-identifications and 78 accessions were inactivated. Twenty-four accessions were inactivated due to failure to germinate/not viable, and 54 accessions were inactivated due to duplication.

Additionally, 430 accessions were assigned PI numbers: 156 wild sunflower accessions, 200 accessions for David Brenner which included 45 spinach, 46 Echinochloa, 30 Amaranth, and 71 Chenopodium and 74 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, 122 new passport files were uploaded to the GRIN-Global database in 2021. We were unable to digitize additional legacy archival documentation this year due to a staffing shortage 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-mass to conform to document naming conventions that more easily support future upload to the GRIN-Global database.

D. Order processing (S. Estrada)

The GRIN-Global public website has improved accessibility to germplasm information and the ability to search for desired crop characteristics. This year, the order processing team continued to refine the use of GRIN-Global order actions, attachments, and local order 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 both NCRPIS teams (curatorial personnel, seed storage, pathology) and other NPGS personnel (i.e., APHIS, GRIN-Global feedback) to monitor a germplasm order more easily as it progresses through the pipeline towards fulfillment. Documentation related to orders is attached directly to the corresponding GRIN order via the Order Wizard's attachment tab, thus accessible to internal NPGS users. External users may also add attachments (usually an import permit, shipping instructions, or Excel file request list) through 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 2021, 2,271 orders were entered into GRIN-Global. A total of 2,345 orders containing 70,727 items were completed. Of these, 1,863 entered the order processing system via the GRIN-Global Public Website. This year, there was an average number, 814 requests, (44%) of non-research, non-educational (NRR) orders submitted for consideration. We anticipate this number will drop to less than 10% next year thanks to the introduction of the NPGS-wide NRR web order processing wizard in late 2021 which will dramatically cut down on the amount of NRR requests submitted through the public website and processed directly by Ames. A detailed summary of NCRPIS distribution activity is summarized in the table below which illustrates various internal use purposes, and in Appendix Table 3. A total of 41,700 items were shipped to external cooperators in 1,096 requests (834 domestic) even though order processing capacities and operations were reduced throughout the year because of the COVID-19 pandemic.

	2021 - NCRPIS Germplasm Distributions Summary										
				Grand Tota	ıl		Distributed				
		Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order
	Distribution	1,154	58%	47,068	68%	41	1,078	98%	41,360	76%	38
a	Non-research, non-educational	816	41%	9,592	14%	12					
External	Observation / evaluation	18	1%	349	1%	19	18	2%	340	1%	19
Ĕ	Repatriation	1	0%	1	0%	1					
	Total	1,989	100%	57,010	82%	29	1,096	100%	41,700	77%	38
	Backup	74	22%	2,923	4%	40	74	22%	2,923	5%	40
	Germination	183	54%	5,632	8%	31	183	55%	5,632	10%	31
rnal	Phytosanitary Testing	35	10%	3,219	5%	92	34	10%	3,201	6%	94
Internal	Replenishment/regrow	47	14%	853	1%	18	39	12%	767	1%	20
	Transfer	1	0%	1	0%	1	1	0%	1	0%	1
	Total	340	100%	12,628	18%	37	331	100%	12,524	23%	38
	Grand Total	2,329	100%	69,638	100%	30	1,427	100%	54,224	100%	38

Shipped orders:

A total of 2,329 external orders were processed. Of these, 1,427 (61%) were shipped and 902 orders were cancelled. External orders were cancelled for a variety of reasons including: 816 (35%) were NRR, 39 requestors were unable to secure an import permit, and 85 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. The COVID-19 pandemic significantly delayed all international distributions this year.

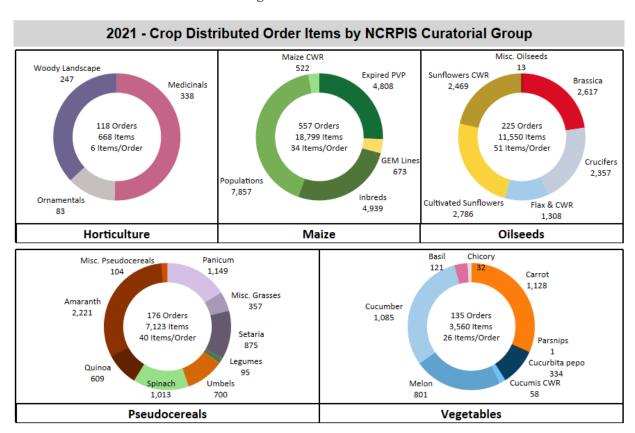
Domestic orders accounted for 76% of all externally distributed orders and 54% of the distributed items (Appendix Table 3), indicating that U.S. requestors received fewer items per order on average. Maize was the most highly distributed crop within the United States. We continue to see a high demand for oilseeds crops, mainly sunflower, by international requestors.

2021 - NCRPIS External Germplasm Distribution Summary by Curatorial Group

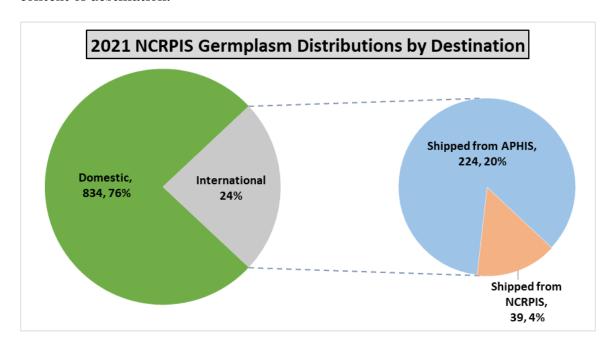
	Orders		Order Items		Avg. Items per Order	
Curatorial Team	U.S.A.	Int'l	U.S.A.	Int'I	U.S.A.	Int'l
Horticulture	106	12	424	244	4	20
Maize	448	109	10,835	7,964	24	73
Oilseeds	147	78	5,147	6,403	35	82
Pseudocereals	123	53	3,804	3,319	31	63
Vegetables	98	37	2,503	1,057	26	29
Grand Total	834	262	22,713	18,987	27	72

Order distributions were also summarized by curatorial group, as seen in the pie charts below. Vegetable and Horticulture curatorial groups saw high demand for a few crop maintenance groups while Oilseeds, Maize, and Pseudocereals requests were more balanced across crop groups. The largest orders, ca. 800 - 3,000+ items, were for maize lepidoptera bioassays and root phenotyping of maize landrace populations. See specific curator's sections for detailed information.

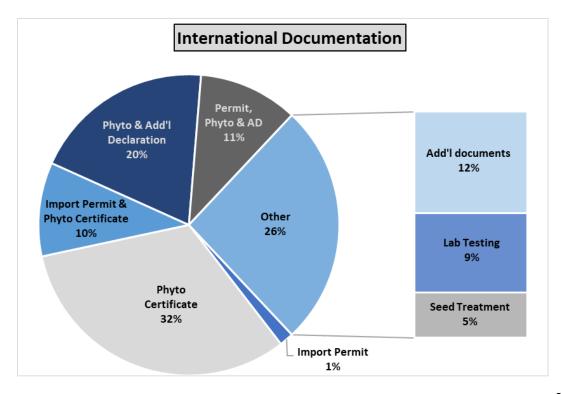
International orders accounted for 24% of those shipped and for 46% of all distributed items. Approximately 4 out of 5 international requests were transferred to APHIS in Maryland for phytosanitary certificate issuance prior to export. The E.U. now requires a phytosanitary certificate for all plant and plant part shipments so most international requests shipped directly from NCRPIS were sent to Canada or Mexico in accordance with the USMCA agreement.



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



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



The order processing team was busy this year with fewer resources available to satisfy germplasm distributions. Almost ninety percent of all international orders required issue of a phytosanitary certificate prior to export and 22% of international distributions were shipped with an import permit. Import permit restrictions vary in complexity.

E. Seed Storage (L. Burke, A. Sonner)

The seed storage area was staffed by two full-time, permanent federal employees (Lisa Burke and Ashley Sonner), and three part-time student employees during 2021.

The seed storage team stored 1,687 inventory lots, including 592 original seed lots. Of the increase lots, 959 were produced in Ames and 125 were produced outside of Ames. Across all stored inventory seed lots, seed quantities of 2,720 lots were reviewed, and any discrepancies with GRIN-Global information were corrected in the GRIN-Global database. 579 samples were prepared and transferred to the -20C freezer for long-term storage.

In 2021, 1,090 seed orders, including those for distribution, observation, germination, transfer, and backup were filled. The NCRPIS distributed 44,699 packets to meet distribution and observation requests. There were 1,029 lots sent to the National Laboratory for Genetic Resources Preservation (NLGRP) for backup, involving both accessions new to the NLGRP and additional seed quantities for previously deposited accessions. 1,713 accessions were packaged for shipment to Svalbard.

With the aid of student workers, 40,486 packets from 3,745 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 2021, 510 accessions received PI numbers and 1,098 inventory samples were relabeled and moved to the chronologically correct location.

Ashley Sonner continued imaging original samples in 2021. Original samples of 375 accessions were scanned in 2021. Images were uploaded to GRIN-Global utilizing the attachment wizard. With the purchase of the Dino-Lite in early 2021, Ashley was able to implement another method. Using the Dino-Lite, Ashley has been able to photograph small seeds (such as Chenopodium) more efficiently and capture taxonomically important details such as the hairs on the Agastache seeds. Ashley has worked on special requests such as imaging original plant parts prior to being destroyed.

Curatorial Group	Original samples imaged
Maize	2
Oilseeds	233
Horticulture	134
Pseudocereals	6

Imaging Protocol	Number of times protocol used in 2021
Large seed	134
Small seed	205
Dino-Lite	36



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Figure 1. Example of small seed protocol

Figure 2. Example of large seed protocol



Figure 3. Example of small seed protocol using the Dino-Lite

Lisa Burke continued to participate in the development of GRIN-Global. She served on the GRIN-Global Advisory Committee as chairperson. She chaired 20 meetings in 2021, each running between 1 and 2 hours. 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. Continued focus was made on clarifying codes and adding descriptions for proper code usage.

Lisa Burke continued as the station's CPR/AED/First Aid instructor. She provided training for two-year CPR/AED/First Aid certification to 11 NCRPIS student workers and 20 staff members. Due to COVID-19, these classes were conducted online with skills certification completed in a socially distance setup to comply with the pandemic situation. Each session was entered into the National Safety Council database and certificates of completion provided for each participant. Cooperative efforts with campus staff to improve the CPR/AED/First Aid training continues.

F. Germination (L. Pfiffner)

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

In 2021, the germination lab completed germination or Tetrazolium (TZ) testing on 133 orders containing 4,133 inventories.

Type of Order	Number of Orders	Number of Inventories
Regeneration	52	915
Maintenance	39	2,709
Original	9	247
Re-germ	26	212
TZ	6	39
Experiment	1	11
Total	133	4,133

This is a 54% decrease from the 7,707 inventories tested in 2020. The cause of the decrease is student schedule interruptions caused by the COVID-19 Pandemic.

Maintenance germination tests were conducted on accession taxa as summarized in the following table.

Genus	Number tested
Amaranthus	210
Brassica	51
Cichorium	128
Coriandrum	112
Cucumis melo	289
Dalea	12
Daucus	50
Helianthus	149
Hypericum	111
Linum	243
Ocimum	6
Pastinaca	8
Perilla	20
Petroselinum	110
Securigera	52
Spinacia	37
Zea	1,121

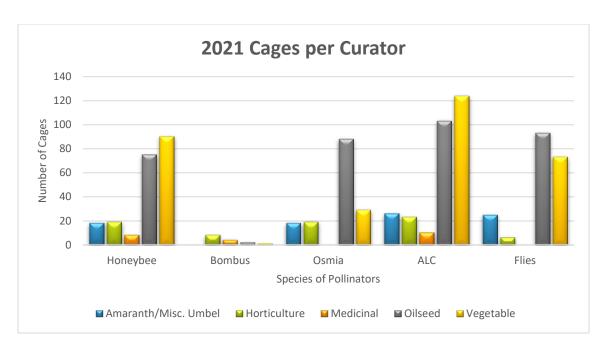
Several Association of Official Seed Analysts (AOSA) webinars and virtual meetings were attended as continuing education to maintain certification to stay in good standing and vote as a member of AOSA.

VII. CURATORIAL AND SCIENTIFIC TEAM REPORTS:

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

Overview:

A total 862 insect pollinators were supplied throughout the pollination season. This number does not include the multiple doses of alfalfa leafcutting bee (ALC) or flies that were added on a weekly basis, only the initial request. The graph below displays the total number of insect pollinators provided. Bee pollinators (minus the ALC and dipterans) were supplied a single time to 358 accessions for controlled pollination of 439 cages.



ALC and fly-pollinated cages are tabulated and reported separately due to multiple distributions of those insects to the same cages over the pollination season. Except for several weeks in during the summer, every cage gets at least a single dose of ALC and flies on a weekly basis. ALC and flies were used on similar plant types and in the summer both Blue Bottle Flies and Common House Flies were introduced together. During the Spring and Fall, only Blue Bottle Flies were used in cage pollinaitons.

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°F. 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 bomubs colony can be used in numerous cages throughout the summer.

Because we are more proficient at managing honeybees, they are the most used pollinator at the station. We can use a nuclues hive 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 for honeybees and a pollen patty mix every other week for continual brood production.

Health requests were made by the entomology crew or other curators throughout the summer in bombus and honeybee colonies. Nucs failed for multiple reasons: queen failure, weakened hive, age, damage, etc. Once notified or observed, the nucleus hive was replaced with another to maintain pollination in that cage. There were 60 honeybee health requests during the pollination season. These numbers are not shown in the graphs as that would be misleading to the total number of cages per curator.

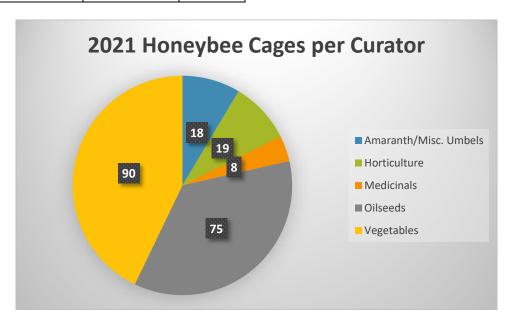
Total cage numbers are not included in the following graphs or charts because of duplicate plantings, multiple nucs per cage or accessions which were relocated from greenhouse to field. A total 862 cages with 748 accessions had insect pollinators supplied to them throughout the pollination season. The graph below displays the total number of accessions pollinated per project.

Honeybee Pollination:

Honeybees were used to pollinate 210 cages in the field including health requests and duplicate accessions. The following table does not include health requests or duplicate accessions, only single honeybee requests.

2021 Honeybee Pollinator Deliveries to Regeneration Cages

Crop Group	Total # of Accessions	# of Genera	# of Accessions/Genera
Misc. Umbels	16	4	12 Carum, 2 Foeniculum, 1 Daucus, 1 Erynigium
Horticulture	10	5	3 Cornus, 3 Salix, 2 Viburnum, 1 Dirca, 1 Spiraea
Medicinals	8	2	6 Agastache, 2 Monarda
Oilseeds	75	1	75 Helianthus
Vegetables	88	5	24 Cichorium, 24 Cucumis, 21 Ocimum, 18 Daucus, 1 Cucurbita
Total	197	17	



Overwintering:

All but eleven three-story hives were left outside during the winter 2020/2021 at three locations and were wrapped with forty-pound roofing paper. Eighteen three story parent colonies were located at the NCRPIS with six in the caged in area designated for hives and twelve at another protected location. Twelve additional colonies were placed at three locations at one off station site, for a total of 30 outside three story colonies. Fourty-one two story parent colonies, 11 three story colonies and 78 double



story nucleus hives were placed into the overwintering room. It was observed that 32% of the double story nucleus hives were lost prior to putting them into the indoor facility and 15% of the parent colonies were lost prior in the fall of 2020. However, we did not lose any of the outside colonies prior to wrapping them. All parent colonies stored inside were removed from the room starting on March 8th, and all outside colonies unwrapped on March 3rd,

the nucleus hives were removed from the over-wintering room on March 9th. In the winter of 2020/2021, 47% of the wrapped three-story colonies left outside survived, 75% of the inside colonies survived and 15% of the double story nucleus colonies stored indoor survived. While the hives were in the over-winter room, they were fed a syrup/sugar patty combination in February and March to improve their survival in the spring.



All nucleus hives which were not used in cages for pollination were fed HFCS, an additional super 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 overwinter room or wrapped. Feeding will begin February/March of 2022 to assist with survival of hives into the spring. In the fall of 2021/2022, we place a total of 138 double nucleus hives, 48 two story colonies and 7 three

story colonies into the over-winter facility. Hives were wrapped at three locations a total of 24 three story colonies (6; 6; 12) with forty-pound roofing paper.

Based on local beekeepers counts, mite numbers were lower in 2021. Hives were not sampled prior to applying mite treatment. In mid-September, all colonies and double nucleus hives were treated with Formic Acid (Mite Away Quick Strip®) to prevent any varroa mite build up over the winter. Hives were not sampled after treatment to confirm 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 2021, 30 five frame nucleus hives were purchased from a local supplier, 30 threepound packages from two local beekeepers (15 packages each) and 50 Italian queens from a California supplier. These were used supplement over-winter losses and to supply spring nucs used for cage pollinations. The queens arrived by USPS mid-April, the packages were picked up and put into full size equipment in mid-April and the nucs were placed into full size equipment prior to pick up in May. 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 and adhering bees. All 50 queens survived and were used for "spring" nuc production.



Queen-rearing:

In early May we selected six resilient, over-wintered parent colonies and set them up as "cell builder colonies" for queen production during the summer 2021. The first queen grafts were done on June 1st, the first two grafts only produced approximately 12 cells per week, however later in the summer the queen production improved to a weekly average of 30 cells. The last graft was done at the end of July. As in the last two years, because packages/nucleus hives were purchased locally, we had no issues of aggressive bees.

Feeding:

Starting in March through early April, all parent colonies and nucleus hives were given a sugar patty and pollen patty and then four feedings of HFCS. In October to mid-November, all hives were fed weekly. In 2021, we were again able to mix the Fumagilin – B® medication used to treat dysentery (nosema) with the syrup, the last two feedings in the fall which all hives received was medicated.

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 17th, 550 gallons of HFCS was purchased for supplemental feeding during the summer and into the spring of 2022. 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. The new "winter/spring" feeding system of using granulated sugar and HFCS showed to be beneficial to the bees and increase hive survival.

In 2021, 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 this method sustained the hives until they could be relocated outside and fed HFCS.



Wax moth:

As with the past four years, for wax moth control during the summer, all stored supers with "cleaned" frames were stacked at right angles to each other to prevent adult moth migration in the equipment room. Starting in June through September, the lights in the equipment room were left on during working hours (8 hours; five days). All equipment removed from the field as "dead hives" was placed in the back room of the shop, this in the past has been the initiation for a late season outbreak of moth in some boxes of frames. However, there was no noticeable wax moth larva in the fall.

Bee yard registry:

As in 2020, 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 directly onto Google maps. For 2021, only locations to be used in 2021 had to be confirmed in the system. The IDALS registry assists pesticide applicators in locating bee-yards and in obtaining contact information of appropriate beekeepers prior to spraying.

Bombus pollination:

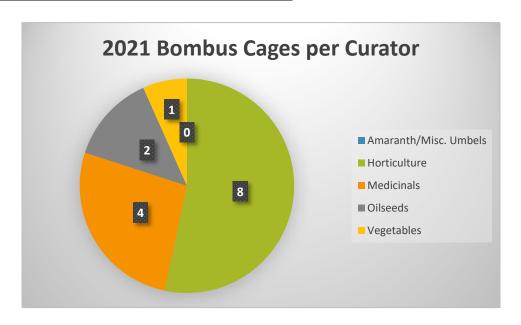
Five "mini-research" colonies of Bombus impatiens were purchased from a commercial supplier and used for pollination at the station. Bombus was used to pollinate 15 field cages with 9 accessions. A single Bombus hive was used for pollinating more than one cage with a minimum lapse of 24 hours between sites to prevent pollen contamination. For some cages, loose worker bees refused to return to the hive and had to be retrieved by hand and released when the hive was switched to a different location. While in holding, hives were stored in a



23° C rearing room and loose bees in cups were supplied with sugar-soaked cotton wicks as a food source while being held. In the Cucurbita and some Helianthus cages, a single Bombus hive was combined with a honeybee nuc for more efficient pollination because of the amount of vegetation.

2021 Bombus Pollinator Deliveries to Regeneration Cages

	Total # of		
Crop Group	Accessions	# of Genera	# of Accessions/Genera
Misc. Umbels			
Horticulture	2	2	1 Dirca, 1 Heuchera
Medicinals	4	1	$4\ Monarda$
Oilseeds	2	1	$2\ Helianthus$
Vegetables	1	1	1 Cucurbita
Total	9	5	

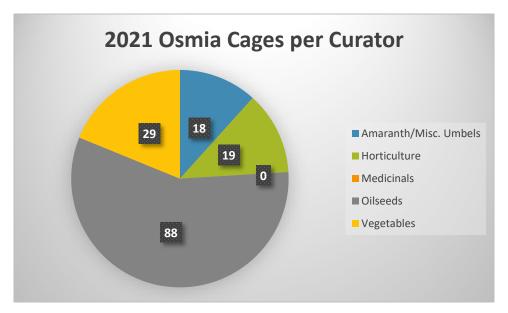


Osmia cornifrons/O. lignaria pollination:

Osmia sp. were used to pollinate a total of 154 cages containing 152 accessions in five fields and four greenhouses.

2021 Osmia Bee Pollinator Deliveries to Regeneration Cages

Crop Group	Total # of Accs	# of Genera	# of Accessions/Genera
Misc. Umbels	18	3	14 Carum, 2 Coriandrum, 2 Pimpinella
Horticulture	17	5	10 Aronia, 3 Salix, 2 Viburnum, 1 Dirca, 1 Heuchera
Medicinals			
Oilseeds	89	8	77 Brassica, 4 Linum, 3 Camelina, 1 Hesperis, 1 Lepidium, 1 Matthiola, 1 Sinapis, 1 Thlaspi
Vegetables	28	2	27 Daucus, 1 Cucumis
Total	152	18	





Osmia pupae arrived in March from a previously used supplier based in Idaho and in April from a new supplier in Washington state. A total of 3,800 pupae were shipped (2,300 Idaho and 1,500 Washington). It was determined pupal cocoons needed to be opened prior to placing into the cages for the early greenhouse pollinations based on an absence of adult bees. This practice will be continued in the future for Osmia use in the greenhouse. In the spring of 2021, 164 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 2022 season.

In November of 2021, Osmia tubes were sorted and counted. Approximately 166 pupae were collected which will be used in the spring of 2022. To get a more accurate count, all Osmia cocoons were

removed from the straws. Our numbers were lower than past years because previous counts were made by looking through the paper tubes and making estimations on total ALC cocoons and possible counting pollen disks or other types of pupae in the past. An additional 2,300 Osmia were ordered from the same two commercial suppliers for delivery in mid-February/early March of 2022.

Alfalfa leafcutting bee (ALC) Megachile rotundata:

ALC bees were purchased as pupae in leaf cells from a single supplier for use throughout the 2021 pollination season over a variety of crops. Arriving on April 20th, the bee cells were held in a refrigerated storage unit until scheduled for placement in the warm incubation unit and bee emergence boxes. The cocoons were from a Canadian source, which have fewer parasites and parasitoids than found in U.S. cells.

All ALC were stored in Precision® incubators and initial emergence was done in a Thermo-Scientific Environmental Growth Chamber (EGC). Bees were available weekly throughout the year for use in plant regeneration cages in the field and greenhouses from December 2020 into 2022.

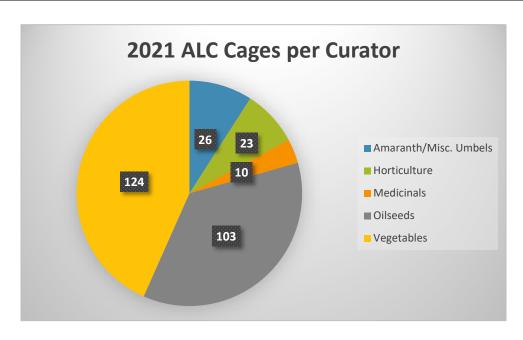
The 2021 pollinations were completed using the remaining tubs of the 2020 cocoons first and finishing with the 2021 cocoons. There was a period in March and April, prior to the arrival of the 2021 cocoons, in which



bees were unavailable for cage pollination and were not used until early May. In 2021, 2,234 total ALC deliveries were made to a total of eleven fields and four greenhouses with 286 cages containing 233 accessions and 27 genera.

2021 Alfalfa Leafcutter Pollinator Deliveries to Regeneration Cages

	# of	# of	# of	# of	
Crop Group	Deliveries	Accessions	Locations	Genera	Time Period
Misc. Umbels	163	23	3	7	Dec. (20) – Oct.
Horticulture	57	15	6	6	May – Aug.
Medicinals	83	10	3	2	June – Sept.
Oilseeds	873	98	5	9	Feb. – Oct.
Vegetables	1,058	87	4	3	Nov. (20) – Sept.
Total	2,234	233	21	27	Nov. (20) - Oct.



Numbers of active ALC-supplied cages and frequency of bee delivery vary seasonally and by cage structure/location and individual accession characteristics. In normal pollination situations, ALC bees/cells are only provided to crops in the field during the summertime months. However, at the station ALC are used outside of the normal time frame. From November 2020 through October 2021, greenhouse cages were supplied weekly with bees and field cages started in early May and the number of weekly active cage increased through late-July and then declined with the last field cages supplied through late-October.



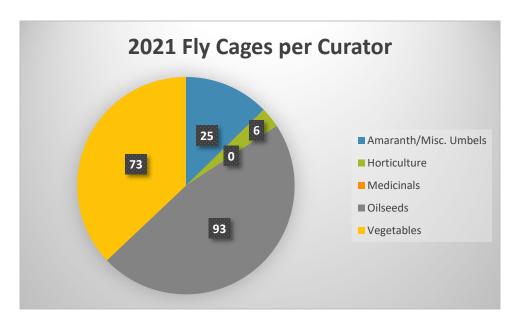
From late August to Mid-October, ALC were placed into cages of *Helianthus sp*, *Aronia sp*, *Agastache sp* and several field vegetables cages. Under normal conditions ALC are not the major pollinator of some of these accessions nor are they used this late in the season in field cages. Because we had no fall greenhouse pollinations, yet a low number of bees continued to emerge, we chose to place them into field cages rather than just setting them free outside.

Flies (Blue Bottle Flies and Houseflies):

Fly pupae of Calliphoridae and *Musca domestica* were purchased from two suppliers and incubated for weekly use from early March 2021 through mid-October 2021 for greenhouse and field pollinations. From June through August, 13 orders of 20,000 house fly pupae were purchased and from late May 2021 through mid-September 2021, 189 cups of blue bottle pupae were purchased. In 2021, 1,379 fly deliveries were made to seven fields and five greenhouses with 197 cages holding 157 accessions of 16 genera.

2021 Fly Pollinator Deliveries to Regeneration Cages

C C	# of	# of Accessions			Time Period	
Crop Group	Denveries	Accessions	Locations	Genera	Time Period	
Misc. Umbels	148	22	3	6	March-Oct.	
Horticulture	16	2	2	2	April – Aug.	
Medicinals						
Oilseeds	705	91	4	7	March – Oct.	
Vegetables	510	42	4	1	March – Sept.	
Total	1,379	157	13	16	March - Oct.	



From mid-March 2021 through late August 2021, greenhouse cages were supplied weekly with flies. The 2021 field requests for flies started in early April and the number of weekly active cage increased through mid-August and then declined with the last field cages supplied through early-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 have fly pollinators introduced also have 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 three 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 late September 2020, new Epi-pens were purchased with end-of-the-year spending. The distribution and use of the new pens was based on the stipulation that in 2021 all station personal would complete the Epi-pen training in the spring of 2021. In mid-March, K. Grooms (nurse at ISU Occupational Medicine) was contacted about required training and if a PowerPoint presentation had been created by ISU Environmental Health and Safety (EH&S) on anaphylactic shock and Epi-pen training or if the Epi-pen website training would continue to be used for training staff. 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.

Plans for 2022:

Changing of signage:

To assist station staff in the delivery of shipped pollinators in case of entomology staff being absent and the deliverer being unfamiliar with the storage location of an insect, new insect signage in the entomology building will be updated during the winter of 2021/2022. Because different pollinators are stored in different locations under different conditions to promote their survival and longevity, posting will be created listing what the pollinator is and where it is to be placed including a picture description posted directly on the location.

Safety information or warning signage has not been updated in many of the labs since 2011. Since much of this information is either outdated or a different action is carried out now, this signage will be updated to 2022 standards.

<u>Training videos</u>:

In the fall of 2019, all pollinator activities were videotaped by IT staff to be used as training videos, some of these were then updated/improved in the summer of 2020 by K. Judson and entomology staff. However, because of the need of more professional videos required by the HEC grant, a major focus in 2022 is to work with F. Douglass in either updating present videos or create new ones showing all aspects of the pollination duties and the introduction of pollinators into cages. In the future, these videos will be used as training videos for both PI staff or by educators to teach individuals the work done within the USDA for germplasm production and more specific the pollination activities carried out at NCRPIS to produce that seed.

B. Plant Pathology (C. Warfield, N. Pal)

C. Warfield joined NCRPIS in March 2021 and N. Pal announced her departure from NCRPIS effective January 2022 after accepting a position with USDA-APHIS in Ames, IA.

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. In addition, they provide support for curators and their crews in addressing plant health concerns and providing disease diagnostics and management recommendations.

Maize:

During August, field inspections of 129 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. Similarly, 142 GEM plots were inspected for the same group of diseases. The disease pressure was quite low throughout the growing season. The most prevalent diseases in terms of incidence of infected plants were gray leaf spot (Cercospora zeae-maydis), common rust (Puccinia sorghi) and common smut (Ustilago maydis) observed on ears, stalks, and/or tassels. Northern corn leaf blight (Exserohilum turcicum), and Physoderma were also noted. In terms of common diseases of phytosanitary concern, none were observed.

A spring greenhouse inspection of six maize accessions took place in June and a fall greenhouse inspection of 10 accessions in October. Common and southern rust were prevalent in one accession during the fall inspection. A low incidence of gray leaf spot was present, but no diseases of phytosanitary concern were found.

Oilseeds:

Two field phytosanitary inspections, the first in June and the second in July, were carried out for 163 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. One plant with basal stalk rot was determined to be infected with *Sclerotinia sclerotiorum* and was removed from the plot. Alternaria leaf spot was prevalent in most plots. A high infestation of Gorgone checkerspot butterfly caterpillars and associated leaf damage were observed in multiple plots (cages) during the July inspection.

Flax increase plots (51 accessions) inspected in June and July, in general, had no diseases present. Stem dieback or wilt was observed on a few plants. Alternaria and *Fusarium* were isolated. Either fungus may have been present as a primary pathogen or secondary saprophyte, but no further spread was observed.

Three phytosanitary inspections were conducted in May, June, and July for all 81 Brassica napus 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. Stem fasciation was observed on one plant, but phytoplasma infection was ruled out via PCR testing. A few plots had plants with leaf damage due to Gorgone checkerspot butterfly caterpillars. No black rot or other diseases of phytosanitary concern were observed.

Cucurbits/Vegetables/Herbs:

Routine disease testing for squash mosaic virus (SqMV) was conducted on all cucurbit seedlings prior to transplanting, as has been done since 1993. Of the 22 accessions tested by ELISA in 2021, there was no evidence of SqMV.

Multiple disease inspections of the cucurbit cages took place during July. Chlorosis and mottling of the lower leaves were prevalent symptoms throughout the plots but appeared to be a nitrogen, and possibly magnesium, deficiency rather than a disease issue. Angular leafspot caused by *Pseudomonas syringae* pv. *lachrymans* was suspected in one plot but ruled out via PCR. Pathogenicity assays were unable to reproduce the symptoms. No other diseases of phytosanitary concern were observed.

No diseases were observed for the 21 *Ocimum* and 20 *Daucus* accessions inspected. A high incidence of stem fasciation was noted in most *Cichorium endivia* plots. A subsample of plants with and without fasciation were tested via PCR for phytoplasmas but all were negative. Discoloration and leaf spots were observed on one chicory plant, and Neopestaliotiopsis was isolated. No diseases of phytosanitary concern were found.

Carum, Celosia, Echinocloa, Foeniculum, Setaria, Erygnium:

Phytosanitary inspections were conducted in the field for 27 total accessions. No diseases of phytosanitary concern were noted. Cercospora leaf spot was found on *Celosia*, and Helminthosporium leaf spot was found on *Echinocloa*.

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

Greenhouse inspections were conducted in spring and fall. No diseases of phytosanitary concern were noted among the 108 accessions scouted in the spring or the 81 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 *Wheat streak mosaic virus* (WSMV). The majority of seed health assays performed use ELISA as the detection method.

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 prepared letters to assist seed requesters in obtaining waivers from regulatory officials in their respective countries. Approximately 102 seed orders required an AD and 15 orders required a waiver letter in 2021.

Research:

Monarda:

Powdery mildew is the most common and serious disease associated with Monarda. While powdery mildew resistance is a key trait of interest in breeding programs, only a handful of commercially available cultivars exhibit high disease resistance. Fortysix Monarda accessions established at the NCRPIS farm were visually evaluated for powdery mildew susceptibility in a preliminary survey to assess the value, feasibility and methodology necessary for conducting a formal resistance screen. Variability in powdery mildew incidence and severity was observed among plants within a given accession (row), and the pattern of infection on individual plants was variable. This variability was likely influenced by multiple factors including environmental exposure, row position and plant age. We determined field screens using established, mature plants may have limited value in identifying or characterizing powdery mildew resistance as a population for a given accession. However, the variability observed among plants within an accession, if real, suggests individual plants with higher degrees of powdery mildew resistance may be present within the population. If identity preserved, these individuals could be useful as a potential source of genetic resistance in a breeding program depending on the degree of resistance. An alternative approach to using visual disease ratings in a field screen to enable more precision in identifying and characterizing meaningful differences in susceptibility may be inoculations of detached leaves under controlled environmental conditions and digitized evaluation of infection progression. It was determined through DNA sequencing the powdery mildew infecting the Monarda in the field plots was Golovinomyces monardae. Henbit (Lamium amplexicaule), an annual weed found throughout the Monarda plot and farm was also infected with powdery mildew. We hypothesized the henbit may be a source of infection for the Monarda; however, the powdery mildew fungus infecting the henbit was identified as Neoerysiphe sp. through DNA sequencing.

Pantoea Validation Study:

The pathology team participated in a validation study of a qPCR method for the detection of *Pantoea stewartii* subsp. *stewartii* as part of an ASTA Seed Science Foundation (SSF) funded project. Drs. Charles Block and Silvina Arias, affiliated with the ISU Seed Science Center, organized the study. This qPCR method is intended to be used as a follow-up, or confirmation step, for the NSHS *Pantoea stewartii* ELISA assay Mz10.1. The Stewart's wilt ELISA assay occasionally produces false positives, mainly on seed grown in tropical or sub-tropical regions (e.g. Hawaii, Puerto Rico, Mexico). The PCR assay was originally designed by Pal et al. (2019) to distinguish true positives from false positives caused by *P. stewartii* subsp. *indologenes*. The goal

of the validation study was to ensure the qPCR assay could be reproduced in multiple labs and eventually added as an amendment to the NSHS ELISA assay.

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

Acquisitions:

Thirty accessions were acquired in 2021: 13 Amaranthus, 10 quinoa-related, and 7 Dalea.

The Amaranthus accessions (Ames 35636 to Ames 35648) were donated by Dr. Katherine Waselkov of California State University, Fresno, with USDA Plant Exploration Office funding. Dr. Waselkov worked with her students in a field biology class she teaches to collect seeds of Amaranthus species including our first two accessions of the rare Amaranthus obcordatus. Four of these Waselkov accessions were regenerated in the fall 2021 greenhouse planting.

The ten quinoa related accessions arrived in two separate donations. The first was received from Dr. Eric Jellen of Brigham Young University. Jellen donated four accessions (Ames 35661 to Ames 35664) including the rare species *Chenopodium cycloides* and *C. nitens*. Jellen also donated a *C. ficifolium* which is of current research interest as a representative of quinoa's "B" genome. David Brenner donated the second group of six accessions (Ames 35761 to Ames 35766) including DB 2020146 which was segregated from PI 658740 for having unusual deciduous seed coats, and an obscure wild-collected weed *Chenopodiastrum simplex* from Ames, Iowa.

The seven *Dalea* accessions (Ames 35721 to Ames 35727) were donated by Laura Fischer Walter of the University of Northern Iowa's live plants from well-documented Iowa locations. They were transplanted into field cage footprints for seed production. Some established poorly and the success of these regeneration efforts will not be known until seed is harvested in 2022 or 2023.

Collection Maintenance:

Spinacia:

Our spinach regeneration cooperator, Beiquan Mou of the USDA-ARS in Salinas, California regenerated 37 accessions of spinach in 2021 and shipped the seed lots to us in early 2022. Mou planted 15 accessions in 2021 for harvesting in early 2022. We regenerated a wild spinach accession here in Iowa with a seed harvest in early 2021, and in the fall planted a second wild spinach for harvesting in early 2022. We are grateful for the substantial effort and commitment from the Salinas group driving our spinach germplasm maintenance. In addition to increasing seeds Mou takes notes on the presence of rare dioecious plants for us to post publicly in the GRIN-Global spinach descriptors.

The staff at the NCGRP in Fort Collins, CO sent PDF scans of storage information forms for spinach accessioned there since 1961. We extracted information for GRIN

narrative fields and attached the forms to 56 accessions so they can be seen by public GRIN-Global users. PI 699766 is an example.

<u>Chenopodium</u>:

We are growing many wild species of *Chenopodium* now that the entire genus is a research priority for improving *Chenopodium quinoa*. Sixty-one seed lots of wild *Chenopodium* were harvested in 2021. Our success propagating wild *Chenopodium* species has improved with a new dormancy breaking protocol. A video of this method, involving sliding the seeds across a sharpening stone to grind a hole in one face of the seed, was produced by Forrest Douglass (Douglass and Brenner 2022) after researchers expressed interest. The video is available on YouTube.



Wild *Chenopodium* seed scarified with our new method using a sharpening stone to scape a hole in the seed coat on one flat side of the seed. The seeds are about 1 mm wide and were imaged with the new microscope.

Miscellaneous maintenance progress:

We had a successful 74-row amaranth observation planting in the field. Notes on adaptation to Iowa field conditions will be added to GRIN-Global.

Characterization/evaluation/taxonomy:

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

Updating GRIN-Global 2021

Count	Name	Description
13	Taxonomic Re-IDs	Taxonomic changes were made in five genera. Seven of the changes were in <i>Chenopodium</i> .
46	Observations	Observations were loaded into GRIN-Global mostly for the new <i>Amaranthus</i> male sterility crossing descriptors.
138	Citations	Links to research publications were loaded onto cited accessions in GRIN-Global.
206	PI numbers	Permanent "PI" number identifiers were assigned to accessions with temporary Ames numbers. They include 71 <i>Chenopodium</i> accessions and a mix of other genera.
264	Seed lots stored	Most of these were grown for regeneration in 2019 or 2020. They include 97 (37%) in the quinoa group, 50 (19%) <i>Amaranthus</i> , 44 (17%), <i>Setaria</i> , 31(12%) Umbels, 17 (6%) spinach, and small numbers of accessions from five other groups.
1,690	Images loaded	Including 1,292 (76%) loaded by Samuel Flomo. Sam made great progress with our image loading backlog.

New microscope:

A new stereo microscope with a digital camera was purchased for improving taxonomic determinations and for seed imaging. The microscope is especially useful for the seeds of wild *Chenopodium*, with taxonomically diagnostic microscopic traits.

Image donations:

Two researchers donated images of accessions to load into GRIN-Global. Dr. Jellen of Brigham Young University donated 106 images of wild Chenopodium seeds taken under a microscope (PI 698415 is an example). They are helpful for confirming taxonomic identifications. Dr. Sandra Branham of Clemson University donated images of 379 of our spinach accessions growing in field conditions, in North Carolina (PI 699766 is an example).

Amaranthus:

New descriptors were added to the observation section of GRIN-Global and populated with observations on how amaranth accessions would function in producing hybrid seeds in a cytoplasmic male sterility (CMS) hybrid-seed system. Accessions used as males either maintain male sterility or restore male fertility, and this is now documented in GRIN-Global. Three accessions are male fertility restorers and 13 are male sterility maintainers as males in crosses with our standard CMS male sterile as a female.



The DB 2020139 plants on the left are progeny of a cross between cytoplasmic male sterile PI 686465 and PI 628779 on the right. The stunted misshaped progeny are from a genetic incompatibility that PI 628779 and related accessions have with most other *Amaranthus*. This incompatibility may someday be useful for maintaining seed purity in areas where weedy amaranth pollen contaminates genetic purity of grain amaranths.

Apiaceae:

An unusual accession that arrived as a taxonomically unidentified Apiaceae was reidentified to *Daucus carota*, carrot, with expert help by Fernando Martinez Flores and Kathy Reitsma. This accession (Ames 30304, Tun312) was collected by expert *Daucus* collectors in Tunisia in 2009 as one plant, since only one umbel arrived in the original packet. The collectors were misled by the seeds which lack the bristles of typical *Daucus*, so they collected it as an unidentified Apiaceae. In 2021 when the plants were growing, the taxonomy was corrected. The accession is non-uniform and it especially segregates for seed types, bristled and non-bristled. The non-bristled trait is potentially useful in improving cultivars since non-bristled seeds would flow better in planting or seed cleaning. Unfortunately, flowering is un-synchronized, resulting in many small harvests. After the final harvest in 2022, the accession will be transferred to Kathleen Reitsma, the Carrot Curator.



Seeds of Daucus carota, Ames 30304, Tun312, imaged by Ashley Sonner.

Panicum:

Dipak Santra and his group in Nebraska planted the proso millet collection to study climate adaptation in the High Plains. They discovered that only 210 (30%) of the 701 accessions they planted matured seeds early enough to be adapted there and be directly useful for plant breeding. The group is actively working with and publishing on our germplasm.

Commercial development:

Dryland genetics released two new proso millet varieties DLG 40 and DLG 240 yielding 20 to 40% more than 'Huntsman' in the high plains area of Colorado and Nebraska (Anonymous 2021). Patrick Schnable (Iowa State University). Plant breeders involved with this acknowledged that the PI station germplasm was "extremely helpful" in the development of these new varieties.

Outreach and Presentations:

Brenner presented on cytoplasmic male sterile amaranths at the American Society of Agronomy meeting in Salt Lake City (Brenner, 2021), and again online at a virtual meeting organized in Yucatan (Brenner & Turriza-Escalante, 2021).

Service:

Brenner led a field trip at the American Society of Agronomy meeting in Salt Lake City on November 7, 2021: This encompassed a foray in the nearby mountains of the Unita-Wasatch-Cache National Forest.

https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Session/22156

Brenner reviewed one journal article.

Plans for 2022:

Amaranthus duplication:

We are continuing a multi-year study of duplication in the Amaranthus collection especially accessions from the Peruvian LSK collection that arrived and were accessioned more than once. After duplication is confirmed by side-by-side grow outs, accessions are merged under one active accession number.

Unidentified accessions:

Four taxonomically unidentified accessions are currently being grown for seed increases and identification (Ames 25708, Ames 29103, Ames 29113, Ames 31286). The most unusual is Ames 29103 an accession presumed to be in the carrot family, but the seedlings have only one cotyledon which is typical for plants in the monocotyledon group of plant families, and not for the carrot family. However, the seeds and first true leaves are consistent with plants in the carrot family. A seedling's image with just one cotyledon is posted below.



A taxonomically unidentified accession, Ames 29102, has a monocotyledon first leaf which is very unusual for plants in the carrot family which generally have paired dicotyledons.

Petroselinum (parsley):

The seed viability team performed a maintenance viability test on the parsley distribution seed lots. Viability had fallen since the previous test, 116 accessions (62% of the parsley) have less than 70 percent viability. Many of these 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. We will start this new regeneration cycle with a summer 2022 planting for summer 2023 harvests. The accessions with temporary accession numbers should receive PI numbers before regeneration. Similarly, a large maintenance germination run on *Panicum* is in

progress and is expected to reveal some low viability seed lots that will need fresh regenerations and replacement of 60-year-old seed lots.

Setaria:

I plan to install descriptors in GRIN for the four kinds of tall "giant" weedy *Setaria viridis*: brown bristle, green bristle, red-purple bristle, and white bristle.

Publications about our germplasm:

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D. Horticulture (J. Carstens, A. Sherwood)

The Horticulture project currently holds 3,990 accessions representing 191 genera (Table 1.0). Significant NC7-medicinal collections include: Actaea (44), Agastache (100), Echinacea (199), Calendula (83), Hypericum (226), Monarda (172), Prunella (54), and Tanacetum (53). Significant NC7-ornamentals collections include: Alcea (34), Malva (55), Phacelia (53), Potentilla (127), Sphaeralcea (90), and Thalictrum (52). Significant NC7-woody landscape collections include: Aronia (110), Betula (167), Cornus (219), Euonymus (60), Fraxinus (505), Gymnocladus (90), Rhus (101), Salix (63), Spiraea, (106), Staphylea (45), and Ulmus (44). Jeffrey Carstens serves as curator and Andrew Sherwood as technician.

Table 1. Active accessions maintained in the NC7 horticulture collections (medicinals, ornamentals, and woody landscape) as of December 31, 2021

Management group	Genera	Accessions
NC7-medicinals	35	1,133
NC7-ornamentals	56	778
NC7-woody landscape	100	2,079
Total	191	3,990

Acquisitions:

During 2021, a total of 54 accessions were acquired including 18 medicinal, 3 herbaceous ornamental, and 33 woody landscape accessions. An additional 12 acquisitions (Hesperostipa (1), Penstemon (1), Thuja (1), Geum (1), Dalea (7), and Verbena (1)) were acquired through NCRPIS horticulture team collection activities (3) or through other collaborators (9) and transferred to other NPGS repositories for curation.

Targeted acquisitions were focused on collecting *Aronia* (4) in Illinois funded by the USDA-ARS Plant Exchange Office. A NCRPIS Collection Trip Report (Aronia) was published to GRIN-global for each accession (10) acquired during that exploration.

Collaboration continued with the Iowa Department of Natural Resources to sample *Pinus strobus* from remnant specimens in Iowa resulting in the acquisition of six accessions. The goal of this project is to eventually create a seed orchard utilizing only Iowa ecotypes to supply germplasm for future restoration projects.

The following acquisitions merit recognition:

- Sassafras albidum (Ames 35740 and Ames 35741) was collected in the northwestern edge of the species native range. This species is being researched by the National Laboratory for Genetic Resources Preservation to better understand seed quality and storage behavior potentially leading to improved *ex situ* conservation methods for taxa known to be recalcitrant or short-lived in storage.
- Salix serissima (Ames 35657) is a boreal species and an obligate wetland species with a concentrated abundance and distribution in the northeastern portions of the U.S. and in Canada from Newfoundland to British Columbia and south to New Jersey, Illinois, and Colorado. Disjunct populations occur in Montana, Wyoming, Colorado, and South Dakota. Only four occurrences of this species occur in South Dakota where two populations occur on Black Hills National Forest lands, a large population at McIntosh Fen (Pennington County), and a small population on Middle Boxelder Creek (Lawrence County). Genetics for this accession originate from McIntosh Fen. This site has significance tracing back to an expedition in 1874 led by Lt. Colonel George Armstrong Custer. Photos of the site are listed in the GRIN-Global accession page that were taken in 1874 by N. Winchell, 1985 by D. Ode, and 2021 by J. Carstens. Genetics of this accession represents the extreme western edge of the species native range.
- *Monarda fistulosa* (Ames 35736) is an early flowering variant of *Monarda fistulosa* that tends to be localized to xeric habitats with a short stature and pubescent leaves. In comparison to local *M. fistulosa*, this accession tends to flower approximately five weeks earlier.
- Echinacea laevigata (Ames 35728) is a federally endangered species that was recently acquired from a newly discovered population in Georgia. This brings the current collection of *E. laevigata* to 11 accessions originating from Georgia (2); North Carolina (3); South Carolina (5); and Virginia (1).

Maintenance:

Regenerations:

Existing plantings that consist mostly of *Aronia, Agastache, Monarda, Betula, Hypericum*, and *Spiraea* were increased via controlled pollinations and harvested. A total of 37 accessions were increased as seed. A total of 12 *Salix* accessions were regenerated clonally by shoot cuttings. Statistics in Table 2.0 (Number Harvested Regen) also includes the number of original seed samples harvested to keep records of harvest dates in nature. A total of 34 accessions were harvested as LV to serve as potential distributions to cooperators interested in genetic analysis as opposed to depleting seed inventory.

Germination for future regeneration was attempted on 45 accessions focused on Aronia, Agastache, Cornus, Sorbaria, Staphylea, Symphoricarpos, and Viburnum accessions.

A total of 48 accessions were transplanted to the field mostly focused on *Agastache, Aronia, Monarda, Salix, Spiraea*, and *Ulmus*. One accession of interest is *Monarda* spp. Ames 35227, which is currently an undescribed species of *Monarda* that seems to inhabit floodplain forests and is extremely rhizomatous. Leaves are very rugose, but yet closely resemble *M. fistulosa*. A single plant in a single season can quickly cover a 5' x 5' area. The hybrid of *Salix humilis* x *Salix discolor*, discovered in Ames 32385 and assigned as *S.* x *conifera* Ames 35549, is also of interest.

A total of four large hoop houses (30' x 50') were constructed by the horticulture project to aid in the regeneration of taxa that require substantial space for optimum growth/flowering. A total of eight hoop houses are now available for use. Currently these houses contain *Salix caprea*, *Cornus mas*, *Euonymus atropurpureus*, and *Physocarpus opulifolius*. New accessions of *Cornus alternfolia*, *Staphylea trifolia*, *Cornus mas*, and *Salix eriocephala* were germinated in 2021 for future hoop house plantings.

Availability and Backup:

Currently, approximately 71% of the medicinals, 71% of the herbaceous ornamentals, and 54% of the woody landscape accessions are available.

Currently, approximately 76% of the medicinals, 77% of the ornamentals, and 47% of the woody landscape accessions are backed up at the National Laboratory for Genetic Resources Preservation (NLGRP) in Ft. Collins, Colorado.

Viability Testing:

Seed viability assessments were made on a total of 203 horticulture collection accessions including increase (16%), maintenance (66%) and original (18%) inventories. Maintenance tests were executed on available *Hypericum* accessions (91). A total of 34 accessions tested below 30% viable and 8 accessions tested at 0% viable. Except for one sample, all samples were stored in 4C. Storage in -18C storage may likely result in extended storage on *Hypericum* species.

Research was executed on *Dirca decipens* (Ames 35698), a taxa that is new to the NPGS collection and is also unknown as to its storage capabilities. In 2021, seeds of Ames 35698 were split into three treatments:

- 1. seed direct sown
- 2. fruit direct sown
- 3. excised seed + gibberellic acid

Initial results approximately five months after planting indicated excised seed plus gibberellic acid resulted in the best germination at approximately 10%, followed by seed (6%) and fruit (0%). However, after a cold stratification of 90 days direct seed sown had the best total germination (38%), followed by whole fruit (14%) and excised embryos (11%) (Figure 1.0).

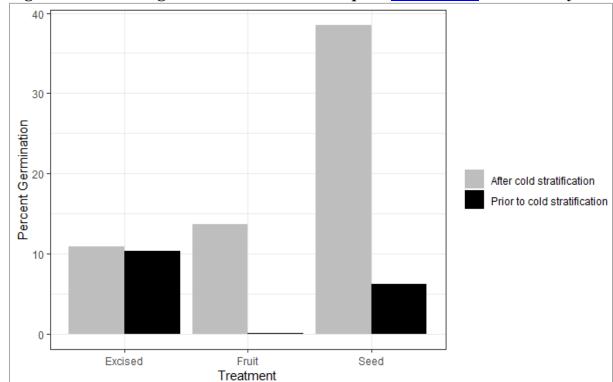


Figure 1.0. Percent germination of Dirca decipens Ames 35698 as of 27 May 2022

Distribution:

Distribution figures for the horticulture collections are summarized in Table 2 and 3, below, and Appendix Table 3. For the combined horticulture program, we distributed 122 external orders to 104 requestors totaling 668 items from 480 accessions. We cancelled 325 orders from 316 requestors representing 1,952 items. Most of the orders were cancelled because they were requested for home gardening or other non-research use and/or commercial sources could meet the needs of the request. In addition to the 122 distribution orders, 3 orders to 3 requestors totaling 29 items from 10 accessions represented herbarium specimens sampled at NCRPIS or in nature. Orders in 2021 were requested for a variety of uses including breeding, additions to botanical gardens/arboretums, disease screening, seed testing for the Association of Official

Seed Analyst exam, anthropological research, and restoration projects requiring known provenanced germplasm.

Table 2. Taxa most distributed from the NC7 horticulture program in 2021

Taxa	Most distributed (greatest to least)				
Medicinals	Echinacea				
	Agastache				
	Calendula				
	Tanacetum				
	Monarda				
Ornamentals	Phacelia				
	Potentilla				
	Anemone				
	Althaea				
	Malva				
Woody landscape	Pinus				
-	Salix				
	Fraxinus				
	Cornus				
	Spiraea				

Table 3. External domestic and foreign germplasm distributions for the NCRPIS horticulture program 2017 through 2021

	No. of	No. of	No. of Items	No. of Accs		
Crop	Year	Orders	Recipients	Distributed	Distributed	
Medicinals	2017	5 3	44	387	233	
	2018	44	36	218	169	
	2019	5 3	47	404	293	
	2020	44	44 42 267		196	
	2021	36	36 338		269	
	Average	46	41	323	232	
Ornamentals	2017	27	26	174	160	
	2018	46	40	117	93	
	2019	22	21	63	55	
	2020	30	28	108	100	
	2021	21	21	83	67	
	Average	29	27	109	95	
Woody Landscape	2017	71	56	367	146	
	2018	82	68	327	164	
	2019	72	58	206	154	
	2020	59	48	202	138	
	2021	78	61	247	144	
	Average	72	58	270	149	

Characterization/taxonomy:

During 2021, six horticulture accessions *Aronia* (4), *Heuchera* (1), and *Monarda* (1), were renamed based on a publication and morphological characteristics, respectively. No PI numbers were assigned. Communication with taxonomist Melanie Schori (USDA-ARS), resulted in the addition of three new taxa to GRIN-Global database including *Dirca decipiens* (Ames 35698), *Monarda fistulosa* f. *albescens* (Ames 35745), and *Heuchera alba* (Ames 34945).

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 3 mother trees from each accession, replicated 5 times totaling 720 trees. The main goal is to identify superior accessions of *G. dioicus* germplasm. *G. dioicus* has recently become one of the more popular, widely planted, urban street trees.

We continue to capture observation data on nine *Betula nigra* accessions (145 trees) in a common garden study to identify potential elite lines 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 superior accessions of *H. quercifolia* including fall color, growth habit, floral display, and cold hardiness.

A total of 971 records were attached in GRIN-Global as either images (782) or as documents (189) including publications, collection trip reports, viability cards, permits, field maps, passport data, and USDA NRCS Release documents. A total of 15 records were created for accessions that are USDA-NRCS Plant Materials Releases that readily provides a plant description document on the GRIN-Global public webpage.

A total of 36 observations were captured including ploidy level and fruit soluble solids for 12 *Aronia* accessions.

Substantial effort was placed on capturing observation data for *Monarda* accessions including *M. austroappalachiana* (1); *M. bradburiana* (1); *M. didyma* (1); *M. fistulosa* (14); *M. fistulosa* var. *menthifolia* (1); and *M.* sp. (1). A total of 35 traits were captured for each accession including plant habit, inflorescences per stem, plant height, plant width, population variability, stem branches, chlorosis, inflorescence profusion, bract color, first flower date, last flower date, days in bloom, floral quality, seed ripening date, stem surface, petiole length, petiole pubescence, adaxial leaf surface pubescence, abaxial leaf surface pubescence, leaf margin, leaf tip shape, leaf base shape, corolla upper lip shape, leaf length, leaf width, inflorescence diameter, corolla tube length, capitulum diameter, calyx width, calyx length, corolla color, leaf area, leaf

length/width ratio, upper corolla lip length, and lower corolla lip length. Data and associated images are pending addition to GRIN-Global.

Enhancement:

An interspecific combination between *Agastache foeniculum* x *Agastache scrophularifolia* was acquired in 2020 as Ames 35585 and prompted efforts to manually recreate this cross. In 2021, *A. foeniculum* (Ames 35586) originating from southeastern Montana and *A. scrophulariifolia* (Ames 35234) originating from southeastern Minnesota was crossed via isolated hand pollinations. Plans are to write a description of the hybrid indicating known parentage, 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:

In 2021, the horticulture project did not distribute plants for regional trials. Select accessions of *Magnolia acuminata* (1), *Betula lenta* (2), and *Populus grandidentata* (1) are currently being grown for future distribution.

Posters, Presentations, and Seminars:

In 2021, all activities involving conferences, tours, etc. were cancelled due to COVID-19 restrictions.

Conclusions:

The 2021 growing season was one of the driest seasons on record at NCRPIS. We estimate approximately 160-200 hours more than an average year were spent watering. A total of 4,154 linear feet of temporary fencing was installed taking just over 100 person hours to install. The fencing is now warranted due to a substantial presence and continual damage to plantings from deer and/or rabbits. Some accessions have been set back approximately 5-8 years as damage requires new growth in order for flowering to occur. Due to the presence of fencing in almost all fields, the time required to accomplish simple, daily tasks has drastically increased in order to work around, enter and exit, and fix fencing. A variety of fence types are being tested and range in cost from \$1.00-5.50 per linear foot of fence.

Plans for 2022:

For 2022, seed increases will be attempted for 50 horticulture accessions. Significant time will likely be spent reviewing plant inventory and removal of successfully increased accessions. We will need to continue the erection of more fencing. Several accessions could be assigned PI numbers and paperwork to complete inactivations. Plans are also in place to make interspecific crosses between *Monarda fistulosa; Monarda clinopodia;* and *Monarda didyma*. Assuming COVID-19 restrictions may allow travel, we intend to sample *Fraxinus* species in the southwestern U.S. and *Monarda lindheimeri* in Texas. Plans also include continued acquisition of N. American native *Salix* and *Ulmus thomasii*. Collaboration is scheduled to occur with the Iowa Department of Natural Resources Prairie Resource Center and the University of Northern Iowa to target collections of native Iowa herbaceous species and also the acquisition of *Morus rubra* with South Dakota State University.

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

Project Management:

Curators Mr. Mark Millard and Dr. Vivian Bernau are assisted by a full time USDA-ARS Agriculture Science Research Technician, Ms. Samantha Armintrout. Samantha joined the maize curation team in April 2021. ISU Agricultural Specialist Mr. David Zimmerman resigned in April 2021. There are currently no plans to fill this position.

Acquisitions:

In 2021, 124 new accessions were acquired. These included 19 populations and inbreds developed by Dr. Jim Brewbaker (University of Hawaii), 10 GEM lines, 80 PVPs expiring in 2022, two historic inbred lines (AKh42, Va84), and 13 accessions submitted to the Journal of Plant Registrations.

Maintenance:

There were 19,935 accessions of *Zea* in the active collection at the NCRPIS as of December 31, 2021. The maize curators maintain an additional 41 accessions from the *Coix* and *Tripsacum* genera for a total collection of 19,976 accessions (Figure 1). Total collection size was significantly decreased in 2020 with the inactivation of more than 1,500 accessions from the Mangelsdorf-Galinat collection.

There were 15,507 available Zea accessions held at the end of 2021 (77.78% of the total). This represents a steady increase in availability, despite fewer student employees and reduced regeneration activities due to COVID-19. Low viability dictated making 65 accessions unavailable. A total of 343 new distribution lots were made available in 2021. Progress has not and will not be possible without the in-kind regeneration assistance of private companies, the GEM programs in North Carolina and Iowa, and others.

Accession Backups:

A significant 383 accessions were backed up at the NLGRP in Fort Collins in 2021. This compares with 156 in 2019 and 868 in 2020. The number of accessions in the collection with backup samples at NLGRP has increased from 15,533 (78%) to 15,974 (80%). 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.

Regenerations:

A total of 667 regenerations were attempted, compared to 316 in 2020. As of December 2021, 211 harvests had been made during the calendar year. This big increase in regenerations is due to a large number of smaller regenerations being attempted in the Puerto Vallarta winter nursery.

The size of our Ames nursery remained small in 2021 due to COVID-19 restrictions. The hiring of temporary workers in Ames during the summer pollination period

continues to be extremely difficult, despite increasing our hourly wage. Regenerations were limited to PVPs expiring in 2022 and a few populations with low germination.

No Stewart's wilt was observed in any increase plots in 2021, as in every year between 2010-2021. ELISA testing is still necessary on Ames increase lots to meet foreign phytosanitary requirements because the state cannot be declared Stewart's wilt free.



Figure 1. Collection size, availability, and backup status over the last 10 years.

Ames greenhouse increases included ten inbred accessions planted in February 2021. In May and June 2021, we germinated samples of *Z. perennis* (Ames 21875, VIII.B. 11), *Z. diploperennis* (Ames 21890, 10932), and *Zea mays* subsp. *parviglumis* (Ames 28133, FS1885). The *Z. perennis* began flowering in August, and approximately 13,000 cupules were harvested in October. The harvested seed is currently being tested for viability before being made available for distribution. The *Z. mays* subsp. *parviglumis* started flowering in October and 66,242 cupules were harvested between November and December 2021. The *Z. diploperennis* also flowered in October 2021, however no viable pollen was observed from the tassels. The plants flowered again in January 2022 and harvest continued through March 2022.

In January 2021, 75,000 viable cupules of *Zea luxurians* were harvested from a greenhouse increase. Maize CGC member, Wenwei Xu and students, Morgan Molsbee and Macy Molsbee, conducted an in-kind seed increase of *Z. mays* subsp. *parviglumis* in Lubbock, TX and sent back more than 45,000 cupules of the accession (PI 384071, Wilkes 10). Ms. Morgan Molsbee, presented a poster about the increase at the Annual Meeting of the Agronomy, Crop and Soil Science Societies in November 2021.

In total, three wild accessions were made available in 2021. As we move forward with the regeneration of more wild maize, we are looking for feedback from stakeholders on standard traits that should be measured.

A tropical nursery consisting of 116 mid-altitudes to lowland accessions was sent to Semillas Moreno Retis (SMR) in Nayarit, Mexico in the fall of 2020 and harvested in early 2021. Seed was received on the ear in March 2021. Few pollinations were made through the nursery, and thus few ears were harvested from each accession. At the end of the calendar year, 23 seed lots had been processed.

An additional 113 accessions were shipped to SMR for increase in September 2021. Based on issues exporting seed grown outside of the United States to Mexico in 2019, we initially sent material of domestic origin, and made a smaller shipment of Mexicanorigin material later. 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. We will need to continue working this issue and will attempt to resume re-exports of seeds grown in Mexico for the 2022-2023 winter nursery. To review, this nursery was set up to assist in regenerating accessions that would grow best in the tropics. We have seed of several thousand accessions that were grown in Peru, Mexico, and Colombia during 1986-1995.

St. Croix grew 17 accessions (13 populations, two inbreds, two checks) in 2021. Seed was received on the ear in February 2022 and is awaiting processing. 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.

CIMMYT (International Maize and Wheat Improvement Center) grew 24 Peruvian highland accessions in Toluca, Mexico under USDA-ARS contract in 2020. This is the second nursery contracted with CIMMYT. This nursery was received on the ear in June 2021. Processing was completed in January 2022. Our goal population of 100 ears was reached with only one accession, 80-95 ears were sampled on nine accessions, and 50-70 ears were harvested on 11 accessions, and fewer than 50 ears were harvested on three accessions.

<u>Viability Testing</u>:

There were 1,499 accessions tested for viability in 2021 (8% 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.

Most accessions are tested on a 10-year cycle. The collection has current viability test data (<10 years old) on 11,609 accessions (58.1% of the collection) (Table 1). However, 4,954 available distribution lots (31.9% of available accessions) have viability tests older than 10 years, and 2,318 available distribution lots (14.9% of available accessions) have tests more than 15 years old. At the end of 2020, 36.1 of available accessions had viability tests older than 10 years, and 17% of accessions had tests more than 15 years old. The current distribution lots of 24% of the available accessions (and 28% of the whole collection) were grown in 1990 or earlier; we expect to see more accessions being made unavailable with further maintenance viability testing (Figure 2).

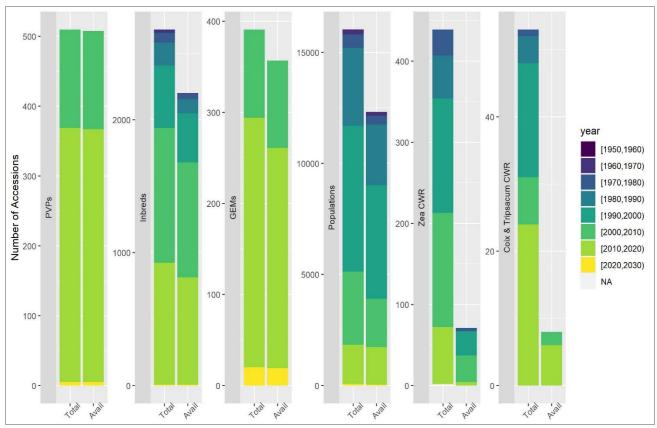


Figure 2. Comparison total to available accessions according to accession type. Each bar is colored according to the approximate age of the current or last distribution lots.

Characterization and Evaluation:

31,377 images on 14,235 accessions were loaded to GRIN in 2021, compared to 1,730 images on 1,208 accessions loaded in 2020. This increased the maize images on GRIN-Global to 42,929 images on 16,629 accessions. There are now images uploaded to GRIN-Global for 83% of accessions in the maize collection (Figure 3). This was the focus of curator Mark Millard's work in 2020, and it has been noticed and appreciated by maize community stakeholders.

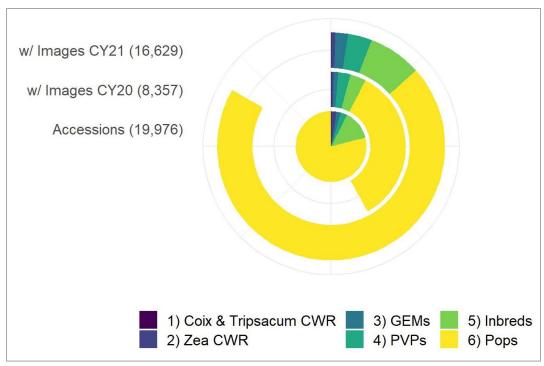


Figure 3. Proportion of the maize collection with images uploaded to GRIN-Global at the end of CY20 (middle ring) and CY21 (outside ring).

Reduced staffing and field activities 2021 have reduced the annual data collection on the Maize Curation team to 21% of the previous year's activity. There were 4,735 data points on 350 accessions loaded to GRIN-Global in 2021. This compares with 22,067 data points on 1,504 accessions loaded to GRIN-Global in 2019 and 36,697 data points on 6,200 accessions loaded to GRIN-Global in 2020. We imaged 400 accessions in 2021 compared to 861 accessions in 2019, and 1,701 accessions in 2020. With continued COVID-19 restrictions, field regenerations have been limited to high priority inbreds and PVPs, which do not generate as many data points as populations. Furthermore, student help on the maize crew through CY2021 has been reduced from 4-5 FTE to one FTE through the academic year. This significantly reduces our ability to process local, contract, and in-kind regenerations in a timely manner.

Germplasm Management:

In 2021, 59 maize accessions were inactivated. Inactivation of an accession occurs when it has been determined by the curator of the Crop Germplasm Committee that the accession cannot or should not be maintained as part of the active inventory of the North Central Regional Plant Introduction Station (NCRPIS). Accessions can also be inactivated if they are duplicates of an earlier received accession—this was the case for 53 of the maize accessions inactivated.

PI numbers were assigned to 148 maize accessions in 2021. 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 Intellectual Property Rights (IPR) restrictions apply to the material.

Distribution:

Overall, order items filled from the maize collection items increased by 29% in 2021 relative to 2020 (Figure 4). Both total and foreign packet distributions (items) were above the five-year average. The number of items filled of wild *Zea* species nearly doubled relative to 2020 (95% increase), and the number of items filled of populations increased by 87% relative to 2020. The number of items filled for GEM lines and inbreds, decreased relative to 2020 (22% and 9%, respectively). Distributions of expired PVPs increased by 28%, reflecting the increased number of PVP certificates that expired in 2020. PVPs were included in 43% of all *Zea* orders and 38% of foreign *Zea* orders.

Almost all orders are now entered by requestors in GRIN-Global. Expired PVP-lines continue to be a major maize distribution category followed by NAM inbred parents, the Goodman-Buckler inbred diversity set, and all other inbred lines. Targeted requests for fewer accessions continue to comprise the bulk of the orders. Handling this number of packets would be very difficult without GRIN-Global and the hard work of Ms. Stacey Estrada, Ms. Lisa Burke and their teams in order processing and seed storage.

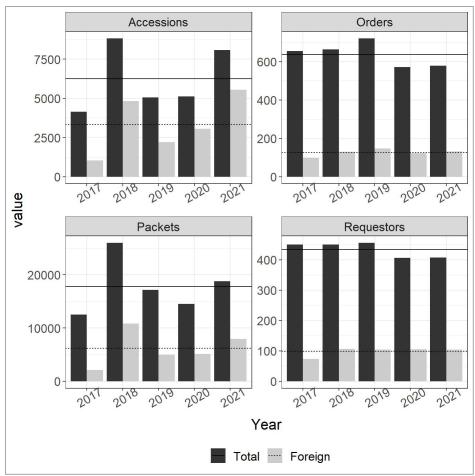


Figure 4. Distribution statistics over the last five years for the maize collection. Total distributions are represented by the black bars with the average across five years represented by the black lines. Foreign distributions are represented by grey bars and the dotted lines.

Plans for 2022:

In 2022, as in recent years, attending to regenerations and regeneration processing will need to take precedence. Without viable seed, distribution and resulting research cannot be done. To better focus these regenerations, we look forward to updated viability status for outstanding maize populations from the germination team.

Nursery processing will be a priority in 2022. Our highest priority is processing PVP accessions prior to their expiration dates so they can be shipped to stakeholders and included in breeding nurseries. An additional 94 (of 118) seed lots grown in the 2020-2021 Puerto Vallarta nursery (received March 2021) require processing. Seventeen seed lots grown in St. Croix at the Germplasm Research and Introduction Unit (received February 2022) require processing. Seed lots of 113 accessions generated in the 2021-2022 Puerto Vallarta nursery are expected to be received in April 2022.

NSL and Ames numbered accessions will continue to be reviewed, and PI numbers assigned. Over 1,007 Ames-numbered and NSL-numbered accessions need to be reviewed and considered for permanent PI numbers in 2022. Accessions that have not been successfully propagated will also continue to be reviewed for inactivation.

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

Project Management:

Curator Dr. Laura Marek was assisted by full time Agronomy Department staff, Grace Welke, ISU Agronomy Assistant Scientist III, and by Jeff Schwartz, USDA-ARS Agricultural Research Technician. John Reinhardt, ISU Farm Equipment Operator, works with the NCRPIS Oilseed Project December through March. The Oilseeds Project is also supported by a team of hourly student workers. Due to COVID-19 pandemic effects on operations, Oilseed regenerations were decreased 30% compared with 2019 and, as in 2020, regenerations were focused on fewer very straightforward taxa. We did not attempt any miscellaneous asters, *Euphorbia* nor *Cuphea*.

Acquisitions:

The oilseed project received one new accession in 2021, a wild sunflower *Helianthus* petiolaris collected in southeastern Arizona.

Maintenance:

General statistics about availability and management of the collections are presented in Appendix Tables 1 and 2. Protocols put in place in 2020 to provide for safe interactions during the COVID-19 pandemic continued during 2021. Selected details for oilseed accessions increased during 2021 are noted below.

Helianthus, Ames regenerations:

Cultivated *H. annuus* accessions are 93% available for distribution. Regenerations are managed to ensure that core collection accessions and other accession subsets of interest to specific stakeholder groups are available. In 2021, 43 cultivated *H. annuus* accessions were regenerated and seeds were harvested from all plots.

Wild annual *Helianthus* accessions are 96% available and wild perennial accessions are 87% available. Thirty-three wild annual sunflower regenerations were grown in the field in Ames in 2021 and seeds were harvested from all plots. Processing of harvested seed is underway. Eight perennials were transplanted to the field and seeds were harvested from seven of the plots. The unharvested accession appeared to have plants of more than one species; we will continue to observe this plot in 2022.

Typically, several oilseed accessions requiring long seasons or short days to flower are increased in a NCRPIS greenhouse during the winter. No sunflowers were started in the greenhouse for 2020-2021 and 2021-2022 seasons; all available space was needed for winter flowering type *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 left to right: large (10x10x20 ft) screened cage, small (7x7x20 ft) screened cage, rows with bagged heads. Cultivated sunflowers are grown using all three techniques; wild sunflowers are always grown in one of the two cage options.

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

The miscellaneous asters are 34% available. No regenerations were attempted at Ames in 2021.

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

We continue to partner with the National Arid Lands Plant Genetic Resources Unit (NALPGRU), Parlier, CA to regenerate wild taxa requiring a longer growing season than is reliably obtained in Ames as well as those species that do not grow well in midwestern humidity and heavy soils. The Parlier location uses cages purchased by the NCRPIS and can grow up to 40 NCRPIS oilseed accessions per year. Seeds are germinated in Ames and live seedlings shipped to Parlier in late March and early April. Parlier staff transplant the seedlings and manage plant growth. Following the Ames protocol, plots are caged before flowering, pollinator insects are introduced

(honeybee services supported with Ames resources), and seed heads are harvested as they mature. Harvested material is shipped to Ames for threshing and processing.

In 2021, we were fortunate to be able to continue to send seedlings to California and the Parlier location was able to handle the regenerations for the most part as they do in a typical year. Seedlings of 39 wild sunflower accessions were shipped. One miscellaneous aster, an *Encelia californica* population sent in 2020, was maintained. Seeds were returned for all 2021 *Helianthus* accessions. The *Encelia* flowers over the winter and seeds will be returned when a significant number have been harvested in 2022.

The Parlier staff record basic field observations (transplant, flowering, and harvest dates and takes some images) but they do not have the resources to record standard descriptor data such as ray and disc flower color, plant height, and branching characteristics nor to take all images. Phenotypic information is a valuable component associated with each accession and it is important to capture the observation data. In addition, it is critical to assess species identity. Typically, the curator and an oilseeds technician travel to Parlier in September and record data. Careful examination of the plants is conducted for species confirmation. In 2021, the curator traveled alone. Without a technician's assistance, less accession imaging was possible but important details and the confirmation of species identification were carried out.

We have an excellent partnership with the NALPGRU staff, ensuring successful regenerations of many wild taxa. We are most grateful for the dedicated efforts of Dr. Claire Heinitz, Curator. Mr. Jerry Serimian, long time agricultural research technician at Parlier retired in September 2020. Dr. Heinitz worked to get the position refilled, and Mr. Juan "John" Jimenez started in May 2021.

Brassicaceae regenerations:

Brassicaceae accessions are 90% available.

Regenerations have been prioritized for the low viability *Brassica* accessions whose status was brought to our attention during maintenance viability testing started in 2016 and completed in 2020. Germination of some lots fell by as much as 80% in the 6 or 10-year interval since prior testing. A total of 460 lots were determined at 50% or less viability over the testing interval and an additional 388 fell between 50 and 70%. Regeneration of 280 of the low viability accessions were conducted between 2018-2021, starting with the lowest viability accessions. Seed is requested from the Ft Collins back up lot for accessions with very low viability, roughly 150 accessions to date.

Most of the accessions with low germination distribution lots are *B. napus* (83%), and most of those are of winter flowering type, requiring a vernalization period to induce flowering. In past trials, most winter flowering type accessions do not survive the winter in the field in Ames; therefore, we start winter flowering types in the greenhouse and induce flowering in an NCRPIS vernalization space. In December 2020, 80 *Brassica* accessions were started, three of which did not germinate, and 76 accessions were kept in the greenhouse until mid-February 2021 when they were moved to the vernalization rooms and then transplanted to the field during the third week of April. One accession remained in the greenhouse because it flowered without

vernalization and was caged and grown to harvest there. All field plots were harvested, and seed processing is underway. Seventy-three of 75 'probable' winter flowering type *Brassica* accessions started in late November 2021 germinated for growth, vernalization and spring 2022 transplanting to the field.

NCRPIS greenhouse 2 (FGH-2) is managed in the winter to provide conditions that approximate a Mediterranean climate allowing us to regenerate Brassicaceae accessions native to that region, and to grow other Brassicaceae taxa which flower very early in the growing season in Ames. In fall 2020, we started 21 accessions, 12 of which did not germinate and will be inactivated (10 Alliaria petiolata and two Matthiola incana). Seven of the remaining accessions were harvested: two accessions of Camelina microcarpa and one each of Camelina laxa, Lepidium fremontii, Matthiola ovatifolia, Sinapis alba, and Thlaspi arvense. One accession of Erysimum gomez-campoi did not flower and was held over to 2022. In fall 2021, 13 accessions were started, three of which did not germinate. Plants of Berteroa incana (two accessions), Camelina laxa, Camelina sativa, Eruca vesicaria ssp. sativa, Erysimum gomez-campo, Erysimum witmannii, Lepidium sativum (two accessions), and Thlaspi arvense are growing in FGH-2.

Linum regenerations:

Cultivated flax accessions are 99% available and 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 these accessions were of uniform seed age. The NCRPIS viability lab determined that seed viability had started to decline for some distribution lots and increased maintenance germination testing for this crop completing the current viability testing cycle in 2019. Based on the resulting data, 45 accessions of cultivated flax are now being regenerated every year, with priority given to accessions with lowest viability with the long-term goal that all distribution lots have 80% or higher viability. In 2020, however, the planned cultivated flax regenerations were postponed due to our need to decrease fieldwork because of pandemic restrictions. In 2021, we again regenerated 45 cultivated flax accessions; seeds are being processed. In 2020, we started six wild flax accessions and harvested seed from two. All accessions were left in the field to overwinter. Plants from all six wild flax accessions overwintered from 2020 regrew and seeds were harvested from five of the accessions. Plants from the plot which did not flower were transplanted to the greenhouse because the field they were in was scheduled for re-working.



Figure 2: Wild flax, Ames 34946, regeneration of the sample of the rare *Linum lewisii* Appalachian strain (collected in West Virginia).

<u>Cuphea regenerations:</u>

No Cuphea field regenerations were set up in 2021. 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 *E. lagascae* and its accessions are 93% available. No *Euphorbia lagascae* accessions were attempted in 2021. Six *Euphorbia* accessions are maintained as clones in the greenhouse and distributed as vegetative cuttings.

Distributions:

A total of 231 unique orders containing 12,051 oilseed packets were shipped in 2021. 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 has been able to continue to fill and ship most seed orders during the pandemic, international shipments were halted for months during 2021 and a backlog of inspections for issuance of phytosanitary certificates for international shipments still exists. Oilseed orders shipped in 2021

decreased compared with 2020 and roughly 50% fewer items were distributed. In 2021, 85% of the 2019 number of oilseed packets were shipped, approaching prepandemic levels of distributions.

Table 1: Summary: Oilseed Crops 2021 Distributions. Because some orders contain items from more than one crop category, the total number of unique orders is less than any totals calculated from the table.

	shipped		international	international	international	domestic	domestic	domestic	total
Crop	orders 2021	packets	orders	packets	requestors	orders	packets	requestors	requestors
Brassicaceae	120	4972	34	1633	29	85	3339	67	106
Cuphea	3	5	0	0	0	3	5	3	3
Euphorbia	0	0	0	0	0	0	0	0	0
Helianthus	101	5750	50	4596	36	58	1157	51	71
Linum	14	1315	6	667	6	8	648	8	14
misc asters	3	9	2	4	2	2	5	2	4

Helianthus:

More than half (63%) of the total *Helianthus* items distributed in 2021 were sent to seed companies or other commercial entities, 90% of which were sent to international destinations, reflecting current locations of most of the commercial sunflower breeding programs. Programs associated with public institutions, primarily universities, and national institutes around the world received 28% of the distributions. Sunflower packets were sent to support breeding programs (52%; the category 85% of seed companies identified as their research purpose), genetic studies (23%), and pathology research (20%), with smaller numbers for botanical or taxonomic investigations, bioremediation, anthropological research, and several specific categories of plant physiological research. Fifty-four percent of the total distributed packets were of cultivated *H. annuus* accessions, 38% were wild annual sunflower species including wild *H. annuus* and 8% were wild perennial sunflowers.

Brassicaceae:

The genus Brassica accounted for 53% of the 2021 Brassicaceae distributions (4,972 total packets, 1,892 unique accessions). The Brassicaceae genus with the second largest number of packets distributed was Eruca, 1,309 packets sent, ~26% of the total, including four separate orders for the entire $E.\ vesicaria$ ssp. sativa collection. The diversity present in the Brassicaceae collection (262 taxa in 21 genera) supports a very wide range of research purposes including genetic studies, details of which vary widely, pathology research related to disease resistance, varietal development and other breeding related discovery.

<u>Linum</u>:

Ninety-three percent of the flax packets distributed in 2021 were cultivated flax accessions. Distributions were almost equally divided between domestic (51%) and international (49) requesters. One large international order (494 accessions) was sent to a program developing improved fiber quality and the largest domestic order (583 accessions) was sent to a breeder looking at lipid and agronomic traits.

Cuphea:

Cuphea accessions were distributed in 2021 to two different breeding programs looking at pathogen host range interactions and as part of a display at the National Arboretum.

Euphorbia:

No Euphorbia accessions were distributed in 2021.

Miscellaneous asters:

Miscellaneous aster accessions were distributed in 2021 for a breeding program and for seed reference and identification collections.

Research Activities:

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

Helianthus:

Loren Rieseberg's lab at the University of British Columbia developed the GB_UBC pre-breeding lines with support from The Global Crop Diversity Trust. The Crop Trust provided funding for an international evaluation effort of these lines, "Evaluation of Sunflower Pre-Bred Lines for Stress Resistance and Associated Trade-off with Yield", managed by the University of British Columbia group with partners in Chile, Argentina, Africa, India, Israel and Ames. We contribute to the partnership by providing seeds for the international locations and recording descriptor data during the regeneration process. Funding was received in 2017 (\$25,000) for two years with a no-cost extension to ensure use of all funds the last of which were used in January 2021.

Brassicaceae:

Evaluations are being conducted on the 675 accession NCRPIS B. rapa collection for flowering type (winter or spring). 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 FGH-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 2020-2021, 100 accessions were evaluated of which 80% were spring type. In early December 2021 another set of 100 accessions were started. Observation priority was determined based on viability data; lowest viability accessions were evaluated first so that those accessions could be incorporated into regeneration priorities. After the 2021-2022 set are scored, roughly 284 accessions will remain to be screened. Scoring is final for 291 accessions and of those, 75% are spring type, whereas the B. napus collection is about 80% winter type. About 50 accessions need to be re-tested.

Professional Activities:

Meetings and Presentations:

<u>January</u>: Participated virtually in the National Sunflower Association's 42^{nd} Annual Research Forum in Fargo, ND.

April: Attended most sessions of the R F Baker Plant Breeding Symposium (Agronomy, ISU) via zoom.

<u>June</u>: Gave presentation about USDA sunflower germplasm resources for a class about genebanks at the University of Moldova via zoom.

October: Participated virtually in the International Sunflower Association sponsored Sunflower-Pollinator Interactions web conference, Toulouse, France.

November: Attended the Tri-Societies meeting in Salt Lake City the 9th through the 13th, participating in all Crop Science C-08 (Plant Genetic Resources) sessions and additional sessions of interest whenever possible. Marek presented an invited talk, "Cultivated Sunflower Pre-breeding Success Stories", in the C-8 Prebreeding Symposium. In addition, because USDA scientists were still not allowed in person meeting attendance, L. Marek presented a talk for Clare Coyne the Cool Season Legume curator at NE-9 entitled "Pea seed protein concentration in USDA plant genetic resources" and moderated the Frank Meyer Medal session and award presentation in her absence.

Training:

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

Publications:

Guidini, R., Braun, H., Korah, M., Marek, L.F., Mathew, F. 2021. Greenhouse data suggest growth state impacts Phomopsis stem canker severity associated with *Diaporthe gulyae* on sunflower (*Helianthus annuus* L.). Plant Health Progress. 22(4):470-473.

https://doi.org/10.1094/PHP-12-20-0108-RS

Active Grants:

Crop trust:

In 2017 the ISU Curator for the Oilseeds project received a \$25,000 grant from the Global Crop Diversity Trust administered through the University of British Columbia to support regenerations of the Canadian developed GB_UBC sunflower pre-breeding lines received in 2016 and to provide partial travel support to attend the project's annual meetings at the Plant and Animal Genome conferences in San Diego, CA. The last of the funds were spent to support workers in January 2021.

Collecting:

L. Marek was an ISU authorized administrative PI for a domestic collecting grant managed by David Brenner for which all documents were completed in 2021.

Service Activities:

Tours:

There were no live tours in 2021 due to the pandemic.

Interview:

L. Marek participated in an interview with the National Geographic journalist Sarah Gibson about the use of crop wild relatives to make the sunflower crop more resilient.

Journal peer review:

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

CSSA Genetic Resources C08:

L. Marek served on the Frank Meyer Medal committee member.

Plant Germplasm Operating Committee (PGOC):

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

G. Vegetables (K. Reitsma, B. Chapman, C. Hopkins)

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

The Vegetable project is led by Curator Kathy Reitsma. The project was down a fultime technician and with the retirement of Cindy Clark January 4, 2021, after 21 years of service as the team's ISU Agricultural Specialist I. We were able to hire a new ISU Agricultural Specialist II, Brandyn Chapman, and he reported for work October 11, 2021. The station experienced recruitment and retention challenges with part-time, temporary student employees throughout the year – especially at critical times such as planting/transplanting, caging, pollinating, and harvesting. These challenges are likely to continue into 2022.

									Total			
					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	254	89	89	231	28	26	260	91	0	179
NC7-cucumis.cucs	1401	0	1332	95	95	1230	143	28	1335	95	0	1098
NC7-cucumis.melo	3250	22	1926	59	60	2906	283	35	2606	80	363	1054
NC7-cucumis.wilds	318	0	220	69	65	245	73	0	225	71	0	74
NC7-cucurbita	981	1	721	73	74	886	90	5	830	85	0	298
NC7-daucus	1564	0	1282	82	81	967	567	30	1362	87	0	703
NC7-ocimum	106	0	100	94	93	93	13	0	100	94	0	76
NC7-parsnips	73	0	58	79	79	52	19	2	58	79	0	33
Totals	7978	23	5893	74	74	6610	1216	126	6776	84	363	3515

Acquisitions:

Cucurbita pepo PI 603279, 'Whitaker' (breeding line NY 247) was received as an expired PVP. It is a compact bush-type with a complicated breeding history of four species (C. pepo, C. ecuadorensis, C. martinezii, and C. moschata). Embryo culture was used to make the interspecific cross with C. ecuadorensis. Resistant lines were intercrossed to combine resistance to different viral diseases and to overcome the genetic barrier to transferring homozygous resistance to zucchini yellow mosaic virus from C. ecuadorensis to C. pepo. 'Whitaker' is reportedly resistant to zucchini yellow mosaic virus, cucumber mosaic virus, and papaya ringspot virus (watermelon mosaic virus -1) and produces parthenocarpic fruit when conditions are unfavorable for good pollination.

Seed of 21 of 22 Cucumis melo accessions comprising the International Cucurbit Powdery Mildew Initiative (ICPMI) melon race differential host set were received. Differential line ICPMI 1 10 (PI 414723) will be increased in The Netherlands in 2022 and the seeds deposited with the NCRPIS once regeneration and quarantine procedures are completed. The NCRPIS will be responsible only for distribution of the seed (25 count per accession) material to requestors. Dr. Ales Lebeda (Palacky University, Faculty of Science, Department of Botany, Olomouc-Holice, Czech Republic) coordinated the process of purification, multiplication, and deposition of the differential set. The following publications provide additional background information on the development of the ICPMI differential set.

- Lebeda, A., Sedlakova, B., Kristkova, E., Widrlechner, M., Kosman, E. 2021. Understanding pathogen population structure and virulence variation for efficient resistance breeding to control cucurbit powdery mildews. Pl. Pathol. 70:1364-1377. doi: 10.1111/ppa.13379
- Lebeda, A., Kristkova, E., Sedlakova, B., McCreight, J. 2018. <u>Initiative for Uniform Cucurbit Powdery Mildew Race Determination and Denomination:</u>
 Status of Race Differentials. Cucurbit Genet. Coop. Rep. 41:17-19.
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Maintenance:

Data for vegetable crop regenerations attempted and number of accessions harvested in 2021 are summarized in the appendices in "Table 2: NCRPIS Accessions (Accs) Germinated, Regenerated, Made Available, Backed Up."

Regenerations:

The NCRPIS Vegetable Project regeneration program was scaled back in 2021 in part to accommodate COVID-19 mitigation measures, but also due to personnel issues.

<u>Cichorium</u>: Regenerations were attempted on 25 <u>Cichorium endivia</u> accessions having low seed viability, and harvests were made on 24 (one accession failed to bolt and flower). Four accessions had inadequate seed quantities harvested due to poor plant populations or because few plants bolted and flowered. These accessions will be regenerated again in 2022 with resulting seed increases bulked with the 2021 increase lots to replace existing distribution lots with declining seed viabilities.

<u>Cucumis sativus</u>: Availability of the cucumber collection is at 95% with only 69 accessions unavailable for distribution. The unavailable 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. Cucumber field cage regenerations in 2021 focused primarily on 18 accessions where controlled pollinations were compromised due to wind damage to cages during a derecho on 10 August 2020. We were able to salvage some of the fruit from the 2020 cages, but many were not mature and seed quantities were inadequate after processing. Seed increases from the 2021 field cages will be bulked with the 2020 increase lots after viability testing in April 2022.

<u>Cucumis melo</u>: Regenerations were attempted on five accessions of melon in field cages in 2021. Four accessions were breeding lines donated to the NPGS by Dr. Jim McCreight, Research Leader at USDA-ARS, Crop Improvement and Protection Research, Salinas, CA prior to his retirement in January 2021. A fifth melon accession having a low seed quantity on the distribution lot was successfully regenerated from original seed. These seed lots will be made available for distribution after viability testing and storage in the spring of 2022.

<u>Cucumis spp.</u>: Three wild melon accessions were started in the greenhouse in December of 2019 and transplanted to field cages in 2020 and 2021 for continued regeneration when plants failed to produce enough fruit/seed in the greenhouse and field cages. The *C. africanus* accession thrived in the 2021 field cage producing 841 fruits (4.5 cm x 7 cm) ensuring a very successful regeneration. The perennial *C. heptadactylus* and *C. hirsutus* accessions have dioecious (separate male and female) plant 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 fruit. Plants of each accession were dug from the field cage at the end of the season, potted, and maintained in the greenhouse over the winter. These plants will again be transplanted to field cages in 2022 for continued regeneration. Cuttings were taken of the female plants to boost their numbers in the population. Still, this process may need to be repeated for multiple years until enough seed is secured. (A previous

regeneration effort on the *C. heptadactylus* required six years to secure enough seed for back-up and distribution.) 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 all seed lots in the -20 °C freezer may improve long-term seed viability and availability of this species.

<u>Cucurbita</u>: Six accessions of pumpkin/squash were regenerated in 2021. Five accessions required a longer growing season to mature fruits and were grown in the greenhouse over the fall and winter. PI 420328, a hull-less seeded landrace pumpkin from Turkey was unavailable due to a low seed quantity and regenerated in a field cage. This accession was requested by a researcher in an evaluation of NPGS Cucurbita germplasm for possible resistance to whiteflies and whitefly-transmitted diseases. The accession was regenerated in anticipation of possible increased interest in the accession generated by an article pending publication in early 2022. All seeds have been processed and should be available after viability testing and storage in April 2022.

<u>Daucus</u>: Regenerations focused on wild species of carrot including 28 perennials and 26 biennials. Regeneration protocols for many of the wild species are still under development (there is little in literature to provide guidance). The perennial accessions (22 *D. crinitus* and six *D. setifolius*) from Morocco, Portugal, and Spain were planted in the greenhouse in early November 2019 and plants remained in the greenhouses through 2020 and 2021 where we had better control over environmental conditions. Germination was very poor and sporadic resulting in small plant populations for most accessions. Five accessions began flowering in May 2020 while others did not flower until fall 2020. Most of the accessions experienced a dormant period and began to flower again early in 2021. Flies and alfalfa leaf cutting bees (ALC) were introduced into the cages to effect pollination, and multiple harvests were made as the seeds matured. Overall, the perennial accessions proved challenging to maintain and produced relatively few seeds. We will continue to develop better regeneration protocols for these species.

Twenty-six accessions were planted as biennials in November 2020 for regeneration in field cages in the summer of 2021. Six accessions were mixed populations of annual and biennial plants, 19 were biennials, and one behaved as an annual with nearly all plants in its population bolting in January 2021. The annual plants were maintained in greenhouse isolation cages to which flies and ALC were introduced to effect pollination. Biennial plants were vernalized, and the roots transplanted into field cages in April 2021. Flies, ALC, and honeybees were introduced to cages to effect pollination, and multiple partial harvests were made on all cages as seeds matured. The seeds have all been processed, but cleaning is still in progress.

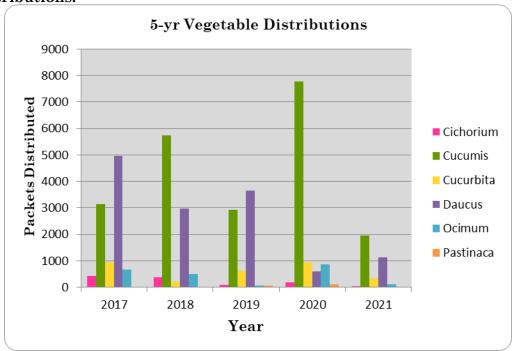
<u>Ocimum</u>: Twenty-one accessions of basil were regenerated in field cages in 2021. Harvests were made on all accessions with successful regenerations on 17. Four accessions were still flowering heavily in September and many seeds were not mature at harvest. Seed cleaning is still in progress.

In addition to the Ames carrot regenerations, seed increases were received of cultivated biennial accessions - five 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.

As NCRPIS accessions are regenerated, backup seed samples are sent to the NLGRP in Ft. Collins. Backup samples of 107 accessions from the Vegetable Project were sent to the NLGRP in 2021. 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 363 *Cucumis melo* accessions for deposit in the Svalbard seed vault with a total of 3,514 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 2021, 3,560 items (packets) involving 2,322 accessions were distributed to fulfill 143 orders (98 domestic, 45 foreign) for 116 unique recipients. The total number of vegetable crop distributions has declined over the past two years in part due to research being limited by COVID-19 restrictions, but also due to the completion of evaluations of *Daucus carota* germplasm 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 2021 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.

Non-Research Requests (NRR) continued to make up a significant portion of orders received by the NPGS, but the implementation of the "Non-Research Request (NRR) review process" at the national level in August 2021 has significantly reduced the work associated with processing such orders at the sites. Specific modifications were made to GRIN-Global and triggers were added to help identify NRRs, to aid reviewers of the orders and automatically email requestors of the reviewers' decision. NRR requestors have an opportunity to provide additional information and request a reassessment of the orders they submitted. From January 1 to August 2021, 77% (462 of 581 orders) requesting genera curated by the NCRPIS Vegetable Project were classified as NRRs. This national-level order review process has provided a more efficient, streamlined, and consistent handling of NRRs and significantly reduced the workload for order processing staff and curators at the local level.

Germinations:

In 2021, 666 vegetable inventory lots were tested for viability (Appendix Table 2), with most of the testing attributed to maintenance germinations and new increase 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 2020, 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 *Squash mosaic virus*, 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 is planned as part of CucCAP2 which received four-year funding beginning in 2021. The project's website, https://cuccap.org, posts a list of publications resulting from the research, and provides access to cucurbit genomics tools and databases via the Cucurbit Genomics Database website. All phenotypic data generated in the evaluation process will be referenced in or made available via the GRIN-Global database, and enhanced lines developed through the process may be made available through the NPGS.

Publications/Posters:

Grumet, R., McCreight, J.D., McGregor, C., Weng, Y., Mazourek, M., Reitsma, K., Labate, J., Davis, A., Fei, Z. 2021. Genetic Resources and Vulnerabilities of Major Cucurbit Crops. Genes. 12(8):1222. Special Issue Horticultural Crop Genetics and Improvement. Published August 7, 2021. https://doi.org/10.3390/genes12081222

Lebeda, A., Sedláková, B., Křístková, E., McCreight, J., den Hertog, M., Reitsma, K. Development, Characterization, Multiplication, Deposition, Availability and Utilization of Melon Differential Set for Determination of Virulence Variation of Cucurbit Powdery Mildews (*Phodosphaera xanthii* and *Golovinomyces orontii*). Cucurbit Genetics Cooperative Report 44. Publication pending.

Plans for 2022:

Regenerations:

Vegetable Project regeneration plans for 2022 will again be scaled back primarily due to USDA-ARS COVID-19 mitigation measures. Student recruitment and retention also continue to be a concern and may impact regeneration efforts. Cole Hopkins continues with the project as a half-time ISU Agricultural Specialist I who is shared with the Farm Management Project. Brandyn Chapman, the new ISU Agricultural Specialist II will be able to experience his first full regeneration season from planting to harvest for chicory, cucumber, pumpkin, carrot, and basil as well as become more familiar with the GRIN-Global database and other aspects of the job. Several of the genera to be regenerated in 2022 must be started in the greenhouses in January through April, but the cucurbit crops (cucumber, pumpkin/squash) are not planted until May so we will be able to adjust the project's regeneration plans as needed.

Biennial *Daucus* could not be planted in time for the 2022 field season so focus was shifted to annual species which do not require vernalization to induce flowering. Thirty-five *Cichorium* accessions will be planted for regeneration – five of which did not produce an adequate quantity of seed during the 2021 season, and 30 which have low viability or seed quantities. Approximately 20 *Ocimum* accessions with low viability, low seed quantity, or older distribution seed lots will be planted for field regeneration. Four of the Ocimum regenerated in 2021 did not produce enough seed so will be grown again in 2022. Thirty *Cucurbita pepo* and approximately 50 *Cucumis sativus* will be planted for field regeneration.

Two perennial, wild *Cucumis* (*C. heptadactylus*, and *C. hirsutus*) planted in 2019 continue to grow in the greenhouse and female plants) but will again be transplanted to field cages in 2022. Both accessions produced few fruits in the 2021 field cages. If

an insufficient quantity of seeds results from the 2022 field planting, the roots will again be transplanted to pots in the greenhouse in the fall.

Characterization:

Many years of fruit characterization data on cucurbits still remain to be converted and loaded into GRIN-Global. Cindy Clark made significant progress renaming digital image files of seeds, plants, and fruits that were acquired since 2013, but no images were loaded before her retirement on January 4, 2021. Loading these images to GRIN-Global 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 GRIN-Global. Review of accession passport data will continue for the cucurbit and *Daucus* collections in preparation for assigning PI numbers to many of the Ames-numbered accessions in the collections (414 *Cucumis*, 88 *Cucurbita*, and 99 *Daucus*).

Evaluation:

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

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

H. Research Leader Activities (D. Peters)

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 Germplasm Enhancement of Maize (GEM) Project and contributes to the coordination and execution of activities which support those objectives. Dr. Peters also served as the Coordinator of the Hatch-funded Multistate NC7 Project.

In 2021, COVID-19 pandemic associated precautions continued to add complexity for university and federal operations. Health and safety concerns were paramount; addressing these was guided by both ISU and USDA-ARS. Practices included such as sanitation, safe distancing, wearing of masks when in proximity to others (inside

buildings in the field), and maximum telework were implemented. Field activities continued at a reduced level across all curatorial and the GEM projects. Student hiring was reduced proportionately, and our stats indicate that fewer accessions were regenerated. Travel was restricted to mission critical 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 was significantly reduced. Scientific and curatorial personnel generally met with colleagues virtually as the station was closed to visitors and tours. Participation in scientific meetings was shifted to virtual format. Distribution of seed to U.S. requestors continued, but international requests requiring APHIS handling were substantially delayed.

Fiscal 2022 appropriations were fully available in early quarter 3 of 2022 following several continuing resolutions. Delays in release of funds create uncertainty for program planning and complicate completion of hiring and procurement, exacerbating 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 labor costs were used to replacement or repair aging curational regeneration equipment. Acquired materials included replacement of 230 aging, controlled pollination cages which are critical for seed increases of many collection accessions.

David Peters was rapidly immersed in the non-GEM activities of the genebank and learning the ropes of research unit administration. He served as the Coordinator of the Midwest GEM Project from July 2016 to December 2020 and continued to manage the project until October of 2021 when the new GEM Cooperator, Adam Vanous, assumed responsibility for the project.

The current five-year project plan period of Hatch Multistate Project NC-007's is 10/01/2017-9/30/2022. Dr. Peters is the NC-7 Project Coordinator and worked with the NC-7 participants to draft and submit the new project plan in November of 2021. The project plan was accepted and will cover the period of 10/01/2022-9/30/2027.

2021-2022 Plans:

The ARS CRIS projects located at the NCRPIS are scheduled to be rewritten. These will be developed in coordination with ARS Office of National Programs in the first half of 2022 and submitted for full panel review.

Review of the lease agreement between ISU and ARS immediately after the derecho storm in August 2020 revealed that the lease needs to be reviewed and updated. This will ensure that 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.

The VMEK Metrix optical sorter will continue to be used to experiment with sorting 'recipes' for a number of crops to explore quality improvement of seed lots. It offers a lot of promise.

We hope to reengage use of the QSorter from the Swiss company, QualySense, purchased with USDA-ARS Midwest Area and HQ support, captures 3D images and 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; the company plans to locate a research and development team in Ames.

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.

We continue to focus 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. We will continue to use the ORISE program to hire contract employees to cover some aspects of our activities; plans are use this program to help fulfill development of learning/training objects to serve the objectives of the Higher Education Challenge Grant devoted to management and utilization of plant genetic resources.

01/01/2021 to	Table 1. o 12/31/2021		NCRPIS Acc	essions (A	ccs), Acqu	ired, Avail	able
CURATOR	GENUS_CROP	Number Accs	Number Accs Acquired		Number 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%	58%
	NC7-grass.panicum	936	0	0.0%	911	97%	97%
	NC7-grass.setaria	1117	1	0.1%	1047	94%	91%
	Subtotal Grasses:	2510	1	0.0%	2327	93%	91%
	NC7-legume.melilotus	1006	0	0.0%	870	86%	86%
	NC7-legume.misc	311	7	2.3%	165	53%	53%
	Subtotal Legumes:	1317	7	0.5%		79%	79%
	NC7-pseudocereal.amaranth	3353	14	0.4%		97%	97%
	NC7-pseudocereal.celosia	61	1	1.6%		64%	65%
	NC7-pseudocereal.perilla	25		0.0%		96%	96%
	NC7-pseudocereal.portulaca	13		0.0%		77%	77%
	NC7-pseudocereal.quinoa	663		1.4%		61%	52%
	Subtotal Pseudocereals:	4115	24	0.6%	3721	90%	89%
	NC7-spinach	4113	1	0.0%		76%	75%
	NC7-umbels	1196		0.2%	823	69%	68%
	Brenner Total:	9552	1000	0.0%	8221	86%	85%
Carstens	NC7-medicinals	1133		1.6%	V	71%	70%
Carstens							
	NC7-ornamentals	778	3	0.4%	552 1129	71%	70%
	NC7-woody.landscape	2079		1.6%		54%	53%
3/ 1	Carstens Total:	3990	54	1.4%	2488	62%	61%
Marek	NC7-asters	459				34%	35%
	NC7-brassica	2019		0.0%		91%	88%
	NC7-crucifers	1307	0	0.0%		87%	87%
	NC7-cuphea	638	0	0.0%		80%	80%
	NC7-euphorbia	210	0	0.0%		47%	49%
	NC7-flax	2839		0.2%		99%	99%
	NC7-flax.wilds	167	0	0.0%		77%	74%
	NC7-sun.cults	2647	1	0.0%		93%	90%
	NC7-sun.wilds.ann	1705	7	0.4%		95%	96%
	NC7-sun.wilds.per	903	0	0.0%		87%	85%
	NC7-sun.wilds.sp	2	0	0.0%	0	0%	0%
	Subtotal Wild Sunflower:	2610	7	0.3%	2412	92%	92%
	Marek Total:	12896	22	0.2%	11550	90%	89%
Bernau	NC7-maize.coix&tripsacum	41	2	4.9%	8	20%	15%
& Millard	NC7-maize.gems	401	7	1.7%	395	99%	91%
	NC7-maize.inb	2697	26	1.0%	2170	80%	82%
	NC7-maize.pop	15797	8	0.1%	12268	78%	77%
	NC7-maize.pvp	603	80	13.3%	601	100%	100%
	NC7-maize.teosinte	437	0	0.0%	73	17%	16%
	Subtotal Zea:	19935	121	0.6%	15507	78%	77%
	Bernau & Millard Total:	19976	123	0.6%	15515	78%	77%
Reitsma	NC7-chicory	285		0.0%		89%	89%
	NC7-cucumis.cucs	1401	0	0.0%		95%	95%
	NC7-cucumis.melo	3250		0.7%			60%
	NC7-cucumis.wilds	318		0.0%		69%	65%
	NC7-cucurbita	981	1	0.1%		73%	74%
	NC7-daucus	1563		0.0%		82%	81%
	NC7-ocimum	106		0.0%		94%	93%
	NC7-parsnips	73		0.0%		79%	79%
	Reitsma Total:	7977	700			79%	74%
NCRPIS To		54391	255		43667	80%	74%

Year 2021 Table 2. 01/01/2021 to 12/31/2	Year 2021 Table 2. 01/01/2021 to 12/31/2021				NCR	PIS Accessi	ons (Accs)	NCRPIS Accessions (Accs) Germinated, Regenerated, Made Available, Backed Up	, Regenera	ted, Made	Available, l	3acked Up			
CURATOR	CURATOR GENUS_CROP	Number Accs	Number Percent Accs Accs Number Germed 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		2%	8	4		0	0	5		0	0	285	%06
	NC7-grass.misc	142	က	2%	0	0	0	0	0	2	2	0	0	66	%02
	NC7-grass.panicum	936		1%	13	13		0	0	8		95	0	921	%86
	NC7-grass.setaria	1117	2	2%	16	14	0	0	0	36			0	982	88%
	Subtotal Grasses:	2510		1%	37	31		0	0	51		1		21	91%
	NC7-legume.melilotus	1006		%0	9	0	0	0							93%
	NC7-legume.misc	311		22%	00	2	0	0	0 0						71%
	Subtotal Legumes:	1317	11	2%	6	23	0	0		9			0		87%
	NC7-pseudocereal.amaranth	3353	285	8%	92	22	0	0			31	9		3266	%26
	NC7-pseudocereal.celosia	61	1	2%	1	1	0	9				0	0	44	72%
	NC7-pseudocereal.perilla	25	20	%08	1	1	0	0				0	0	24	%96
	NC7-pseudocereal.portulaca	13		%0	1	-	0	0	0 0	0			0	12	92%
	NC7-pseudocereal.quinoa	663		%9	81	73	0	٥					0	362	25%
	Subtotal Pseudocereals:	4115	GT3	%8	160	86	0	0			21	9	0	3708	%06
	NC7-spinach	414		13%	16	I	0	0	0					406	%86
	NC7-umbels BT.4.1.			21%	13	127					169			824	%69
	NG7	9992		100/	239		11			N		999	14	000	0/00/
Carstens	NC7 omomentals	1133	601	10%	n c		11	1 0		32	25			809	40%
	NC7 woody landeans	9070		020	98		7 26	C						200	170%
	Carstens Total:			4%	45	75	49					0	0	2447	%1%
Marek	NC7-asters			%0	0	0	0	0					0	184	40%
	NC7-brassica	2019	-	%9	80	77	0	0	0 0	82	79	9	0	1991	%66
	NC7-crucifers	1307	27	2%	0	7	0	0	0 0		0		0	1188	91%
	NC7-cuphea	638		%0	0	0	0	0	0				0	583	91%
	NC7-euphorbia	210		3%	0	0	0	0	0 (66	47%
	NC7-flax	2839	28	10%	44	45	0			7				2837	100%
	NC7-flax.wilds	167		1%	9 9	0	0	0	0					141	84%
	NC7-sun.cults	1705	108	%9	43	43	0	-	0	147				7.777	84%
	NC7 sun milds non	001		0%0	00	900					24	200		287	95%
	NC7-sun wilds en	606		%0	O										%0
	Subtotal Wild Sunflower:	2610	15	7%	78	39					7	-1.		237	91%
	Marek Total:			%9	251	211	0	1	0 1	334	2	2			%06
Bernau	NC7-maize.coix&tripsacum			10%	0	0	0	0	0					15	37%
& Millard	NC7-maize.gems	401		10%	1	1	0	0	0				0		%98
	NC7-maize.inb	2697		13%	11	30	0	3	0			4	0		62%
	NC7-maize.pop	15797		%9	138	165	0		0				0	2	84%
	NC7 maize.pvp	003	190	110%	497	0	0 -			102	113	0110		0/1	35%
	Subtotal Zea.	19935	1495	70%	667	77.6	-			3.4	383	100		15974	80%
	Bernau & Millard Total:			8%	667	277	1		0				0		%08
Reitsma	NC7-chicory	L		45%	25	24	0	0					0		91%
	NC7-cucumis.cucs	1401		%1	17	17	0	0		9	9		0		95%
	NC7-cucumis.melo	3250	25	%6	5	4	0	0	0 (0	36	0	2	80%
	NC7-cucumis.wilds	318		3%	0	က	0	١	0 (15	0	0	225	71%
	NC7-cucurbita	981		4%	7	9	0		0	0	0		0	831	85%
	NC7-daucus	1563		2%	0 0	40	0		0	39	588	0	0	1362	87%
	NC7-ocimum	106	10	9%	21	12			0	4	4 0	0		100	94%
	Not-parsings Reitema Total	70	9	2170	75	115				197	107	363		6777	1970
NCRDIC Total		M.	6	70%	1979	297	A CA	96		1161	276	-	7.7	45900	70878
T OF TATO AT	ora:	10010	2000		0.01	5	20	3		1011	2		10000	20101	200

Subtotal Cs NC7-grass. NC7-grass. NC7-grass. NC7-grass. NC7-grass. Subtotal Ca NC7-pseud NC7-cuphea NC7-	lloa tus tus elosia eerilla oortulaca tuinoa		External Number Nu		Domestic Distributions	tions		Foreign Distributions	etribintione		External	External Domestic and Foreign Distributions	Foreign Di	stributions
	loa tus maranth elosia oerilla oortulaca puinoa	lection 315		Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
	and a trus trus amaranth celosia perilla portulaca quinoa	315		Accs	Orders	Recipients	Items	Accs	Orders	Recipients	Items	Accs	Orders	Recipients
	tus amaranth celosia perilla portulaca quinoa		35	28	10		322	283	3	8	357	284	13	13
	ntus amaranth celosia perilla portulaca quinoa	142	0	0	0				0	0			0	0
	ntus amaranth celosia perilla portulaca quinoa	936	776	736	9		373	355	4 1	4		737	13	12
	tus amaranth celosia perilla portulaca quinoa	2510	1666	1588	37	28	7	655	15	14	2381		525	24
0	amaranth celosia perilla portulaca quinoa	1006	10	10	4			76	4	4			00	00
	amaranth celosia perilla portulaca quinoa	311	2	5	2	23		0	0	0	5	5	.01	63
0 Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z	amaranth celosia perilla portulaca quinoa	1317	15	15	9	9	81	92	10	70	96	6	11	11
	celosia perilla portulaca quinoa	3353	413	358	39	32	18	1336	16	14	25	18	55	46
NC7-pseud NC7-pseud NC7-pseud Subtotal Ps NC7-umbels NC7-umbels NC7-umbels NC7-umbels NC7-woody.1 NC7-asters NC7-cuphea NC7-cuphea NC7-cuphea NC7-flax will NC7-maize.C NC7-maize.	perilla portulaca quinoa reals:	61	24	23	3	3		10	2	2			5	70
NC7-pseud Subtotal Ps NC7-pseud Subtotal Ps NC7-umbels NC7-umbels NC7-woody.1	portulaca quinoa rreals:	25	22	15	4	4	35	24	2	2		24	9	9
Subtotal Ps NC7-pseud Subtotal Ps NC7-medicir NC7-medicir NC7-curbels NC7-curbes NC7-curphea NC7-maize	quinoa reals:	13	11	9	4	4		2	23	2			9	9
Subtotal Ps NC7-madicir NC7-medicir NC7-woody.] NC7-woody.] NC7-asters NC7-asters NC7-cuphea NC7-cuphea NC7-cuphea NC7-cuphea NC7-cupher NC7-cu	reals:	699	238	165	36	27	371	165	80	80			44	35
NC7-spinach NC7-umbels NC7-umbels NC7-asters NC7-asters NC7-tasters NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w Subtotal Wi NC7-maize.C NC7-maize.		4115	402	292	10		2226	1537	34	32		1	104	68
NC7-umbels NC7-cmedicin NC7-asters NC7-asters NC7-brassica NC7-crucifer NC7-cuphea NC7-cuphea NC7-flax.wil NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-maize.c NC7-maize.c NC7-maize.c NC7-maize.c NC7-maize.c NC7-maize.c		414	186	327	14	14	32	32	4	4			18	18
NC7-medicin NC7-asters NC7-asters NC7-brassics NC7-cuphea NC7-cuph		1196	435	225	12		265	261	9	9			18	18
NC7-medicin NC7-woody.1 NC7-asters NC7-brassics NC7-crucifers NC7-sun.w NC7-sun.w NC7-sun.w NC7-maize	Brenner Total:	9552	3805	2722	123		3319	2561	61	58	7	4380	184	157
NC7-orname NC7-asters NC7-asters NC7-cucifer NC7-cuphorl NC7-cuphorl NC7-flax wil NC7-flax.wil NC7-flax.wil NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-maize.c NC7-maize.c		1133	147	121	28	28	191	177	8	00		269	36	36
NC7-woody.1 NC7-asters NC7-crucifer NC7-crucifer NC7-crucifer NC7-flax NC7-flax NC7-flax.wil NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-sun.w NC7-maize.C NC7-maiz		778	33	31	17	17	90	36	4	4		19	21	21
NC7-asters NC7-brassicc NC7-cuphea NC7-cuphea NC7-flax NC7-flax wil NC7-sun.wl NC7-maize.C	e.	2079	244	142	77	09	3	3	1	П			78	61
NC7-maize.pv NC7-maize.gv NC7-cuphorbia NC7-flax.wilds NC7-flax.wilds NC7-sun.cults NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.gv NC7-maize.gv NC7-maize.gv NC7-maize.gv NC7-maize.gv NC7-maize.gv NC7-maize.gv	Carstens Total:	3990	424	294	106	88	244	216	16	16	899	480	122	104
NC7-brassica NC7-crucifers NC7-cuphorbia NC7-flax.wilds NC7-sun.cults NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.cois NC7-maize.cois NC7-maize.co		459	D	5	2	21		4	ಣ	60			Ω	īΟ
NC7-crucifers NC7-cuphea NC7-flax wilds NC7-flax wilds NC7-sun.cults NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.coix NC7-maize.coix NC7-maize.coix NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		2019	1754	1073	42	36			18	17			09	53
NC7-cuphea NC7-flax.wilds NC7-flax.wilds NC7-sun.wilds NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.coix NC7-maize.coix NC7-maize.coix NC7-maize.br NC7-maize.br NC7-maize.br NC7-maize.br		1307	1560	463	47	38	788	564	17	17	2348	716	64	55
NC7-euphorbia NC7-flax NC7-flax NC7-sun.cults NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.coix NC7-maize.coix NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		638	က	က	2	2	0	0	0	0			2	22
NC7-flax wilds NC7-sun.culfs NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-maize.coix NC7-maize.coix NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		210	0	0	0	0	0	0	0	0			0	0
NC7-maize.pc NC7-muvild NC7-sun.wild NC7-sun.wild NC7-sun.wild Subtotal Wild NC7-maize.coix NC7-maize.gc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		2839	594	1.80	9 0	9 0	638	909	י מ	no c	1232		n c	מ מ
NC7-sun.wild NC7-sun.wild NC7-sun.wild NC7-sun.wild Subtotal Wild NC7-maize.coix NC7-maize.ge NC7-maize.ge NC7-maize.pc NC7-maize.pc NC7-maize.pc		101	000	49	000	99	250	1995	0.0		9826	1502	02	0 2
Subtotal Wild NC7-sun.wild NC7-sun.wild NC7-maize.coix NC7-maize.gc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		1705	296	265	93	18			21				44	35.5
Subtotal Wild Subtotal Wild NC7-maize.coix NC7-maize.gc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.pc		903	194	178	14	14			12	: 1			26	25
Subtotal Wild NC7-maize.coix NC7-maize.gc NC7-maize.pc NC7-maize.pc NC7-maize.pc NC7-maize.te Subtotal Zea: Bernau & R		23	0	0	0	0	0	0	0			0	0	0
NC7-maize.coix NC7-maize.gc NC7-maize.in NC7-maize.pc NC7-maize.pc NC7-maize.te Subtotal Zea: Bernau & R	flower:	2610	490	443	34	28	1978	1098	24		2468	1331	58	48
N Z Z Z Z Z	Marek Total:	12896	5138	3171	147	120	6402	4224	92		11540	5866	239	201
San	sacum	41	12	8	4	4	5	5	3	co			L	7
Z Z Z Z ng		401	280	158	42	28	393	285	11		673	311	53	39
Z Z Z ng		2697	3325	1377	198	158	1613	971	63				261	213
NS S		15797	3900	2972	185		3957	3716	42	34		E.	227	184
ns S		603	3149	561	199		1659	483	49		4		248	145
ng S	Ф	437	168	80	36	33	337	79	12				48	45
, and		19935	10822	5148	446	302	7959	5534	128	101			574	403
	rd lotal:	97.661	10834	9010	448	304	1964	9939	131	104	18/98	SC SC	67.6	408
Reitsma NC7-chicory		285	32	29	9	9 !		0	0	0			9	9
NC7-cucumis.cucs		1401	639	109	20	7.1		3/2	19	15			39	32
NC7-cucumis melo		318	199	38	000	27 00	16	14	10	10	58	50	13	19
NC7-cucurbits		981	315	258	0 00	286		19	9	1 10	334		44	23.5
NC7-daucus		1563	889	444	17	17	239	237	. 10	7.0	1128		22	22
NC7-ocimum		106	54	41	6	6	67	62	2	2	121		11	11
NC7-parsnips		73	1	1	1	1	0	0	0	0	1	1	1	1
	Reitsma Total:	7977	2503	1833	86	75	1057	943	45	41	3560	2322	143	116
NCRPIS Total:		54391	22704	13176	834	290	18986	13483	306	257	41690	21132	1140	847

CURATOR GE Brenner N N N N N N N N N N N N N N N N N N N	GENUS_CROP NC7-grass,echinochloa		Number	Number	Number of		Number of		Number of		Number of		Number of	
	VC7-grass.echinochloa	Number Accs in Collection		of Obs in GRIN for Year	Accs with Obs in GRIN for Year	Number of Obs In GRIN Last Year	Accs with Obs In GRIN Last Year	Number of Obs in GRIN (all years)	Accs with Obs in GRIN (all years)	Number of Accs Imaged	Accs with Images in GRIN for Year	Number of Images in GRIN for Year	Accs With Images in GRIN (all years)	Number of Images in GRIN (all years)
		315	4	0	0	0	0	1167	305	8	23	36	84	21
	NC7-grass.misc	142	0	0	0	0	0	290	113	0			34	œ
	NC7-grass.panicum	986	0	0	0		0	4256	936				368	584
	NC7-grass.setaria	1117	0	0	0		0	4668	1078				359	492
	Subtotal Grasses:	2510	4	0	0		0	10381	2432	က	10	-		1652
	NC7-legume.melilotus	1006	0	0	0		0	7485	966			13	220	329
0 2 2 2	NC7-legume.misc	311	0	0	0		0	547	244			31	88	130
0 2 2	Subtotal Legumes:	1317	0		0		0	8032	1240		26	44	308	459
0 2 2	NC7-pseudocereal.amaranth	3353	75	က	19	1185	96	56044	3327		259	512	15	2487
o z z	NC7-pseudocereal.celosia	61	0		0		0	164	56			8	23	5
S Z Z	NC7-pseudocereal.perilla	25	0		0		0	98	25		3	7	24	56
S Z Z	NC7-pseudocereal.portulaca	13	0	0	0		0	10	4		1			31
	NC7-pseudocereal.quinoa	699	29	2	2		450	1888	451					985
	Subtotal Pseudocereals:	4115	104	37	21	21	546	58192	3863	15	446	876		3616
	NC7-spinach	414	0	0	0	846	411	8883	413			œ		200
	NC7-umbels	1196	1		0		1		1146			353		738
	Brenner Total:	9552	109	က	21	3303	958		9094	2	7	-	3	6965
	NC7-medicinals	1133	0	0	0		5	11981	462				635	1437
Z	NC7-ornamentals	877	0	0	0		0		101				203	363
Ž_	NC7-woody.landscape	2079	-	98	12		50	4997	877		167	454	1088	3421
	NG7 actom	9990	1	ac	0	٥	00		1440	7	919	700	ET	276
Marek	NC7-ascers	9010		808	77	1939	166	49359	1996	47	# 6°	07	161	1184
N	NC7-crucifers	1307	0		. 0		0	7325	889	cc	39	00		870
N	NC7-cuphea	638	0	0	0		0	4260	278		0			60
N	NC7-euphorbia	210	0	0	0	0	0	0	0	1	1	1	7	1
ž	NC7-flax	2839	0	0	0	0	0	1717	285				51	138
ž	NC7-flax.wilds	167	0	0	0	0	0	852	82				22	22
ž	NC7-sun.cults	2647	18		0		0	104316	1826					117
4	NC7-sun.wilds.ann	1705	2		0		0	40118	1307					245
4	NC7-sun.wilds.per	903	co (0		0	13850	630	17			16	457
7	NOT-sun.wilds.sp	9610	0 10					53968	1937		0 10	0	666	207
	Marek Total:	12896	93	9	27	193	166	6	7994	6	6	6		4169
Bernau NC	NC7-maize.coix&tripsacum	41	0		0		4		4					2
rg.	NC7-maize.gems	401	37	74	41	1976	358	11885	400	4	306	7	Cij	1424
A	NC7-maize.inb	2697	88	220	14	14487	1701	104902	2562	43	1433	5822	1517	1999
4	NC7-maize.pop	15797	2		152		3543	202177	13403		11	2	18	30233
4	NC7-maize.pvp	603	52	216	143	44	551	29241	685	148	09	199		4457
	NC7-maize.teosinte	437	0	0	0	76	43	271	125	I	2007	9	114	123
16	Rornan & Millard Total.	19999	183	4735	350		0610		17179				16699	42904
Reitsma NC		285	0	0	0		0		279			5	262	918
	NC7-cucumis.cucs	1401	1	0	0	0	0	26149	1377	-	0	0	920	1231
N	NC7-cucumis.melo	3250	0	0	0	0	0	12286	3196		4	4	653	1078
N	NC7-cucumis.wilds	318	0	0	0	0	0	089	286	2	0	0	75	118
ž	NC7-cucurbita	186	1	0	0	0	0	2999	970	7	22	61	153	329
ž	NC7-daucus	1563	0	0	0	0	0	19502	1358	0	eo (8	763	3243
Z	NC7-ocimum	106	0	0	0	0	0	635	86	0	0	0	14	21
Z.	NC/-parsnips	7077	0	0	0	0	0	193	7697		0	0 1	9241	603
T W CAMBOA	Keitsma lotal:	1161	N	0	0	0	0	21169	1639	31	G	14	2841	6939

