NCRPIS

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North Central Regional Plant Introduction Station Manual of Operations

October 2014

USDA United States Department of Agriculture

Agricultural Research Service

Iowa State University

Of Science and Technology

Manual of Operations

U.S. Department of Agriculture - Agricultural Research Service

North Central Regional Plant Introduction Station

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Introduction

The US Department of Agriculture - Agricultural Research Service (USDA\ARS) National Plant Germplasm System (NPGS) is comprised of a network of cooperating institutions, agencies, and research units in the Federal, State, and Private sectors. The NPGS is part of the ARS National Program 301 (Plant, Microbial and Insect Genetic Resources, Genomics and Genetic Improvement) and is federally funded through the Department of Agriculture, as approved by the US Congress.

As one of the units in the NPGS, The North Central Regional Plant Introduction Station (NCRPIS) has the role of furnishing genetic and bioinformatic tools, phenotypic and genomic information, and well-documented genetic raw materials to enhance American agricultural productivity to ensure a high quality, safe supply of food, fiber, feed, ornamentals, and industrial products. The NCRPIS is a collaborative effort between the USDA-ARS and Iowa State University. We are located in Ames, Iowa, southwest of the main campus.

This Manual of Operations provides guidelines related to day-to-day activities at the NCRPIS.

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Section 1A

Germplasm acquisition policy

Given limited resources in terms of personnel, facilities, and funding, germplasm repositories in the NPGS must focus their collections. In general, NCRPIS only acquires germplasm of taxa for which we are designated as the NPGS "priority site."

In supporting other NPGS sites, we occasionally acquire seed or vegetative samples of priority species of other NPGS sites for evaluation at Ames. An example would be an accession that is being evaluated in the regional ornamental testing program.

When deciding whether to acquire additional accessions of a particular crop or crop group, crop-specific curators, often in consultation with the relevant CGC, are guided by the NPGS Germplasm Acquisition Policy that was approved by the PGOC in 2009, with additions in 2010 and 2013 and is contained in the Manual of Procedures for the National Plant Germplasm System. Accessions are acquired when they fill "gaps" in a crop or crop group's genetic diversity, or, when the accessions are of particular interest to the user community.

Section 1B

Accessioning germplasm

Detailed information on accessing germplasm at NCRPIS is contained in the document "Procedures for Accessioning Germplasm" that is maintained on the NCRPIS Intranet site. General information is described below.

Germplasm generally cannot be accepted with intellectual property restrictions (IPR). Exceptions that apply to NCRPIS collections include SMTA (standard material transfer agreement associated with the ITPGRFA), Crop Science Registered (CSR), and Plant Variety Protection (PVP) accessions. PVP and CSR germplasm is deposited at the NCGRP in Ft. Collins, CO, and held there until the IPR has expired. Requestors of this material prior to IPR expiration are referred to the donor by NCGRP staff. When the IPR has expired, the germplasm is transferred to the appropriate active NPGS site for maintenance and distribution. Expired PVP germplasm may have additional IPRs.

Accessioning decision key:

Germplasm is received from private donors, national and international organizations, and through collection efforts by curatorial personnel and their partners. This material is accessioned (or "logged in") at NCRPIS through the various steps detailed in following dichotomous key.

1. Germplasm originates outside the U. S. and lacks a quarantine certificate: If "Yes," go to 1a. If "No," go to 1'.

    a. This germplasm is first shipped to a USDA/APHIS unit in Beltsville for quarantine inspection.

    b. Upon its return to the NCRPIS, go to 2.

1'. Germplasm originates in the U.S., or foreign germplasm with a quarantine certificate:

    a. Go to 2.

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2. Determine whether germplasm has been genetically engineered: If "Yes" or "Unknown," go to 2a. If "No," go to 2'.

    a. For "improved" accessions (including breeding lines, genetic stocks, and cultivars), information from the donor should be solicited to determine whether the germplasm has been genetically engineered and, if so, whether the event has been registered with the EPA and USDA. If it has not been registered, deny acceptance. Genetically engineered germplasm needs to be labeled as such and special storage and maintenance conditions apply. A special notation should be made in the inventory status comment field and the site narrative of the GRIN record. Additional IPR restrictions may be associated with genetically modified germplasm which may impact distribution. The germplasm donor may clarify.

    b. When these processes are complete, go to 3.

2'. Germplasm has not been genetically engineered.

a. Go to 3.

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3. Germplasm is propagated vegetatively: If “Yes,” go to 3a. If “No,” go to 3'.

a. Forward vegetatively propagated germplasm to the relevant crop-specific curator as soon as possible.

b. Any accompanying information is prepared separately for data processing, as detailed in 3’ for germplasm propagated by seed.

3'. Germplasm is propagated by seed.

a. Seed packets and accompanying information are kept together during data processing and are stamped with their date of arrival at NCRPIS. Taxonomic identity is verified by checking the GRIN database for valid taxonomic classification.

b. When these processes are complete, go to 4.

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4. GRIN lacks taxonomic information for the taxa, or NCRPIS is not designated by NPGS as the primary maintenance site for the species: If “Yes,” go to 4a. If “No,” go to 4'.

a. The GRIN taxonomist (located at the USDA-ARS National Germplasm Resources Laboratory) and crop-specific curators are contacted to determine the valid taxonomy and most appropriate maintenance site. If necessary, the seed is then shipped to that site.

b. When these processes are complete, go to 5.

4'. GRIN includes taxonomic information and the NCRPIS is the primary maintenance site for NPGS.

a. Curatorial responsibility is determined by referring to the NCRPIS’s “Curator Assignments by Genera” list. Curators consult with the Germplasm Program Assistant to decide which of the following germplasm login procedures to use.

b. When these processes are complete, go to 5.

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5. “Identifiers” (ID’s) are included on packets or accompanying information: If “Yes,” go to 6. If “No,” go to 5a.

a. The germplasm is assigned a temporary “Ames number” until the curator determines whether or not the material can be maintained and should receive a PI number. Ames numbers are assigned sequentially to accessions arranged alphabetically by genus and taxa. An inventory lot is added to each accession (according to the NCRPIS Revised Inventory Code maintained on the NCRPIS Intranet site). The Ames number and inventory lot are written on the packet and recorded in the accompanying records (paper or electronic) for cross-reference. Passport information is extracted from the records accompanying the germplasm and recorded on GRIN. Donors will be contacted for additional information when necessary. Accessions are recorded in the “Ames Number

Logbook" that is maintained by the Program Support Assistant. Once the login process is complete, permanent barcode labels are printed and affixed to packets.

b. Original seed packets frequently have information (written or attached) that should be captured and attached to the accession record. As needed, the PSA and/or seed storage staff members capture a digital image record and may annotate the record. This may also be accomplished by delegated staff under the supervision of seed storage staff. c. When these processes are complete, go to 6.

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6. NPGS ID's are present: If "Yes," go to 7. If "No," go to 6a.

a. The ID's are compared on GRIN with all ID's for accessions of the same genus. If a similar ID is found, the curator will determine if the material duplicates an existing accession, or whether it should be considered a new accession.

b. When these processes are complete, go to 7.

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7. PI numbers have been assigned: If "Yes," go to 7a. If "No," go to 7'.

a. The accession records will be updated to the "NC7" primary maintenance site. A NCRPIS inventory lot is added to each accession's record (according to the NCRPIS Revised Inventory Code), which also shows the date the accession was received. The accession number and inventory lot are written on the packet and recorded in the accompanying records for cross-referencing. PI accessions are recorded in the "NSL / PI / Old Ames Numbers Logbook." Once the login process is complete, permanent barcode labels are printed and affixed to packets.

7'. PI numbers have not been assigned: Go to 7'a.

a. Accessions with NPGS site-specific ID's (e.g. W6, NA, or G numbers) are generally assigned a temporary "Ames number" to simplify maintenance according to the instructions under Step 4. The only exceptions to this rule are for accessions already numbered by NCGRP and those accessions that are the priority of other active sites. Accessions with "NSL numbers" will be maintained at the NCRPIS under those ID's. The accession records will be updated to the "NC7" primary maintenance site. An NCRPIS inventory lot is added to each accession's record (according to the NCRPIS Revised Inventory Code), which also shows the date the accession was received. The accession number and inventory lot are written on the packet and recorded in the accompanying records for cross-referencing. NSL accessions received should be recorded in the "NSL / PI / Old Ames Numbers Logbook." Once the login process is complete, permanent barcode labels are printed and affixed to packets. Go to 7'b.

b. A PI number can be requested of DBMU staff for a new accession for which complete provenance information is known, viability information is known and acceptable, and the quantity received supports immediate distribution. The curator submits a request form to the PSA for PI number assignment, which requires Coordinator approval.

Section 1C

Distribution guidelines

The objective of distributing NPGS of germplasm is to provide materials in the form of seed, DNA, vegetative tissue, and/or other plant parts such as pollen, to scientists, educators, producers, and other bona fide users, domestic and foreign, in a timely manner for research and education purposes.

Conditions and Considerations for Distribution:

Plant germplasm is distributed from NPGS active collection sites to scientists, educators, producers and other bona fide research and education entities. The NPGS Curator and/or Research Leader will, in accordance with current NPGS policies and procedures, determine the legitimacy of a request when necessary.

Distributions to fulfill requests for repatriation of subsamples of germplasm collections to a country or community of origin, especially following natural or man-made catastrophes, are considered a high priority.

Although distributions for research, education, and repatriation are of the highest priority, the NPGS also encourages various seed-saver organizations and public gardens to conduct germplasm conservation activities that engage many individuals and groups throughout the country. Elements of the NPGS cooperate with seed-saver organizations and public gardens and may store germplasm for and distribute germplasm to such organizations.

Distribution of germplasm from NPGS collections to fulfill requests from individuals seeking free germplasm strictly for home use is generally considered an inappropriate use of limited resources and conflicts with USG policy of not competing with commercial enterprises. Requestors can be asked, in an appropriate manner, to justify the use of specific NPGS germplasm instead of suitable commercially available germplasm. Curatorial staff members consider such requests in context of purpose and availability of suitable commercial or NPGS germplasm.

Curators consider germplasm adaptation, invasive potential, and whether the plant poses toxicity or health issues to humans or animals with respect to the requestor's stated intended use. Appropriate procedures must be followed for germplasm governed by forms of intellectual property protection (IPR) or threatened or endangered status. Users may be directed to commercial sources of germplasm when appropriate.

NCRPIS Distribution Policy:

In accordance with NPGS plant germplasm distribution guidelines, NCRPIS will supply germplasm to scientists, educators, producers and other bona fide research and education entities.

In terms of educational entities, curators may distribute germplasm to public elementary and secondary schools for specific educational activities, provided the germplasm is available and appropriate for the intended use. Educators are typically advised to utilize commercially available germplasm for conventional purposes. The number and diversity of accessions and the quantity of

materials shipped for such purposes are at the discretion of the curator who has responsibility for those particular accessions. It is anticipated these distributions will be limited in number.

When germplasm availability permits and the materials are appropriate for the intended use, requested germplasm may be provided to public gardens and seed saver organizations.

Germplasm for repatriation requests should generally be distributed, provided the material is available in adequate amounts.

Germplasm intended for strictly home or personal use, including home schooling and such activities as Boy Scout and Girl Scout projects, should generally not be distributed. These requestors should receive the Non Research Request (NRR) letter by US mail or via email explaining why NCRPIS cannot supply the requested materials.

Our goal is to initiate orders for research requests within two weeks of receipt and to initiate orders for non-research requests within three weeks of receipt. Curators should respond to queries regarding germplasm orders within 10 days of first being contacted. Requests for vegetative samples are handled case-by-case by the relevant crop-specific curator and are designed to facilitate the safe and rapid movement of such material. If we are unable to provide the germplasm requested, we will then attempt to provide the requestor with alternate sources. The requestor is notified regarding any request that the NCRPIS cannot fill.

Although there is no charge for the germplasm, requestors may be asked to provide shipping costs, especially when expedited domestic or international services are requested. Phytosanitary inspection costs have traditionally been supported by ARS-NPGS funds. This will change in 2015 when requestors will be asked to use APHIS' PCIT system to support phytosanitary inspection fees.

Requestors have, in the past, been asked to report on the performance of the material we provided through our Accession Performance Report (APR) system. Those requestors who failed to return APRs were to be reminded by letter of their responsibility to do so. Because of resource constraints, distribution of hard copy APRs was discontinued. We plan to re-implement a modified APR system as a web-based application via the GRIN-Global System in order to capture valuable information concerning customer service as well as germplasm utilization.

Requests that cannot be filled within six months due to lack of response to requests for information from the requestor or inability to secure an import permit should be cancelled and a message sent to the requestor informing them of the request status. Implementation of the GRIN-Global System will automate electronic notification when the status of a request changes.

Determination of NRR status:

In an effort to manage non-research requests, NCRPIS developed a Non-Research Request letter in 2004 and most recently updated it in 2013. The NRR letter (which is usually sent as an email message) is sent to requestors that request germplasm for home use or for uses that do not otherwise meet the criteria for distributions outlined above. The object of the letter is to provide requestors with information about our mission and the genetically variable nature of our accessions, while directing them toward commercial sources of germplasm.

The Program Support Assistant (PSA) will make an initial determination of NRR status and whether the order is a first-time or repeat request. In cases where a request is obviously for home or personal use, the PSA will cancel the order and communicate with the requestor using the NRR letter (copying the pertinent curators via email) explaining why NCRPIS cannot provide the plant materials.

The requestor is asked to provide clarifying information if they believe denial is in error and the request will be reconsidered upon receipt of such information.

Indications that a request should be classified NRR and not be filled include, but are not limited to:

- The requestor states that the materials are intended for home or personal use.
- The requested or equivalent germplasm is available from commercial sources.
- No clear research or educational purposes are stated.
- Requests are vague, such as when no accessions or no clear criteria for selecting accessions are specified.
- The requestor asks for extremely diverse germplasm of a number of taxa.
- The requestor lacks the ability to grow/maintain the requested germplasm.
- The requestor asks for an unreasonable number of accessions.

Curators will receive and review copies of all NRR letters that apply to their specific collections. If a curator believes that a denied request should be filled, she/he will work with the GPA in resolving the issue.

Where an apparently non-research request does not clearly fall under home or personal use status, the GPA will forward the order to the curators of the requested germplasm for their consideration. If all of the curators elect not to supply the requested germplasm, the GPA will cancel the order and communicate with the requestor via the NRR letter, as described above. Curators should work through the GPA on communications until the GPA has informed the requestor of disposition of the request.

Curators may disagree regarding the validity of a particular request. Each curator may elect to send all, some, or none of the requested accessions within her/his respective collections if they have valid reasons for disagreeing with the non-research classification decision. Curators are encouraged to share their knowledge regarding a requestor and decision process / rationale when it will be helpful to their colleagues.

NRR communications:

We utilize five categories of form-letter communications with non-research requestors. These letters are generally sent as email messages, but can be sent via the U.S. Postal Service when email communication is not possible or feasible.

1. Requestor receives a one-time distribution of germplasm and receives an NRR letter referring her/him to other germplasm sources.
2. Requestor is denied germplasm for a first-time request and receives an NRR denial letter referring her/him to other germplasm sources.
3. Requestor is denied germplasm for a new request after having already received an NRR letter and receives a second NRR denial letter referring her/him to other germplasm sources.
4. Requestor provides additional information after receiving an NRR denial letter and we continue to deny the request and send a second NRR denial letter.

5. Requestor provides additional information after receiving an NRR denial letter and we send the germplasm with or without an NRR letter, depending on the circumstances. If, after reconsideration, the requestor appears to be a legitimate researcher, the request will be processed as a normal research request and an NRR letter will not be sent.

Designation of backup curators for NRR requests:

Each curator should designate an alternate person (e.g. seed storage manager, GPA, or technician) to make non-research order decisions when the curator is absent for more than 3 days and will be unable to respond to germplasm orders via email.

Requests for declaration of non-GMO or non-GEO status:

The NPGS does not warrant germplasm as free of non-genetically modified (non-GMO) or non-genetically engineered (non-GEO) status. We provide information about our maintenance methods, any adventitious presence (AP) testing results if available, and information such as the age of a seedlot if relevant. Requestors are free to test material for AP if they choose to receive it, and are encouraged to share AP test results with the curator.

Section 1D

Germplasm and information distribution

Processing germplasm requests:

Requests for germplasm, seed lists, and accession-specific information arrive by mail, fax, telephone, email, and through GRIN.

Requests are recorded in the GRIN Order Module, where a system order number and a local order number are assigned. These order numbers are noted on all documentation (electronic and/or hard copies), for purposes of cross-reference.

Requests for seed lists and accession-specific information are also recorded in GRIN as IO orders.

When the requestor has not identified specific accessions, the curator responsible for the respective taxa may designate accessions to be sent or may submit suggestions to the requestor for a final decision, depending on the nature of the request.

When requested accessions are not available, the curator may:

1. elect not to send the requested accessions.
2. designate substitute accessions.
3. suggest substitutions to the requestor for a final decision, depending on the nature of the request.

The Germplasm Program Assistant will make a preliminary assessment of the legitimacy of each request in accordance with the guidelines in Section 1-C of this manual. Requestors submitting apparently inappropriate orders will be notified via standard Non Research Request (NRR) form email or letter that we cannot supply the requested germplasm and are offered the opportunity to appeal that decision. Curators responsible for the accessions requested will receive a copy of the denial letter and should review the request to determine if the denial should be reconsidered.

Apparently legitimate requests are forwarded to the respective curators for a determination of which accessions and the quantities of plant material to be shipped. Requestors should be contacted regarding any questionable requests to confirm that the germplasm is to be used for bona fide research or educational purposes. Undergraduate and graduate students who request germplasm should be asked to provide the principal investigator/advisor's contact information. The program assistant or curator who contacts a student for this information should confirm the intended purpose and student supervision with the PI or advisor. This will also aid in soliciting feedback on experimental results, publications, and germplasm performance.

Germplasm requests may range from a few specific accessions, a representative sample of a crop, all accessions in a genus, or material with specific traits. In the absence of specific instructions, standard distribution quantities will be provided for all available seed samples unless the curator changes the distribution quantities for the order due to low inventories or other reasons.

Seed packets are prepared and addressed with computer-generated stick-on labels. The packets are forwarded to the seed storage area for processing and are filled following procedures outlined in the Seed Storage Handbook (http://ncrpis-intranet/supportteams/seeds/Shared%20Documents/Seed_Storage_Handbook%202014.doc). When an order is complete, the envelopes are sealed and the order is shipped in either a box or a padded envelope.

For crop collections where inventory accuracy is difficult to maintain due to seed shape or size, seeds may be prepackaged to improve accuracy and overall efficiency of seed-order fulfillment. Crop collections where a subset of the accessions has a regular, predictable order history may also be prepackaged for efficiency.

When inventory lots are particularly large, requests for seed samples in excess of standard distribution quantities for replicated evaluation tests or other special purposes will be considered on a case-by-case basis by the curator, especially when needed for Crop Germplasm Committee (CGC) directed efforts.

Phytosanitary requirements:

All germplasm provided to cooperators outside the U.S. must follow phytosanitary regulations specific to samples transferred between the U.S. and the importing country. USDA-APHIS (Animal and Plant Health Inspection Service) is contacted before such orders are filled for information regarding the importing country's phytosanitary regulations. When required, APHIS provides a phytosanitary certificate to accompany seed samples attesting to freedom from specified pests and pathogens. The Iowa Department of Agricultural and Land Stewardship provide the same service for vegetative samples. We generally do not distribute vegetative samples outside of the United States. The NCRPIS Plant Pathologist may also be required to provide additional declarations based on field inspections or laboratory assays.

When we are unable to meet phytosanitary requirements, we contact the requestor indicating that we are unable to meet the requirements as written, but are willing to supply seeds if the requestor can obtain a waiver of these requirements from officials in their country.

Shipping costs:

For expedited shipping requests, the requestor may be asked to provide a shipping number to provide for shipping costs. Currently, the NPGS supports the cost of phytosanitary inspections by APHIS. This procedure is subject to change as requestors may be asked to cover phytosanitary inspection costs in the near future.

Accession performance reports:

As mentioned in Section 1C of this manual, requestors in the past were asked to report on the performance of the materials we provided through our Accession Performance Report (APR) system. Because of resource constraints, distribution of APRs was discontinued in 2008. Plans are to re-implement a modified APR system as a web-based application via the GRIN-Global System. Details on that implementation are still in development as of August 2014.

Section 1E

De-activating germplasm

At the curator's discretion, accessions may be inactivated using the "NCRPIS Nomination to Inactivation File" form in accordance with the instructions "NCRPIS Procedures for Accession Inactivation". Such germplasm, ID's, and database records will be inactive and germplasm will not be accessible under a different ID on the database.

An accession may be inactive because:

- it was never received.
- it duplicates an accession already in the NPGS.
- it cannot be maintained ex situ at the NCRPIS.
- it is of questionable origin and/or importance to the system (the latter may include commercial germplasm, currently available from private companies, that was not sent to us by the developer).

Germplasm with PI numbers can be inactivated only if it cannot be maintained. Seed packets of inactivated accessions are maintained in cold storage separate from the active collection. The accession is classified as "null" or "inactive site", but information regarding it is maintained on the database.

Section 1F

Reaccessioning or removing germplasm

Re-identifying germplasm:

Occasionally accessions are received that have been misidentified or mislabeled during collection, processing, or transfer. Some of these misidentified accessions are discovered during receiving and storage operations, while others are discovered during regeneration or viability testing. In any case, it is the curators' responsibility to ensure that the accessions in their collections have been identified properly.

At the curator's discretion, accessions may be re-identified using the "NCRPIS Taxonomic Re-identification" form in accordance with the instructions "Procedures for Taxonomic Re-identifications." The form and instructions are maintained on the NCRPIS Intranet site. Appropriate use of this form covers taxonomic re-identifications based on comparisons of a particular accession to the current taxonomic system. Taxonomic re-identifications based on synonymy, unpublished names, misspellings, or multiple and ambiguous taxonomic classifications that can be resolved with the donor's assistance before the accession is loaded into GRIN, should be handled at that time. When questions of taxonomy arise, they should be resolved with the assistance of the USDA-ARS National Germplasm Resources unit, which is the final authority for taxonomy used in the NPGS.

Requesting PI numbers:

At the curator's discretion, accessions that were identified with Ames numbers upon receipt may be assigned PI numbers. This can be accomplished by using the "PI Number Assignment Form" in accordance with the instructions "Procedures for PI Number Assignment." The form and instructions are maintained on the NCRPIS Intranet site.

Candidate accessions must be prepared before PI numbers are assigned. Such preparations can include proofing and updating of all passport data, determining that there are no duplicate accessions, and growing or viability testing the accession. Growing or viability testing the accession will help determine if the accession is viable and can be maintained. Growing the accession will also aid in determining if the taxonomic identification is correct and, if the accession is a cultivar, how well the accession represents the cultivar.

Combining duplicate accessions:

At the curator's discretion, accessions that are duplicates of one another can be combined into one accession. This procedure is initiated by using the "Duplicate Accessions Form" in accordance with the instructions "Procedures for Duplicate Accessions." The form and instructions are maintained on the NCRPIS Intranet site. This procedure differs from that of duplicate accessions that are simply to be inactivated. Only duplicate accessions that are to be combined and remain in the active collection should be addressed with the use of the Duplicate Accessions Form. Seed storage, computer, and login personnel are encouraged to bring questionable accessions to the curator's attention. The combining process will begin after the curator determines that the accessions are, in fact, duplicates of one another.

Inactivating accessions:

There are occasions when accessions need to be inactivated. Examples include:

- The accession was never received.
- Duplicate IDs were given to a single plant population and the germplasm will be combined under a single ID.
- Disposing of excess material following a transfer.
- The germplasm is not viable or cannot otherwise be maintained.
- The accession represents a noxious weed, disease source, or other threat.
- Recordkeeping errors.
- When an accession needs to be inactivated, the curator will complete the "NCRPIS Nomination to Inactive File" form according to the instructions found in "Procedures for Accession Inactivation." The form and instructions are maintained on the NCRPIS Intranet site. The completed form will be submitted to the NCRPIS Research Leader for approval prior to changing the GRIN status to inactive or disposing of plant materials.

Section 2A

Seed processing - storage

The following steps describe how seed lots are stored and physically located in the seed storage facilities.

Following "log-in," the first inventory lot of an accession received at the NCRPIS (which is generally original seed) may be placed into the -18 ^{o}C freezer or into cold storage rooms (4 ^{o}C at 25% to 40% relative humidity) depending on the crop. For freezer storage, the seeds are repackaged and stored in trays that are labeled as to crop and the tray location inside the freezer. Information on the accession and where the inventory lot or lots are located are entered into the GRIN database (Germplasm Resources Information Network).

If a sufficient number and volume of original seeds are received, the first inventory lot may be stored inside the freezer, as described above, and a second inventory lot may be created, which is referred to as the “51 lot.” Inventory lots designated as “51 lots” are stored inside a cold room with the general collection and are distributed immediately after germination tests and/or according to curatorial discretion. Additional inventory seed lots may be placed into the 4 ^{o}C cold room jar assigned to the accession or in a location at the end of that crop’s collection.

Before seed lots are stored, hundred seed weights are calculated and total weight of seed in each inventory seed lot is documented. These seed inventory data are recorded in the GRIN.

If it appears that a seed lot consists of inferior quality seed, those seeds will not be stored until the cause(s) is (are) determined.

The inventory file is programmed to alert curators to re-grow accessions when seed quantity or germination/viability percentage drops below a particular threshold value. When seed quantity drops below the threshold value, the inventory lot is categorized as "requiring regeneration," regardless of its germination percentage. Regeneration quantity thresholds vary from l,000 seeds for some genera, 1,500 with the ornamentals, and 10,000 for grasses and brassicas. When a regeneration quantity threshold is reached, no further distributions from that inventory lot are allowed, except as determined by the respective curator.

The number of seeds maintained at NCRPIS for inventory seed distribution lots may vary considerably according to the curator's judgment. The storage container used for an accession depends on seed size. One-gallon jars are used for cucurbits, sunflower, and maize. One-quart jars are used for taxa with medium-sized seed. Small-seeded accessions, such as amaranth, *Celosia*, and *Cuphea*, are stored in one-pint jars.

For small-seeded accessions, one quart of seed is sufficient for future distributions. For larger-seeded accessions, extra seed beyond the standard container is stored at the end of that crop’s collection. The locations of the jars and the extra seed are recorded in GRIN

Seeds are stored in clear polymer or glass jars with screw on lids. These jars are arrayed on a slide-out shelving system in the 4 ^{o}C cold rooms. The shelving system is constructed of 24' x 36" (60 cm x 90 cm) 20-gauge steel shelves, each with 425 lb. capacity. The shelves are mounted on a Knape and Vogt Model 8500 progressive full extension drawer slide. The slides are connected to 1.25" (3.2 cm) rail steel angle posts, and mounted on a Tab movable carriage platform to increase space efficiency.

Provided that quantities are sufficient, when seeds from newly-regenerated accessions are stored, a subsample is removed from each accession that is not yet "backed-up" at NCGRP (National Center for Genetic Resources Preservation. These subsamples are shipped to NCGRP. Replacement backup samples are sent to NCGRP when newly-regenerated seed lots are of better quality than what is currently stored at NCGRP. “Quality” generally refers to seed germinability or estimated viability.

Material that has been inactivated is physically maintained in the north 4 ^{o}C cold room or in the -18 ^{o}C freezer. Inactivated materials are repackaged and held in trays for reference and possible reactivation in the future.

Detailed information on the NCRPIS seed processing and storage program is contained in the NCRPIS Seed Storage Handbook at http://ncrpis-intranet/supportteams/seeds/Shared%20Documents/Seed_Storage_Handbook%202014.doc.

Section 2B

Seed processing - germination

Historically, the goal at NCRPIS has been to monitor seed viability of distribution lots every five years (for some genera such as *Cucumis*, every ten years) as calculated from the date of the last germination test for each seed lot. Limited personnel resources and a large number of accessions create challenges meeting this goal and it is important that the curators and germination manager coordinate their efforts to ensure that the viability of high-priority accessions is monitored in a timely fashion. Based on expertise with their crops, curators may designate longer or shorter testing intervals for particular taxa.

With some genera, accessions exhibiting less than 70% germination are selected for regeneration. With others, such as *Cuphea*, *Beta*, *Daucus*, and some Cucurbita, wild or domesticated accessions often have intrinsically lower germinability than named varieties, so different thresholds apply.

We conduct our germination tests according to the Association of Seed Analysts (AOSA), ISTA (International Seed Testing Association), or IBPGR (International Board for Plant Genetic Resources) standards, modifying rules for particular problematic crops or wild species as needed to obtain maximum germinations. Specific germination procedures for particular crops are included in the Appendices.

Section 2C

Germplasm regeneration

Seed regeneration methods and schedules are developed by the curators on a crop-specific basis. These methods are designed to produce healthy, viable seeds or clonal materials that maintain, as closely as possible, the genetic composition of the original samples as received by NPGS. Methods reflect differences in plant adaptation, reproductive biology, and phytosanitary concerns and are outlined in the Appendices at the end of this document.

Research was conducted at NCRPIS to develop sound, crop-specific methods for estimating target seed quantities for regeneration. These methods are generally based on concepts outlined in Sackville Hamilton and Chorlton (1997) "Regeneration of Accessions in Seed Collections: A Decision Guide." The general approach is based on our knowledge of seed longevity under NCRPIS storage conditions and of historical patterns of distribution. Preliminary results for rapeseed, cucumber, domestic sunflowers, and maize suggested regeneration targets ranging between about 10,000 and 25,000 seeds.

Section 2D

Management of insect pollinators

The following species of insect pollinators are utilized at the NCRPIS for controlled pollinations of germplasm within field and greenhouse cages:

- Honey bees (*Apis mellifera* L.)
- Osmia bees (*Osmia lignaria* or Blue orchard bee and *Osmia cornifrons* or Hornfaced bee)
- Bumblebees (*Bombus impatiens*)
- House flies (*Musca domestica* L.)
- Blue bottle flies (*Calliphora* sp.)
- Alfalfa leafcutter bees (*Megachile rotundata*)

HONEY BEES (*Apis mellifera* L.):

Traditionally honey bees have been used to pollinate a wide variety of crops; this holds true at NCRPIS as well. Honey bees have been used to pollinate many accessions of most curatorial holdings with the exception of cultivated type Helianthus and Zea mays. Honey bees are used in ca 400 cages annually.

Honey bees are social insects (many bees live and work together in a single colony with one queen whose primary function is to lay eggs); they forage best from 60 to 90 F (15 to 32 C). They are considered aggressive and may sting when they feel threatened. Rearing of honey bees is well established but costly due to the equipment and continuous care required.

At NCRPIS, honey bees are maintained for pollination in small queen-right colonies referred to as "nucleus hives" or "nucs". Nucs are constructed of pine wood; boxes are 16.8 cm high x 50.8 cm long x 27 cm wide overall. The bottom board features a unique sliding entrance which allows the bees to fly either within the cage only or outside of the cage (Ellis et al, 1981). This slide also allows for the bees to be held within the nuc box for short periods of time (e.g. for transport of nuc or pesticide applications to caged plants). Because of limited food supplies for bees within the cages, a feeding hole in the nuc lid allows for supplementary feeding.

Each nuc contains six 15.9 cm frames. Two of the frames contain honey and pollen, three frames contain brood; the final frame contains empty drawn comb. In addition there are ca 2000 to 5000 workers and a mated queen bee.

Nucs are obtained through one of two methods. The first is the division of an over-wintered two story nuc in the spring several weeks after the hives are moved outdoors. One of the stories maintains the queen from the previous year, while the second story will have a purchased queen introduced shortly after the stories are separated.

The second method of obtaining nucs is to use three story honey bee hives referred to as "parent colonies"; the majority of nucs produced annually are created with this method. From April to August, nucs are established from parent colonies as follows: remove two or three frames of brood and adhering bees, provide two stored frames of honey and pollen and one or two frames of empty comb,

and insert one ripe queen cell produced via standard queen-rearing techniques (Spivak and Reuter, 1997). Before nucleus hives are inserted into the cages for pollination, it is ensured that they are queen-right (i.e. the queen bee is producing new brood or eggs and young bee larvae).

Once nucs are ready for use and have been requested by curatorial staff, they are transported to field cages early in the morning. Nucs are placed in the northwest corner of medium field cages or in large cages with the cage opening positioned at the north end in either the northwest or northeast corner. In southern facing large "sunflower cage", the nuc is placed in the southeast or southwest corners. This encourages bees to traverse the entire cage as they are attracted to the southeast side, closest to the spring/summer sun. The nuc is placed entirely inside of a cage in both designs. Beekeepers must enter the cages to feed hives.

The nucs are fed a 3:1 solution of high fructose corn syrup (HFCS) and water on a weekly basis through the field pollination season. Pollen patties (mix of bee pollen, soy flour, and undiluted corn syrup) are provided every other week.

At curatorial direction, nucs are removed from cages, and transported to bee yards where the bees are allowed to forage freely until needed for another cage or prepared for winter.

At the end of the field pollination season, all colonies are prepared for over-wintering. Several feedings of diluted corn syrup are provided to assure adequate food stores within the hives. In December, hives are moved to an indoor over-wintering facility. This facility is maintained at ca 45 F (6 C), RH 60 %, 24 hour dark.

In both the spring and the fall as hives are prepared for the upcoming season, they are examined for diseases such American foul brood and mites. Affected hives are treated appropriately. All hives are given two or three feedings of "medicated" diluted corn syrup containing Fumagilin-B® both in the spring and fall.

Diluted HFCS - In a 30 gallon mixing tank, place ca. one gallon of hot tap water. Next fill the mixing tank to the 20 gallon mark with HFCS. Fill the tank to the 30 gallon mark (10 gallons) with hot tap water. With the orange plastic stir rod or auger-type stirrer mix the contents of the tank for ca. two minutes. Feed containers can then be filled using the faucet on the mixing tank. HFCS is stored in either one or both of the 1050 gallon storage tanks in the shop and behind the shop. In the winter, all syrup must be stored either in the inside tank or in barrels in the shop. The contents of the tank must be circulated daily for ca. five minutes to prevent the syrup from sugaring and plugging the drain spout.

Medicated syrup mix - Medicated syrup is prepared by first placing 163 grams of Fumagilin-B granules in 2 gallons of warm water and stirring in a commercial mixer for five minutes. Note: the Fumagilin-B gets quite foamy while mixing and falls out of suspension if not mixed at the proper temperature or for the proper amount of time. Once the Fumagilin-B is premixed, add this mixture to 30 gallons of diluted corn syrup in the mixing tank and stir another five minutes. An auger-type stirrer that attaches to a 2" heavy duty electric drill was designed for this mixing. Make sure the auger is below the air line, as mixing in air with the syrup will cause it to foam.

Tylan® mix - Use one bag (907 g/32 oz.) of powdered sugar to 9.07 g of Tylan®. Put the mixture in a one gallon bucket with lid and shake it for a few minutes to mix well; this keeps antibiotic dust to a minimum. Several times while administering the treatment, the mix is stirred using a hive tool.

Pollen patty mix - If the weather is cold (early spring), the patty should be a light consistency, and the pollen should be as moist as possible for the longest time possible. Later in the season and as the colonies get more established, the consistency of the mixture is not as critical. Use a five gallon bucket to mix the pollen patties. In the five gallon bucket, mix 25 "cups" of pollen, 15 "cups" of pollen

substitute and 2 gallons of undiluted high fructose corn syrup. Using the auger-type stirrer attached to a 2" heavy duty electric drill, firmly hold the bucket between your legs and mix the contents until they have formed a moist doughy consistency. Extra syrup may need to be added if the mix is too dry.

Powdered Sugar Roll - To sample for varroa mites and not harm the bees a "powdered sugar roll method" can be used. In a screened lidded quart Mason jar place approximately 100 bees brushed from a brood frame, add 1 Tbs. of powdered sugar and replace lid. The jar should be shaken and turned for 30 seconds and set aside for approximately one minute. The sugar contents are then shaken out through the lid into a white plastic pan and all dislodged mites can be counted. Once finished, the bees in the jar can then be placed back into the hive. This sample method should be done at least twice per parent colony with a sample taken from two different brood chambers. In the spring a count of 10 mites/100 bees should indicate treatment and in the late summer a count of over 15 mites/100 bees should indicate mite treatment.

OSMIA (*Osmia lignaria* or Blue orchard bee and *Osmia cornifrons* or Hornfaced bee):

Osmia bees are excellent pollinators of early blooming plants such as Brassicaceae and fruit trees. They are used in up to 100 cages annually at NCRPIS.

They are a solitary bee species (individual female bees perform all the work required to maintain their own nesting cells) that work from 50 to 90 F (10 to 32 C) during the months of April through June. These bees are non-aggressive. Rearing is established for this bee; they are low cost and require little care.

Osmia bees rear their young in waxed paper straws (7.25 mm diameter and 152.4 mm long); the females use mud to create barriers between the up to eight cells formed in each straw. Straws are inserted into cardboard tubes (7.94 mm diameter and 152.4 mm long) to protect the developing larvae from parasitization. These straws are placed inside domiciles constructed from PVC pipe for protection from the elements.

Two different sizes of PVC domiciles are used at NCRPIS; small domiciles are used inside of field cages and large domiciles are used to obtain an annual increase of bees. Small domiciles are constructed from 5.1 cm diameter PVC pipe cut 25.4 cm in length. The front of the domicile is cut at a 45 degree angle to protect the straws from rain; an end cap at the opposite end retains the straws within the pipe. Large domiciles are constructed from 7.6 cm diameter PVC pipe cut 27.9 cm in length. The front of the large domicile is also angled; in addition a piece of mesh screen covers the opening to prevent injury to the straws by birds and other predators. Both sizes of domiciles are fitted with two 6.4 cm eye-bolts on the top of the pipe; eye-bolts are used to secure the domiciles in place in cages or increase areas. A 1.2 m section of rebar bent at a 90 degree angle 0.9 m from the bottom of the bar is used to hold small domiciles in cages. The domicile is suspended from the rebar by sliding the eye-bolts onto the short section of the rod. The rod is inserted to one side at the center of the cage with the domicile entrance pointed to the south-southeast. Large domiciles are simply suspended from tree branches with twine run through the eye-bolts; domicile openings are directed toward the south-southeast.

One peculiarity of Osmia is that once these bees have emerged from a domicile in a particular location, if the domicile opening is repositioned even a slight distance, the bees will abort use of the domicile and will die soon after. These bees cannot be confined to their domiciles without causing death either. Because of this habit of the Osmia, if movement of domiciles is required prior to the end of pollination (e.g. for pesticide application to caged plants), the old domicile must be retired from use and a new Osmia domicile or a different bee must be introduced to the treated cage.

The ca 3500 Osmia bees used at the NCRPIS annually are obtained through two methods. 1500 bees are purchased from U. S. suppliers located in California/Oregon. In addition, ca 2000 bees are obtained from the nest cells produced in the field cages as well as outlying increase sites.

Osmia domiciles are removed from the field in early July, once temperatures are too warm for bees to continue working. Domiciles must be handled gently to prevent dislodging developing larvae from pollen balls within the nest cells. The domiciles are placed in a rearing room maintained at ca 70 F (23 C) and 50 % RH until November. In mid-November the straws are removed from the domiciles and examined for completed cells. Groups of straws containing 100 bee pupae are wrapped in 2-ply tissues and then placed into large garbage bags with water-dampened sponges (two sponges per bag). The garbage bags containing the sorted straws are stored at 40 ^{o}F (4 ^{o}C) for the winter and are opened weekly for the exchange of CO_2/O_2 and to remoisten the sponges. In February, the straws are removed from winter storage and placed in PVC domiciles in preparation for the coming field season. Small domiciles receive ca 24 bee pupae in a bundle of 15 total cardboard tubes. Large domiciles receive ca 32 bee pupae in a bundle of 23 cardboard tubes. Domiciles are maintained at 40 ^{o}F (4 ^{o}C) until use. Domiciles are moved from cold storage to cages immediately as the first bees will emerge from domiciles within 24 hours after they are brought to warm temperatures.

BUMBLE BEES (*Bombus impatiens*):

Bumble bees are known to pollinate many plants; however they excel at pollination of plants with trumpet-shaped flowers due to their long tongues. At NCRPIS, bumble bees are used primarily for pollination of Cucurbita sp., Baptisia sp., Potentilla sp. and Calendula sp. as well as other "challenging accessions" not easily pollinated by other NCRPIS insects in ca 32 cages annually.

Bumble bees are social insects; they forage at 55 to 90 ^{o}F (13 to 33 ^{o}C) in all types of weather. Unlike other bees, bumble bees will continue to work in cool rainy conditions. These bees are mildly aggressive when they feel threatened. Rearing is possible, but difficult. Colonies are available through several commercial sources.

NCRPIS purchases "mini-research colonies" consisting of a queen and up to 30 workers. The furnished domicile consists of a cardboard box containing a plastic chamber which rests on top of a plastic feeding bag. The chamber houses the nesting area; bees are provided with upholsterer's cotton within which they build the brood nest. A cotton wick bridges the area between the nest and the feeding bag below. The feeding bag contains HFCS. Initially bees can only exit the box once a sliding tab has been opened and lightweight mesh fabric is removed. Once a hive has been opened, bees can be allowed to enter/exit the box as appropriate by setting the slides as recommended by the supplier.

If the queen-right colony is found to be too aggressive in working tender flowers, the use of a "drone-only colony" is recommended. Drones (male bees) tend to have a more "mellow" disposition; they cannot sting.

Bumble bee hives are placed entirely within the cage regardless of cage size. They are placed within a protective 60 quart plastic tub to reduce weather damage to the cardboard box. When bumble bees are no longer needed in a cage, the slide is set to "entrance only" and the hive is collected between the hours of 9 PM to 7 AM.

Bumble bees are allowed to forage freely when not in cages or are stored in a rearing room at 70 °F (23 °C) and 50 % RH. They are not stored in any way over the winter; individual colonies are allowed to die off naturally.

HOUSE FLIES (*Musca domestica* L.):

BLUE BOTTLE FLIES (*Calliphora* sp.):

Two species of flies are used at NCRPIS to pollinate umbelliferous, horticultural and oilseeds crops (e.g. *Anethum*, *Angelica*, *Biscutella*, *Brassica*, *Calendula*, *Camelina*, *Coriandrum*, *Crambe*, *Dalea*, *Daucus*, *Erysimum*, *Eruca*, *Hypericum*, *Hyoscyamus*, *Isatis*, *Matricaria*, *Petroselinum*, *Pimpinella*, *Sinapis*, *Spiraea*, *Thlaspi*, and *Torilis*) in field and greenhouse cages. Flies are used in ca 40 greenhouse cages in the winter and ca 20 greenhouse cages in the spring/summer; ca 80 field cages are supplied with flies in the summer.

Flies are considered "incidental" pollinators. As they move around flowers in search of nectar or rest on flower sets, they move pollen on their body hairs from one flower to another. When flies are included with honey bees in cages containing the above mentioned plant species, pollination is more effective than when only honey bees are present (Wilson et al, 1991).

Flies will work plants at average temperatures of 70 to 90 °F (21.5 to 32.5 °C). Both species of flies may be less active in inclement weather; blue bottle flies may be less active at very warm temperatures. Flies are non-aggressive toward humans, but may be considered "irritating". Rearing of flies is well established and pupae are low cost to purchase.

At NCRPIS, both species of flies are purchased as pupae from outside sources. House flies are purchased from a commercial source in California; blue bottle flies are purchased from a commercial source in Idaho. House fly pupae cannot be stored for an extended length of time, so weekly shipments are received for cage pollinations. Blue bottle fly pupae can be stored at 32 °F (0 °C) for three weeks before adult emergence is significantly reduced; regular shipments (received every two to three weeks) are scheduled at the beginning of each pollination season (winter greenhouse and summer field).

Based on past NCRPIS research, ca 200 fly pupae are placed in each cage weekly (Wilson et al, 1991). In general flies will live 2 to 3 weeks but weekly replenishment ensures an adequate population for pollination at all times (e.g. in case of less desirable environmental conditions). Fly pupae are incubated for 2 to 3 days in 0.2 liter paper cartons with screened lids at 80 °F (26.5 °C) 30 % RH before placing in cages to ensure higher percent adult emergence. Fly pupae holders constructed of 0.9 liter plastic containers weighted with plaster are placed inside each cage to receive the pupae. Adult flies also may use these containers to rest in during the evening or inclement weather. No further care is provided to the flies once they are placed in the cages.

ALFALFA LEAFCUTTER BEES (*Megachile rotundata*):

The alfalfa leafcutter bee (ALC) is a solitary bee which historically has been used for pollination of forage legumes and more recently utilized for blueberries. At NCRPIS ALC bees were brought in primarily as a supplement or replacement for honey bees; their use began on a small scale in 2004 and has increased to ca 300 cages annually as of 2013. In general the ALC bees seem to be most effective pollinating small to medium flowers of a "flat" nature. Germplasm pollinated by ALC includes *Agastache*, *Angelica*, *Brassica*, *Biscutella*, *Calendula*, *Coriandrum*, *Crambe*, *Cucumis*, *Cuphea*, *Daucus*, *Erysimum*, *Eruca*, wild-type *Helianthus*, *Isatis*, *Linum*, *Melilotus*, *Ocimum*, *Potentilla*, *Sanvitalia*, *Sinapis* and

Tanacetum. These bees have been used in both late winter/spring greenhouse cages and in summer/fall field cages.

The ALC have been found to be good companion pollinators to (i.e. present in the same cage as) honey bees, flies, and Osmia. In Brassica cages containing both Osmia and ALC bees, the Osmia work in cool mornings while the ALC work in the warmer afternoons when the temperatures are too warm for Osmia activity. It appears that the presence of ALC bees in the same cages as honey bees may motivate the latter to work harder than if the honey bees are in the cage by themselves. Because the ALC are motivated to collect pollen to feed offspring, they may effect more pollination than flies on the same flowers.

ALC will work at temperatures of 80 ^{o}F (26 ^{o}C) or above as they prefer dry sunny climates; these bees will not pollinate as well in cool cloudy or rainy weather. The ALC is non-aggressive, but will bite if accidentally squeezed; the bite produces a stinging sensation. Rearing of ALC bees is well established and the pupae are low cost to purchase.

ALC bees are purchased from commercial suppliers in Canada or the western U. S. Bees arrive as late instar larvae enclosed in leaf cells; cells are sold in gallon quantities with one gallon containing ca 10,000 cells. High quality cells will result in ca 80% bee emergence (Logan UT Bee Lab, 2004). The ALC cells are stored in screen trays or plastic screened incubation containers (32 oz./950 ml rectangular plastic boxes 4 cm high x 23 cm long x 16 cm wide, with screen inserts in the bottom of the box and in the lid) at 40 ^{o}F (4 ^{o}C). Cells should be kept in layers of 3.8 cm or less to prevent reduced bee emergence; greater cell depths allow overheating which kills larvae in the bottom layer of cells.

The ALC bee cells require ca 30 day warm treatment before all bees will emerge (International Pollination Systems, 2004). After removing incubation containers from cold storage, containers are placed for one week at room temperature, approximately 70 to 75 ^{o}F (ca 21 to 24 ^{o}C). They are then moved to an 86 ^{o}F (30 ^{o}C) dark incubator for two weeks. Next the cells are transferred to an 86 ^{o}F (30 ^{o}C) rearing room which provides light for 5 hours per day. The cells from up to three incubation containers are placed in a single wood emergence box (30 cm long x 25 cm wide x 20 cm high outside with interior depth of 7 cm) which is attached to two plastic collection dishes (26 cm diameter x 7 cm high) via 3 pieces of thick wall flexible plastic tubing (I.D. 5/16", O.D. ½", wall 3/32", 20 cm long) placed in the front of the box and connected to rigid plastic tubing (O.D. 1.6 cm, 8 cm long) in the dish lid. Within several days of cell placement in the emergence box, bees will begin exiting from their cells. Attracted by the light in the rearing room, the bees move from the box through the flexible plastic tubing and into the collection dish where they are provided Binderboard® (wood cell blocks 13.2 cm long x 10.2 cm wide purchased through Pollinator Paradise, Parma, ID) and several 2.5 cm long cotton wicks soaked in 5% sucrose solution to keep them calm until they are collected mid-afternoon daily. Bees on wicks are placed in vented 120 ml plastic cups; with each cage receiving a "cup of bees". Ca 20 to 40 bees are released to each cage depending on the number of open flowers present. Because female ALC bees (brown eyes) are thought to do more pollination than male bees (green eyes), it is important to include both sexes in all cages.

ALC bees do not normally emerge from their cells before springtime, so bees used in late winter greenhouse cages must be replenished weekly from January until late March/early April. After April bees are replaced ca every other week depending on the bee activity/life span noted in each cage. Depending on the quality of cells received in the annual early spring shipment (i.e. if cells have very low percent infestation by parasitoids), ALC bees are emerged for late fall/early winter greenhouse cages into January of the following year. During this time, bees must be replaced weekly.

Developing ALC bee cells are subject to parasitism by several species of small Hymenoptera including *Pteromalus*, *Monodontomerus*, *Tetrastichus*, and *Melittobia* (Peterson et al, 1992). In order to control these parasitoids, traps consisting of black lights shone over open dishes of soapy water are placed in all ALC rearing areas. Traps are cleaned weekly.

Domiciles are important in extending ALC bee life span and activity level. The domicile design used in cages consists of a commercial Styrofoam nesting blocks with pre-drilled holes of 0.635 cm diameter and 6.75 cm depth (the size preferred by ALC bees, purchased through Northstar Seed Ltd, Neepawa, Manitoba) cut into smaller blocks 14 cm long x 9 cm wide; the Styrofoam blocks are placed in a simple pine wood frame with a plastic-coated wire handle ca 75 cm long which is wrapped around the metal cage frame to one side at the center of the cage so the cell block faces to the south – south-east for rapid warm up from the morning sun.

It is not feasible for us to collect high quality ALC bee cell increases in our geographic location due to the large populations of parasitoids present. Any cells created by bees in the Styrofoam nest blocks are discarded in the fall.

Insect cages:

The cage screening is "natural"-color Lumite, the "medium cage dimensions are 6.1 m long x 1.5 m wide x 2.1 m high for the "medium" cages, and 6.1 m long x 3.1 m wide x 3.1 m high for the larger "sunflower cages." With the "sunflower cage", one end has an opening with flaps secured with a Velcro closure or plastic clasps. In the "medium cages" there is a zipper opening at both ends of the cage. The cage frame is galvanized pipe (1.3 cm diameter). In the "sunflower cage," connects are Kee Klamp® clamps: 4 three way corners, 6 T-ends, 3 cross keys, and 2 two way corners. For the "medium" cages, all joints are held together by the assembled cage being pushed into the ground and have no Kee Klamp® connects.

Once the framework is assembled, the screening is draped over the frame so that equal amounts of excess screening lies on the ground on all sides. The excess screen is buried in a furrow ca. 13 cm deep directly beneath the outside edges of the cage frame. The screen is pulled tight when soil is mounded over the edges in the furrow. As the soil settles, the screen is pulled down firmly over the frame.

Cages are erected prior to flowering. In some crops susceptible to insect feeding and insect-vectored diseases, the cages are erected immediately after the plants are transplanted to the fields. The cages remain around the plants until harvest. Immediately before harvest the screening is removed, cleaned if necessary, rolled up with a Rol-Zit roller, and stored. The cage frame is partially disassembled into two halves and stored near the field.

References:

Logan UT Bee Lab, USDA-ARS Bee Biology and Systematics Laboratory, Logan, UT. Personal communication with all lab personnel. April 1, 2004.

Ellis, M.D., G.S. Jackson, W.H. Skrdla, and H.C. Spencer. 1981. Use of honey bees for controlled interpollination of plant germplasm collections. HortSci. 16:488-491.

International Pollination Systems. 2004. A Calendar of Incubation for Alfalfa Leafcutting Bees. http://www.pollination.com/managed/albincubationcalendar.cfm

Peterson, S.S., C.R. Baird, and R.M. Bitner. 1992. Current Status of the Alfalfa Leafcutting Bee, *Megachile rotundata*, as a Pollinator of Alfalfa Seed. Bee Science 2:135-142.

Spivak, M. and G.S. Reuter. 1997. Successful queen rearing. Short course by University of MN Dept. of Entomology and University of MN Extension Service.

Wilson, R.L., M.P. Widrlechner, and K.R. Reitsma. 1991. Pollination methods for maintaining carrot germplasm collections. FAO/IBPGR Plant Genetic Resources Newsletter, 85:1-3.

Section 3

Computing and information management

Equipment:

The NCRPIS is equipped with approximately 57 desktop and 19 laptop computers, although this number fluctuates slightly with personnel and program changes. Every permanent staff member is equipped with at least one computer and several computers are available for temporary employees. Other hardware for computing/information management includes telecommunications between NCRPIS and GRIN, bar-coding equipment, and miscellaneous data collecting devices such as Pocket-PC's and Windows tablets. As of July 2014, NCRPIS had 18 physical servers. More detailed information about NCRPIS computer hardware can be found in the ISSP.

Software:

Standard client software for the NCRPIS operations includes Windows 7 or Windows 8 operating system. Standard software includes: Microsoft® Office Professional, Oracle, Adobe PhotoShop, Adobe Acrobat, Microsoft® SQL Server, Retrospect, and Track-It!. Regular Windows updates are automatically applied at the workstation level. A managed Symantec Endpoint Protection system is used to ensure regular antivirus and malware scans as well as real-time scanning of files.

Network:

The NCRPIS is part of the Iowa State University Active Directory Domain. All network wiring to the jack is maintained and upgraded by Iowa State University and a monthly fee is charged for each jack. All computers have a 1 Gigabit per second connectivity to the domain. The station is behind a Cisco Adaptive Security Appliance (ASA) firewall and all jacks on campus and on the farm are on an isolated network behind the firewall. Users are able to connect to isolated network resources when on travel or teleworking via Cisco VPN connectivity through the ASA.

Documentation:

To encourage more consistent use of software packages and other NCRPIS procedures, and to reduce errors, procedures and standards are documented and posted to the NCRPIS Intranet in Adobe Acrobat format. These procedures and standards are often embedded within NCRPIS committee meeting minutes.

Data entry:

Data entry to GRIN is accomplished utilizing in house Oracle Forms or the WEB based GRIN system. Bulk entry of data is often accomplished utilizing ODBC connections to the GRIN database or via FTP file transfer. Some of these protocols will change with the implementation of GRIN Global.

Section 4

Germplasm characterization and evaluation

Ensuring that accessions have the correct taxonomic identification is a vital role for curators. Accessions that have incorrect or questionable identifications have little value. Likewise, documenting an accession's genetic, morphological, phenological, chemical, and other traits greatly increase its potential value in a germplasm collection and the efficiency and efficacy of selecting accessions for research, breeding, or educational activities. Confirming plant identification and documenting traits are accomplished during germplasm characterization and evaluation. These activities are described in some detail in the Manual of Procedures for the National Plant Germplasm System.

When accessions are grown for initial increase by a curator, data on various plant characteristics important for the confirmation of accession identity and future monitoring of accession identity should be recorded. The list of traits, generally referred to as a "descriptor list," will be developed by the curator in consultation with the appropriate Crop Germplasm Committee. Some traits important to the curator for monitoring accession integrity may not be important, per se, to the CGC or user community.

The curator has the responsibility to determine which characterizations can be accomplished in the normal increase or regeneration process and which must be done by cooperating scientists. The CGC may develop overall plans for characterization not included in the curator's program.

In developing descriptor lists, which are documented in the GRIN database, curators and CGCs should generally follow the guidelines provided by Bioversity International (formerly known as International Board for Plant Genetic Resources (IBPGR) and International Plant Genetic Resources Institute (IPGRI)). Besides identifying traits, curators and CGCs also specify the parameters used to measure the traits. In characterizing Hypericum taxa, for example, plant form is characterized as forb or herb, shrub, subshrub (herbaceous with a woody base), or tree. Leaf shape is characterized as lanceolate to oblong, linear, orbicular to obovate, scale-like, or other (which is described in the comments field). Descriptor lists are not static, but continue to evolve as we learn more about the crops we curate and as technological advances allow us to better measure traits.

The NCRPIS entomology and plant pathology evaluation programs generate host-plant resistance data for certain crop-disease and crop-insect combinations.

Observations (including photographic or scanned images) made on plants during regeneration, phenotyping, or other activities are entered into the GRIN system for each accession being observed. These data and images are available to internal GRIN users and are also available through the public GRIN portal. Characterizations, like descriptor lists, are not static and data may be added to a particular accession's records over the course of years.

As part of the NC7 Regional Woody Ornamental Trials, trial‑site cooperators record observations on plant characteristics and performance at their sites. One, five, and ten‑year summaries of accession performance are provided to the NC7 Woody Landscape curator for interpretation. The curator prepares an overall summary of each accession's zone of ecogeographical adaptation, or lack thereof. Common disease or insects are noted whenever they may affect an accession's ability to survive.

Section 5

Germplasm enhancement

Germplasm enhancement programs currently include those conducted by the amaranth curator (improved amaranth cultivars), the GEM Project coordinator (introgression of exotic maize germplasm to increase genetic diversity), and by the pathologist (population improvement of wild sunflower for leaf diseases). We supply seeds for enhancement programs with sunflowers and maize, and curate the resulting enhanced germplasm that is publicly released.

Enhancement activities (non-GEM Project related) center around improvement for specific attributes identified by stakeholders that limit the ability of the germplasm to contribute, and usually in collaboration with these researchers. These efforts are highly focused, and secondary in importance to other curatorial objectives.

Much information about the GEM Project can be found on their website, http://www.public.iastate.edu/~usda-gem/.

Section 6

Research related to plant genetic resource management.

In general, research pursued at the NCRPIS seeks to improve the NCRPIS's efficiency and efficacy in the areas of:

- Conserving diverse crop plant genetic resources and their wild relatives,
- documenting and providing associated information,
- producing and providing healthy propagules,
- encouraging germplasm utilization, and
- germplasm enhancement.

The research effort is diffused throughout the NCRPIS. Most NCRPIS staff members are involved in some form of research, much of which seeks to generate data with immediate practical utility.

NCRPIS scientific and curatorial personnel seek to intensify the integration of molecular genetic technologies, systematic and statistical genetic principles, computer-assisted image analysis, and improvements in information management and communication technologies into plant genetic resource management. This underlying theme provides direction and impetus to much of the research conducted at NCRPIS. The precise foci for research, however, vary from year to year, and our research program is described below in general terms. More specific and timely information can be found in the NCRPIS annual reports.

Acquisition:

Genetic improvement is contingent on access to useful genetic variation. In order to develop quality collections representative of the breadth of genetic and ecogeographic diversity of crops and their wild relatives, it is necessary to survey both indigenous and cultivated distributions of a given taxon. Through a process of gap analysis, and based on informed stakeholder input, curators determine collection development needs and seek to incorporate new genetic variation into their collections via plant exploration or exchange. Considerable effort is devoted to studying herbaria, flora records, maps, gazetteers, and engaging expert partners to recommend and secure new germplasm. The Crop Germplasm Committees (CGC) facilitate these efforts.

Documentation and Information Management:

Automation of information capture and management of associated information is essential for effective and efficient plant genetic resource workflows. Publicly providing information increases the value and hence the utility of plant genetic resources. NCRPIS staff members utilize various electronic data capture and transfer technologies. They are highly involved in the development (programming, analysis and testing) of the GRIN-Global System, a genebank information management system designed to replace the legacy Germplasm Resource Information Network (GRIN), utilized by the National Plant Germplasm System. GRIN-Global is being adopted by several international genebanks, and is becoming the global standard for genebank information management.

Crop morphology, phenology, genetics, phytochemistry, and utilization:

Two of the five primary tasks of the NPGS are germplasm evaluation and documentation. NCRPIS curatorial staff members capture detailed morphological and phenological observations of accessions during seed increases and other grow-outs and document those observations by entering the data and digital images in GRIN / GRIN-Global. While NCRPIS does not maintain laboratories for biochemical and DNA analyses, we collaborate with scientists and research institutions to characterize biochemical and genetic profiles for the germplasm that we maintain. Links to research articles and other published information relevant to our accessions are posted on individual accession web pages in GRIN / GRIN-Global, where it is publicly accessible. Characterization and information availability increases the value and utilization of the accessions by providing a measure of potential value as parents in breeding programs and sources of constituents such as phytochemicals for industrial, medicinal, and nutraceutical applications.

Taxonomy:

Correct taxonomic identification of accessions is critical to ensure that plant taxa and populations are properly represented in the NPGS and that recipients can be confident of the nature of the genetic resources requested, and that their investment in basic and applied research activities is well-directed. Plant taxonomy is undergoing a marked evolution as genetic and biochemical analytical tools allow increasingly precise determinations of phylogenetic relationships. With support from the ARS National Germplasm Resources Laboratory and collaborating scientists, NCRPIS curators remain abreast of taxonomic developments that relate to their collections and re-identify or reclassify accessions as needed, to ensure that our accessions are properly identified. In recent years, substantial changes in nomenclature have affected NCRPIS taxa, including *Actaea*, *Alcea*, *Althaea*, *Baptisia*, *Potentilla*, *Sphaeralcea*, and others.

Reproductive biology:

Understanding floral initiation, development, morphology and phenology; self-fertility and incompatibility; and pollinator requirements are critical in effectively regenerating plant germplasm. NCRPIS curators, scientists, and curators study their crops' characteristics and needs to develop and refine regeneration practices.

Seed dormancy and germination:

Improved understanding of germination requirements and dormancy breaking methods for many species is a research priority. Knowledge in this area impacts our ability to accurately assess collection condition, and to provide useful information to germplasm requestors to enhance the success of their efforts. Effective protocols are needed to accomplish dormancy breaking and germination for many taxa. Objectives include refining viability assays, determining dormancy mechanisms, and developing effective seed germination protocols. Current projects include studies on dormancy characteristics and germination dynamics in *Actaea*, *Hypericum*, and *Fraxinus*, which are high-priority

genera within the horticultural collections. The effects of storage temperatures and time in storage are being determined for long-term storage of *Betula* and *Calendula*.

Plant pathology:

The plant pathology program at NCRPIS has three main functions. The first is to maintain healthy plantings and to provide healthy propagules to germplasm requestors. The second is to develop and refine plant pathological study methodologies. The third is to identify and document the characteristics of the accessions that we maintain. Our plant pathology program evaluates Helianthus germplasm for host-plant resistance to Alternaria leaf blight and studies the mode and frequency with which Erwinia stewartii, Stewart's wilt, is transmitted via maize kernels. The pathologist monitors health of seedlings and seeds, and develops methods for detection and quantification of pathogens such as *Pantoea stewartii*, *Acidovorax avenae*, *Clavibacter michiganensis* subsp. *nebraskensis*, and others. Research findings contribute to our ability to provide high quality, disease-free germplasm to requestors, and to address challenges to food production and germplasm movement.

Entomology:

The entomologist conducts research to establish improved methods for rearing pollinators and maintaining health of the pollinator colonies. Honeybee and Mason bee colonies are maintained. Six insect species are provided to curatorial staff on demand for controlled pollination of caged accessions. Collaborations with curators are designed to better understand appropriateness of pollinators for specific taxa in order to obtain quality increases of sufficient quantities, and to better maintain the original genetic profile of an accession.

Crop production systems:

The NCRPIS farm staff (directed by the Farm Superintendent), in conjunction with various crop-specific curators, the entomologist and other staff, continually updates and improves various management practices via applied research. Recent projects include design and construction of photoperiod control structures to accomplish reproduction of short day accessions in our long day environment; design and construction of shade houses to improve plant growth of woody ornamentals; experimentation with LED lighting systems in greenhouses to improve plant growth and seed yields.

NC7 Regional Ornamental Trials:

The NC7 Regional Ornamental Trials is an ongoing program involving germplasm evaluation, documentation, and utilization. The horticulture program coordinates the trials, which is a long-term, multi-location evaluation of perennial species for their merit as landscape plants for the North Central Region. Horticultural personnel focus their scholarly efforts on:

- the development of collections that represent diversity of key woody ornamental species,
- evaluation and characterization of this germplasm in collaboration with research partners,
- assessment of characteristics that impact adaptation and/or invasive potential,
- reproduction of the germplasm, and

- conservation of the germplasm.

Transfer of information to facilitate germplasm utilization and diversification of the landscape is a key objective to realizing the value of this research.

Section 7

NCRPIS Staffing (See Organizational Chart, Appendix 1)

CURATORIAL AND ADMINISTRATIVE

A. Research Leader-Coordinator (Category 4 USDA/ARS position):

- Coordinates and leads the NCRPIS overall and is responsible for successful accomplishment of site goals; prepares and administers budgets.
- Supervises, directs, and guides a variety of personnel.
- Plans and conducts personal and team research.
- Facilitates technology transfer and pursues professional advisory and consulting activities.

B. Program Manager II (ISU P & S position – NC7 RRF funded):

- Directs daily farm activities, including all aspects of support for plant growth and cultivation, farm and greenhouse management.
- Coordinates and supervises two field-lab technicians and hourly employees.
- Ensures communication of physical and labor resource needs and facilitates resource sharing among Station personnel.
- Interviews and selects temporary labor force; involved with public relations with ISU, USDA and external officials, visitors to the station, vendors, and building contractors.
- Constructs and maintains farm facilities and/or equipment.
- Provides advice to scientific, curatorial, and technical staff.
- Maintains safe and healthy conditions at the NCRPIS farm.
- Participates in a wide range of technical matters with internal and external stakeholders.

C. Horticulturist (Category 4 USDA/ARS position):

- Serves the local, regional, national, and international scientific community by providing germplasm for research.
- Pursues research on plant genetic resource management and germplasm viability.
- Serves as a resource person for the crop-specific curators, assists Research Leader-Coordinator with horticultural crop germplasm.
- Plans and organizes certain NCRPIS programs.
- Develops and curates herbaceous ornamental and medicinal plant germplasm collections.
- Coordinates with GRIN personnel to enter and disseminate information regarding the NPGS collection.

- Provides periodic informal and/or formal training, instruction and guidance to other staff, scientists, and students.

D. Horticulturist (Category 3 USDA/ARS position).

- Serves the local, regional, national, and international scientific community by providing woody ornamental plant germplasm for research.
- Pursues research on plant genetic resource management and plant-pollinator relationships.
- Serves as a resource person for internal and external stakeholders, with a special focus on preserving ash (*Fraxinus*) due to the Emerald Ash Borer issue.
- Assists Research Leader-Coordinator with horticultural crop germplasm; plans and organizes certain NCRPIS programs.
- Directs a regional trial program for woody ornamentals; and curates woody ornamental germplasm.
- Coordinates with GRIN personnel to enter and disseminate information regarding the NPGS collection.
- Supervises temporary employees and a USDA/ARS Agricultural Research Technician if feasible, and provides periodic informal and/or formal training, instruction and guidance to other staff, scientists, and students.

E. Entomologist (Category 3 USDA/ARS position):

- Develops optimal insect pollination methods for entomophilous germplasm, and methods to ensure pollinator health. Provides pollinator insects for regeneration activities.
- Facilitates technology transfer and is involved with professional advisory and consulting activities.
- Identifies and controls insect pests affecting increase plantings. Supervises one Agricultural Research Technician and temporary employees.
- Reports on activities and findings.
- Serves on internal committees to facilitate exchange of information and technologies between teams and develop cross-functional solutions.

F. Plant Pathologist (Category 4 USDA-ARS position):

- Pursues a research program to evaluate plant germplasm for host-plant resistance to viral, bacterial, and fungal diseases (seed-transmitted diseases are emphasized), and to develop tools and methods to identify pathogen on field and greenhouse plantings.
- Conducts field inspection for phytosanitary certification; identifies seedlots with seedborne pathogens using ELISA and molecular methods.
- Advises curators and researchers regarding disease control strategies for field and greenhouse plantings, and develops those strategies.

- Reports results of research in publications and at scientific meetings.
- Lectures touring scientists and the general public regarding research and service program.
- Supervises several hourly employees. Serves on national committees tasked with seed health concerns and phytosanitary issues.
- Supervises an Agri Research Science Technician (plant pathology), a Biol Research Sci Technician (Seed Storage), and temporary employees.

G. IT Specialist/Software Applications Development – (GS 12/14 USDA/ARS position):

- Develops software solutions to enhance our ability to document and manage plant genetic resources, and to share information with stakeholders to facilitate germplasm utilization.
- Recommends/selects technical equipment to improve processes and efficiency in information capture and management.
- Ensures integrity of network systems.
- Supervises a Network Security IT Specialist.

H. IT Specialist – Network Security – (GS 9/11 USDA/ARS position):

- Oversees all NCRPIS information equipment and network systems, including installation and periodic upgrading.
- Ensures integrity and security of network and database systems, in conjunction with ARS OCIO and ISU cybersecurity infrastructure.
- Selects information technology applications and equipment.
- Recommends IM training of staff, and assists staff with IM concerns.

I. Crop-specific curators:

At the NCRPIS, a curator is assigned to each of our major crops or "related crops" groups. Curatorial positions may be supported by either by USDA-ARS or NC-7 RRF funds. The curators have earned a B.S., M.S., or Ph.D. in plant breeding, botany, horticulture, plant physiology, or a related field. Curation involves applying a broad knowledge of agronomy and/or horticulture, botany, plant genetics, physiology, ecology, pathology, and entomology, and deep knowledge of the collections entrusted to them.

Our objectives are to A) conserve, ex situ, the full spectrum of genetic diversity found in particular crops and their wild relatives and associated information; and B) facilitate and encourage the use of these genetic resources by bona fide researchers and their incorporation into crop improvement programs. The following specific duties are involved:

- In cooperation with local, national, and international colleagues, curators maintain and increase NCRPIS germplasm accessions according to the best genetic, agronomic, and/or horticultural principles and practices.

- Curators develop crop plant collections representative of the breadth of their ecogeographic and genetic diversity, and their wild relatives (secondary and tertiary gene pools).
- Curators characterize and evaluate NCRPIS germplasm accessions with respect to their taxonomic identity, diagnostic morphological and/or molecular traits, and agronomically or horticulturally important features, including adaptation, invasive potential, and biotic and abiotic stress tolerance.
- Curators maintain and manage computerized crop-specific databases (either local or on GRIN/GRIN-Global) that include inventory data, passport information, characterization and/or evaluation data, and relevant research publication citations.
- Curators cooperate with computer coordinators in compiling seed lists or other more specific catalogs of particular accessions for local use, or on request from users.
- Curators act as scholarly researchers and "information clearinghouses" for their assigned crops.
- Curators confer frequently and effectively with the chairs and members of relevant CGC's regarding key curatorial matters, e.g., priority tasks, gaps in the collection, etc.
- Curators serve as ex officio members of relevant CGC's and ad-hoc committees dedicated to advancing NPGS objectives.
- Curators communicate frequently and effectively with researchers and educators who request and use specific germplasm, sometimes recommending particular accessions or research directions.
- Curators provide technically sound advice to colleagues and the user community.
- Curators each supervise several to many temporary employees.

1. CAT 3 Maize (Zea) geneticist/curator (GS 9/11 USDA-ARS position funded):
    - Curates the *Zea* collection. Supervises a USDA-ARS Agri Research Science Technician, multiple temporary employees, and provides guidance and direction to an ISU Agricultural Specialist. Manages and maintains computerized crop-specific databases (either local or on GRIN/GRIN-Global) that include inventory data, passport information, pedigree information, characterization and/or evaluation data, and relevant research citations.
    - Compiles seed lists or other more specific catalogs of particular accessions for specialized or local use, or on request from users. Serves as the subject matter expert resource for maize germplasm research needs.
    - Collaborates with investigators to expand collection holdings to develop and provide comprehensive collections from key crop origin areas, and to fully characterize them.
    - Maintains and manages computerized germplasm databases from data entry, through formatting, to verification. Identifies and facilitates adoption of technical innovations to support germplasm management.
    - Serves as the NPGS GRIN Advisory Committee Liaison from the NCRPIS with users of GRIN at other sites, and with the DBMU at Beltsville, MD, and as the Business Analyst for the GRIN-Global Project.

2. Curator III – Vegetable Crops (ISU P & S position – NC-7 RRF funded position):

- Curates *Cichorium*, *Cucumis*, *Cucurbita pepo*, *Daucus*, *Ocimum*, and *Pastinaca* at the NCRPIS. Supervises an Ag Specialist I (ISU P & S position – RRF funded position).
- Duties similar to those of the first two bullets of the maize curator, with additional considerations for the intricacies of vegetable crop species and their pollinators.
- Collaborates with investigators to expand collection holdings to develop and provide comprehensive collections from key crop origin areas, and to fully characterize them.

3. Curator III - Oilseed Brassicas and Sunflower Crops (ISU P & S position - NC-7 RRF funded):

- Curates oilseed brassicas, sunflowers, and flax.
- Develops collections through exploration and exchange.
- Supervises an Asst. Scientist III (ISU P & S position) and provides guidance and direction to a USDA-ARS Agri Research Sci Technician and numerous temporary employees.
- Duties similar to those of the first two bullets of the maize curator, with additional considerations for the intricacies of oilseed crop species and their pollinators.
- Collaborates with investigators to expand collection holdings to develop and provide comprehensive collections from key crop origin areas, and to fully characterize them.
- Collaborates with molecular and other investigators to develop genomic information on elements of the collection.

4. Curator II - Pseudocereal and Specialty Legume Crops (ISU P & S position – NC-7 RRF funded):

- Curates amaranths, chenopods, sweet clover, crown vetch, and other legumes; warm season grasses, primarily Panicum miliaceum and Setaria italica.
- Develops collections through exploration and exchange.
- Duties are similar to those of the first two bullets of the maize curator, with additional considerations for the intricacies of many diverse crop species and their pollinators, and a wide array of stakeholders.
- Supervises an Agricultural Specialist ((ISU P & S position – NC-7 RRF funded) and temporary employees.

J. Agricultural Research Science Technicians:

1. Insects (GS 5/9 USDA/ARS position).

- Manages and maintains colonies of bees and flies to provide adequate numbers of insect pollinators for seed increases.
- Builds and maintains hives, maintains pollinating supplies, and helps improve and develop pollination techniques.

- Assists with experiments to improve insect pollinating techniques for entomophilous species and evaluations of host-plant resistance to insects.
- Maintains insect colonies.
- Performs laboratory bioassays of phytochemicals.
- Reports to the CAT III Entomologist.
- Supervises temporary employees.

2. Plants/Pathology (GS 5/9 USDA/ARS position).

- Assists Plant Pathologist with all phases of laboratory, field, and database activities dedicated to collection maintenance, evaluation, producing and providing healthy plant propagules.
- Supervises temporary employees.

3. Plants/Oilseeds Curation (GS 5/9USDA/ARS position)

- Assists Oilseed Curation program with all phases of laboratory, field, and database activities dedicated to collection maintenance, evaluation, producing and providing healthy plant propagules.
- Supervises temporary employees.

4. Plants/Maize Curation (GS 5/9 USDA/ARS position)

- Assists Maize Geneticist/Curator with all phases of laboratory, field, and database activities dedicated to producing and providing healthy plant propagules.
- Supervises temporary employees.

5. Plants/Ornamentals Curation (GS 5/9 USDA/ARS position)

- Assists Cat 3 and Cat 4 Horticulturists with all phases of laboratory, field, and database activities dedicated to collection maintenance, evaluation, producing and providing healthy plant propagules.
- Supervises temporary employees.

K. Biological Science Lab Technicians (GS-9 USDA/ARS positions).

1. Seed Storage Manager (GS 5/9):

- Provides quality assurance for all seed entering and leaving seed storage; verifies accuracy of data on seed lots (e.g., trueness to type and inventory amounts).
- Determines availability of individual seed lots during annual storage.
- Fills seed orders and submits them to the Program Support Assistant for shipping.

- Interacts with curators to facilitate efficient seed handling processing, and storage.
- Provides statistical summaries on collection inventory and viability status.

2. Germination (GS 5/9):

- Conducts viability testing of the collections when first stored and at appropriate intervals to determine maintenance needs.
- Captures digital images of newly acquired germplasm and associated information. Enters and manages all information in GRIN or GRIN-Global.
- Provides support for the GS-9 Biological Science Technician in seed storage.
- Assists with filling seed orders, storing lots, inventorying of new and previously maintained lots, enters data into the Germplasm Information Network (GRIN) and collaborates with curators and other support staff in the maintenance of NCRPIS inventory lots.

3. Term Seed storage support (GS 5/9):

- Provides support for the GS-9 Biological Science Technician in seed storage. Assists with filling seed orders, storing lots, inventorying of new and previously maintained lots, enters data into the Germplasm Information Network (GRIN) and collaborates with curators and other support staff in the maintenance of NCRPIS inventory lots.

L. Research Unit Secretary (Program Support Assistant) (GS 5/7 USDA/ARS position):

- Provides secretarial and administrative support to RL/Coordinator and other scientists, including typing, purchasing, payroll, travel voucher preparation, scheduling of meetings for RL and arranging transportation and lodging.
- Maintains office supplies and inventory.
- Assists with ARMPS package and budget preparation; operates personal computer, employing standard software packages, and RMIS and other e-mail applications.
- Tracks expenditures and budgets throughout the FY.

M. Program Support Assistant (GS-4/6 USDA/ARS position).

- Assists the Secretary, Program Manager, and all station personnel with a wide variety of administrative, purchasing and logistical tasks or requirements, including time card entry and management of safety training records.
- Assists temporary employees with job application and job entry processes.

N. Program Support Assistant (GS 5/6 USDA/ARS position).

- Prepares seed orders and completes related data entry in GRIN/GRIN-Global. Produces shipping slips and labels, prepares packets, packages all seed shipments, and completes order processing on GRIN; interacts with local and national germplasm staff.
- Coordinates communication of orders involving multiple curators and/or sites, phytosanitary requirements, import permit requirements and their issue, mailing and receiving accession performance reports, including preparing annual summary reports of seed distribution.
- Receives new germplasm from collection and exchange efforts. Reviews and incorporates passport and provenance information. Requests Ames and PI numbers for new germplasm from DBMU personnel.

O. Two Field Lab Technicians III (ISU NC7-RRF funded).

- Assists Farm Superintendent with technical support, including vehicle maintenance and minor repairs, facilities and equipment maintenance, plot preparation, seeding, cultivating, and harvesting.

P. Two Ag Specialist I (ISU P & S positions – NC-7RRF funded).

- One assists vegetable curator with all phases of germplasm maintenance, processing, characterization, record-keeping, data management, equipment and field plot maintenance.
- One assists the pseudo cereals and legume curator with same tasks, taxon-specific.
- Guides and directs temporary employees.

Q. Ag Specialist II (ISU P & S positions – NC-7RRF funded).

- Assists the maize geneticist/curator with all phases of germplasm maintenance, processing, characterization, record-keeping, data management, equipment and field plot maintenance.
- Develops process improvements.
- Guides and directs temporary employees.

R. Biological Aides, temporary, part-time (GS1/3, USDA/ARS).

- Assists the curatorial staff with germplasm maintenance, processing, record-keeping, data entry, field and/or greenhouse plot maintenance, bee-keeping activities, plant pathology laboratory and field tasks, seed storage and order fulfillment tasks.

GEM PROJECT

The Germplasm Enhancement of Maize Project is integrated with the maize curation project and other NCRPIS activities. Its mission is to introgress useful genes and traits from exotic/unadapted maize genetic resources that will diversify the genetic base of U.S. maize production, thereby supporting its sustainability.

It is directed by a CAT IV Maize Geneticist, and supported by two Agri Science Research Technicians, an Agronomist (IT), and numerous temporary employees. The Ames based component of GEM is closely tied to its sister program in Raleigh, NC. While the Ames program focuses primarily on 25% tropical germplasm, the Raleigh program focuses on 50% tropical germplasm.

Section 8

Health and safety

All NCRPIS employees receive annual training for operating farm equipment and for understanding and using material safety data sheets ("MSDS"). Employees required to wear respirators and dust mask are fit-tested annually and trained in the proper use and care of the respirator. Fit tests for paper dust masks are conducted by the Program Manager; fit tests for half- or full-face respirators are conducted by the Iowa State University Department of Environmental Health and Safety. All employees, except for certain clerical and administrative personnel, must wear steel-toed safety boots while at work and engaged in activities that require them. The Program Manager is responsible for documenting that the above training has been completed, and that relevant procedures are followed.

An extensive effort has been devoted to development of JSA (Job Safety Assessments) and PPE (protective equipment) documentation. Employees complete JSA and PPE training in addition to hands-on training specific to tasks, processes, and equipment. These records are maintained electronically at the station.

Because our staff frequently is in close contact with honey bees, kits containing an antihistamine and epinephrine are maintained to treat hypersensitive reactions. Annual training in the safe use of Epipen® auto-injectors for individuals suffering life threatening allergic reactions to bee stings is provided online via https://www.epipen.com/about-epipen/how-to-use-epipen.

All NCRPIS laboratories follow the Chemical Hygiene Plan developed in cooperation with Iowa State University Department of Environmental Health and Safety personnel. It is the responsibility of each laboratory supervisor to implement this general plan, and specific relevant procedures, in their laboratories. Safety training modules are available via the ISU EHS website, and via USDA-ARS AgLearn online system. Specific courses are also offered at ISU and taken on an as needed basis.

Every full-time NCRPIS employee is covered by Iowa State University's Occupational Medicine Program, which checks general health annually, and monitors specific health risks due to certain hazardous materials more frequently.

Periodic safety inspections are conducted by ISU and USDA/ARS safety personnel to assure that the NCRPIS meets or exceeds current OSHA standards.

Occupant emergency plans and emergency instructions are maintained and posted on bulletin boards in the building and are also available to staff electronically.

Section 9

Facilities

Land:

The NCRPIS presently maintains 120 acres with approximately 70 acres cultivated annually for seed regeneration, accession characterization, horticultural plantings, and disease and insect evaluations. To maintain an adequate level of seed regeneration, we will require the current acreage for at least the next decade.

Tropical maize accessions and those from the southern United States are difficult to maintain and regenerate in Ames. We work with private companies to grow out these accessions for regeneration in Puerto Rico and Hawaii. External collaborators also provide regeneration services for other crops nationally and internationally, and other NPGS sites provide regeneration assistance, as needed.

Buildings:

Field headquarters and germplasm processing and information building (ISU-RRF; 1981): The 60' x 225' steel frame building is insulated and heated, and houses rooms and equipment for seed drying, threshing, germinating, and documentation before storage. The farm staff, curators, technicians, clerical staff, program assistants, and biological aides have offices in this building.

Seed storage rooms (ISU-RRF; 1953 and 1990): The NCRPIS has three cold storage rooms, all maintained at 4 ^{o}C and 28% to 35% relative humidity. The rooms are 18' x 50', 18' x 30', and 50' x 75' and are connected by a common space that, in turn, connects to the field headquarters. A 26' x 15' 9" freezer is maintained at -18 ^{o}C and is accessed through the seed storage common room that also connects to the cold rooms. The freezer was constructed in 1993. The 4 ^{o}C cold storage and -18 ^{o}C freezer space are very limited and expansion of facilities is a short-term priority.

Entomology building (USDA/ARS; 1991): The 40' x 104' steel-frame building that houses three insect-rearing rooms, a work lab, a diet prep room, two offices, an equipment storage area, a woodworking shop, and an unheated storage area for honey bee equipment.

Machine shed shop (USDA/ARS; 1979): The 50' x 180' steel frame building includes an insulated and heated 50' x 50' shop and one office for farm staff.

Equipment building: A 60' x 100' steel-frame storage building for large farm equipment was constructed in 2005.

Greenhouses: We presently have four greenhouses located at the NCRPIS site:

- One 30' x 60' (USDA/ARS; 1968) aluminum frame greenhouse sheathed with polycarbonate and connected to a headhouse (ISU-RRF; 1953),
- One 30' x 96' (ISU-RRF, 1985) quonset style house covered with two layers of plastic film,
- One 30' X 100' (ISU-RRF, 1991) greenhouse, and
- One 20' x 40' greenhouse was constructed in 1993 and is attached to the Entomology building.

- We have 4700 square feet of space for our research program in the Agronomy Department greenhouse on the ISU campus. In the headhouse of the Agronomy Department greenhouse we have two 10.5' x 11' wet labs, one 10' x 12' storage room, and access to the general work area.

Office and laboratory space:

Iowa State University provides us with 13 rooms on the ground floor of Agronomy Hall. Four of these rooms are wet labs, seven are offices, one is a computer room, and one is a small conference room.

Vernalization rooms:

Two 20' x 20' (6 m x 6 m) vernalization rooms are located in an underground root cellar (ISU-RRF; 1953). Their walls and ceilings are constructed of 8" (20.3 cm) thick concrete. One room is equipped with fluorescent grow lights on the ceilings and walls. Temperature is maintained at 5 ^{o}C. These rooms are used for vernalization of seedlings and overwintering or short-term storage of plant materials.

Miscellaneous structures:

There are two shade houses (ISU -RRF; 1985) for woody ornamentals, each 30' x 60'. One shade house has ground-level planting beds filled with a peat-based soil mix and protected by a rabbit fence. The other house has a gravel floor for containerized plant production. A frame storage building (ISU-RRF; 1953) is used for overwintering bees used in our pollination program.

Section 10

Crop Germplasm Committees (CGC's) and other advisory committees

As of 2014, the NPGS utilizes network of experts organized into 42 Crop Germplasm Committees (CGCs). The CGCs provide advice vital to the efficient and effective function of the NCRPIS's plant genetic resource management program. The NCRPIS Research Leader and crop-specific curators serve as ex officio members of the following CGCs: Clover and Special Purpose Forage Legume, Crucifer, Cucurbit, Forage and Turf Grass, Herbaceous Ornamental, Leafy Vegetable, Maize, Medicinal and Essential Oil, New Crops, Root and Bulb Vegetable, Sunflower, and Woody Landscape Plant.

Each year, the curators present progress reports to their pertinent CGCs and consult with CGC members on issues related to germplasm acquisition, conservation, evaluation, and distribution for their collections. For those genera with no established CGC, the curator should identify and consult with individuals or organizations with technical expertise on the management and utilization of their collections.

Leadership individuals of all NPGS units participate in the Plant Germplasm Operations Committee (PGOC), a body established to share information, develop technical and operational guidelines, and advise the ARS National Program Staff and other administrators. The Research Leader and Horticulturist are active participants in the PGOC and its subcommittees.

The NCRPIS is also advised by a Regional Technical Advisory Committee (RTAC), which has certain responsibilities specified by the NC-7 Multi-state Research Project. The NC-7 RTAC is one of four such groups that act as a body of germplasm experts who represent their State Agricultural Experiment Station (SAES) Directors and other organizations through regular annual meetings and ongoing communications regarding issues affecting the four Regional Plant Introduction Stations' ability to fulfill their mission.

Members provide advice on germplasm and resource management issues, and assist in identifying opportunities. Representatives from all four of the RTAC's assist in evaluating and ranking proposals for plant exploration proposals, and make funding recommendations.

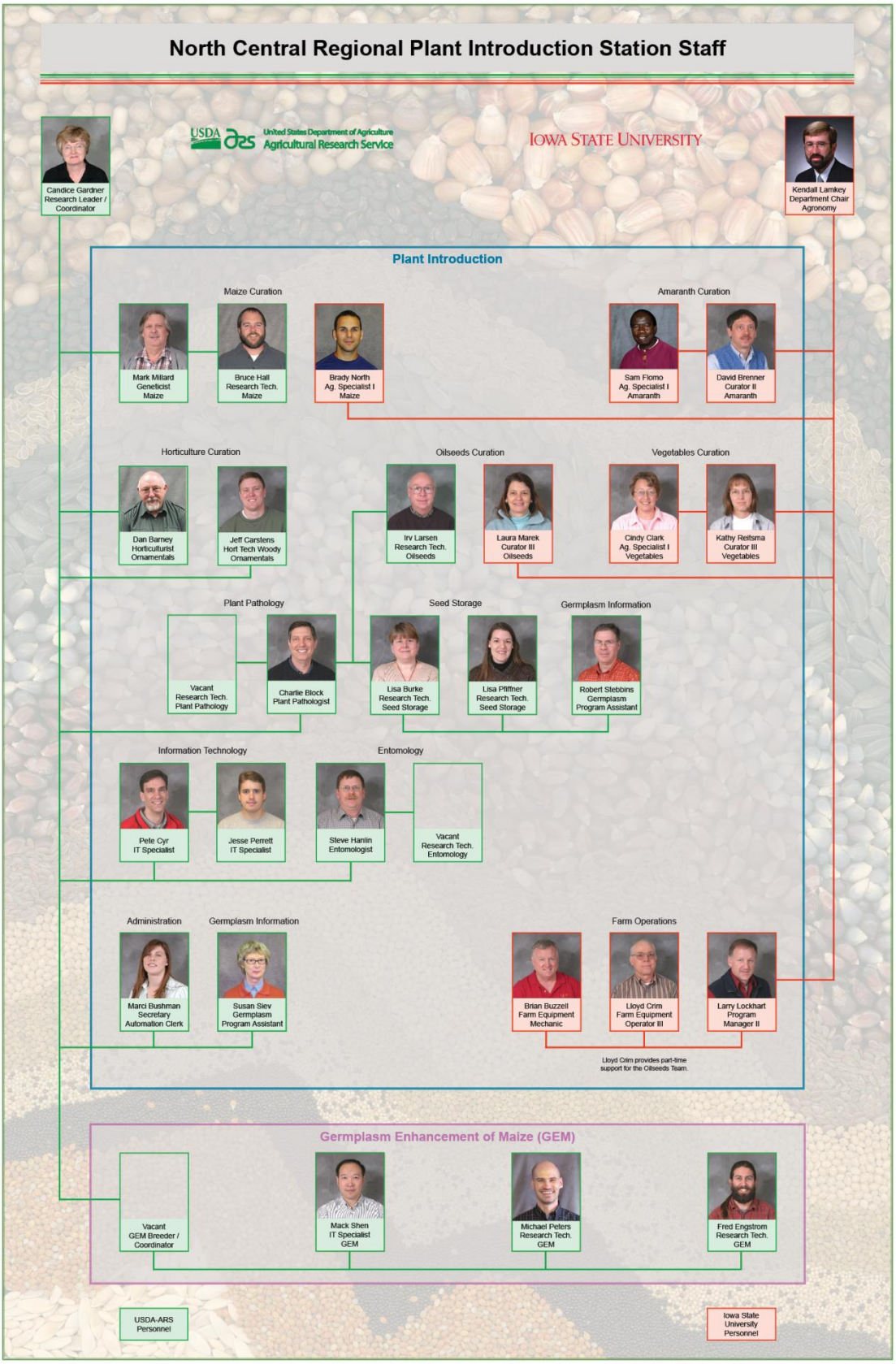
Section 11

Reports

The NCRPIS staff submits either formal or informal annual reports to all the committees listed in Section 10 of this manual. The annual report for the NC-7 Regional Research Project is comprehensive and serves as a definitive reference for NCRPIS's annual activity. Every five years, a project renewal document is prepared for the NC-7 Regional Research Project.

All the USDA/ARS Category 1 (Research) and Category 4 (Administrative/Service) scientists prepare annual reports for either the RL/Coordinator or the Associate Area Director as part of their annual evaluations. Standard USDA AD 421 or AD 417 reports are submitted for each CRIS (5 in total), Hatch Project (1), and/or Specific Cooperative Agreement (currently 3) administered by the NCRPIS.

Appendix 1


North Central Regional Plant Introduction Station Staff
USDA ARS United States Department of Agriculture Agricultural Research Service
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David Brenner Curator II Amaranth
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Oilseeds Curation
Irv Larsen Research Tech. Oilseeds
Laura Marek Curator III Oilseeds
Vegetables Curation
Cindy Clark Ag. Specialist I Vegetables
Kathy Reitsma Curator III Vegetables
Plant Pathology
Vacant Research Tech. Plant Pathology
Charlie Block Plant Pathologist
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Lisa Burke Research Tech. Seed Storage
Lisa Pfiffner Research Tech. Seed Storage
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Larry Lockhart Program Manager II
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Appendix 2

NCRPIS Viability Standards Protocol

Introduction:

This appendix to the NCRPIS Operations Manual provides an overview of viability testing, as conducted at the NCRPIS. It includes historical references to earlier procedures to facilitate interpretation of older records.

As of 2014, the NCRPIS manages approximately 52,600 germplasm accessions of crop plants and their wild relatives. More than 90% of these accessions are maintained as seed samples, with most accessions exhibiting orthodox storage characteristics. For quality assurance, we periodically monitor seed lot viability to determine initial seed quality and to document changes in viability over time. These viability assessments help curators prioritize regenerations and, if necessary, make new collections to replace nonviable accessions.

We generally monitor seed viability with germination assays. Other test procedures are sometimes needed to clarify whether non-germinating seeds are non-viable or whether their dormancy has not yet been broken (FAO/IBPGR, 1992). Non-germination viability tests are also be used for taxa, such as Actaea and Fraxinus, that are difficult to germinate in the laboratory or have prolonged and/or complicated dormancy requirements. For purposes of this manual, "viability" refers to general tests of viability, including germinability. The term "germination" is construed narrowly to refer to assessments that use radical or radical and shoot emergence as indicators of viable seed.

In developing viability assays, we gain information about each accession's seed dormancy characteristics and optimal germination protocols. Such information is important in developing guidelines for seedling establishment and general plant culture. Initial viability tests also allow us to refine seed production, harvesting, and cleaning methods. Repeating viability tests on specific seed lots over long periods of time provides the information needed to compare different storage methods and to optimize storage protocols for particular crops and accessions.

Responsibility for Conducting Viability Tests:

According to the "Manual of Procedures for the NPGS" (NPGS, Version 1.1 11 March 2013), curators are responsible for maintaining and protecting germplasm collections. Part of those duties includes gathering, maintaining, and processing all data needed to manage these living collections.

Curators at the NCRPIS are responsible for managing viability testing of seed samples for their priority genera. Curators have the final responsibility in choosing samples to test, testing intervals, and methodologies, overseeing testing, and record keeping. They are assisted in these efforts by the germination manger (who normally conducts the germination testing and data entry into GRIN) and seed storage manager. At the curator's discretion, pertinent viability data collected at other NPGS sites can be captured and transferred to the NCRPIS.

Many resources are available to help curators manage seed viability. Selected literature references are cited at the end of this appendix. An Agronomist (AGR) for seed science and germination manager can provide seed-physiology and viability-testing expertise to curators. The germination and

seed storage staff serve as liaisons to the Seed Science Center at Iowa State University. The RL, curators, and GPA serve as liaisons to the NCGRP, as appropriate.

Conducting Germination Tests:

Whenever possible, seed lot viability is estimated using replicated germination tests conducted according to protocols set by the Association of Official Seed Analysts (AOSA, 2014) and the International Seed Testing Association (ISTA, 2005). Many plant species, however, have no AOSA or ISTA standards. Other valuable references include the IBPGR compendium (Ellis et al., 1985), Deno (1993), and Phillips (1985). Additional publications that may be useful in seed viability testing are listed at the end of this appendix.

Curators assess the germination protocols used for their collections. For some taxa, modifications to the protocols may be needed in order to achieve the maximum germination potential of each accession. The need to experiment with dormancy- breaking and germination protocols is particularly important for many crop wild relatives, for which germination data are lacking. Modifications include pregermination treatments such as scarification, warm and/or cold stratification, and treatment with plant growth regulators, to overcome physiological dormancy, hard seed coats, and/or germination-inhibiting substances in or on the seeds (ISTA, 1993).

Development and documentation of new protocols should include cooperation and open exchange of information between the curator involved and the viability laboratory staff. The curators will document the results of germination protocol tests (both effective protocols and those that are not effective) in GRIN. GRIN serves as a database of our germination procedures (as well as procedures that are not effective) and provides guidance in developing protocols for new taxa added to our collections.

As mentioned above, non-germination viability assays are used primarily for special-case taxa at the NCRPIS. However, curators and the viability laboratory staff should be aware of and further explore these assays when appropriate to the taxa and circumstances. Whenever possible, numbers of seeds and replications should match the standards for germination tests and the data should be recorded and summarized in GRIN.

The NCRPIS had recommended that the AOSA, ISTA, and USDA-ARS National Center for Genetic Resources (NCGRP) testing procedures be loaded into the GRIN database. The ARS Database Management Unit made NCGRP's germination methods available on GRIN in 2002. Copyright laws prevent the posting of AOSA and ISTA rules.

Identifying Samples that Require Germination Testing:

Germination testing requirements vary for the different germplasm inventory categories described below.

Original inventory lots - Germination tests may be conducted on original seed lots if there is sufficient seed and at the curator's discretion. If there are not sufficient seeds for germination testing or other destructive viability assays, non-replicated data obtained during regeneration activities should be entered into GRIN. The circumstances for backing up original seed vary and are based on what the curator wants. For materials that they have collected, curators may choose to back the seed up without performing germination testing.

Distributable inventory lots: Depending on seed quantity, a germination test is conducted on newly-regenerated inventory lots prior to seed storage. This initial assay establishes the base-line viability and allows the curator to estimate the quality of the seed increase lot. Follow-up germination tests are then conducted at scheduled intervals determined by the curator. Traditionally, NCRPIS has used a 5-year interval guideline to monitor the viability of distributable lots. Because of the large number of accessions maintained at NCRPIS and limited personnel resources, longer intervals may be needed for some accessions. Based on expertise with their crops, curators may also designate longer or shorter testing intervals based on knowledge of seed physiology. Close coordination between the curators and germination manager is needed to ensure that the viability of high priority accessions is monitored in a timely fashion.

Bulked distributable inventory lots - When two or more seed inventory lots (all having germination data no more than five years old) are combined to create a new bulked lot, a calculated germination value can be determined by averaging the individual lots' most recent germination values, weighted by the number of seeds in each lot. The date recorded for this calculated germination value should be the same as that of the oldest data used in the calculations. For example, if you are bulking three seed lots having respective viability test dates of October 2010, June 2012, and August 2014, the recorded test date would be October 2010. Presently, Oracle forms developed at NCRPIS facilitate calculation of weighted bulk seed lot viability assays. New tools to assist in this process remain to be developed for GRIN Global.

Non-distributable inventory lots - Seed inventory lots in this category include, but are not limited to, reference lots and lots with limited seed quantity, low viability or low population size. The viability of non-distributable inventory lots is monitored at the curator's discretion. Non-replicated germination data may be generated when using such lots for regeneration, and that data may be entered into GRIN at the curator's discretion.

Experimental lots - Lots grown or maintained for experimental purposes are tested for viability at the curator's discretion.

Problematic accessions - Viability tests of problematic accessions are conducted at the curator's discretion. Literature searches and/or laboratory and greenhouse experiments may be necessary to identify effective viability assays and dormancy-breaking/germination protocols. Non-germination viability assays include staining with 2,3,5-triphenyl tetrazolium chloride (TZ), cutting seeds open to examine embryos, x-rays of embryos, and other methods.

Some seed lots that score highly on non-germination viability assays do not germinate normally or produce viable seedlings. In dealing with problematic seed lots, such as those that do not germinate normally or whose dormancy increases with age, results of non-germination viability assays must be evaluated carefully and correlated with germination and/or regeneration trials to ensure accurate estimations of the regenerability of the seeds involved.

Initiation of processes and record keeping:

Maintaining accurate GRIN records of seed quantities on hand and seed lot viability involves:

- Creating and using inventory lot codes,
- entering an order on GRIN for seeds to be used in germination testing,
- carefully following and documenting viability assay protocols used in the tests, and
- entering germination data into the GRIN data entry forms.

The recommendations below apply to most cases and serves as a starting point for alternatives that may be needed for particular situations. Regardless of methodology, curators are responsible to ensure that the GRIN database accurately reflects the conduct and results of viability assays.

Newly-acquired accessions have inventory lots assigned to them by the germplasm program assistant at the time of receipt. Curators generally create a new inventory lot when they regenerate an accession from seed or conduct observations or experiments. These inventory lot identifiers are used to trace the history of the seed being tested, distributed, or increased. How the seed lot germinated, how the lot fared in the field, what its harvest yield parameters were, and what the quality of the increased seed is (as roughly measured by a viability test) are all documented by entering the data into GRIN.

Whenever seed needs to be removed from the seed storage facilities, the curator, seed storage manager, or germination manager enters an order for the seed lots to be tested. Seed orders for viability testing will be coordinated between the seed storage manager, germination manager, and curators. Seed lots stored in the freezer can be pulled by seed storage personnel, germination manager, curators and technicians. This is to insure accurate tracking of original seed. The locations of individual seed lots are indicted in the location fields of the inventory table of the GRIN database.

Problematic accessions or individual seed lots that fail to germinate after repeated tries can be brought to the attention of the seed storage manager and germination manager. Experiments can be collaboratively developed and scheduled to identify methods for breaking seed dormancy or to test the viability of the seed lot via TZ staining or other methods.

GRIN:

The GRIN system is critical to our plant collections. In addition to documenting accession acquisition, inventory, maintenance, and distribution, GRIN also documents viability testing protocols and viability test results. Information pertinent to plant germplasm viability testing and data recording can be found in the GRIN operator's manual. The GRIN-NPGS Data Dictionary at http://www.ars-grin.gov/npgs/dict/prod/dd.html explains terms used in GRIN and how the system operates.

Normally, viability assays are replicated with 4 replications of 50 seeds each being standard (2 replications of 100 seeds each are allowed for small-seeded accessions). Germination of all replications for a particular seed lot should be started on the same date. GRIN maintains data fields for normal seedlings, abnormal seedlings, and dormant seed. Viability is estimated as the sum of the normal seedlings and dormant seeds. A vigor rating can be entered, but this is rarely done because of the level of expertise required to make such ratings. The sample size is the total number of seeds used and is calculated as the sum of all the seeds used in each replication. All replications should contain the same number of seeds. A comment field allows the tester to summarize observations on each replication, such as unusual seedling phenotypes.

The specific test used to evaluate the seeds is identified by an "environment name" (ENAME) which is designated by the data base primary key "environment number" (ENO). NCRPIS curators determine the environment names used at NCRPIS, but should follow the naming convention "NC7.GERMS." at the beginning of the name. A crop name or group of crops should come next. The next portion of the name may include temperature regimen, lighting regimen, and materials used in the test. "NC7.GERMS.cucumis.standard" (ENO 490382) and "NC7.GERMS.HORT.KNO3.15C.1WK PRECHILL" (ENO 490506) are examples presently used at NCRPIS.

Data archives:

Some viability data remains archived in paper and electronic form, and has not yet been entered into GRIN.

Resources:

AOSA. 20414. (Rev. Ed.) Rules for testing seeds. J. Seed Technol. 16(3). S

Deno, Norman C. 1993. Seed germination theory and practice, second edition. Norman C. Deno, State College, Pennsylvania.

Dirr, Michael A. 1998. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses, fifth edition. Stipes Publishing Company, Champaign, Illinois. H

Dirr, Michael, A. and Charles W. Heuser, Jr. 1987. The reference manual of woody plant propagation: from seed to tissue culture. Varsity Press, Inc., Athens, Georgia.

Ellis, R.H., T.D. Hong, and E.H. Roberts. 1985. Handbook of seed technology for genebanks. Vol. II. Compendium of specific germination information and test recommendations. IBPGR, Rome. S

Emery, Dara E. 1988. Seed propagation of native California plants. Santa Barbara Bot. Garden, Santa Barbara, CA.

GRIN Database Dictionary at http://www.ars-grin.gov/npgs/dict/prod/dd.html

IPPS. 1984 - date. Combined Proceedings of the International Plant Propagators= Society. Bound volumes 34-48. (Vols. 31-40 are indexed.)

ISTA. 2005. International rules for seed testing. Seed Science & Technol. 21, suppl. S

Macdonald, Bruce. 1986. Practical woody plant propagation for nursery growers, Vol. I, Chapter 1. Timber Press, Portland. H

Phillips, Harry R. 1985. Growing and propagating wild flowers. C. Ritchie Bell and Ken Moore eds., The University of North Carolina Press, Chapel Hill.

Rock, Harold W. 1981. Prairie propagation handbook. 6th ed. Wehr Nature Center, Hales Corners, WI.

U.S. Forest Service. 1974. Seeds of woody plants in the United States. Agriculture Handbook No. 450, U.S. Department of Agriculture, Washington, D.C.

Young, James A. and Cheryl G. Young. 1986. Collecting, processing and germinating seeds of wildland plants. Timber Press, Portland.

Young, James A. and Cheryl G. Young. 1992. Seeds of woody plants in North America, revised and enlarged edition. Dioscorides Press, Portland, OR.

Appendix 3

Annual species of

Amaranthus, *Chenopodium* and allied genera, *Echinochloa*, *Panicum*, *Perilla*, *Portulaca*, and *Setaria*

Germination:

Sow the seeds ca. 0.5-1 cm deep in greenhouse soil, or, sow first on wet blotter paper and then transplant with forceps to soil. Seedlings generally emerge less than one week after planting. Germination percentage is usually over 80%, so plant thinly. The ideal germination temperature is 20 ^{o}C at night and 30 ^{o}C during the day, but temperatures above 15C are adequate. Some of the wild species (especially *Amaranthus australis*, *A. cannabinus*, *A. quitensis*, and *A. pumilus*) have seed dormancy that can be broken with a month of cool-moist treatment at 4 ^{o}C, on moist blotter paper, and then the seeds germinate well at warmer temperatures.

Seedlings:

With light and warmth the seedlings will grow rapidly. Transplant them outside at two or more weeks of age, but allow a week of hardening off before transplanting.

Mature plants:

Amaranths will grow well at temperatures above 20 ^{o}C; heat of even 50 ^{o}C apparently will not injure them. High soil fertility increases yields.

For most *Amaranthus* accessions we apply slow release fertilizer (Scotts 15-9-12 Osmocote® Plus) when the inflorescence first emerges, and not before; this delayed fertilizing reduces plant height. For other accessions it is best to fertilize early because the plants stay small even with fertilizer. This early fertilizing is especially beneficial to *Chenopodium quinoa* accessions, and accessions of the Love-Lies-Bleeding type *Amaranthus caudatus*.

Cultivation in greenhouses:

It is safest to grow for seed in the greenhouse (Brenner and Widrlechner 1998). Our harvests begin ca. 2 to 4 months after planting, when the seeds are starting to shatter. Plant spacing and pot size can be adjusted to yield many small plants or fewer large plants in the same area. We grow 100-200 plants, 40 to 100 cm tall, in 0.8 m2 (6 ft2), to harvest ca. 40,000 to 120,000 seeds (20 to 60 gm). The plants are grown in plastic greenhouse flats of the 1020 style (10 X 20 inches) with drainage holes, but without pots.

The flats are lined with one or two layers of newspaper and filled with a commercial growing media ®Sunshine LC1 mix. They are watered through 5/8" 15 mil emitter line drip tape, with two inch outlet spacing; flow rated at 1.5 gpm per 100 ft., and pressure controlled with Senninger Irrigation Inc. 0.4 to 30.3 LPM PRO75HL12 regulators. Watering is initiated manually but shut-off with Nelson auto shut-off water timers. Clear plastic bags (Associated Bag Item Number 22-3-14), supported by PVC pipes

(and enclosing a space 88cm wide, 54 cm deep, and 112 cm tall), prevent pollen from spreading between accessions if crossable species are in the same room (Brenner and Widrlechner, 1998).

Aphids, thrips, spider mites, and mice will feed on amaranths grown in the greenhouse, and supplies are kept on-hand to control them.

Field conditions:

In the field, seed yields are much greater than in the greenhouse. However, seed production in the field was stopped in the mid-1990s because the greenhouse method is more consistently successful.

The plants are planted in the greenhouse about 3 weeks before transplanting. Transplanting occurs from the third week of May through the first week of July; the end of May is ideal. We transplant pairs of plants 15 cm (0.5') apart in the row, with 0.9 m (3') alleys. This spacing allows workers to bag inflorescences (with Lawson 421 bags) to prevent outcrossing.

Without this precaution, ca. 5% to 30% outcrossing is expected (Jain 1982). Lygus bugs, amaranth weevils, web worms and aphids can infest field plantings (Wilson 1989), but are seldom at harmful levels.

Photoperiod:

Most accessions require short day lengths of less than 12 hours for blooming which generally requires curtains to exclude long-day lighting from neighboring greenhouse projects. In Iowa field conditions, many will not mature seeds before frost since our daylengths are too long for timely flowering. In the greenhouse we use local winter daylength, with supplemental light for 8 hours during the middle of the day.

We plant on about September 1, and can replant by mid-January for two seed harvests. Zabka (1961) studied duration of the short-day treatment. If the short-day treatment is discontinued, flowering ceases and vegetative growth resumes. Some short-day plants will eventually flower under long-day conditions in a greenhouse, when they are four months old or older. Many of the wild species accessions stop growth in short day lengths, as seedlings, so they are grown in long days then brought to short-day lighting for flowering (especially weedy temperate *Amaranthus powellei*, *A. retroflexus*, *Echinochloa*, and *Setaria*).

Harvest:

Harvest when the seeds are hard enough to not macerate when rolled between the fingers. Clip off the seed heads and dry them in fine mesh bags in a drier with forced air flow.

Seed Cleaning:

The first threshing is by rubbing the seed heads by hand inside a five-gallon bucket to free the seeds from the stems. Many seed lots require additional threshing with rubber-covered wooden blocks, and sometimes screening to remove twigs. The chaff and immature seeds blow out with air column separators. Most seed lots require some final sorting by hand with spatulas to remove impurities.

References:

Brenner, D.M. and M.P. Widrlechner. 1998. *Amaranthus* seed regeneration in plastic tents in greenhouses. FAO/IPGRI Plant Genetic Resources Newsletter 116: 1-4.

Jain, S.K., H. Hauptli, and K.R. Vaidya. 1982. Outcrossing rate in grain amaranths. Journal of Heredity 73:71-72.

Wilson, R. L. 1989. Studies of insects feeding on grain amaranth in the Midwest. Journal of the Kansas Entomological Society 62(4):440- 448.

Zabka, G. G. 1961. Photoperiodism in *Amaranthus caudatus*. I. A re-examination of the photoperiodic response. American Journal of Botany 48:21-28.

Appendix 4

Annual Apiaceae excluding *Daucus*

(*Anethum*, *Ammi*, *Bifora*, *Carum*, *Caucalis*, *Coriandrum*, *Cuminum*, *Foeniculum*, *Orlaya*, *Pimpinella anisum*, *Torilis*, and *Trachyspermum*)

Introduction:

Most of these are winter-growing plants in Mediterranean climates with some adaptation to growing as summer annuals in our temperate Iowa summers. They are direct seeded as early as possible in the field, where they are frost-hardy. In the case of *Coriandrum*, early planting correlates with the largest and best harvests, although later planting will still result in a harvest. If the seed lots are poor we regenerate them in the winter greenhouse to reduce risk of loss.

The exceptions are: Carum, which is either annual or biennial, both forms are adapted in Iowa, but the biennials remain in the field over winter to flower in their second year. *Cuminum* is grown as a winter crop in Parlier, CA since it is not adapted to our summer field or our winter greenhouse environments. *Foeniculum* is a perennial, but is grown as an annual. The cultivated *Foeniculum* accessions are adapted for direct seeding. Wild accessions can be longer-season and need to be started in the winter greenhouse then transplanted into the field. *Torilis japonica* needs to over-winter before it flowers well and it frequently dies in the winter field, so our success with it is sporadic; the other *Torilis* species that we have grown are reliable.

Pests:

Occasionally plants are infected with the aster yellows virus; these are uprooted and removed to reduce the disease spreading. Swallowtail butterflies over-winter in the south and then fly north laying eggs before the pollination cages are constructed, which develop into caterpillars that feed on these plants. Some years are worse than others for this problem. Hand removal of caterpillars is generally adequate control, but treatment with DiPel® may be needed. Aphids are a problem, but infrequently.

Planting:

Rub the seed units to separate schizocarps into the two mericarps, to reduce crowding after the plants emerge. Plant as early as possible in springtime. Planting has been done successfully as early as March 9, but more frequently after April first. Plant two rows of seeds ¾ inch deep and two feet apart to run the length of standard 7-foot wide and 20-foot long pollination cages. Erect the cages after emergence is verified.

Pollination:

Pollinate with honey bees and flies when the flowers emerge.

Harvest:

Harvest into fine mesh bags at seed maturity. Harvested plant counts are generally taken during harvest and the plants uprooted and accurately counted. Anethum and Foeniculum produce primary, secondary, and tertiary umbels; the best and largest seeds are in the primary umbels; the secondary and tertiary umbels have progressively smaller, poorer quality seeds. Time the harvest, or make multiple harvests, to harvest the primary umbels at their best maturity. If primary and secondary umbels provide sufficient harvested seed, do not harvest the tertiary umbels. Do not harvest from broken fallen branches that dry out, since their seeds are immature.

Seed Cleaning:

After drying, the seeds are removed from the stems often by stomping on the un-opened harvest bag, and stem materials are removed by hand following transfer of bag contents to a container such as a 5-gallon bucket. The harvested seeds are blown in an air column separator, and then manually picked to clean condition. Seeds that are joined together (schizocarps) are kept that way as much as possible since it is believed that they maintain viability in storage longer than separated seeds (mericarps).

References:

Small, E. 1997. Culinary herbs. NRC Research Press. Ottawa; 710 pages.

Appendix 5

Brassicaceae

(*Alliaria*, *Alyssum*, *Berteroa*, *Biscutella*, *Brassica* (primarily oilseed genera), *Camelina*, *Christolea*, *Crambe*, *Enarthrocarpus*, *Eruca*, *Erucastrum*, *Erysimum*, *Goldbachia*, *Iberis*, *Isatis*, *Lepidium*, *Matthiola*, *Parrya*, *Sinapis*, *Stevenia*, *Thlaspi*, and × *Brassicoraphanus*)

Planting:

For each accession, enough seed is germinated to yield at least 40 seedlings; however, we proceed with fewer plants if less seeds germinate and there is no or little remaining inventory. Seeds are germinated on water or 2% potassium nitrate saturated blotter paper in clear plastic germination boxes in growth chambers set for 25/15 ^{o}C, light/dark, 12 hour cycles. *B. juncea* and *B. tournefortii* seed receive a pretreatment of ten days at 10 ^{o}C. Other Brassicaceae species germinate better after a pretreatment of at 4 to 5 ^{o}C for seven to ten days or longer. Seedlings with good root growth and emerged cotyledons are planted in flats (generally of the size to hold 2.25 inch peat pots) and kept in the greenhouse until weather conditions permit field preparation and a period of hardening the plants outside before transplanting to field plots. Field plots are sized to accommodate caging and screening (generally 7 x 7 x 20 foot cages) to provide controlled pollination.

Some species and types within species (e.g. *Brassica napus* and *Brassica rapa* winter-type canolas, others) require a vernalization period with a controlled maximum temperature and light period before plants will flower in addition to any seed pre-chilling treatment that might be required. Seedlings of these accessions are moved to the farm vernalization rooms after they have about four mature leaves. The vernalization rooms can maintain a temperature of 5 to 8 ^{o}C and light is provided from grow lights set for short light/long dark periods. Generally at least four weeks of vernalization is necessary and some accessions require six to eight weeks of chilling to induce flowering.

Tags containing identifying information including cage number, accession number, parental lot code, and increase lot code are attached to the cages (one tag outside, several harvest tags inside). The information is bar coded allowing for pollinator requests and tracking and field note taking with PDAs.

Pre-plant incorporated Treflan® is used as an herbicide for field plantings. Appropriate pest control treatments are applied as needed during the growing season (aphids, caterpillars, white moths are common pests in Brassicaceae). Plants are monitored during growth and outliers are removed as appropriate.

Some Brassicaceae species require a longer growing season than Ames can provide and/or require cooler temperatures and shorter days during flowering. These accessions are grown in pots in the greenhouse and otherwise handled as for field increases including caging for controlled pollination. For example, *Thlaspi arvense* flowers very early in the spring and has an extensive weedy presence in the farm fields in Ames. The only way we can ensure the genetic integrity of accessions is to regenerate in the winter greenhouse.

Pollination:

Pollination insects are added to cages after about 10% of the plants are flowering. The type of pollinator is dependent on time of year. Osmia bees are active in cooler weather and are used early in

the field season; alfalfa leaf cutter bees and honey bees are used later in the season, although honey bees are generally not used with the Brassicaceae. Flies are the primary pollinator used in cages in the winter greenhouse.

Harvest and Seed Processing:

Seeds are harvested as the pods mature and turn brown, often resulting in several harvests per accession. Plant material is harvested into bags and one harvest tag is put inside the bag and one is secured to the outside of the bag. Harvested material is dried for five to ten days at 30 to 32C as needed and stored in a cool, dry area until processing. Processing begins after fall field work is complete or on days when the weather does not permit outside activity. Plants are hand-threshed and cleaned using air-column separators followed by hand-picking as needed to remove remaining debris and/or broken and immature seed. One-hundred seed weight is determined and the total number of seed is calculated from the weight of the clean-seed sample. Seed viability is determined on four 50 seed samples (see Viability Appendix for details) before the cleaned seed is submitted to Seed Processing for storing.

Characterization:

Flowering date, the arrangement of the pods/siliques on the stem, plant height, and harvest date(s) are recorded at harvest. At harvest, 15 to 20 pods/siliques are collected into an appropriately sized envelope and when time permits, the silique length and width, beak length, seed number, seed color, and locule number are recorded from 10 siliques. In addition, images are taken of plants, flowers, pods/siliques and mature seeds. These data entered into the Germplasm Resources Information Network (GRIN) database and are available at: http://www.ars-grin.gov/npgs/searchgrin.html.

Resources:

US Canola Association webpage: www.uscanola.com

Canola Council of Canada webpage: www.canolacouncil.org.

Appendix 6

Cichorium

Planting:

Sow seeds in the greenhouse in early January directly into 3 Rootrainer™ pots (32 cells per flat) labeling each flat with the accession number and parental lot code. Thin the seedlings to one per cell, leaving 60-90 plants in total. Fertilize weekly with a commercial liquid fertilizer (20-10-20). Allow the plants to dry and wilt 6-8 weeks before expected field planting date (mid to late February). The foliage is trimmed to 1" (2.5 cm) above the crown, and plants are counted. Let pots dry until soil is barely moist under the surface. Vernalize the plants in the middle room of the cave (4-5o C, 50-70% RH) for 40-60 days. A fungicide spray is applied at the beginning of vernalization and reapplied as necessary to prevent Botrytis during vernalization.

One week before the expected field transplanting date, move the roots outside to a protected area and water so that the plants develop new foliage. Re-count the plants and assign a pollination cage number. (Plants can be transplanted into the designated cage area before cages are constructed as insect pests cause little damage.) Remove plants from each book cell, and transplant them into furrows dug in three evenly spaced rows in a 7' x 7' x 20' (2.1 m x 2.1 m x 6 m) pollination cage positioned so that alleys are 15' (4.5 m) wide. Plants are watered immediately after transplanting to the field, and additional watering is required during drought. Mulch the plots with chopped paper to preserve moisture and reduce weed competition. The cages are covered with Lumite screening when plants show evidence of bolting or flower initiation.

Pollination:

Plantings are scouted weekly for flowers. A small queen-right colony (nuc) of honey bees is placed in the cage for pollination as flowers begin to bloom. Alfalfa leaf cutting bees have also been found to be useful pollinators of *Cichorium*. Pollinators remain in cages for several weeks. (See section on insect pollinators for additional information.)

Harvest:

Hand clip dried plants into small-mesh harvest bags. Shattering of seeds from capitula may occur, so multiple harvests may be necessary. Plant material is dried 2-4 days in a forced air dryer at 85 ^{o}F (30 ^{o}C).

Fruit Cleaning:

Seeds are removed by placing small amounts of dried plant material through a brush thresher which also removes plant debris. Light, immature seeds and plant debris are removed in an air-column separator. To remove any remaining low-quality seeds and impurities, the lots are picked by hand. Germination tests are performed on each seed lot following the Association of Official Seed Analysts (AOSA) Rules for testing seeds for species that have standards; modification of germination rules may be necessary for seeds exhibiting dormancy (as discussed in the Viability Standards Guidelines). Seed increases are inventoried and stored in 1 quart (1 liter) glass jars at 4 ^{o}C and 25% RH.

Characterization:

It is preferable to reserve a field for two consecutive years to record first and second year notes on the biennial collections. To achieve proper chicon development and because some biennial accessions may not survive severe winters at the NCRPIS, alternate methods for recording certain first and second year notes must be developed.

First year notes: vegetative traits recorded include: growth habit; foliage color; mature leaf surface texture, laminal serration, erectness, length, and width; whether the roots are fibrous or fleshy; percentage of annuals bolting.

Second year notes: inflorescence traits recorded include: days to flower, corolla color, plant height at harvest, percentage of biennials bolting, and weight per 100 seeds.

Appendix 7

Miscellaneous legume genera

Coronilla, *Dalea*, *Galega*, *Marina*, and *Securigera*

Germination:

Scarify the seeds with sandpaper, to allow imbibition. Germinate on moist blotter paper in plastic germination boxes. The boxes are at room temperature, with daytime lighting during germination. The seedlings are transplanted into wooden boxes or pots for greenhouse growing, or into Rootrainer™ pots for later transplantation to the field.

Seasonal timing and culture:

This is a diverse group; however the following protocols work for most of them.

The safest method, especially for un-adapted accessions, is to keep them in a greenhouse for the entire crop cycle. The yellow-flowered perennial species such as *C. glauca* and *C. minima* are not winter hardy. For the *Dalea* and *Securigera* sow in early March, then transplant into wooden 24 X 16 X 4 inch boxes. Pollinate within greenhouse cages. Our greenhouse cages for this are marketed as camping equipment to exclude mosquitos from cots "Rectangular Mosquito Nets". The pollination can be either by honey bees or Osmia sp. bees. The annual *Coronilla* species are planted in October and given cool conditions in the winter greenhouse: 5-13m ^{o}C nights with no artificial lighting. This method allows them to be grown with the *Melilotus* planting, and would also vernalize any accessions which are biennial.

The *S. varia* accessions are winter hardy in our area and adapted to grow in the field. We sow *S. varia* in early March in a greenhouse and transplant into the field in late May. This species blooms during the first year, and more strongly in the second year. A black plastic mulch sheet controls weeds and reduces rhizomatous spreading. In the field, *S. varia* flowering occurs from early June to frost, but the flowering almost ceases during the hot part of the summer. McKee et al. (1972) report that crownvetch will flower with day lengths of 15.5 hours or greater after a cold thermo-induction. The time between pollination and seed maturity is 50 days of warm weather, which is unusually long (Al-Tikrity et al. 1974) so, in our area, flowering after mid-July is too late for seed maturity before frost.

Many but not all Galega accessions are adapted. They over winter well, but set seed poorly until the third field year when the plants are at full strength. Within field cages they are susceptible to leaf hopper damage, so we grow them without cages, and are therefore limited to one accession on the farm at-a-time for reasons of pollination isolation.

Pollination:

Securigera varia has self-incompatible flowers (Baluch et al. 1973). *Securigera varia* populations require large population sizes in order to have enough different S incompatibility alleles for pollination. In many cases we are uncertain if the original seed stock came from more than one individual, and has diverse alleles. These population size questions will be of concern if controlled pollination becomes a priority. Most of our distribution seed lots are open pollinated and date to before the 1990s.

Honey bees have successfully pollinated *S. varia* and *Dalea* inside of cages. Anderson (1959) found that honey bees visit 'Penngift' crownvetch flowers reluctantly, but do pollinate them; bumble bees might be better pollinators (Anderson 1959). The annual species, such as *C. cretica*, *C. scorpioides* will self-pollinate in the greenhouse. An accession of *Coronilla coronata* set seed well with confined Osmia bee pollination, but not with wild un-caged wild bees.

Harvest:

Begin harvest when the seeds are hard. The *S. varia* fruits shatter readily and must be harvested promptly, especially in the field. Dry the harvest with forced air in fine mesh bags.

Seed Cleaning:

The seeds are threshed by rubbing with rubber-covered wooden blocks. The larger chaff is removed by screens, fine chaff and the immature seeds are blown clear.

Characterization:

The traits measured include: flower color, and weight per 100 seeds.

References:

Al-Tikrity, W., W.W. Clarke, G.W. McKee, M.L. Risius, and R.A. Peiffer. 1974. Days from pollination to seed maturity in crownvetch. Crop Science 14:527-529.

Anderson, Edwin J. 1959. Pollination of crownvetch. Gleanings in Bee Culture 87:590-593.

Baluch, S.J. M.L. Risus, and R.W. Cleveland. 1973. Pollen germination and tube growth after selfing. Crop Science 13:303-306.

McKee, G.W., M.L. Risius, and A.R. Langille. 1972. Flowering of crownvetch as affected by thermo-inductive treatment, photoperiod, plant age, and genotype. Crop Science 12:553-557.

Appendix 8

Cucumis

Planting:

Cucumis: Plant 1-5 seeds directly into eight 3" (7.6 cm) peat pots in 7" x 14" x 3" (17.8 cm x 35.5 cm x 7.6 cm) wooden flats in the greenhouse in early May. Two pot labels with the accession number and parental lot are placed in pots in opposite corners of the flat. Seedlings are tested for the presence of squash mosaic virus using ELISA, and infected seedlings are eliminated. If necessary, thin the remaining seedlings to leave three plants per pot. This practice yields 24 plants, which is generally sufficient for regeneration. Normally, 24 cucumber plants will set sufficient seed. It is preferable to have up to 32 plants for melons and for cucumbers producing long, narrow fruit with small seed cavities.

Cucumis melo: Special precautions are taken with all *C. melo* regenerations to identify (and remove) *Acidovorax citrulli*, bacterial fruit blotch (BFB) contamination and prevent its spread to adjacent accessions. Seed lots are direct seeded into eight 3" (7.6 cm) peat pots in 36 cm x 21.5 cm x 9 cm plastic flats in the greenhouse, and individual plastic flats are isolated from each other by a vertical barrier system consisting of a 2' tall by 8' long polycarbonate panel (5/16" thick) with 18" × 24" side walls attached perpendicularly every 24" to create four compartments on each side. The barrier system is attached to the top of an 8' × 3' greenhouse bench top to stabilize the unit. The compartments are open on the front and the top, and one flat of melon seedlings is placed in each compartment.

To further reduce the chance of spreading the disease, a bottom-watering system is used, where each flat containing the peat pots is placed in a second flat which serves as a reservoir for water. The reservoirs are carefully filled with water so as not to allow the foliage to become wet. Seedlings are observed daily for symptoms of BFB. Any seedlings positive for BFB are destroyed before the accession is transplanted into field cages. Field plantings are scouted regularly for evidence of the disease.

Transplanting:

In late May when plants have grown to the two to three leaf stage, they are treated with Companion (a beneficial microbial inoculant drench) at least 24 to 48 hours before transplanting to the field cages to provide added protection against root pathogens and to promote root growth. Plants are hardened-off outdoors for two to three days on trailers fitted with a Lumite screen cover to protect the plants from harmful insects.

Eight hills with three plants each are transplanted 2' (60 cm) apart in the center of a 7' x 7' x 20' (2.1 m x 2.1 m x 6 m) pollination cage; cages are separated from each other by 15' alleys. The cage frame is immediately covered with a Lumite screen, which protects the plants from a variety of diseases by excluding the insect vectors. The plants are mulched with chopped paper to preserve moisture and reduce weed competition.

Plants are watered immediately after transplanting to the field, and additional watering is required during drought. One application of a commercial liquid fertilizer (20-10-20) soon after transplanting is beneficial to improve plant establishment and performance.

Pollination:

Cages are scouted every 2-3 days for flowers. When both staminate and pistillate flowers appear on an accession, a small queen-right colony ("nuc") of honey bees is placed in the cage for pollination. Alfalfa leaf-cutting bees (introduced into cages on a weekly basis) have also proven to be effective pollinators in field and greenhouse grown Cucumis. Pollinators remain in the cages until the end of August. (See section on insect pollinators for additional information.)

Wild species of Cucumis:

Most wild species of *Cucumis* require a longer growing season than cultivated varieties, and must be regenerated under greenhouse conditions. Seeds are planted directly to 18.9 liter pots in the greenhouse. Plants are thinned to 3 - 5 per pot and trained to trellises as they grow. Supplemental lighting is used and plants are fertilized weekly with a commercial liquid fertilizer (20-10-20).

For accessions regenerated in greenhouse cages, insect pollinators (honey bees, bumblebees, alfalfa leaf-cutting bees) are introduced when both staminate and pistillate flowers are present. Which insect pollinator is used will be determined by pollinator availability and plant species to be pollinated.

Hand pollination is required to effect pollination of accessions not grown in isolation cages. During the afternoon, locate pistillate floral buds that will open the following morning and close them with a small metal single prong hair clip. After locating all such pistillate buds in an accession, close at least five staminate buds for each pistillate bud to provide for sib pollination the next morning. Pollinations begin at 9:00 a.m. by collecting an accession's staminate buds closed the previous afternoon, and distributing them among the pistillate buds bound in the same accession. Check each bud for damage which may have exposed the stamens or pistils to pollination contamination by insects. Label each pollination with a 2.5 cm x 2.5 cm paper tag labeled with the date and accession number. Unclip the pistillate flower, peel the petals off the staminate flower to expose the anther, and brush the pollen onto the pistil. Repeat this procedure for each of five staminate flowers. Close the petals around the pistil and secure again with a hair clip to prevent insects from contaminating the pollination.

Monitoring For Diseases:

Cucumis accessions are monitored by the plant pathologist during regeneration for the presence of disease. One or two applications of a fungicide may be necessary to control Anthracnose during the growing season. Seed borne diseases are of particular concern - specifically bacterial fruit blotch (*Acidovorax citrulli*) on *Cucumis melo*. An effective seed treatment is available for bacterial fruit blotch (presented below). Seeds of an accession exhibiting symptoms of bacterial fruit blotch during regeneration are treated with HCl during the seed extraction and cleaning process.

Harvest:

Fruits are harvested as they mature, from August through October.

Seed Cleaning:

Fruits are sliced, and seeds are extracted by hand and washed in a screened tray. A wall-mounted, hand-crank meat grinder can also be used to extract seeds from the small fruits produced by

the wild species of *Cucumis*. Seeds are then floated in water to remove light, immature seed and debris. (Some viable seeds of *Cucumis melo* will float, so wash as much debris as possible through the screened tray and do not float them). After seeds have been dried in dryer carts (ca. 32 ^{o}C for 24-36 h), they are separated by rubbing between gloved hands, and blown in an air column separator to remove any remaining low-quality seed and impurities. Hand picking of some damaged seed may be required. Germination tests are performed on each seed lot following the Association of Official Seed Analysts (AOSA) Rules for testing seeds for species that have standards; modification of germination rules may be necessary for seeds exhibiting dormancy (as discussed in the Viability Standards Guidelines). Seed increases are inventoried and stored in 1 quart (1 liter) glass jars at 4 ^{o}C and 25% RH.

***Cucumis melo* seed treatment for bacterial fruit blotch:**

Day 1: Fermentation Treatment:

Slice melons in half. Scoop seed and juices into clean 5 gallon (19 liter) plastic bucket, making sure all seed is scraped down from sides of bucket and into the fruit juices. Cover with air tight lid and place in warm area for 24 hours of fermentation. (We leave the buckets in the Wet Processing Room at approximately 25 ^{o}C.)

Day 2: HCL Treatment:

Fill a large plastic bucket with 18.9 liters of water. Add 500 ml hydrochloric acid (12.1 Normality HCl), and stir well. Set aside.

Pour fermented seed mixture into clean screened drawer, wash thoroughly with water, and float off light, immature seed and debris. Pour cleaned seed into clean 18.9 liter bucket. So as not to waste the acid solution, add just enough of the pre-mixed 1% HCl solution to completely cover seeds, stirring well and making sure all seeds are scraped down from the bucket side into the solution. Allow seeds to soak for 15 minutes, stirring an additional two times. After 15 minutes, pour off the acid and rinse the seed well (3-4 washes with fresh water.) Pour clean seed into a clean screened dryer-cart drawer and place in dryer cart to dry for 24 to 48 hours. Remove dried seed from dryer cart drawer and place in appropriately labeled paper bag.

Characterization:

The traits measured include: date of first staminate/first pistillate flower; plant habit; fruit shape, length and width (min-max), surface color and texture, flesh color and thickness (min-max), spine color, and weight per 100 seeds. Digital color images of representative fruits help characterize each accession, and aid in its proper taxonomic identification.

Appendix 9

Cucurbita

Planting:

In early May, plant 1-5 seeds directly into each of eight three-inch (7.6 cm) peat pots that are placed into 7" x 14" x 3" (17.8 cm x 35.5 cm x 7.6 cm) wooden flats located in the greenhouse. Two pot labels with the accession number and parental lot are placed in pots in opposite corners of the flat. Seedlings are tested for the presence of squash mosaic virus using ELISA, and infected seedlings are eliminated. If necessary, thin the remaining seedlings to leave three plants per pot, which yields 24 plants, a number generally sufficient for regeneration. In late May when plants have grown to the two to three leaf stage, they are treated with Companion (a beneficial microbial inoculant drench) at least 24 to 48 hours before transplanting to the field cages to provide added protection against root pathogens and to promote root growth.

Plants are hardened-off outdoors for a few days on trailers fitted with a Lumite screen cover to protect the plants from harmful insects. Plants are transplanted into 5' x 15' x 40' (1.8 m x 4.5 m x 12.2 m) field cages in late May. Eight hills (three plants each) are transplanted in two rows (4 hills each) with 5' (1.5 m) spacing between hills, and mulched with chopped paper to preserve moisture and reduce weed competition.

Plants are watered immediately after transplanting to the field, and additional irrigation is required during droughts. One application of a commercial liquid fertilizer (20-10-20) soon after transplanting is beneficial to improve plant establishment and performance.

Pollination:

Cages are scouted every 2-3 days for flowers. When both staminate and pistillate flowers are present on an accession, a small queen-right colony ("nuc") of honey bees is placed in both the north and south ends of the cage for pollination. *Bombus* are also effective pollinators. Pollinators remain in the cages until the end of August. (See section on insect pollinators for additional information.)

Harvest:

Fruits are harvested as they reach mature color or after a killing frost. Sequential harvests may be necessary on accessions exhibiting variable fruit maturity. To produce the highest quality seed, the fruits are stored in a cold dry room (in the middle room of the cave) for as long as possible and processed before fruits rot.

Seed Cleaning:

Fruits are split open with a hatchet and seed is extracted by hand. Pulp is washed off the seeds in screened boxes and the seeds are dried in dryer carts at 30-34 ^{o}C for 24-36 h. Dried seeds are rubbed between hands and blown in an air column separator to remove light, immature seeds. Hand picking of some damaged seed may be required. Germination tests are performed on each seed lot following the Association of Official Seed Analysts (AOSA) Rules for testing seeds for species that have standards;

modification of germination rules may be necessary for seeds exhibiting dormancy (as discussed in the Viability Standards Guidelines). Seed increases are inventoried and stored in 1 gallon (3.8 liter) plastic jars at 4 ^{o}C and 25% RH.

Dryer Carts:

A special drying cart was designed and built by the NCRPIS staff to facilitate drying cucurbit seeds. The cart cabinet (48" h x 48" w x 24" d; 1.2 m x 1.2 m x .6 m) contains 18 drawers (3.5" h x 14" w x 24" d; 8.9 cm x 35.5 cm x 60 cm) with wire screen bottoms. The cabinet has a false bottom of .25" (.63 cm) masonite pegboard to allow air from the blower to pass to the drawers. The blower is a Dayton 7.75" (19.6 cm) squirrel cage type, powered by a .33 HP electric motor. The cart is mounted on 3" (7.6 cm) hard rubber tires for easy transport.

Characterization:

Traits recorded include pollination dates; plant habit; fruit shape, length and width (min-max), flesh thickness (min-max), flesh color, surface color and texture, weight per 100 seeds. Digital color images of representative fruits, with peduncles attached, help characterize each accession, and also aid in their proper taxonomic identification.

Appendix 10

Cuphea

Planting:

For each accession, enough seed is germinated to yield at least 40 seedlings; however, we proceed with fewer plants if less seeds germinate and there is no or little remaining inventory. Seeds are germinated on water saturated blotter paper in clear plastic germination boxes. The boxes are placed in growth chambers set for 25/15 ^{o}C, light/dark 12 hour cycles. Seedlings with good rood growth and emerged cotyledons are planted in small flats (generally of the size to hold 2.25 inch peat pots) and placed on the greenhouse mist bench. After a few days on the mist bench, seedlings are placed on tables in the greenhouse until weather conditions permit field preparation and a period of hardening the plants outside before transplanting to field plots. Plants are watered after transplanting and as necessary until established in the field.

Plots are sized to accommodate caging and screening (generally 7 x 7 x 20 foot cages) to allow controlled pollination. Some Cuphea species require a longer growing season than Ames can provide and/or require cooler temperatures and shorter days during flowering. These accessions are grown in appropriately sized pots in the greenhouse and otherwise handled as for field increases including caging for controlled pollination.

Tags containing identifying information including cage number, accession number, parental lot code, and increase lot code are attached to the cages (one tag outside, several harvest tags inside). The information is bar coded allowing for pollinator requests and tracking and field note taking with PDAs.

Pre-plant incorporated Treflan® is used as an herbicide for field plantings and appropriate pest control treatments are applied as needed during the growing season. Plants are monitored during growth and outliers are removed as appropriate.

Pollination:

Pollinators are added to cages usually when about 25% of the plants are flowering. Pollinators are left in the cages until mid-September or until more seed pods are shattering than maturing.

Harvest and Seed Processing:

Plants are hand harvested as seed matures during the growing season, as often as once a week. One harvest tag is put inside the harvest bag and one is secured to the outside of the bag. Harvested material is dried at 30 to 32 ^{o}C as necessary and stored under cool, dry conditions until processed. Processing begins after fall field work is complete or on fall days when the weather does not permit outside activity. Plants are run through the belt thresher and the seeds are processed with screens and air-column separators. Large volumes of *Cuphea* seed can be effectively cleaned using the spiral separator. If necessary, remaining debris and/or broken or immature seed are removed by hand.

One-hundred seed weight is determined and the total number of seed is calculated from the weight of the clean-seed sample. Seed viability is determined on four 50 seed samples (see the Viability Appendix for details) before the cleaned seed is submitted to Seed Processing for storing.

Characterization:

The following traits are recorded: flowering date, harvest date(s), plant height and habit, and lower and upper petal color and number. In addition, images are taken of plants, flowers and mature seeds. These data entered into the Germplasm Resources Information Network (GRIN) database and are available at: http://www.ars-grin.gov/npgs/searchgrin.html.

Appendix 11

Daucus and *Pastinaca*

Planting:

Sow seeds of *Daucus* and *Pastinaca* in the greenhouse in mid to late October directly into 3 Poly Flats (25 tree band pots per flat). Label each flat with accession number and parental lot code. Thin the seedlings to one per cell, leaving 60 -100 plants in total. Fertilize weekly with a commercial liquid fertilizer (20-10-20). Allow the plants to desiccate and wilt 6-8 weeks before expected field planting date (mid- to late February). The stems and foliage are trimmed to 1" (2.5 cm) above the crown, and plants are counted. Let pots dry until soil is barely moist under the surface. Vernalize the plants in the middle room of the cave (4-5 ^{o}C, 50-70% RH) for 40-60 days. A fungicide spray is applied at the beginning of vernalization and reapplied as necessary to prevent Botrytis.

One week before the expected field transplanting date, move the roots outside to a protected area and water so that the plants develop new foliage. Re-count the plants and assign a pollination cage number. (Plants can be transplanted into the designated cage area before cages are constructed as insect pests cause little damage.) Remove plants from each pot, and transplant them into furrows dug in two to three evenly spaced rows in a 7' x 7' x 20' (2.1 m x 2.1 m x 6 m) pollination cage positioned so that alleys are 15' (4.5 m) wide. Mulch the plots with chopped paper to preserve moisture and reduce weed competition. The cages are covered with Lumite screening when plants show evidence of bolting or flower initiation. Insect are introduced into the cages as umbels begin to flower.

Note: Both annual and biennial plants may be present in some genetically variable accessions of *Daucus*. Bolting annual plants within an accession are separated from the biennial plants before vernalization and placed into greenhouse isolation tents. The umbels are hand pollinated by gently rubbing them together, or insect pollinated (house fly, blue bottle fly, alfalfa leaf cutting bees) in the greenhouse to develop sib-pollinations within an accession. Isolation tents (1/2-inch PVC pipe frame, 50" x 20" x 30" [127 cm x 51 cm x 76 cm] covered with a fine mesh polyester screen) prevent contamination of the greenhouse pollinations. To preserve the accession's genetic variability, the seeds from the annuals regenerated in the greenhouse are combined with the seeds harvested from biennial plants in the field cage prior to seed lot inventory and storage for distribution.

Seeds of annual *Daucus* are planted in Rootrainer™ pots (book-cell) flats, each labeled with accession number and parent lot code, in the greenhouse in March. Thin the seedlings to one per cell, leaving 60 -100 plants in total. Fertilize weekly with a commercial liquid fertilizer (20-10-20). Remove plants from each cell, and transplant them into furrows dug in two to three evenly spaced rows in a 7' x 7' x 20' (2.1 m x 2.1 m x 6 m) pollination cage positioned so that alleys are 15' (4.5 m) wide. Mulch the plots with chopped paper to preserve moisture and reduce weed competition. The cages are covered with Lumite screening when plants show evidence of bolting or flower initiation. Insect pollinators are introduced into the cages as umbels begin to flower.

Pollination:

Cages are scouted weekly for flowers. Houseflies, bluebottle flies, and/or alfalfa leaf cutting bees are introduced into a cage as plants begin to flower. A small queen-right colony (nuc) of honey bees is placed in the cage when forty percent or more of the plants are flowering. Fly pupae are added every 3-5 days to supplement the honey bee pollinations. Alfalfa leaf-cutting bees (introduced into cages

on a weekly basis) have also proven to be effective pollinators in field and greenhouse grown *Daucus*. Pollinators remain in the cages for several weeks. (See section on insect pollinators for additional information.)

Harvest:

Hand clip brown, dried umbels into fine mesh polyester bags in sequential harvests from late August through October. Dry the umbels for 2-4 days in a forced air dryer at 30 ^{o}C.

Fruit Cleaning:

Seeds of *Daucus* and *Pastinaca* can be removed from umbels by hand-picking or belt-threshing. A series of graduated hand sieves are then used to separate the seeds from plant debris. A more thorough removal of spines from seeds of some *Daucus* species using gentle abrasion between gloved hands or a rubbing block may be necessary to further facilitate seed cleaning.

An air-column separator is used to remove light, immature seeds and plant debris. To remove any remaining low-quality seeds and impurities, the seed lots are picked by hand.

Germination tests are performed on each seed lot following the Association of Official Seed Analysts (AOSA) Rules for testing seeds for species that have standards; modification of germination rules may be necessary for seeds exhibiting dormancy (as discussed in the Viability Standards Guidelines. Seed increases are inventoried and stored in 1 quart (1 liter) glass jars at 4 ^{o}C and 25% RH.

Characterization:

It is preferable to reserve a field for two consecutive years to record first and second year notes on *Daucus* and *Pastinaca*. Annual and biennial plants may occur within one accession of *Daucus* due to genetic variability. All notes can be recorded on the annual plants in one growing season (note the percentage of annual/biennial plants). Some biennial accessions may not survive severe winters at the NCRPIS, so an alternate method for recording second year notes must be developed.

First year notes: The characters recorded include: seedling vigor; vegetative traits such as foliage color, leaf pubescence, leaf type, mature leaf erectness, petiole diameter, mature leaf length, and mature leaflet width; root traits such as storage root shape, length, diameter, weight, surface texture, core size, and color of the epidermis, parenchyma, xylem and phloem; percentage of annuals that bolt.

Second year notes: The characters recorded include: days to anthesis, fertility, umbel size, umbel shape at full anthesis and mature fruit, flower height, petal color, and anther color; seed traits such as spine size (if present), seed length, and weight per 100 seeds; percentage of bolting plants.

Appendix 12

Helianthus (sunflower)

Regeneration practices are described separately for cultivated sunflower (*Helianthus annuus*) and for the wild sunflower species.

Cultivated *Helianthus annuus*

Planting:

Cultivated sunflower accessions are usually regenerated in the field either in groups of four, 25 foot rows spaced four feet apart or in plots of an appropriate size to be caged and screened (generally 10 x 10 x 20 foot cages) to provide controlled pollination. Accessions are caged if they are branched (most restorer lines, most ornamental accessions), traditionally open-pollinated lines or populations or if we have very low inventory. Seeds are direct-seeded in the field or started on water saturated blotter paper in clear plastic germination boxes, depending on inventory amounts. Field planted seeds are treated with a fungicide containing the active ingredient metalaxyl to control downy mildew [*Plasmopara halstedii* (Farl.) Berl. And deToni]. If seeds are started in germination boxes, the boxes are placed in a growth chamber set for 12 hour light/dark cycles of 30/20 ^{o}C. Seedlings with good root growth and emerged cotyledons are planted into Rootrainer™ pots or other suitable containers and placed on the greenhouse mist bench for a day if seedlings are very small. Seedlings are then placed on tables in the greenhouse until at least two sets of true leaves have emerged and weather conditions permit field preparation and a period of hardening the plants outside before transplanting to field plots. The target population size is 100 plants per accession for rows and 40 to 50 plants for cages; however, we proceed with fewer plants if less seed geminate and there is no or little remaining inventory.

Tags containing identifying information including cage or row number, accession number, parental lot code, and increase lot code are attached to the first plant in each row (two tags) or onto the cages (one tag outside, several harvest tags inside). The information is bar coded allowing for pollinator requests and tracking and field note taking with PDAs.

Pre-plant incorporated Treflan® is used as an herbicide for field plantings and appropriate pest control treatments are applied as needed during the growing season. Plants are monitored during growth and outliers are removed as appropriate. The plant pathologist inspects the plantings for diseases at least two times prior to flowering. The main goal is to ensure that there are no downy mildew-infected plants in the production field. Other diseases of phytosanitary interest are apical chlorosis (*Pseudomonas syringae* pv. *tagetis*) and virus infections. Suspect plants are rare, but any suspects are removed. All plots are inspected again later in the season for general diseases.

Long-season cultivated Helianthus accessions are regenerated in the greenhouse in Ames. Seeds are germinated in boxes as described above and seedlings are transplanted to large pots which are watered by drip irrigation. The growth regulator paclobutrazol (we generally use Bonzi®) is applied as a drench treatment when plants are about 1.5 feet tall to accessions expected to grow taller than four or five feet.

Pollination:

For sunflowers planted in rows, heads are bagged with unbleached cotton bags or with Delnet® bags when the petals of the ray flowers open but before stigmas emerge and are receptive. The cotton bags are closed with drawstrings; delnet bags are secured with twist ties. Using a cheesecloth-covered cotton swab, or 'ghostie', a bulk of pollen is collected into an envelope from all pollen-shedding heads in a row. The ghostie is used to apply the bulk pollen to all receptive stigma plants in the accession. This process is repeated approximately every three days for two to four times per plant during the time period that the plants are producing fertile pollen. A new ghostie is used for each accession each day of pollination. The bagging date and pollination dates are recorded for each plant on a tag secured around the stem.

For caged sunflowers, pollination insects, usually honeybees, are added soon after flowering begins.

Harvest and Seed Processing:

Head bags are left on plants until harvest to protect developing seeds from bird predation. Heads are harvested by row and harvest begins when the back of the heads turn yellow and the bracts turn brown (after growth stage R-9, Schneiter and Miller, 1981). One tag from the first plant in the row is placed inside the bag and the second identifying tag is secured on the outside of the bag. Heads from caged plants are put into the number of harvest bags necessary to hold all heads with identifying tags inside and on the outside of the bag(s). Harvested heads are dried at 30 to 32 ^{o}C for seven to ten days as needed and stored in a cool, dry area until processing. Dried heads are hand shelled and seeds and light weight debris are removed using screens and air column separators. Accessions are handpicked to remove remaining debris and poor quality seeds. Seed viability is determined on four 50 seed samples (see Viability section for details) before the cleaned seed is submitted to Seed Processing for storing.

Wild *Helianthus* species, annual and perennial

Planting:

Seeds of wild sunflowers are placed in small beakers and soaked in 3% hydrogen peroxide (H_2O_2) for five minutes with occasional stirring. The seeds are transferred to a small mesh strainer and rinsed with cool, running tap water before being returned to the rinsed beaker and soaked in 25 ppm Ethrel® overnight at room temperature. Ethrel® is removed by rinsing the seeds in small mesh strainers and rinsed seeds are placed on water saturated blotter paper in clear plastic germination boxes. The germination boxes are pretreated for 7 to 14 days or longer at 4 ^{o}C. During the cold pre-treatment, boxes are checked periodically to ensure the seeds do not dry out nor become overrun with pathogens. After the cold pretreatment, germination boxes are placed in growth chambers set for 12 hour light/dark cycles of 25/15 ^{o}C.

Seedlings are planted in Rootrainer™ pots or other suitable containers when they show good root growth and emerged cotyledons. Seedlings are generally kept in the greenhouse mist bench for a few days before being placed on tables. After the seedlings are of a suitable size (generally at least two true leaves fully expanded) and the weather is appropriate for field preparation, plants are moved

outside to harden off for a few days before transplanting to the field. Seedlings are planted in field plots of an appropriate size for caging (generally 10x10x20 foot cages). Plants are spaced in three rows with a target population size of 40 to 50 plants.

Tags containing identifying information including cage number, accession number, parental lot code, and increase lot code are attached to the cages (one tag outside, several harvest tags inside). The information is bar coded allowing for pollinator requests, tracking and field note taking with PDAs.

Pre-plant incorporated Treflan® is used as an herbicide in field plantings and appropriate pest control treatments are applied as needed during the growing season. Plants are monitored during growth and outliers are removed as appropriate.

Long season wild sunflowers are sent as seedlings to our alternate growing location at the USDA-ARS NPGS Arid Lands Unit in Parlier, CA. Plants are caged and screened and pollinator insects added after flowering begins as in Ames. Harvested material is shipped to Ames for processing. The curator and the sunflower technician visit Parlier once per season, generally in late September, to take descriptor notes and images.

Pollination:

When at least several plants of an accession are flowering, a nucleus of honey bees is placed in the cage and left until the plants are no longer producing viable pollen.

Harvest and Seed Processing:

Because of their indeterminate growth habit, wild sunflowers are harvested into bags as seed heads mature, generally four to six or more times during the season. One harvest tag is put inside the bag and one is secured to the outside of the bag. The harvested sunflower material is dried at 30 to 32 ^{o}C for seven to ten days as needed and stored in a cool, dry area until processing.

Wild *Helianthus* heads are run through a belt thresher one to three times and then threshed material hand screened. The seeds are put in a blower to remove chaff and undeveloped seeds and then handpicked to remove remaining debris. Seed viability is determined on four 50 seed samples (see Viability Appendix for details) before the cleaned seed is submitted to Seed Processing for storing.

Both cultivated and wild Helianthus

Characterization:

Data are collected for many traits including days to flower, harvest dates, flower color, head diameter, and various seed traits during regeneration and seed processing. In addition, images are taken of plants, flowering heads and mature seeds. These data are entered into the Germplasm Resources Information Network (GRIN) database and are available at: http://www.ars-grin.gov/npgs/searchgrin.html.

Resources:

National Sunflower Association webpage: www.sunflowernsa.com.

Heiser, C.B., D.M. Smith, S.B. Clevenger, and W.C. Martin. 1969. The North American sunflowers (*Helianthus*). Memoirs Torr. Bot. Club 22:1-218.

Rogers, C.E., T.E. Thompson, and G.J. Seiler. 1982. Sunflower species of the United States. Natl. Sunflower Assoc., Bismarck, ND.

Schneiter, A.A., and J.F. Miller. 1981. Description of Sunflower Growth Stages. Crop Sci. 21:901-903.

Appendix 13

Lamiaceae (*Agastache*, *Monarda*, *Origanum*, and other NC7 non-*Mentha* mints)

Planting:

Sow seeds or nutlets containing seeds in the greenhouse during mid-January through mid-March, depending on the crop. A variety of starter containers can be used for the mints, ranging from standard 1020 nursery flats filled with 36-cell inserts for small seedlings, such as Origanum, to 18-cell inserts for larger seedlings, such as Monarda. Book flats are also suitable. Larger square tube pots, ranging from 2x2x6-inch to 4x4x12-inch, may be useful when the plants will be kept containerized for long periods of time. In general, strive to grow about 32 plants for large crops, such as Monarda, and 50-76 plants for smaller crops, such as *Origanum*.

Generally, a commercial peat moss-based potting soil works well for mint accessions. If additional drainage is desired, amend the potting soil with clean, washed sand or perlite. Do not use vermiculite. Regardless of the container used, ensure that all flats are labeled with bar-coded pot stakes and/or self-adhesive pot labels.

For accessions with a history of poor germination or when seed quantities are severely limited, you may choose to start seeds on blotter paper in germination boxes in a growth chamber using a protocol of 20/30 ^{o}C day/night temperatures and 16 hour photoperiod. Stratifying imbibed seeds at 4-5 ^{o}C for 5-10 days improves the germination of some accessions. Transplant germinated seeds/seedlings to one of the containers described above.

Pinch the plants back if they begin to flower before transplanting to the field cages. In cloudy spring weather, the seedlings benefit from supplemental light and relatively high fertility levels.

Greenhouse-grown seedlings are typically transplanted into field cages between mid-May and early June, once the possibility of frost has passed. In general, transplant into two or three evenly-spaced rows inside a 7' x 7' x 21' (2.1 m x 2.1 m x 6.4 m) pollination cage. Cages are separated from one another by 15' (4.5 m) alleys. Mulch the plots with chopped paper to preserve moisture and reduce weed competition. Cover the cage frames with a screen just prior to flowering.

Plastic seed tubs approximately 18x24 inches in size may be used for *Prunella* for long-term container culture and seed regeneration, particularly for cold-tender accessions. For *Prunella*, it is sometimes possible to obtain seed the same year as sowing. Sow the seeds during January through February. When most of the seedlings have developed two true leaves, transfer the flats to a cold room at 5 ^{o}C for 11 to 14 days. Light during that period is beneficial, but not required. After vernalization, return the flats to the greenhouse pending long-term container culture or transplanting to the field.

Overwintering:

Most accessions of *Agastache*, *Monarda*, and *Origanum* are perennial and can be successfully overwintered in the field. Overwintered plants need to be checked in the spring to ensure that a high proportion of plants have survived the winter. If this is the case, cage the accession just prior to flowering in the second year.

Grow accessions that are known or suspected to be cold tender, such as *Origanum vulgare* subsp. virens, in pots, rather than the field. Pot size depends on the size of the mature plants and

number of plants in each pot. Grow the plants outdoors during the summer, either in full sun or inside a shade house. For accessions that cannot tolerate any frost, transfer to the cave in late fall before the first predicted frost. For frost-tolerant but winter-tender accessions, allow the plants to remain outdoors for one to several light frosts (temperatures no lower than about -2 ^{o}C) before transferring them to the cave. Transfer the plants to a greenhouse in early to mid-January.

Pollination:

During the harvest year (generally beginning the second growing season for mints) scout the cages every 2-3 days for flowers. For most genera, place a small queen-right colony (nuc) of honey bees into the cage for pollination just as the flowers begin to open. These plants are generally quite attractive to honey bees. For *Monarda* and other mint-family genera with long corolla tubes, *Bombus* are generally more effective pollinators than are honey bees (see section on insect pollinators for additional information). Keep pollinators inside the cages until flowering has finished or harvest is to begin.

Harvest:

When the infructescences are brown and papery, cut them off into small-mesh polyester bags. The nutlets of some species are quite small and the use of double-bagged paper bags may be necessary. Shattering can also complicate harvest. Whole plants can be harvested, but processing whole plants makes seed cleaning more difficult. Perennial mints are typically harvested twice a week from late August until the occurrence of a hard frost. Dry the plant materials for 4-7 days inside a forced-air dryer at 30-32 ^{o}C. The harvest of annual mints is usually completed by the end of September.

Seed Cleaning:

Dried plant tissue is belt-threshed, processed by a Clipper air-screen cleaner, and then an air-column separator removes light, immature nutlets and debris. Small samples may be hand-threshed and cleaned in an air-column separator, without processing in a Clipper. Hand picking is required to remove damaged or moldy nutlets, and debris left by the mechanical separators. Germination tests and storage are performed according to the protocols described elsewhere in this manual.

Characterization:

The traits measured during regeneration include: flowering date; leaf number, size, texture, and color; stem size; growth habit; fruit set; corolla color; plant height at harvest, and 100-seed weights.

Appendix 14

Linum (flax)

Planting:

For each accession, enough seed is germinated to yield 40 (wild flax species) to 200 (cultivated flax) seedlings. However, we proceed with fewer plants if less seed germinate and there is no or little remaining inventory. Seeds are germinated on water saturated blotter paper in clear plastic germination boxes. Wild flax accessions are germinated at room temperature (generally 20 to 22 ^{o}C); cultivated flax accessions are germinated in growth chambers set for 30/20 ^{o}C, light/ dark, 12 hour cycles. For some wild flax species, germination boxes are held at 4 ^{o}C for seven to ten days or longer before germination at room temperature.

When sufficient seed inventory is on hand, cultivated flax accessions may be direct seeded into field plots. Seedlings with good root growth and emerged cotyledons are planted into flats (generally of the size to hold 2.25 inch peat pots) or Rootrainer™ pots and placed on the greenhouse mist bench. After a few days on the mist bench, seedlings are placed on tables in the greenhouse to grow until weather conditions permit field preparation and a period of hardening the plants outside before transplanting to field plots. Field plots are sized to accommodate caging and screening (generally 7 x 7 x 20 foot cages) to provide controlled pollination.

Some wild flax species require a longer growing season than Ames can provide and/or require cooler temperatures and shorter days during flowering. These accessions are grown in appropriately sized pots in the greenhouse and otherwise handled as for field increases including caging for controlled pollination.

Tags containing identifying information including cage number, accession number, parental lot code, and increase lot code are attached to the cages (one tag outside, several harvest tags inside). The information is bar coded allowing for pollinator requests and tracking and field note taking with PDAs.

Pre-plant incorporated Treflan® is used as an herbicide for field plantings and appropriate pest control treatments are applied as needed during the growing season. Plants are monitored during growth and outliers are removed as appropriate.

Pollination:

Most flax species are self-fertile. Nevertheless, for regenerations, all flax accessions are caged and screened. Pollinator insects are added to the wild flax cages after flowering begins; insects are generally not released into cultivated flax cages but cages protect against cross pollination and bird predation.

Harvest and Seed Processing:

Plants are harvested when more than 50% of the capsules have turned brown and before capsules dehisce. The plants are hand harvested into nylon mesh bags. One harvest tag is put inside the bag and one is secured to the outside of the bag. Harvested material is dried five to ten days at 30 to 32 ^{o}C as needed and put into a cool, dry storage area until processing. Processing begins after fall field work is complete or on fall days when the weather does not permit outside activity. Cultivated flax accessions

are run through the belt thresher. Wild flax accessions are generally hand threshed. Seeds are further processed in air-column separators and/or hand-picked as needed to remove remaining debris and immature and broken seed.

One-hundred seed weight is determined and the number of seed harvested is calculated from the total weight of the clean seed sample. Seed viability is determined on four 50 seed samples (see the Viability Appendix for details) before the cleaned seed is submitted to Seed Processing for storing.

Characterization:

Flowering date, plant height, number of plants harvested, and harvest date(s) are recorded. Flower color, size and seed color are also documented. In addition, images are taken of plants, flowering heads and mature seeds. These data are recorded and entered into the Germplasm Resources Information Network (GRIN) database and are available at: http://www.ars-grin.gov/npgs/searchgrin.html.

Resources:

Flax Council of Canada webpage: http://www.flaxcouncil.ca/index.html.

Appendix 15

Medicinals and Herbaceous Ornamentals

Plant establishment:

Annuals - Sow annuals destined for field planting during January through April, depending on the time required for the seeds to germinate and seedlings to reach transplant size. Book flats or 18-cell or 36-cell plastic inserts designed for standard 1020 nursery flats work well for many annual crops. When using the 1020 inserts, purchase those at least 3 inches deep. Except for taxa that germinate, grow rapidly, and do not produce long tap roots, shallow (approximately two-inch deep) peat pots are problematic and should be avoided unless there is a specific purpose in using them. Maintain seedlings inside the greenhouse until conditions are suitable for field planting.

Annuals that are to be regenerated entirely in a greenhouse may be started at any time, taking into account daylength requirements for plant development, flowering, and seed set. As for field planting stock, book flats or 1020 inserts work well as starter containers and are suitable for many crops whose seedlings may or may not need to be transplanted to larger pots as the plants mature.

Biennials and Perennials - We typically sow biennials and perennials during January through April, depending on a particular crop's speed of establishment and growth. Various containers are appropriate, depending on the expected growth rate, root structure and depth, and top growth. To reduce labor needs for transplanting, sow the seeds in containers large enough to grow the plants to field-planting size. Species that develop long tap roots, such as Actaea, may be started in two- to four-inch square by six- to twelve-inch tall pots. Book flats work well for species with moderate root and shoot growth. 18-cell or 36-cell inserts for 1020 nursery flats work well for crops that do not produce long tap roots.

Generally, fields should be set aside for perennial crops for three years. Some crops, however, may require more than three years to reach harvest maturity and to produce sufficient seed for regeneration purposes. Coordinate with the farm manager to ensure that long-term perennials are located where they will remain undisturbed without tying up unnecessarily large field areas.

All Crops - Unless a particular crop has special needs for soil during germination and establishment, a peat moss-based potting soil, such as Sunshine Mix No. 1, is used for sowing seeds. Amend the potting mix with sand or perlite if additional drainage is desirable.

Thinning is faster, easier, and less labor-intensive than transplanting seedlings between pots. When seed inventory allows, and depending on germinability test results for the respective seed lot, sow at least two seeds in each cell. When you know or suspect low germinability, sow up to five seeds per container. When more than one seedling emerges per cell, you may transplant extra seedlings to empty cells within the same flat or remove the extra seedlings to leave one plant per cell. For crops that produce small plants, such as *Matricaria aurea* or *Calendula arvensis*, leaving 2 to 3 seedlings per cell is generally not problematic. For seed increase or phenotyping purposes, remove extra seedlings in a random fashion to avoid selecting only the most vigorous seedlings and shifting the genetic profile of the seed lot.

Start any seed lots labelled "if fail to dead file" (IFTDF) or otherwise suspected to be difficult to germinate in a growth chamber by using germination test procedures for that particular genus. Transplant the resulting seedlings into book flats or other appropriate containers for growing out in the greenhouse, shade house or field.

For each book flat or 1020 flat, place two barcoded pot stakes labeled with the genus and accession number into cells in opposite corners of the flat. When plants are to remain in their containers for prolonged periods, label each container with a barcoded lid label suitable for outdoor use.

The desired population size is typically 32 to 72 plants, depending on containers used for establishment and seed productivity of the crop. Calendula cultivars, for example, often produce relatively few seeds per plant and the larger number of plants is needed for seed increase purposes.

When seed lots available for regeneration are very small, you may need to regenerate from only a few plants. Except where an accession is rare, high-priority, and not reasonably available for re-collection, avoid producing seeds from a single individual. Harvest population size is very important and must be recorded in GRIN for each seed lot increase.

When growing field planting stock, set the flats outside (preferably inside a shade house) to harden off for at least one week before transplanting to the field.

By mid to late May, ensure that field plots are free of weeds, undesirable volunteer plants, and trash. Transplant the seedlings for each accession into an individual field plot (referred to as a cage area or cage plot). Plots for the screen cages for horticultural plots are generally 21 feet long by 7 feet wide and are separated by 15 feet of bare ground between the sides and ends of the plots.

Use sunflower cages for *Alcea*, *Althaea*, *Lavatera*, and other accessions that may exceed 180 cm (6 feet) tall. For perennial crops, you may not need to cage and screen the plants until the second or later year following planting. Depending on winter hardiness characteristics, biennials and perennial crops may require fall mulching with shredded newspaper or other organic mulch after several hard frosts in autumn. Mulches can be applied to any crop shortly after planting to help with weed control.

There are three basic options for producing perennial crops that are known or suspected to be cold tender in Ames.

1. Transplant from establishment containers directly into appropriately-sized pots for long-term culture. Such plants can be:
    a. grown continually in a greenhouse through harvest.
    b. grown inside a greenhouse until the plants are ready to flower, at which point they may be transplanted to a field plot.
    c. grown in pots outdoors in the open or inside a shade house during the summer and overwintered inside the "Cave" at approximately 7 ^{o}C.
2. Field plant as described above. Before damaging cold temperatures occur, dig up the plants and transplant them into suitable containers for continued growth in the greenhouse. This strategy may allow for additional seed harvests when overwintering in the field is not feasible.
3. Field plant as described above. After the plants have entered dormancy, dig them up and transplant into suitable containers for overwintering in the "Cave". Replant into the field the following spring, continuing the process until sufficient seeds have been harvested.

Field plots:

Assign each accession to a one-half or whole cage plot for controlled pollination. For crops that do not cross-pollinate each other, two different crops may be grown in a single plot. Within a cage plot, we normally set out plants in from 1 to 4 rows, leaving extra space in the northwest corner of the cage for

installing and removing bee hives. Mulching with shredded newspaper, bark, or other organic mulch helps reduce moisture loss and weed competition and can help marginally cold-tender perennial crops overwinter in the field. Water plants when transplanting, and as needed, depending on the weather.

Scouting greenhouses, shade houses, and fields:

Observe young seedlings and all greenhouse and shade house plants at least twice weekly for damage related to pests, diseases, improper irrigation, and other problems. Observe established fields for pest and disease damage and other problems at least once weekly during the growing season to scout for problems. As an accession approaches flowering, observe it at least twice weekly to determine when to place pollinators. Once pollinators are in place, continue to observe the cages at least twice weekly to monitor pollinator activity and crop maturation.

Pollination:

Whenever possible, ensure that screens are in place and sealed to prevent entry of pollinators before the flowers open. Any flowers that have opened prior to the screening process must be removed to prevent the possibility of cross-pollination between the accession being regenerated and other pollen sources.

In rare cases, it may be acceptable to leave plants unscreened and allow native pollinators to cross-pollinate the accession. You should use this option only when you are sure that no other sources of compatible pollen are located within approximately seven miles, which is the maximum distance honeybees are known to forage. Given that Ames is culturally diverse with residents from many parts of the world, one should expect that unusual, non-native plants may be found growing within a seven-mile range of the Plant Introduction Station. For that reason, screening of all field-grown plants for controlled pollination is highly recommended.

When an accession has set a sufficient number of open blooms to provide for effective pollination, request that the entomologist place pollinators into the cage to carry out cross pollination. Select the appropriate pollinator species with input from the station entomologist. Honey bees, bumble bees, alfalfa leafcutter bees, Osmia bees, bluebottle flies, and house flies are presently used at NCRPIS for cage pollination. In some cases two or more different pollinators are placed into cages at the same time. For genera with tubular flowers, Bombus are generally more effective than are honeybees as pollinators.

Greenhouse- or shade house-grown crops may require hand pollination. Various flies and alfalfa leafcutter bees are also used for greenhouse and shade house pollination. Small cages are available for screening individual flats or small numbers of pots. Mosquito nets are typically used with the small cages to isolate plants.

Harvest:

Harvest for field-grown crops may begin as early as June and extends into October, depending on the crop. All medicinal, mint, and ornamental crops at NCRPIS are presently hand harvested. Seeds or inflorescences must be harvested before seed shattering occurs, and some crops may require harvesting every 2 to 7 days beginning with the first seed ripening. Collect seed or inflorescences into brown paper

bags or mesh bags that are labelled with genus, accession number, cage, and date of harvest. For small-seeded accessions, tape or otherwise seal the bags to prevent seed loss.

Seed Cleaning, weighing, and storage:

Immediately after harvest, place bags of seed inside a dryer cart or a dryer room held at 30-32 ^{o}C. Allow 7 to 10 days for drying. Following drying, break up the inflorescences by hand, with a rubbing block or using mechanical means, including the belt thresher, hammermill, and brush thresher. Complete cleaning the seeds by using a clipper, air column separator, screens, and hand picking. Specific seed cleaning techniques are documented and kept on file by the curator for each crop. When using new extraction and cleaning methods, inspect the seeds closely under a dissecting microscope after each stage of the process to determine if they are being damaged.

Following cleaning, weigh the seeds to determine hundred seed weight (HSWT) for each seed lot. When quantities allow, weigh three subsamples to determine an average HSWT. Label the seed storage bags with a barcoded label and write on the HSWT, total seed weight, and estimated seed quantity. Deliver the seeds to the seed storage manager.

Documentation:

All stages of the regeneration process, from the time the seeds are ordered until the new seeds are placed into storage, are documented in GRIN. The curator is responsible to ensure that all seed lot inventories, site-specific locations, and inventory actions are entered into GRIN.

Characterization:

Regeneration provides an opportunity to record observations of plant traits. Traits to observe and record include: date of first flower, date of last flower, corolla color, flower diameter, petal number, position of flowers, profusion of flowers, plant character (single-bushy), plant growth habit (prostrate-upright), plant height, and percent flowering. For perennial crops, recording the percentage of plants flowering the first growing season and every year thereafter is useful in predicting future harvests.

As time and personnel resources allow, additional traits may be documented for taxa for which descriptor lists are available. For the medicinals, mints, and ornamentals collections, we presently have descriptor lists for *Actaea*, *Calendula*, *Echinacea*, *Hypericum*, *Monarda*, *Origanum*, *Potentilla*, and *Prunella*.

Appendix 16

Biennials: *Melilotus* and *Petroselinum*

Cultural methods:

Melilotus regeneration is described in greater detail by Brenner (2005). Seeds are sown in mid-October so that biennial accessions can be vernalized in a cool greenhouse with short-day lighting in the winter, and bloom the following summer. Sweetclover weevils are a hazard to plants that over-winter in the field, so greenhouse-raised transplants are preferable. Seeds are hand scarified by rubbing with sand paper until the seed coats begin to lose their gloss, then germinated on moist blotter paper at room temperature in plastic boxes with room lighting. Mondoni et al. (2013) describe a more efficient method for scarification, but the sandpaper method is effective at our scale. If at first the seeds do not germinate they are re-scarified until they germinate

Two seedlings are transplanted into each small (12 cm, 175 cc) Rootrainer™ pot. The advantage of small pots is that they conserve greenhouse and growth chamber space. Our goal is 100 plants per seed-increase. If the parent seed lot is of poor quality, there will be fewer plants. The plants are grown in a cool, 5-13 ^{o}C, greenhouse through the winter, without artificial light. Cool, long-night greenhouse conditions vernalize the biennials for blooming the following summer (Kasperbauer et al. 1962). By the beginning of March the winter-annual accessions are bolting so they can be distinguished from accessions with longer duration and potted-up for greenhouse seed production before the summer arrives. Also at the beginning of March both biennial and annual accessions are placed in cool storage at 4 ^{o}C with short-day lighting to postpone blooming. The USDA-ARS National Lab for Agriculture and the Environment project provides the cool storage in their growth chambers.

Petroselinum seeds are sown in August since some accessions vernalize better from August planting than from October planting. Otherwise the plant handling is similar to *Melilotus*, except that the seeds do not need scarification, and the seed cleaning is the same as for the annual umbels [in another appendix].

Plants can be transplanted into the field in April or May, but preferably in the first week of April. In 2014 Melilotus and Petroselinum plants withstood -4.4 ^{o}C after transplanting, without ill effects, so they are cold tolerant. In the field, one cage of the 7x7x20 foot size is used per accession to accommodate combined population sizes of over 120 transplanted plants.

Melilotus plants are very sensitive to herbicides. We have observed putatively toxic symptoms from field spraying 0.5 km away and we occasionally observe toxic symptoms after use of pesticides in greenhouses. Aphids and caterpillars are our primary pests. *Melilotus* do well without soil fertilization in the field. Unfertilized plants stay small and are more easily managed than heavily fertilized plants.

Pollination:

With important exceptions, *Melilotus* species self-pollinate (Table 2). *Melilotus officinalis*, however, is a poor self-pollinator and only 0-69% of the flowers (mean 19%) are self-fertile (Sandal, 1951). If these plants can be maintained over longer periods, self-compatibility apparently increases with age (Sandal, 1951). If allowed to self-pollinate, accessions will be selected for higher selfing rates because self-compatibility is highly heritable (Sandal, 1951). Inbreeding depression was measured in Melilotus by Hartwig (1942). Plants grown from seeds that had been selfed for one generation were 30%

shorter in M. officinalis, 16% shorter in *M. albus*, and did not set as many seed as did plants from crossed seeds. Plants grown after two generations of selfing had even lower vigor. It is preferable to pollinate with honey bees confined in cages. Facilities for this kind of pollination were developed for *Melilotus* at this station (Ellis, 1981).

Harvest:

Harvesting begins 4-8 weeks after anthesis begins. The seeds should be hard and dry on the plant before harvesting. We generally harvest once. At maturity the plants are uprooted and the seeds are rubbed into 5 gallon buckets. Uprooting allows us to count the plants since it is otherwise difficult to know how many plants are present. Seeds are dried with forced air in fine mesh bags. The largest harvests are prone to mold in their centers, and should therefore be spread thinly in the harvest bags in the forced-air drier carts to allow good air circulation.

Seed Cleaning:

When the pods have dried to brittleness, thresh the seeds from them by rubbing the pods with rubber-covered wooden blocks. Blow the chaff and the immature seeds clear in an air column separator.

Maintenance Characteristics of *Melilotus* species:

Species[1]	Corolla color[1]	Duration[1]	% self-compatible flowers[2]	"hard" seed?[3]
M. albus	white	biennial/annual	50	No
M. altissimus	yellow	biennial	61	No
M. dentatus	yellow	biennial/annual	87	No
M. elegans	yellow	winter annual		No
M. hirsutus	yellow	biennial		No
M. indicus	yellow	winter annual	92	Yes
M. infestus	yellow	winter annual		--
M. italicus	yellow	winter annual	90	yes
M. neapoliatanus	yellow	winter annual		yes
M. officinalis	yellow	biennial	26	no
M. polonicus	yellow	biennial	46	yes
M. segetalis	yellow	winter annual		yes/no
M. siculus = messanensis	yellow	winter annual	86	yes
M. speciosus	white	winter annual	60	yes
M. suaveolens	yellow	biennial/annual	77	no
M. sulcatus	yellow	winter annual	66	yes
M. tauricu	white	biennial	62	no
M. wolgicus	white	biennial	65	no

[1]The corolla color and duration data are from Stevenson (1969).

[2]Percentages of self-compatible flowers are from Mendoza (1946). These averages must be interpreted cautiously because of infra-accession and infraspecific variability.

[3]The "hard seed" data were generated at the NCRPIS. Fewer than 50% of the "hard seeds" germinate without scarification. Even the non-scarified seeds were somewhat scarified during our routine seed cleaning, which may contribute to germinability.

Characterization:

The traits measured include: seedling stem color; flowering date; flower color; tallness; duration; and weight per 100 seeds.

References:

Brenner, D.M. 2005. Methods for *Melilotus* germplasm regeneration. Plant Genetic Resources Newsletter 141:51-55.

Ellis, M.D., G.S. Jackson, W.H. Skrdla, and H.C. Spencer. 1981.

Use of honey bees for controlled interpollination of plant germplasm collections. HortScience 16:488-491.

Hartwig, E. E. 1942. Effects of self-pollination in sweet clover. Journal of the American Society of Agronomy 34:376-387.

Mendoza, A. Berrios. 1946. A survey of self-fertility and of cross-compatibility between species of *Melilotus*. M.S. Thesis, Iowa State College, Ames, Iowa.

Mondoni, A., E.R. Tazzari, L. Zubani, S. Orsenigo, and G Rossi. 2013. Percussion as an effective seed treatment for herbaceous legumes (Fabaceae): implications for habitat restoration and agriculture. Seed Science and Technology. 41:175-187.

Sandal, P. C. 1951. Mechanisms of self- and cross- incompatibility in sweet clover, *Melilotus officinalis* Lam. Ph. D. dissert., Crop Breeding, Iowa State College, Ames.

Stevenson, George A. 1969. An agronomic and taxonomic review of the genus *Melilotus* Mill. Canasian Journal of Plant Science 49:1-20.

Appendix 17

Ocimum

Planting:

Seeds should be sown in mid-March to be transplanted into cages in May. Seeds can be seeded directly into Rootrainer™ pots in the greenhouse, and each flat labeled with the accession number and parental lot code. Seeds can also be started on blotter paper in germination boxes placed inside a growth chamber set to 30/20 ^{o}C day/night and 16-hour photoperiod. A pre-chill treatment of 4-5 ^{o}C for 5-10 days improves the germination of some accessions. Seedlings are then transplanted to Rootrainer™ pots and placed under mist until plants are sturdy. Plants can be pinched back if they begin to flower before transplanting to the field cages.

Remove 50-75 plants from cells and transplant them into three evenly spaced rows in a 7' x 7' x 20' (2.1 m x 2.1 m x 6 m) pollination cage; cages are separated from one another by 15' alleys. Plants are watered immediately after transplanting to the field, and additional watering is required during drought. Mulch the plots with chopped paper to preserve moisture and reduce weed competition. Cover cage frames with a Lumite screen just prior to flowering.

Pollination:

Cages are scouted every 2-3 days for flowers. When flowering begins, alfalfa leaf cutting bees are introduced into the cage for pollination. As more plants begin to flower, a small queen-right colony (nuc) of honey bees is placed in the cage for pollination. Pollinators remain in cages for several weeks. (See section on insect pollinators for additional information.)

Harvest:

In late August through September, when infructescences are brown and papery, cut them off into small-mesh polyester bags. The seeds of some species are quite small and the use of double-bagged paper bags may be necessary. Shattering can also complicate harvest. Whole plants can be harvested, but processing whole plants makes seed cleaning more difficult.

Seed Cleaning:

Dried plant tissue is belt-threshed, processed by a Clipper air-screen cleaner, and then an air-column separator removes light, immature seeds and debris. Hand picking is required to remove damaged or moldy seeds, and debris left by the mechanical separators. Germination tests are performed on each seed lot following the Association of Official Seed Analysts (AOSA) Rules for testing seeds for species that have standards; modification of germination rules may be necessary for seeds exhibiting dormancy. Seed increases are inventoried and stored in 1 quart (1 liter) glass jars at 4 ^{o}C and 25% RH.

Characterization:

The traits measured include: flowering date; leaf number, size, texture, and color; stem size; growth habit; fruit set; corolla color; plant height at harvest, weight per 100 seeds.

Appendix 18

Perennial and winter annual POACEAE (grasses)

Winter annuals:

Apera are winter annuals. They are planted in the fall and kept through the winter in a greenhouse without extended daylength, for flowering when the days lengthen in the spring. We grow them in 24 X 16 X 4 inch wooden flats.

Perennials:

The perennials are the most laborious grasses to grow for seed increase. *Setaria sphacelata* and the allied species are planted in September for growing in Rootrainer™ pots, then transplanted into the field in May, and harvested in fall.

The *Calamovilfa longifolia*, *Tridens flavus*, and *Tridens strictus* are planted in March, and then transplanted into the field for harvesting in October or November. They are winter-hardy so they can be kept in the field for harvesting in later years. However, the *Tridens flavis* from the southern United States generally flower too late for seed production in our area.

Planting:

The seeds are direct seeded into greenhouse soil and after germination potted-up into Rootrainer™ or other pots for the longer term.

Pollination:

Setaria sphacelata is an out-crosser (Hacker, 1967) we observe flies visiting the flowers and presumably pollinating, so the accessions are isolated in different fields at the farm. *Calamovilfa longifolia* and *Tridens* may be outcrossing so they are also pollinated in isolation.

Harvest:

Seeds are ready for harvest when they turn brown and easily fall off the plants. Seeds are hand harvested into fine mesh bags, and dried in forced air. They are threshed by rubbing, and cleaned in the air-column separators and by hand picking.

Characterization:

The plants are imaged, and the taxonomic determination is verified.

References:

Hacker, J.B. 1967. The maintenance of strain purity in the *Setaria sphacelata* complex. The Journal of the Australian Institute of Agricultural Science 33:265-267.

Appendix 19

Spinacia

Cultivated Spinach (*Spinacia oleracea*):

Since 1993 our cultivated spinach seed regenerations are grown in Salinas, California by cooperators at Sakata Seed America working with the USDA-ARS. In August the seeds are planted in plug flats in a Sakata greenhouse, and are ready for transplanting 35 to 40 days later. The seedlings are transplanted into the soil floors of small greenhouse isolation chambers at the USDA-ARS facility in Salinas. The plants are grown in short daylengths, until they are large enough to flower. Then the lights are set to long daylengths, to force flowering.

The USDA-ARS isolation chambers have positive pressure from a compressor, so that pollen cannot drift from chamber to chamber. This prevents pollen contamination. The harvests are generally completed in February. The staff at Sakata rough-cleans the seeds, and sends them to us.

Alternatively spinach can be regenerated in the greenhouse or field in Ames. Plantings in Ames are limited to few accessions per year because of the risk from cross-contamination by drifting pollen from adjacent accessions. In a greenhouse in Ames, 13 spinach plants have been grown per 24 X 16 X 4 inch wooden flat. When direct seeded September 9, and grown in local daylengths until November 21 then given 16 hour days they flowered and were ready for harvesting in late January. Field cages can also work well. The cage screen does not control spinach pollen (which is wind-borne) but does protect the plants from animal feeding. Plant as early in the spring as the soil can be worked. It seems that some accessions are not adapted to our heavy soil, heat, and humidity, since some of the plants die suddenly; so for field regeneration select adapted accessions with ample backup seeds.

Wild spinach (*S. tetrandra* and *S. turkestanica*):

Wild spinach has substantial seed dormancy but is otherwise horticulturally similar to cultivated spinach. The seeds are in spiny and woody seeds units containing about five seeds. We regenerate them in Ames because the spinach regeneration collaborators in California are unprepared to overcome seed dormancy.

We use two main strategies to cope with wild spinach seed dormancy: time and sand-boxes.

The most effective strategy is time. Wait until the seeds are at least 2 years old and preferably more than 10 years old before planting. Seed dormancy diminishes with age.

We plant into "sand boxes" which is an idea from Baskin and Baskin (1976). The seeds remain in sand, in a greenhouse and germinate sporadically for up to three years. Our version of these boxes uses deep, plastic, 42 cm × 31.5 cm × 8 cm (5-12 Nu Tray™) flats nested together. The lower flat has 3.5 cm of potting media to prevent sand from rinsing out of drainage holes and to be a reservoir of soil moisture. The upper flat is filled to within two cm of the top with sand, and is where the seeds are planted. The sand is also easy to transplant from when the seed germinate. We plant at any time from September to January so the early germinating plants have time to grow before they are forced to flower by long spring daylengths. Hot summer conditions break dormancy as is typical of the winter annual species described by Baskin and Baskin (1976). The seeds are primarily August and September germinating, but some germinate at other times.

At germination the seedlings are transplanted into 6-inch pots. The pots are kept in short-day conditions of the farm greenhouse #2 to postpone bolting until the plants are large. When the inflorescences begin to emerge the pots are taken to separate greenhouses to prevent cross-pollination between accessions. At flowering the male and female pots are occasionally shuffled to improve pollen distribution, and the plants are fanned with pieces of cardboard to distribute the pollen. The seeds are harvested into bags of any mesh size, and dried with forced air.

Seed Cleaning:

The seed regeneration group in California delivers rough-cleaned harvest to us. For the seeds grown in Ames, seeds are rough-cleaned by separating from the stems by hand. After rough-cleaning the seeds are blown in an air column separator and handpicked as needed to remove foreign material.

Characterization:

The traits measured include: coloring at the base of the petioles; leaf blade surface texture; leaf blade shape; the frequency of monoecious plants; and weight per 100 seeds.

References:

Baskin, J. M. and C. C. Baskin. 1976. High temperature requirement for afterripening in seeds of winter annuals. New Phytologist 77:619-624.

Appendix 20

Woody Landscape

Woody landscape plant germplasm held at the NCRPIS is divided into two groups: 1) accessions of taxa for which the NCRPIS is the Priority Site for maintenance within the NPGS and 2) accessions of other taxa that we cultivate for evaluation and propagation activities in the NC7 Regional Ornamental Plant Trials.

NCRPIS Priority-Site Species:

Whenever possible, we maintain woody landscape accessions as seed that is produced from controlled or isolated pollination. When that method is not possible, we maintain the accessions as clonal germplasm in permanent field plantings at the NCRPIS or at some other site in cooperation with the NCRPIS if they cannot be maintained at Ames. For some clonal accessions, cryogenic storage of vegetative buds has allowed for long-term backup of accessions otherwise maintained in permanent field plantings.

For clonally-propagated accessions, procedures in "Manual of Woody Landscape Plants," "Plant Propagation: Principles and Practices," the "Proceedings of the International Plant Propagators' Society," "The Reference Manual of Woody Plant Propagation," and other pertinent references are consulted to develop appropriate handling methods on a case-by-case basis.

For seed-propagated accessions, seeds are germinated following recommendations in "Seeds of Woody Plants in North America (revised form of Agriculture Handbook #450)," "Manual of Woody Landscape Plants," "The Reference Manual of Woody Plant Propagation," and other published and online references. We coordinate seed treatments so that seeds germinate in the greenhouse from late January to late April. This timing allows the seedlings to develop during a period of increasing daylength when growing conditions are not excessively hot and drying. During maintenance or initial testing prior to distributing an accession, the optimum population size varies, depending on the breeding system and genetic variation of the accession or species.

All attempts to propagate accessions, either by seed or vegetatively, are recorded in the GRIN database. GRIN is also used to document the protocol used to propagate accessions, the subsequent number of propagules that are transplanted to the field, and inventory updates. The curator reviews these data periodically, as well as reference materials, to improve propagation methods.

Seeds for storage are obtained, whenever possible, from non-clonal plants by controlling pollination inside screened cages, hand pollinations, or isolating flowering accessions from other plants that might cross-pollinate them. Small to medium-sized shrubs are typically pollinated using Apis, *Bombus*, and/or Osmia bees in large field cages (10'× 10'× 21'). Generally, it takes about three years or more to get sufficient seed quantities from field cages. For accessions that flower in the greenhouse, hand pollination, pollination using flies or alfalfa leafcutter bees, or a combination of hand pollination and insect pollination is used to cross-pollinate the crops.

Plants for NC7 Trials:

NC‑7 Trial Site Cooperators assist the curator in identifying accessions that will be distributed for regional testing. Generally, 100 plants of a particular accession have proven suitable for distribution to test sites. We distribute the plants during late March to mid-May to test-site cooperators that have requested the accessions, and distribute excess plants to botanical gardens and arboreta. For taxa that are maintained by another NPGS site, that site's appropriate curator is contacted and asked if they are interested in obtaining the germplasm for long-term conservation within the NPGS.

Other Priority Site Species:

We maintain non-NC7 accessions at NCRPIS only until they have been distributed for regional trials, found unsuitable for testing, or have been transferred to another site for maintenance.

Appendix 21

Zea

As for all crops curated at the NCRPIS, newly-received *Zea* accessions are grown for increase as soon as possible after being received, unless the original grain is of sufficient quantity and quality (more than 2500 kernels and germination higher than 85%) for immediate distribution. Accessions are increased when quantities drop below 2500 kernels or germination is less than 85%.

Planting:

In the field rotation system at the NCRPIS, maize is always planted after another crop. If the field was not tilled the previous fall, or, if weeds have begun growing, the field is opened with a vibra-shank cultivator. Then 112 kg/ha (100 lbs./acre) N in the form of ammonium nitrate is spread on the field with a dry fertilizer spreader. Our soils are sufficiently high in P and K, addition is not required. The fertilizer is worked into the soil concurrently with the application of a pre-plant herbicide combination with a vibra-shank cultivator equipped with a spray rig at herbicide label rates. The ground is worked diagonally or perpendicularly to the direction of planting.

Corn Belt-adapted maize germplasm is direct-seeded in four 10 m rows .9 m apart. The four rows are separated from other blocks by skipping a row between blocks. A cone-belt planter is utilized. If available, 100 kernels are planted per row. These are randomly thinned to at least 50 plants per row or 200 plants per accession.

For long-season, or day-length sensitive corn lines, increases are made in a winter nursery in Puerto Rico. Accessions with few original kernels are increased in the greenhouse at Ames or are started in the greenhouse and transplanted to the field at Ames. Poor quality kernels in low quantity may be started in a germinator set at 25 ^{o}C in a blotter box with fungicide treatment.

Pollination:

Standard pollinating procedures for producing "seed corn" are followed to protect against outcrossing (ears are covered with shoot bags before silk emergence and tassels are bagged as they start to shed pollen).

Populations: Are sib-mated to avoid inbreeding and maintain the population's genetic profile. When most silks have emerged from an ear shoot, the shoot bag is marked, and marked bags are counted. When the count reaches 10% of the stand, or, before the first tassels finish pollen shed, the accession is prepared for pollination. Tassels are bagged in numbers equivalent to the number of silks counted in the accession. Plants which have not been pollinated, or are not marked for the next day's pollination, should be bagged. The tassel bags have the next day's date on the bag. Tassels are marked, when bagged, by removing the flag leaf from the tassel, to ensure that they are used only once. The next day each tassel bag is removed and a marked ear on another plant within the same row is pollinated. Only one ear is pollinated per plant, and each tassel is used only once as a pollen source. An attempt is made to produce at least 100 ears per accession.

Inbred lines: Are self-pollinated. The tassels are bagged before they finish shedding pollen and, when the top ear on the main stalk has the maximum number of silks emerged, it is pollinated. Again, at least 100 ears is the target number for harvest, in order to supply enough kernels for distribution.

After pollination, the ear bag (glassine) is replaced over the silks and a paper bag is slipped over the shoot‑bagged ear and stapled in place. Striped bags identify selfs; plain, unmarked bags indicate sibs.

Harvest:

Before harvest, as time permits, average plant height, ear height, node number above ground level, node number above the ear, ears per stalk, tillers per stalk, and tassel size and branching are measured from ten healthy plants per accession, selected at random. The plant pathologist examines the fields during the seed filling period for a variety of corn diseases and for any novel disease occurrence. This information is useful for phytosanitary declaration and certification purposes. The panel of diseases monitored includes gray leaf spot, Stewart's wilt, Goss's wilt, northern corn leaf blight, southern corn leaf blight, northern leaf spot, eyespot, common rust, crazy top, sorghum downy mildew, head smut, common smut and virus symptoms. At harvest, percent stalk and root lodging are estimated.

Accessions are harvested generally after the ear husks have turned brown. Each ear is husked in the field and wrapped in the ear bag which covered it. Completely rotten ears are discarded and damaged kernels are removed from partially damaged ears. Each row of an accession is placed in one or more separate harvest bags and all bags for an accession are tied together. Harvest bags are composed of a 1 mm knit cloth mesh or a 1 x 4 mm plastic mesh onion bag is used.

Seed Processing:

The harvested ears are put in a drying room kept at 30-32 ^{o}C, with fans circulating the air. When the kernels reach 10-13% moisture (as determined by the "feel" of the kernel or by a moisture meter) they are placed in temporary storage (15m C, 50% relative humidity) until they are shelled and chaff is blown out. By the end of processing, the kernels have equilibrated to 8-10% moisture.

At processing, ears are unbagged, sorted, laid out, and counted by date. They are then examined by the technician or curator for offtypes, with reference to the colors and kernel types on the current regeneration, parental kernels, and previous increases. The ranges for kernel row numbers and ear lengths are recorded. Twenty-five of the best (many kernels, healthy) ears are selected at random and set aside for photographing. The remaining ears are grouped together.

A digital image is taken of every group of selected 25 ears on a flatbed scanner with a ruler and standard color card for reference. Software developed at the NCRPIS is used to capture the barcode id on the tag, link to the appropriate inventory, and store the image on a local file server. Ears are cut in half using a saw, and the ears stood on the cut side on the flatbed scanner; images are captures of the cross section of the ear. The internal structures of kernels cut in the process are highly visible. Seeds are also scanned, both en masse and using a grid which separates individual kernels.

As time permits, average ear shape, ear diameter, ear length, kernel rows, number of kernels per row, kernel thickness, and kernel width can be measured from the images. A separate image is made of unusual or offtype ears. Offtype ears are discarded from inbred accessions. If it is determined that the offtype plants served as pollen parents in sibbed accessions, a new regeneration is attempted from the parental or earlier increase.

Following photography, one of the twenty-five ears is selected to represent the accession, and is marked as such. Every acceptable ear is then cleaned of visually-damaged or diseased kernels. Then one healthy kernel is removed from every ear, including the representative ear, sixteen times to make up sixteen balanced samples of all ears. Eight samples are grouped for shipment to NSSL with the backup bulk sample, and eight are kept for the next two increases at NCRPIS. The remainder of the kernels is shelled with a hand sheller, and bulked together for distribution. If 100 ears were not obtained from a population, the increase will be bulked with a second increase. Germination levels are obtained from the bulk grain in rolled paper towels in a germinator with 20-30 ^{o}C day-night alternating temperatures. Four replications of fifty kernels are germinated.

The clean, dry grain is then transported to cold storage, where it is compared to the original kernels, or to our oldest increases. The inventory is updated by computer, and the grain enters the cold storage room.