



Floridus Milamexa

Seed Collecting & Storage Basics

Goals for Today

- Target species
- Storage & Distribution
- Assessing a Population for Collection
- Identification & Herbarium Specimens
- Seed Collecting Techniques
- Field Documentation
- Post Collection Seed Care
- Storage
- Seed Cleaning
- Long Term Storage

Target Species

- Use Seeds of Success (SOS) protocols
 - Species needed for restoration and rehabilitation projects
- Multiple collections of species on their restoration target list
 - Capture unique populations in each collection



SEEDS
OF
SUCCESS

Target Species

- Collecting teams are encouraged to work with local federal land managers to develop and execute priority target lists
 - Common native workhorse species appropriate for restoration and stabilization



Target Species

- Potential “clients” include:
 - Emergency fire rehabilitation and restoration
 - Waterway stabilization
 - Landfill and corporate land recovery
 - Wildlife habitat
 - Threatened and endangered species **habitat**
 - Roadside revegetation



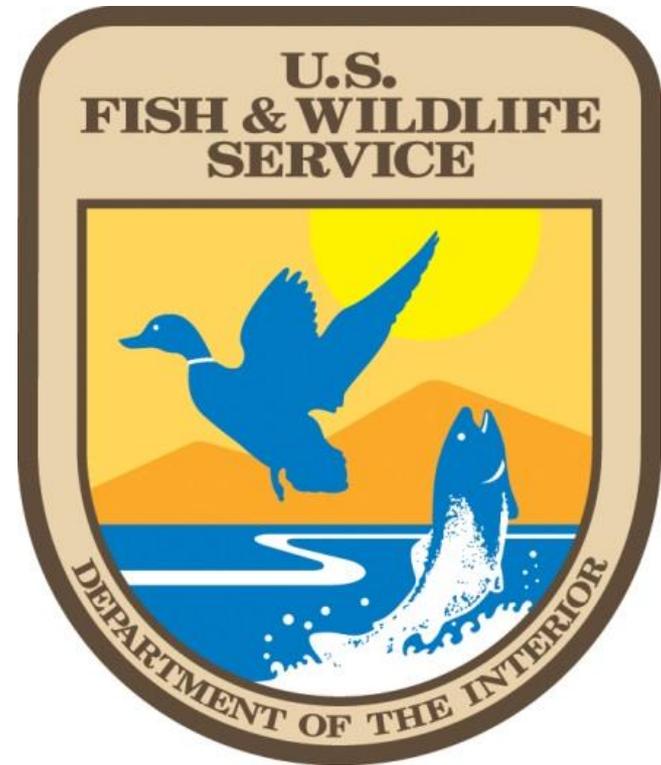
Target Species

- Goal is to make 20 collections across the range of a species
 - Plant Conservation Alliance species list at <http://www.nps.gov/plants/sos>



Excluded Species

- Any native plant species listed as Threatened or Endangered, under the *Endangered Species Act*
- Any Candidate, or any species Proposed for listing, under the *Endangered Species Act*
- Any species listed as G1 or G2 by a State Heritage Program



Excluded Species

- Any species listed as S1 or S2 by a State Heritage Program will not be collected in the state listing it as S1 or S2
- Any species designated as a BLM State Director Sensitive Species that have been ranked G3 or S3 by a State Heritage Program and is included in the CPC network collection



Excluded Species

- Any species included in Appendix I of the *Convention in the Trade of Endangered Species* (CITES)
- Any species not native to the U.S
- Any agricultural or food crop species



Excluded Species

- All species in the genus *Quercus*
- All species in the genus *Vitis*
- All known recalcitrant seeds



Storage & Distribution

- Long-term and working collection needs are being met by the U.S. Department of Agriculture, Agricultural Research Service



Storage & Distribution

- The National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado is managing long-term collections



Storage & Distribution

- Western Regional Plant Introduction Station (WRPIS) in Pullman, WA is maintaining both long-term and working collections for distribution to researchers working on native plant materials development related topics



Permission to Collect

- Landowner permission required for all collections
 - **Even if they are our members, family or friends**
- Document landowner permission on the field data form associated with the seed collection
- Keep written documentation of permission to collect



Assessing Populations for Collection

- An “ideal” collection will be from a large number of individuals (100+) and will contain more than 10,000 viable seeds



Assessing Populations for Collection

- Collections larger than 20,000 viable seeds are preferred
- Maximize the flexibility of the collection and allow for a portion of the collection be held at a second seed bank



Assessing Populations for Collection

- Maximizing the use of the collection means that:
 - Sufficient seed is available for germination and viability testing
 - Samples are available for distribution to users for restoration, education or scientific purposes
 - A substantial amount of seed can be conserved as a long-term safeguard against loss of the wild population

Assessing Populations for Collection

Preliminary Site Visits

- Preliminary site visits are necessary to:
 - Assess the populations
 - Confirm the identification with the collection of herbarium voucher specimens
 - Estimate the likely harvesting date and potential seed production



Assessing Populations for Collection

Preliminary Site Visits

- Collections of a number of different species may be possible from the same site



Assessing Populations for Collection

Preliminary Site Visits

- Ensure that the population is of wild origin, not planted or cultivated
 - Do not collect seeds of native species that were included in a seed mix as part of post fire management in areas that were burned and seeded
 - Native species that were not seeded in those areas could be collected



Assessing Populations for Collection

Preliminary Site Visits

- Small populations (less than 50 individuals) or those that will yield less than 10,000 viable seeds should not be collected with the expectation of seed being transferred to an in-house native plant materials development project, or returned to the collector



Assessing Populations for Collection

Preliminary Site Visits

- Collections of less than 10,000 viable seeds shall be directly transferred to the SOS National Collection
 - *Floridus Milamex* storage facility



Assessing Populations for Collection

Preliminary Site Visits

- Seed development can vary within and between populations of the same species
 - Monitor seed maturation and assess insect damage and empty seeds throughout the population before making the seed collection



Assessing Populations for Collection

Preliminary Site Visits

- It is strongly encouraged that seed collectors return to a population throughout the dispersal period to maximize the genetic diversity of samples
 - Collections taken from the exact same population may be combined into one accession (seed collection reference number) during a single collecting season



Assessing Populations for Collection

Preliminary Site Visits

- Collectors must ensure that no more than 20% of the viable seeds are collected on any given day, and that all combined material is from the same population and uses the same seed collection reference number or accession number
 - Please note that the material was collected on multiple dates on the SOS field data form



Assessing Populations for Collection

Sampling Strategy

- It is important to maximize the number of alleles present within a collected sample by capturing the greatest proportion of alleles represented in the field population



Assessing Populations for Collection

Sampling Strategy

- According to Brown and Marshall (1995), at least one copy of 95% of the alleles occurring in the population at frequencies of greater than 0.05 can be achieved by sampling from:
 - 30 randomly chosen individuals in a fully outbreeding sexual species, or
 - 59 randomly chosen individuals in a self fertilizing species

Assessing Populations for Collection

Sampling Strategy

- The reproductive biology of most target species has not been studied, and the capture of rarer alleles would require a markedly increased sample size
 - Collectors are advised to sample from a single population with individuals of the target species in excess of **50 individuals**, and to look for populations with larger numbers of plants



Assessing Populations for Collection

Sampling Strategy

- Between 10 and 20 collections across a species range are needed to establish seed zone guidelines and ecotype for a species
 - Each of those collections shall be a unique population and should contain more than 10,000 seeds



Identification & Herbarium Specimens

- It is critical to the value of the seed collections that the species is accurately identified
- Voucher material is essential to enable the accurate identification of seed collections



Identification & Herbarium Specimens

- Vegetative material and close-up photographs can **occasionally** be used, but the most useful voucher material for this program is a set of quality herbarium specimens (pressed, dried plant specimens) for each collection
 - Flowers and fruits



Identification & Herbarium Specimens

- Collectors are required to collect herbarium voucher specimens for all seed collections
 - Enter comprehensive identification notes on the field data form including where each specimen was sent and any additional identification notes
- **Do not mount the voucher materials on a herbarium sheet**



Identification & Herbarium Specimens

- Herbarium specimens are valuable additional outputs from the collecting program in their own right
- Collectors should take 3 – 4 representative herbarium specimens for each seed collection made



Identification & Herbarium Specimens

- These specimens can be held at the most appropriate regional, national and international herbaria where they will be available for study or for classification by visiting taxonomists
 - A&M herbarium?



Identification & Herbarium Specimens

- Close-up photographs, especially of flowers or organs that may be damaged by pressing and drying, are welcome and
 - Should have the collection number clearly written on the reverse
 - In the case of digital files, cited in the file name
 - For those species that will not be in bloom during seed collecting time, it is suggested that a herbarium voucher specimen be taken during a preliminary trip to the population or from the same population the following year

Identification & Herbarium Specimens

- Herbarium specimens must be taken from the exact population earlier in the season
 - For the purposes of identification and population monitoring



Identification & Herbarium Specimens

- If a preliminary trip is not made and material for a herbarium voucher specimen is inadequate at seed collection time:
 - Record a representative individual of the population with GPS
 - Herbarium specimens can be taken from those individuals in the following season when vegetative and fertile material would be available



Identification & Herbarium Specimens

Issues: Collection

- The standard Smithsonian herbarium sheet is 11 $\frac{3}{4}$ inches wide by 16 $\frac{1}{2}$ inches long
- If your specimen is larger, consider dividing or folding the specimen so it will fit comfortably on a sheet



Identification & Herbarium Specimens

Issues: Collection

- A specimen that requires more than one sheet is acceptable as long as the label data indicates there are multiple pieces to be mounted on separate sheets
 - Please be aware that these separated pieces still belong to a singular collection



Identification & Herbarium Specimens

Issues: Pressing

- For the majority of vascular plants species no special consideration is made when pressing specimens in the field
 - Try to display the specimen in such a way that all taxonomic features of the specimen can be examined easily



Identification & Herbarium Specimens

Issues: Pressing

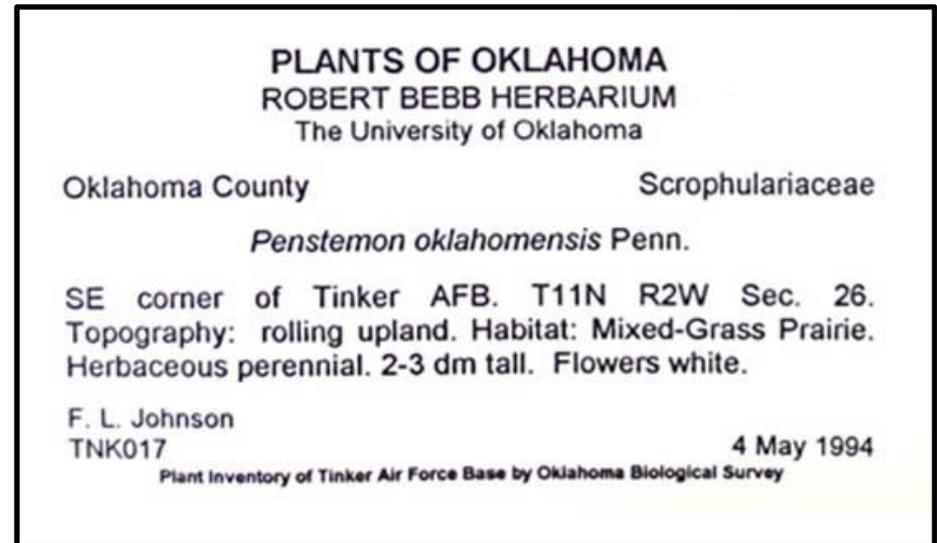
- There are a few exceptions to be aware of and they include:
 - Ferns
 - Large bulky fruits
 - Grasses
 - Seed
 - Large leaves



Identification & Herbarium Specimens

Issues: Labeling

- Labels play a huge role in the significance of a specimen
 - Without a label or with poor/inaccurate label information a specimen is useless as a scientific or historical artifact



Identification & Herbarium Specimens

Issues: Labeling

- A future researcher should be able to use a specimen label to connect the specimen to the place and time of its collection along with the collector and possible determiner of the plant species

Herbarium - University of Iowa (IA)
PLANTS OF U.S.A.

Helodium paludosum (Sull.) Aust. var. **paludosum**

IOWA, Mahaska Co.: Hull State Game Management Area, ca. .5 mi. W of Beacon on G49 and ca. .5 mi. S of G49 along road through reclamation site, on E side of road through fen.
SW 1/4 of NE 1/4, Sec 30, T75N, R16W
41 15'N, 92 43'W

Fen - wet prairie with patches of *Spartina pectinata* and *Calamagrostis canadensis*, and scattered *Bidens* and *Lactuca scariola*. Scattered low hummocks of *Sphagnum fimbriatum* and *S. palustre* mainly associated with *Spartina*.

Diana G. Horton 30816
(With Lon Drake and Carol Thompson)

October 19, 1990

Identification & Herbarium Specimens

Issues: Labeling

- A typical label is approximately a 4 x 4 inch square (the ideal, but not set in stone) and is printed on acid free paper
- The label should, at minimum, contain:
 - Family
 - Genus
 - Species
 - Collection location (as specific as possible)
 - Date of collection
 - Name of the collector(s),
 - Collection number

Identification & Herbarium Specimens

Issues: Nomenclature

- USDA PLANTS Database is the taxonomic standard used by SOS and can be accessed on the web at <http://www.plants.usda.gov>
- Identify collections to the subspecies and/or variety level
 - One goal of the program is to identify the varieties of widespread species that are found in each ecoregion

Seed Collection Techniques

- *Floridus Milamexa* will follow SOS protocols



Seed Collection Techniques

Method

1. Assess the target population and confirm that a sufficient number of individual plants (> 50) have seeds at natural dispersal stage

Rationale

- To ensure that adequate genetic diversity can be sampled from the population, and that the seeds are likely to be at maximum possible viability and longevity

Seed Collection Techniques

Method

2. Carefully examine a small, representative sample of seeds using a cut test and for smaller seeds a hand lens

Rationale

- Estimate the frequency of empty or damaged seeds and confirm that the majority of seeds are mature and fully formed

Seed Collection Techniques

Method

3. Collect mature, dry seeds in either cloth or brown paper bags. Large collections can be made using plastic buckets and then transferred into bags

Rationale

- Ensure the highest possible viability at collection and maximize the potential storage life

Seed Collection Techniques

Method

4. Cleaning should be left to the processing staff at the Bend Seed Extractory for federal partners

Rationale

- Maximize the use of available field time and clean and prepare seeds in controlled laboratory conditions

Seed Collection Techniques

Method

5. Fleshy fruits should be collected directly into plastic bags. Specific advice on ripening and cleaning fleshy fruits is in Section 13, or contact Bend Staff if specific guidance is needed

Rationale

- Fleshy fruits decompose rapidly and poor storage can lead to mold infested seed collections

Seed Collection Techniques

Method

6. Sample equally and randomly across the extent of the population, maintaining a record of the number of individuals sampled

Rationale

- Capture the widest possible genetic diversity from the plant population sampled. Where the population exhibits a pattern of local variation, use a stratified random sampling method to ensure sampling from each microsite

Seed Collection Techniques

Method

7. Collect no more than 20% of the viable seed available on the day of collection

Rationale

- Ensure that the sampled population is not over collected and is maintainable

Seed Collection Techniques

Method

8. Collect seeds from a population throughout its dispersal season, seeds from a population collected in the same year can be combined as one collection, using the same seed collection reference number

- Note the multiple dates of collections on the SOS field data form

Rationale

- Maximize genetic diversity in the collection, capturing early, mid, and late bloomers

Seed Collection Techniques

Method

- 9.
- Collect 10,000 to 20,000+ viable seeds.
 - However, collections of all sizes are welcome
 - The smaller the collection, the less useful it will be

Rationale

- Enable maximum use and study of the collection
- The first 10,000 viable seeds are transferred directly to the SOS National Collection
 - *F. Milamexa* collection

Seed Collection Techniques

Method

10. The first 10,000 seeds of each collection sent to Bend becomes part of the SOS National Collection

- Collections sent to Bend can be cleaned and sent back to collectors if they are needed for native plant materials development research or a re-seeding project

Rationale

- Seed from Bend is then sent to the NCGRP, Ft. Collins, CO for long-term storage and the WRPIS for long-term storage and working collections
 - Anything over 10,000 can be requested back by the collector or shipped to a partner organization for research and development

Seed Collection Techniques

Method

11. For each collection, estimate the viable seed production per fruit, per individual and per population, and note these on the field data form

Rationale

- Document species seed biology, better assess the influence of collecting on the population, and gather information to better document if we are meeting *Standards for Rangeland Health* for native plant communities

Seed Collection Techniques

Method

- 12.** Clearly label all bags (inside and out) with the appropriate collection number
- No other data needs to be included on the label
 - Do **not** write on cotton seed bags with permanent marker; the bags will be reused

Rationale

- To ensure that this unique identifier is attached to each sample of a collection
- All other data will be recorded on the field data form

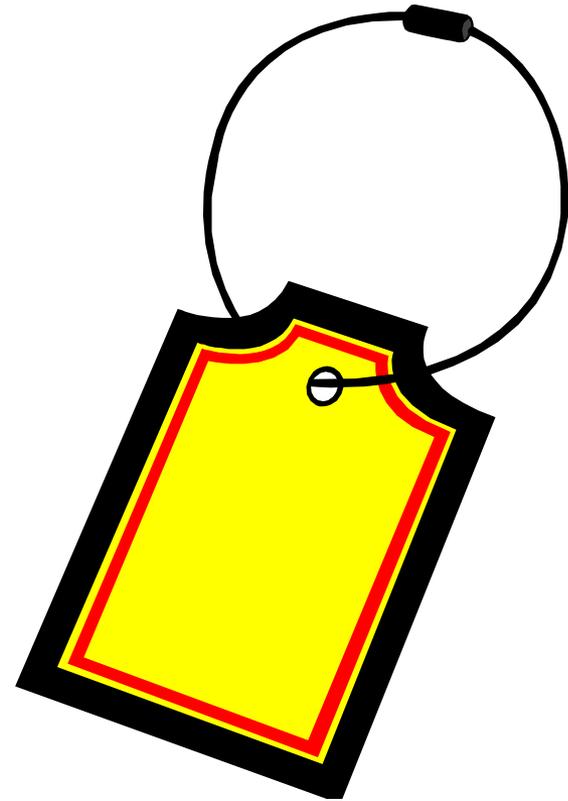
Field Documentation

- Use a copy of the **Field Data Form** for each seed collection made and fill out all the data fields
 - Keep one copy of the completed form for your records and send it whenever you ship seed or vouchers related to the collection
 - Email or send one copy to the SOS National Coordinating Office as soon as possible after the collection has been made to document collection of the species

Seed Collection

Reference Number Format

- Seed Collection Reference Number will include two parts:
 - SOS team code (office mail stop or organization acronym)
 - Collection number



Seed Collection

Reference Number Format

- For example, **OR020-26** for the Burns District Office's 26th collection
- **CBG-25** for the Chicago Botanic Garden's 25th collection
- Seed collection reference numbers should be unique and sequential from year to year, and should never be repeated
 - If the last collection of the previous year was 34, the next year's collection numbering should start with 35

Photos

- Digital photos of the species being collected should always be taken while in the field
- At least three photos should be taken for each collection:
 - Landscape Level/Population
 - Individual plant
 - Material collected (seed)



Photos

- The following naming convention should be used for all SOS photos and each photo should be given a unique picture number (A, B, C, etc):
 - USDA PLANTS Code_Collection Number_Picture Number
- For example Chicago Botanic Garden's collection of *Symphyotrichum lanceolatum* would have photos named the following:
 - SYLA6_CBG-419_A.jpg
 - SYLA6_CBG-419_B.jpg

Post Collection Seed Care

- In general, **keep the seed collections in a cool, dry place**
- Do not freeze seed
- Do not allow collections to overheat
- Do not leave them in a vehicle in full sun



Post Collection Seed Care

- Exposure to sustained high temperatures can badly damage the seed collections
 - Maintain ventilation around the collections at all times and try to park the collecting vehicle in the shade, or at the very least, try to shade the windshield



Post Collection Seed Care

- Damp collections should be spread out on newspaper to dry naturally, either outside in the shade or in a well-ventilated room, as soon as possible



Post Collection Seed Care

Fleshy Fruits

- **Fleshy fruits** may require careful handling and part
- Pack the whole fruits in strong plastic bags with as much air as possible for transport



Post Collection Seed Care

Fleshy Fruits

- Remove as much flesh from the fruits as possible before transit
- This can be done under cool running water using a sieve
- The seeds should then be left to air dry



Post Collection Seed Care

Fleshy Fruits

- Dry carefully on material that will not stick to the seeds
 - Do not use newspaper
- They should then be packed as dry seeds
 - i.e. in cloth bags



Storage

Categories of Seed Storage

- **Orthodox** seeds tolerate removal of water
- **Intermediate** seeds tolerate some water removal
- **Recalcitrant** seeds don't tolerate water removal



Storage

Categories of Seed Storage

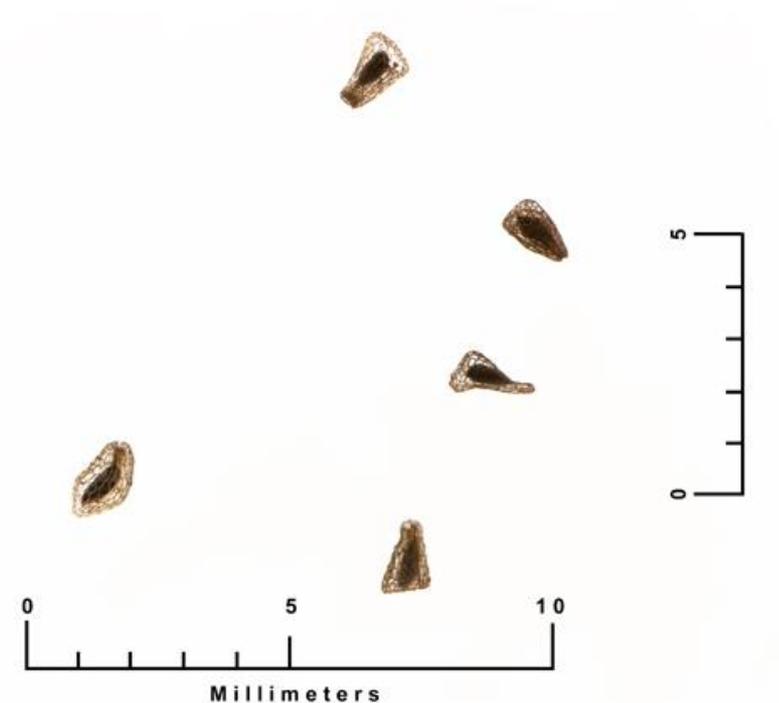
- **Orthodox** seeds can be dried, without damage, to low moisture contents
 - Usually much lower than those they would normally achieve in nature



Storage

Categories of Seed Storage

- Orthodox seed
longevity increases with reductions in both moisture content and temperature
 - In a quantifiable and predictable way
- Can be stored at -20°C



Storage

Categories of Seed Storage

- **Intermediate** seeds tolerate drying to around 40-50% eRH
 - Generally lose viability more rapidly at low temperature
- Cannot be stored at -20°C



Storage

Categories of Seed Storage

- **Recalcitrant** seeds do not survive drying to any large degree
 - Cannot be stored long term



Storage

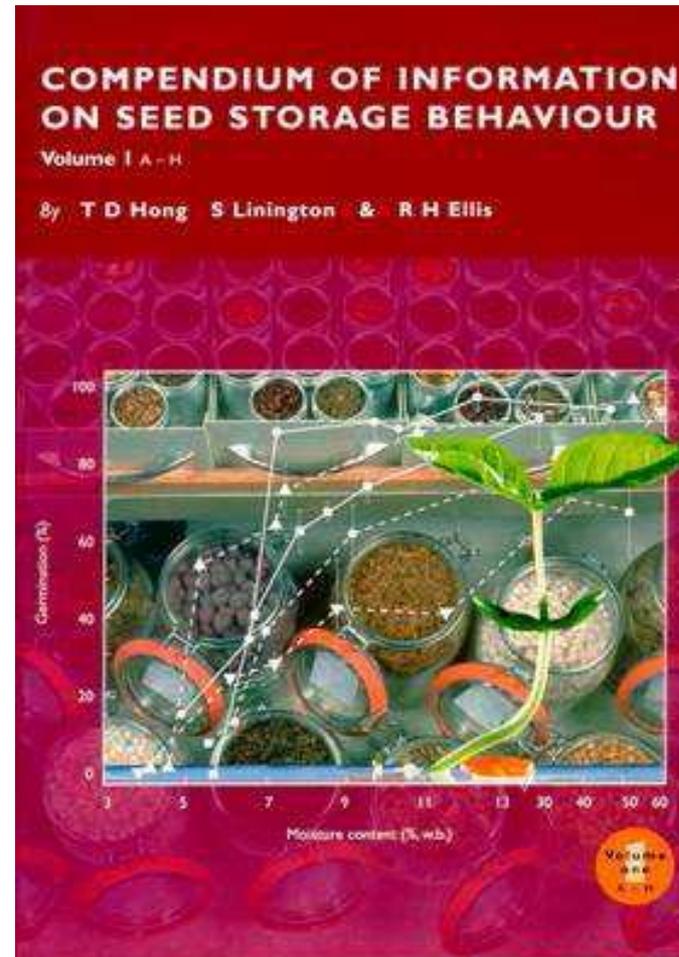
Determining Seed Desiccation Tolerance

- So, how do we know if a species has desiccation intolerant seeds?
 - Taxonomy doesn't really help too much
 - Recalcitrant species are present in 65 families
- **Recalcitrant Groups**
 - Gymnosperms: 12 spp.
 - Dicots: 442 spp.
 - Monocots: 60 spp.

Storage

Determining Seed Desiccation Tolerance

- We can look at the published literature
- ***The Compendium*** lists the seed storage behavior of around 7,000 species



Storage

Determining Seed Desiccation Tolerance

- Kew's Seed Information Database has built upon the Compendium dataset and now includes storage behavior records for >10,647 species

- <http://data.kew.org/sid>

The screenshot displays the Kew Seed Information Database search interface. At the top, there is a breadcrumb trail: "Where am I? > Home > Kew Databases > Seed Information Database". Below this, the page title is "Seed Information Database" and the search prompt is "Search the Seed Information Database". A citation is provided: "Citation: Liu, K., Eastwood, R.J., Flynn, S., Turner, R.M., and Stuppy, W.H. 2008. Seed Information Database (release 7.1, May 2008) <http://www.kew.org/data/sid>".

The search form includes the following fields and options:

- APG Clade:** A dropdown menu.
- APG Order:** A text input field.
- Family:** A text input field with radio buttons for "Use APG" and "Use Brummitt".
- Genus:** A text input field containing "Quercus".
- Species:** A text input field.
- Storage Behaviour:** A dropdown menu set to "(All)".

Below the search form, there are checkboxes for "Only find records with data on:"

- Storage Behaviour
- Weight
- Dispersal
- Germination
- Oil Content
- Protein Content
- Morphology
- Salt Tolerance

Buttons for "Reset" and "Search" are located at the bottom of the search form.

The search results section shows "57 records found." and lists various species of *Quercus* with their associated data fields. The list includes:

- Quercus acuta* Oerst. Recalcitrant?
- Quercus acuticarpa* Camilleri Recalcitrant? 3651g
- Quercus agrifolia* Née Recalcitrant? 2270.46g
- Quercus alba* L. Recalcitrant? 2997g
- Quercus arizonica* Sarg. Uncertain
- Quercus bicolor* Willd. Recalcitrant? 3459g
- Quercus cernis* L. Recalcitrant? 4263g
- Quercus chrysolepis* Liebm. Recalcitrant? 3220.26g
- Quercus coccoloba* Münchh. Recalcitrant? 1931g
- Quercus douglasii* Hook. & Arn. Recalcitrant? 4540.46g
- Quercus dumosa* Nutt. Recalcitrant? 3170g **Disp**
- Quercus ellipsoidalis* Hill Recalcitrant? 1557.93g
- Quercus emoryi* Torr. Intermediate? 191g
- Quercus faginea* Lam. Recalcitrant?
- Quercus falcata* var. *pagodifolia* Elliot Recalcitrant? 783.03g
- Quercus floribunda* Lindl. ex A. Camus Recalcitrant?
- Quercus garryana* Dougl. ex Hook. Recalcitrant? 4369.36g
- Quercus glauca* Thunb. Recalcitrant? 526.32g
- Quercus ilicifolia* L. Recalcitrant? 2311g
- Quercus ilicifolia* Wangerin. Recalcitrant? 648.49g
- Quercus imbricaria* Muhl. Recalcitrant? 1093.99g
- Quercus kelloggii* Newb. Recalcitrant? 4779.8g
- Quercus laevis* Walter Recalcitrant? 1149.67g

Storage

Determining Seed Desiccation Tolerance

- If we analyze that data, we see that the vast majority (89%) of species have desiccation tolerant seeds
- Around 5% are desiccation intolerant



Storage

Determining Seed Desiccation Tolerance

- Remainder are intermediate or with some question marks about their storage behavior
 - It is likely that many of these are in fact orthodox



Storage

Determining Seed Desiccation Tolerance

- To determine seed storage category with certainty, a desiccation screening test must be done
 - Not us



Storage

Predicting Seed Storage Category

- The natural habitat of a particular species can give some indication to its storage behavior
- Typically, the drier the climate, the more desiccation tolerant the species
 - **Recalcitrant** seeds are more common in wet environments



Storage

Predicting Seed Storage Category

- Savannah species are more likely to produce desiccation tolerant seeds
 - In drier habitats, species which shed their seeds during the wet season are more likely to have desiccation sensitive seeds than species that shed seeds during the dry season



Storage

Predicting Seed Storage Category

- Most aquatic species produce orthodox seeds
 - Exceptions are the seagrasses (*Zostera* spp.) and water lilies



Storage

Predicting Seed Storage Category

- Seed morphology provides perhaps the best indicator of storage behavior
 - Desiccation intolerant species tend to have large seeds with 'thin' seed coats



Horse chestnut (*Aesculus hippocastaneum*)

Seed Cleaning

- After a seed collection has been dried it is cleaned to remove empty and poorly-developed seeds, debris and to reduce bulk for effective packaging and storage



Seed Cleaning

- Seed cleaning also reduces the risk of disease
- Releasing the seed unharmed from the diversity of fruit types requires great care and expertise



Seed Cleaning Techniques

Dry Seeds & Fruits

- There are three main techniques for cleaning a seed collection:
- Sieves of different mesh size, and a rubber bung if necessary to separate seeds from bulk material and smaller debris



Seed Cleaning Techniques

Dry Seeds & Fruits

- Using a seed aspirator to remove similarly sized, but lighter/heavier, empty/infested seeds or debris



Seed Cleaning Techniques

Dry Seeds & Fruits

- Hand-sorting, involving piece by piece removal of debris and rubbish



Seed Cleaning Techniques

Fleshy Fruits

- Mature seeds within fleshy fruits may lose viability rapidly, so these collections should be dealt with immediately
 - Treat all fruits as potentially poisonous
 - Wear gloves of a suitable thickness



Seed Cleaning Techniques

Fleshy Fruits

- Open fruits with a sharp knife or scalpel
- Scrape out seeds into a sieve with a mesh size small enough to retain the seeds
 - This operation may be carried out under cool, running water to facilitate seed removal



Seed Cleaning Techniques

Fleshy Fruits

- Wash away any mucilage with warm (never hot) water
- Allow seeds to drain on a nylon mesh or sieve
- Dry slowly under ambient conditions for at least 2 weeks before transferring to a dry room



Seed Cleaning Techniques

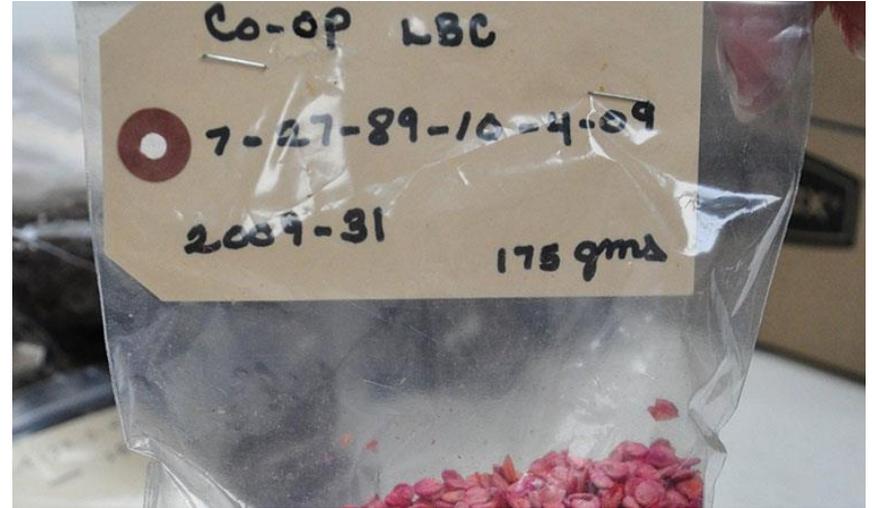
Fleshy Fruits

- Make sure that the collection is clearly labelled
- Do not place wet seeds on paper towels or newspaper as they will be difficult to remove once dry
- After drying, remove any remaining debris using cleaning procedures for dry seed

Seed Cleaning Techniques

After Cleaning

- Label all collections
- Record how long it took to process the collection and include the final cut-test results
- Place the cleaned collection in a dry room or desiccator
- Clean working areas carefully, to prevent cross-contamination



Long Term Storage

- Drying, sealing and freezing will often lead to at least a 100-fold increase in seed storage life



Long Term Storage

- Air dried using desiccant-impregnated dryers to 15% relative humidity maintained at 15°C
 - Seeds lose water to this dry air by diffusion until they are in equilibrium



Long Term Storage

- Few days to several weeks depending on the size of the seeds and other physical characteristics
 - When equilibrium has been reached, the seed is checked for dryness by non-destructive means using a hygrometer



Long Term Storage

- Once dried and processed, the collections are packaged in a variety of different air-tight containers prior to sub-zero storage



Long Term Storage

- We'll be using “vacuum packing technology”



Long Term Storage

- Store in a chest freezer at -20°C





NEXT

FIELD TRIP