

Denver Botanic Gardens

Seed Collection and Processing Protocols



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Introduction

Seed collections provide material for research and *ex situ* conservation in the case of population destruction, extirpation, or extinction. To make seed collections valuable for research and restoration now and into the future, information needs to be collected from each population that allows us to understand baseline data of the collection.

Denver Botanic Gardens partners with the Center for Plant Conservation and the Colorado Rare Plant Initiative to monitor and collect seed from about 70 rare or threatened plant species across Colorado. While rare plant conservation has been a focus of the Gardens for the past couple decades, we are working towards banking seeds of all Colorado native species (rare, common, and in between).

This guide offers collection and data processing instructions for seeds of all species.

The steps outlined in this protocol are designed to achieve three goals:



Goal 1. Take quality data to link with your seed collection.



Goal 2. Make quality and conservative seed collections.



Goal 3. Support the accessioning and storage of a seed collection.

This protocol outlines the steps necessary to make valuable seed collections:

- 1) Identifying the target species and scope of the scouting and collecting trips
- 2) Preparing for the field
- 3) Describing the collection location so that its physical location and habitat characteristics can be well understood by others
- 4) Making a seed collection that does not harm the population with associated descriptors and pictures
- 5) Processing and cleaning the collection

Please note that this protocol focuses on the seed collection process. See ***Field Collecting Protocol for Vascular Plants***, located on the Q Drive, for information about how to collect a voucher specimen (this should be done for all seed collections).

1. Identifying the Target Species and Scope of the Scouting and Collecting Trips

The target species will vary from trip to trip, depending on the blooming and fruiting time of each species. Choose the target species based upon the flowering window (for scouting trips) and the fruiting window (for collecting trips), as well as your goal for the collection (e.g., last year collected, conservation status, distance to site, etc.). Seed collection priority will be for orthodox seeds (seeds that can tolerate drying and freezing conditions of a seed bank).

Scouting trips, while the species is in flower, are beneficial if you have not collected seed from the population in the past. This allows you to more easily identify the species to ensure that the seed you are collecting is what you think it is. A voucher specimen should be collected during the scouting trip.

Some questions you should be able to answer about your collection goals:

- **What is the species of interest?** What are the defining identification characteristics? What is the conservation status? What are the flowering and fruiting windows? Are there any look-alike species that could be confused for the target species?
- **What is the site of interest?** Know the habitat and features of the site. Can it be easily accessed or will it require difficult terrain navigation?

2. Preparing for the Field

Collecting permits and access to private land

- Obtain the necessary collecting permits from county, state or federal agencies
- Secure permission from private landowners
- Carry permits and landowner contact information with you at all times
- Follow all terms and conditions of any granted permits

Reconnaissance of the target site and species

- Research the target species
- Use the Colorado Rare Plant Guide (<http://www.cnhp.colostate.edu/download/projects/rareplants/list.asp?list=master>) and other sources

Colorado Rare Plant Guide

Home | Rare Plant Lists | About The Rare Plant Guide | Information | Colorado Natural Heritage Program

Plant List By Status »
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Master Field Guide List

New profiles can be selected by clicking on the species name. The original 1997 profiles from the Colorado Rare Plant Field Guide (the purple book) are provided as pdfs. Synonyms of scientific names are shown in parentheses. For a complete list of plants tracked by CNHP see [CNHP Tracked Plants](#).
 USFS = USFS Sensitive; BLM = BLM Sensitive

State Scientific Name	State Common Name	Heritage Rank	Feds/State Agency Status	USESA Listing	1997 Profile
<i>Adiantum capillus-veneris</i>	southern maiden-hair	G3			PDF
<i>Aletes humilis</i>	Lanimer aletes	G2G3			PDF
<i>Aletes latifolius</i> (<i>Lomatium latifolium</i>)	Canyonlands aletes	G1G2	BLM		PDF
<i>Aletes lithophilus</i> (<i>Neosaxifraga lithophila</i>)	Rock-loving neoparrya	G3	BLM/USFS		PDF
<i>Alicia sedifolia</i> (<i>Cuba sedifolia</i>)	Stoncrop gilia	G1	USFS		
<i>Ambrosia linearis</i>	plains ragweed	G3			PDF
<i>Amsonia lonchitidis</i>	Jones blue star	G4	BLM		
<i>Anticlia varinatus</i> (<i>Cleodenus varinatus</i>)	Alcove death camas	G2			PDF
<i>Apeis americana</i>	American groundnut	G5			
<i>Aquilegia chrysantha</i> var. <i>rubra</i>	golden columbine	G4T1Q	BLM/USFS		PDF
<i>Aquilegia scopulorum</i> var. <i>marcescens</i>	Mancos columbine	G5THQ			PDF

We've added 31 new species profiles (May 2017)! New profiles can be viewed by clicking on the species name on the [Master List](#) page.
 Your contribution, no matter how small, will help us update the remaining profiles and add the rest of our tracked species. Click [here](#) to support us, and indicate under "Comments" that your gift is to support the rare plant guide.

Related Topics
[CNHP Tracked Plants](#)

Colorado State University
 Disclaimer: Equal Opportunity Privacy Statement

Figure 1. The Colorado Natural Heritage Program provides detailed information on all Colorado rare plant species, as well as the habitat, distribution, and threats and management issues.

- Use Element Occurrence Records provided by the Colorado Natural Heritage Program to find detailed information on populations, including directions to the population, GPS coordinates, size of the population, ownership of the land, etc.
 (Q:\Research\EXTERNAL PARTNERS\CNHP\CNHP Spatial Data\2014 CNHP_Apr2014\Internal\Hyperlink\EO)

Essential collection materials

Preparing supplies for the field is a critical step. If you get caught without the necessary materials for your trip, time and money will be lost. Make a checklist of the following field items before each trip:

- **Physical maps** are a good complement to GPS units
- **Collecting permits** and contact information for landowners
- **Paper lunch bags and coin envelopes** with a pencil for writing all necessary collection information
- **Field notebook** for writing each seed collection and associated data, as well as the associated voucher specimen collection number
- **GPS unit** for marking waypoints of collection locations
- **Camera and camera book** for taking pictures and logging associated data for each collection
- **Hand lens and plant keys** for making identifications in the field
- **Element Occurrence Records** for targeted populations
- **Field press** with a Sharpie attached for writing collection numbers
 - Needed for voucher specimen collections
- **Hori-hori** for digging plants out by the roots
- **Clippers** for collecting branches from shrubs and trees
- **Silica, envelopes, and jewelry tags** for collecting plant tissue

- **Wooden press, straps, blotter paper, cardboard** for transferring specimens from the field press at the end of the day

3. Describing the Collection Location

Physical location

Another person should be able to navigate to any given collection location using the site name and associated information you provide. The information should include political divisions, recognizable landmarks, and precise location information using a GPS. See **Resource 1** for cover data sheet with study site information.

Physical location descriptors:

- Country
- 1st political division, i.e. state
- 2nd political division, i.e. county
- Nearest population center, town, or village
- Directions and distance to the collection site from that town
- Any physical landmarks or landscape features that would help locate the specimen
- GPS coordinates (recorded as decimal degrees), datum and, if possible, uncertainty

Example: USA, Colorado, Phillips County, Frenchman Creek SWA, 5 miles W of Highway 6 and 0.5 miles S of County Road 29. Frenchman Creek SWA is approximately 71 acres and ranges from 1159 m to 1173 m in elevation. 40.59248, -102.39977. WGS84. ~1164 m elevation.

Habitat type

Upon arrival to a study site, take time to familiarize yourself with the **plant community and the substrate** in which the plants are growing (whether terrestrial or aquatic).

- Make note of dominant overstory (trees), midstory (shrubs/sub-shrubs), and understory (herbaceous layer) species.
- Make a physiographic description of the site. This includes **elevation, aspect and slope**.

Example description: Quaking fen dominated by several species of *Carex* grading into upland of shrubby cinquefoil-*Salix* spp. and Quaking aspen-Douglas-Fir-Engelmann Spruce overstory with a mixed forb and grass understory. Low-lying wetland has a thick peat layer that is flooded year-round. Upland soils are of very gravelly sandy loam with 15-30% slopes. Elevation 2750 m.

Summary of minimum required habitat type and physiographic descriptors:

- Plant community type (see **Resource 2** for cover data sheet with plant habitat categories)
- Soil texture (see **Resource 2** for cover data sheet with categories)
- Elevation (from GPS)
- Aspect (from GPS)

- Slope (using a clinometer or visualized along a continuum from flat to a 90-degree angle)

4. Making a Seed Collection

Before collecting

- Make sure that the fruits are mature. Most non-fleshy fruits tend to brown and dry out when they are mature. You can open a fruit and check to see that the fruits are brown and hard, rather than green and fleshy, which would indicate an immature fruit.
- Confirm that collecting seeds from the population will not have negative consequences.
 - No more than 20% of the viable seed of a population of a common species should be collected on any given day. No more than 10% of the viable seed of a population of a rare or threatened species should be collected on any given day.
 - The seed collection should be genetically diverse, meaning that the collection should include seed from 30 or more individual plants (non-clonal). A collection would ideally include seed from 50 or more individuals. If the population is not large enough to achieve this, then do not make a collection, or consider making smaller, multi-year collections.

Collecting seeds

- Collection methods will vary depending on the species
 - The majority of seed collections can be made by stripping or shaking by hand, but some might require cutting or pruning.
 - Dry fruits should be harvested into paper bags or envelopes, while moist, fleshy fruits should be harvested into plastic bags or buckets.
- Collections of rare plants or collections for research purposes should be collected by maternal line. This means that separate coin envelope should be used to collect seeds from each individual plant (i.e., if you collect seed from 30 plants, you should have 30 separate envelopes, with seed from one plant in one envelope).
- Collections of common species can be collected by maternal line or bulked, meaning that all seeds collected from a population can go into one paper bag.
- Sample the population randomly and evenly, making sure to collect from as many plants as possible across the extent of the population
- Keep track of how many individual plants you collect from

- Write down the following information in your notebook and on the seed collection bag or envelope:
 - Species name (if you are sure of identification)
 - Collection date
 - Primary collector's name and any additional collectors' names
 - Site name, including city/county and state
 - GPS points, including elevation
 - Number of plants sampled
 - Include geographic, habitat information (as outlined above), associated species, and approximate size of the population in your notebook



Figure 2. Documenting geographical and ecological information for each collection.

- **Remember to make a voucher and tissue collection with each seed collection. Follow the protocols outlined in *Field Collecting Protocol for Vascular Plants*.**

Taking pictures and recording picture data

Pictures help capture habitat and plant attributes. One person should be responsible for taking photos, recording associated data, and downloading pictures within 24 hours of returning from the field. See **Resource 3** for the workflow associated with taking and processing survey pictures.

- Upon arrival at each site, take several landscape-level pictures that document the habitat type.
 - Capture variation in plant community types
 - Capture variation in topographic and physiographic features
- For each seed collection, take a picture of the plant to capture its habit and features that may be lost during pressing/drying of the voucher.
 - Such pictures can be especially helpful for capturing things like floral tube morphology and coloration.
 - For each picture, enter the photo number, and the information needed to understand what is in the picture. For pictures of plant specimens, you must always put the associated collector and collector number.



Figure 3. Taking pictures that capture plant features, like color and shape, which may get lost when plants are pressed and dried.

5. Post-Collection Handling and Processing

Lab work

- When the collections are brought back from the field, allow them to after-ripen in the field collection bags at room temperature for at least two weeks. This will allow time for slightly immature seeds to mature and dehisce.
- Following after-ripening, the seeds can be cleaned. Seed cleaning methods will vary depending on the species and type of fruit. Finding the cleaning method that works best for the species you are processing might take some time and effort, but there are materials to help. Some of these materials include:
 - Various sizes of sieves (available in the Ecology lab and in the Head House)
 - Textured rubber mats and rubber stoppers
 - Seed boat
 - Forceps
- Once the seed is cleaned, count the number of seeds in the collection. This can be done manually or by using a scale and calculating the number of seeds

Formula for calculating number of seeds:

Average of 5 replicates of 25 seeds = X weight

$X \text{ weight} / 25 = \text{weight of 1 seed}$

$\text{Total weight of collection} / \text{weight of 1 seed} =$
~ number of seeds



Figure 4. Cleaning *Penstemon penlandii* seeds with the help of a textured rubber mat and rubber stopper.



Figure 5. Using the seed boat to clean Asteraceae seeds. This method is useful to remove the pappus on seeds. Sieves can be seen on the left.

Seed Storage

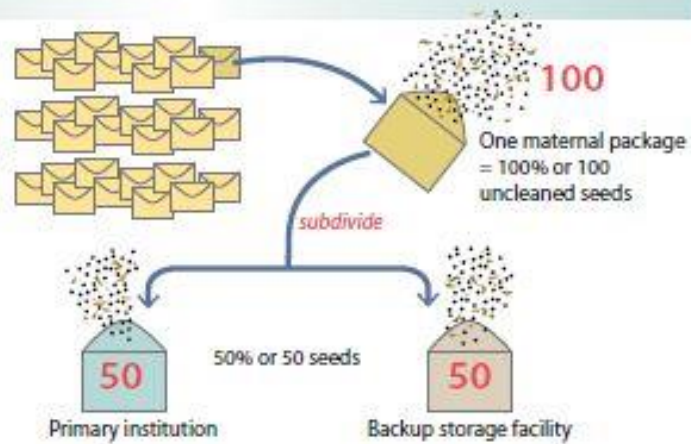
- After the seed is counted and cleaned, the collection will be stored based on the number of seeds in the collection and could be divided among three main types of storage:
 1. **Long-term storage** at DBG, where collections will be sealed in a foil bag, dried at 15°C and 25% relative humidity for at least two weeks, and placed in the freezer.

2. **Back-up long-term storage** at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, CO, where collections will be stored at -20°C.
 3. **Research storage** at DBG, where collections will be sealed and frozen, but will be easily accessible for research purposes.
- Collections should be over 3,000 seeds if split between these three storage locations. If the collection is large enough 40% will go into long-term storage at DBG, 40% will go to NLGRP for back-up storage, and 20% will go into research storage at DBG.
 - If collections are smaller than 3,000 seeds, the entire collection will stay at DBG, and 80% will go into long-term storage and 20% will go into research storage.
 - Maternal line collections: See protocol below from the Center for Plant Conservation

Step 1

Subdivide uncleaned seeds from each maternal line package.

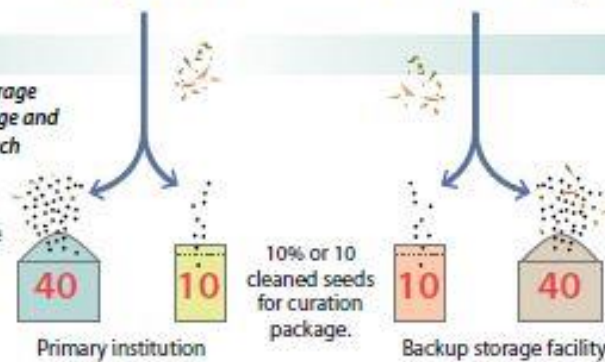
Total accession = packages of seeds from 30 maternal plants



Step 2

Clean seeds from the storage packages to create storage and curation packages for each institution.

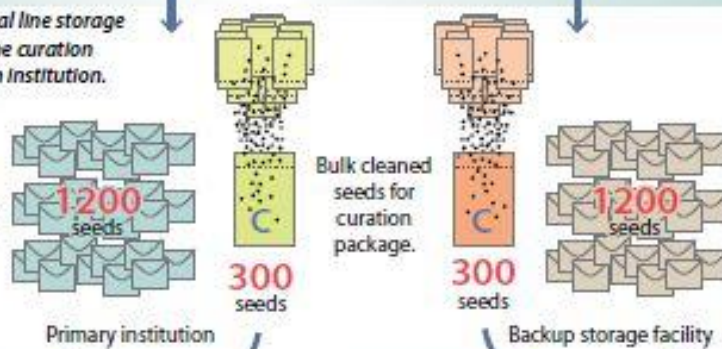
Maternal line storage packages have 40 moderately cleaned seeds.



Step 3

Assemble maternal line storage packages, bulk the curation packages for each institution.

Assemble all storage packages.



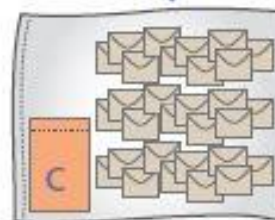
Step 4

Prepare assembled materials for long-term storage.

Dry all packages and store in sealed airtight foil bag.



The primary institution will use the curation package to test germination and propagation.



The backup facility will use the curation package to test baseline viability and longevity in storage.

Additional Resources

Resource 1: Cover data sheet with study site information

Field Work Cover Sheet		
Collector		Date
Associated Collectors		
Study Site Name		
EO Site Number	_____	<input type="checkbox"/> None
Land Ownership		
Owner Type		
<input type="checkbox"/> Private <input type="checkbox"/> USFS <input type="checkbox"/> BLM <input type="checkbox"/> State <input type="checkbox"/> Military <input type="checkbox"/> Indian <input type="checkbox"/> BuRec <input type="checkbox"/> NPS <input type="checkbox"/> Other		
Owner Name (or National Forest, BLM, District, etc.): _____		
Permit:	_____	<input type="checkbox"/> No Permit Required
Location Information		
Survey site name (sub-location)		
State		
County		
Nearest population center, town, or village		
Latitude	Longitude	Slope
Datum	Elevation	Aspect
Directions (Locality)		
Driving and hiking directions and prominent topographical features		

Resource 2: Cover data sheet with habitat information

Ecological Description	
Study and Survey Site Name	Date
Habitat	
<u>Eastern Plains and Foothills Region</u>	
Grasslands: <input type="checkbox"/> Short-grass prairie <input type="checkbox"/> Mid-grass prairie <input type="checkbox"/> Tall-grass prairie <input type="checkbox"/> Foothills grassland	
Shrublands: <input type="checkbox"/> Saline bottomland <input type="checkbox"/> Sand sagebrush prairie <input type="checkbox"/> Mixed foothills shrubland	
Woodlands: <input type="checkbox"/> Pinon pine-juniper woodlands <input type="checkbox"/> Ponderosa pine woodland and savanna	
Wetlands: <input type="checkbox"/> Marshes <input type="checkbox"/> Playas <input type="checkbox"/> Meadows	
Riparian Communities: <input type="checkbox"/> Cottonwood/Willow shrublands and forests	
<u>Rocky Mountain Region</u>	
Grasslands: <input type="checkbox"/> Montane and subalpine grasslands	
Shrublands: <input type="checkbox"/> Sagebrush shrublands	
Woodlands: <input type="checkbox"/> Limber/bristlecone Pine <input type="checkbox"/> Ponderosa Pine	
Forests: <input type="checkbox"/> Douglas-fir <input type="checkbox"/> Lodgepole pine <input type="checkbox"/> Aspen <input type="checkbox"/> Engelmann spruce-subalpine fir	
Alpine tundra: <input type="checkbox"/> Alpine meadows	
Wetlands: <input type="checkbox"/> Marshes <input type="checkbox"/> Playas <input type="checkbox"/> Meadows <input type="checkbox"/> Fens	
Riparian Communities: <input type="checkbox"/> Willow carrs <input type="checkbox"/> Riparian forests <input type="checkbox"/> Alpine riparian communities	
<u>Western Plateau and Canyon Region</u>	
Grasslands: <input type="checkbox"/> Grasslands	
Shrublands: <input type="checkbox"/> Mat saltbush <input type="checkbox"/> Saline bottomland <input type="checkbox"/> Sagebrush <input type="checkbox"/> Mixed mountain shrubland	
Woodlands: <input type="checkbox"/> Pinon pine-juniper woodland <input type="checkbox"/> Pine woodlands	
Wetlands: <input type="checkbox"/> Marshes <input type="checkbox"/> Meadows	
Riparian Communities: <input type="checkbox"/> Cottonwood/Willow Forests	
<u>Disturbed Habitat</u>	
<input type="checkbox"/> Roadside <input type="checkbox"/> Built habitat <input type="checkbox"/> Managed vegetation <input type="checkbox"/> Ruderal vegetation	
Additional habitat/site comments	
<hr/>	
Geology and landform	
Soils: <input type="checkbox"/> Clay <input type="checkbox"/> Silt <input type="checkbox"/> Sand <input type="checkbox"/> Gravel/Pebbles	
Additional soil comments	
Terrain/physiography: <input type="checkbox"/> Flat <input type="checkbox"/> Hill <input type="checkbox"/> Ridge <input type="checkbox"/> Valley <input type="checkbox"/> Saddle <input type="checkbox"/> Depression <input type="checkbox"/> Draw <input type="checkbox"/> Spur <input type="checkbox"/> Cliff	
Additional terrain/physiography comments	
OPTIONAL	
Site history	
Site management practices	
Disturbance history	
Degree of fragmentation or human disturbance (roads, buildings, trails):	
Condition and extent of surrounding landscape	

Resource 3: Plant Identification

A good reference for newer botanists is:

“Field Identification of the 50 most common plant families in temperate regions” located at http://www.sci.sdsu.edu/plants/plantsystematics/Identifying_50_major_plant_families.pdf.

The keys we generally use in the KHD herbarium include:

Ackerfield J. 2015. *Flora of Colorado*. 1st Edition. Fort Worth, Texas: Brit Press.

Flora of North America Editorial Committee (FNA), eds. 1993+. *Flora of North America North of Mexico*. 16+ vols. New York and Oxford.

Weber WA and RC Whitman. *Colorado Flora Eastern Slope: A Field Guide to Vascular Plants*. 4th edition. University of Colorado Press.

Weber WA and RC Whitman. *Colorado Flora Western Slope: A Field Guide to Vascular Plants*. 4th edition. University of Colorado Press.

Resource 4: Taking Pictures

On the Q drive, go to the Interactive Guide to Asset Management. On the Home page choose Digital Asset, Image, Specimen Images, Vascular Plant.