



CHICAGO BOTANIC GARDEN TECHNICAL PROTOCOL FOR THE COLLECTION, STUDY, AND CONSERVATION OF SEEDS FROM NATIVE PLANT SPECIES – *for Seeds of Success*

1. INTRODUCTION

The Chicago Botanic Garden is a founding member of the national *Seeds of Success* (SOS) program, which has undertaken the collection and banking of seed of the flora of the United States. SOS is a conservation partnership undertaken by the Chicago Botanic Garden (CBG), the Bureau of Land Management (BLM), Lady Bird Johnson Wildflower Center, New England Wild Flower Society, New York City Department of Parks and Recreation, North Carolina Botanical Garden, and the Zoological Society of San Diego. The Seeds of Success website can be found at <https://www.blm.gov/programs/natural-resources/native-plant-communities/native-plant-and-seed-material-development/collection>

The purpose of the program is to establish a high quality, accurately identified, and well documented native species seed collection at the population level. Each seed collection should comprise a significant representation of the genetic variation within the sampled population. The collections can act as a basis for off-site (*ex-situ*) conservation and, where and when appropriate, can be used for study and multiplication in the native plant materials development program for restoration purposes.

The Dixon National Tallgrass Prairie Seed Bank at the Chicago Botanic Garden targets collection and banking of seeds from native species in the tallgrass prairie region of the U.S. The initial focus was the preservation of the flora of the tallgrass prairie – an endangered habitat of which less than 0.01% of its former extent remains, but all common habitats within the region are now targeted. By drying the seeds to low moisture content and freezing them in sealed containers, those seeds can remain viable for decades to hundreds of years for many species. A portion of each seed collection is sent to Western Regional Plant Introduction Station (WRPIS) in Pullman, WA which is maintaining both long-term and working collections, which are available for distribution to researchers working on native plant materials development and related topics. Smaller collections are shared with the USDA National Center for Genetic Resources Preservation.

This protocol sets out the procedures for making collections for *Seeds of Success*

2. TARGET SPECIES

The focus of the *Seeds of Success* project at the CBG is the flora of the tallgrass prairie region. Seeds from **one** spontaneous population per species in each of the 12 Omernick Level III ecoregions targeted for collection will be gathered from locations throughout the Midwest and northern Great Plains. Over 500 target species included on our Restoration Target List can be found at <http://www.sciencecollections.org/content/restoration-collection-target-species>. We also accept collections of unique species for which we do not hold collections, but these are a lower priority and collectors should check with the Seed Bank Manager (dsollenberger@chicagobotanic.org) for confirmation to make the collection.

Selection and Ranking Taxa for the Restoration Target List

General criteria used to determine and select taxa important for restoration:

- A. Review the literature (floras, vegetation surveys, etc.) for information indicating the importance of a plant species to the common habitats (prairie, woodland, savanna, marsh, etc.) found in selected ecoregions of the tallgrass prairie region.
- B. Review range maps to confirm the primary distribution of a species occurs within the tallgrass prairie region.
- C. Consider anecdotal information on a species ease of establishment or overall performance in habitat restoration projects.

The taxa selected were ranked with a number **1, 2** or **3** according to the following criteria:

1. This designation was given to conservative taxa that are, according to the literature, considered important to the most common communities (prairie, savanna, marsh) in the ecoregion. Importance may be defined as dominant in or characteristic of a community.
2. This designation was given to conservative taxa that are considered important to a common community as indicated by range maps or other anecdotal information, but not necessarily verified in the literature. Species ranked as a collection 2 priority may also be considered important to a less common community, i.e. fen, riparian flood plain, limestone glade, etc. or they are less conservative taxa but are often used in seed mixes for habitat restoration.
3. This designation was given to less conservative taxa that are considered important to a common community as indicated by range maps or other anecdotal information, but not necessarily verified in the literature. Species with this designation may be considered important to a less common community, i.e. fen, riparian flood plain, limestone glade, etc. or may be used in seed mixes for habitat restoration.

We attempted to be as objective as possible when designating ranks. There was less objectivity involved in designating 3's than 1's and 2's because this designation represented a "catchall" when a species degree of conservatism, importance in the community and/or regional use in habitat restoration came into question. Also, the 3 designation was sometimes given to species known to be conservative in some ecoregions but information other than geographic range data was absent and therefore a 1 or 2 designation was not given. However, there is a possibility of elevating its status if, at some point, more information is provided.

3. SPECIES EXCLUDED FROM THIS PROJECT

The collecting focus of this project is on species needed for restoration and conservation of widespread native species, unless collected under the auspices of an established conservation objective, such as those undertaken by the U.S. Fish & Wildlife Service, the U.S. Forest Service, or the Center for Plant Conservation. The species that are excluded from the project include:

- 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
- 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. (See Appendix 1) BLM Field Office Botanists should carefully coordinate with the CPC Garden that collects in their region to make sure that G3 and S3 species are not overlooked in the collection by both groups, or are not inadvertently collected by both groups
- Any species included in Appendix I of the Convention in the Trade of Endangered Species (CITES)
- Any non-native invasive weed species
- Any agricultural or food crop species that may be growing on BLM lands
- All species in the genus *Quercus*
- All species in the genus *Vitis*
- All known recalcitrant seeds

4. PERMISSION TO COLLECT

CBG staff can assist in obtaining permits for sites that a collector requests. Depending on the land-owner, it may take up to 6 months to get permission, but usually it takes about one month. Use these permits only for collecting seeds for this project please.

5. SAMPLING STRATEGY

For many potential *users* and *uses* of the collection, it is important to maximize the number of alleles present within the sample, by capturing the greatest proportion of those alleles represented in the field population. 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:

1. 30 randomly chosen individuals in a fully outbreeding sexual species, or
2. 59 randomly chosen individuals in a self-fertilizing species.

This analysis suggests that, with care, a single population seed sample collected in this way would possess the potential for re-establishment at that site, and perhaps for establishment at other sites within the natural range of the species.

The reproductive biology of most target species has not been studied, and the capture of rarer alleles would require a markedly increased sample size, so collectors are advised to sample from ***an excess of 50 individuals growing together in a single population*** where available, and to look for populations with a large number of plants.

6. TARGETING THE POPULATION FOR COLLECTION

It is essential that a competent botanist with knowledge of the target species is involved in identifying the most suitable population(s) for sampling. Choosing target populations will be up to the knowledgeable contract botanist. An **“ideal” collection will be from a large number of individuals (between 100 and 500) and will contain between 10,000 and 20,000 seeds,** although we accept collections of 3,000 viable seeds. Collections of 10,000 or greater maximize the flexibility of the collection and allow for a portion of the collection be held at a second seed bank. Maximizing the use of the collection means that:

- sufficient seed is available for germination and viability testing;
- samples are available for supply 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.

Where populations are suitable and the quality and quantity of seed is adequate, it may be possible to make collections of a number of different species from the same site. It is often helpful to make a preliminary visit to the site to assess the populations, to confirm the identification, to estimate the likely harvesting date and potential seed production.

The following points should be considered before harvesting takes place:

- Collectors should try to ensure that the population is of wild origin, not planted or cultivated. For example, 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.
- Seed development can vary within and between populations of the same species. Collectors should take time to monitor seed maturation and to assess insect damage and empty seeds throughout the population before making the seed collection.
- Only regionally common species (no listed threatened or endangered species) should be collected.
- No more than 20% of mature seeds should be harvested **or** if whole fruits are harvested and plants produce a single fruit, the single fruit can be harvested from 20% of the plants producing fruits.
- Seed collections from clonal or rhizomatous species should be made by collecting at widely spaced intervals to increase the chance of sampling from genetically distinct individuals within the population.

There are several species on our restoration collection target list not currently represented in our seed bank because of the difficulty meeting SOS protocols of 50 individuals in a population and/or 10,000 seeds (3000 minimum). Since we would like to receive collections of these species, we felt that a new set of protocols needed to be established to encourage collectors to seek out and make seed collections of small populations and/or populations representing species with naturally low fecundity (reproductive potential or seed set). In order to capture the genetic diversity within these small collections, additional protocols have been developed.

Large collections

1. Bulk collections of at least **10,000 seeds** (3000 seed minimum) from populations of at least **50 individuals** randomly collect across the population without bias for robust, more fecund plants.

Small collections

2. Bulk collections of less than 3000 seeds: Seeds should be collected by **equalizing representation** (collect the same or nearly the same number of seeds from each individual). This method is recommended for species present in numbers of greater than 50 individuals in a population that exhibit naturally low fecundity making it impossible or very difficult to acquire 3000 seeds.

3. Maternal line collections for populations of fewer than 50 individuals: Maternal line collections (seeds from each plant in the population are collected and bagged separately, i.e. for a population of 30 plants, seeds from each plant are placed in separate bags for a total of 30 individual bags) should only be made if there are no known larger populations in the region. **Please contact seed bank staff for approval before making any maternal line collections.**

Assessing populations and selecting a collecting strategy – Outlined in the flowchart below

Scenario: A large population (well over 300 plants) of a species that produces lots of seeds (400-500)/plant. Given 20% of the seeds are collected, 10,000 seeds will easily be attained.

Collecting strategy: Bulk collection.

Scenario: A large population (well over 300 plants) of a species that produces a few seeds (5-10)/plant. Given 20% of the seeds are collected, fewer than 3000 seeds will be attained.

Collecting strategy: Bulk collection equalizing representation.

Scenario: A small population (about 40 plants) of a species that produces either lots of seeds or few seeds/plant. Given 20% of the seeds are collected fewer or greater than 3000 seeds will be attained. No known larger population exists in the region. **Collecting strategy:** Maternal line collection with permission from seed bank staff.

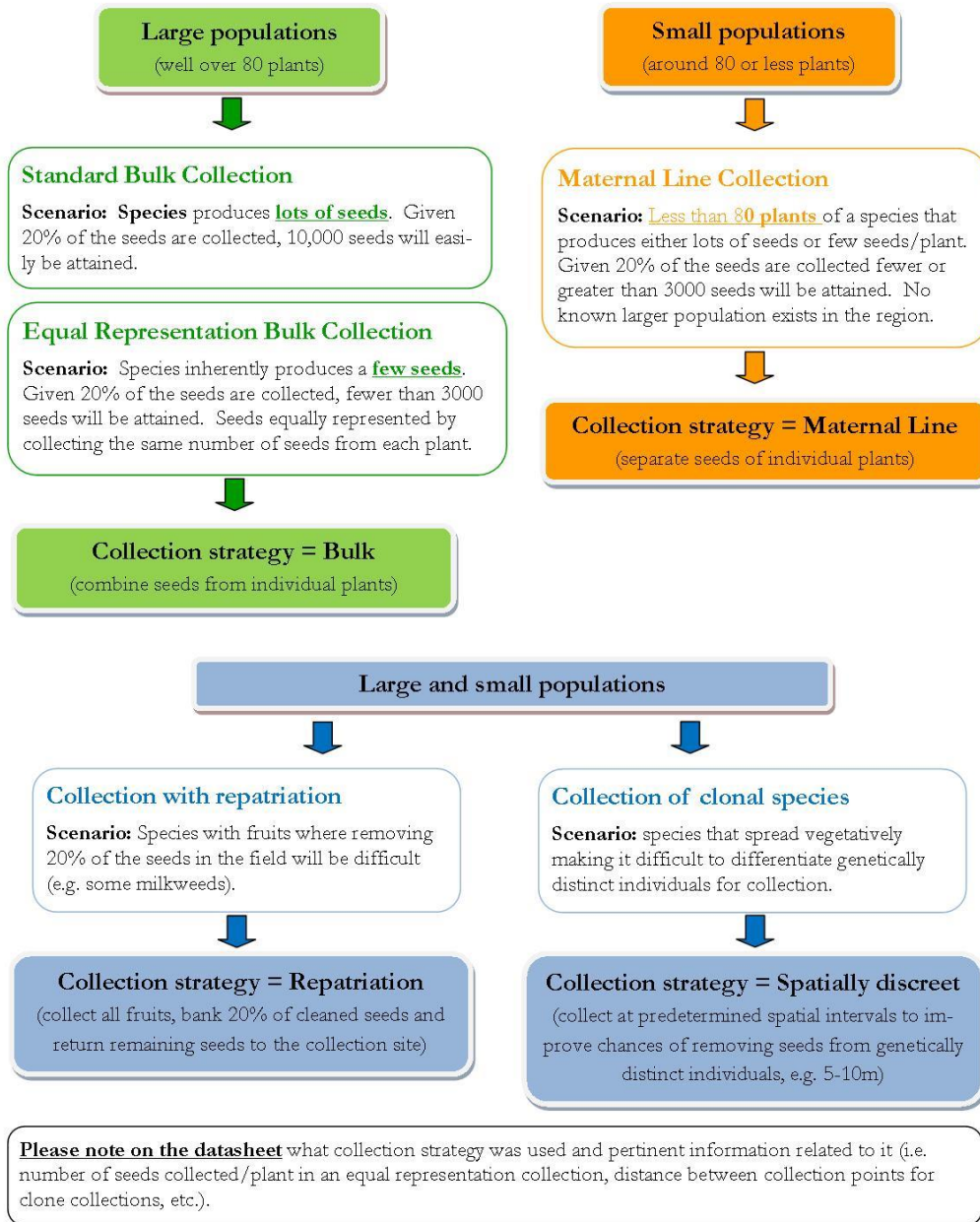
Scenario: A small population (less than 50) of a species with fruits where removing 20% of the seeds in the field will be difficult. Also, fruit production is low so that removing 20% of the fruits in the population will result in a very small sample (example: some milkweeds).

Collecting strategy: Make a maternal line collection of all of the fruits from all plants (instead of just 20% of the plants), remove 20% of the fruits during the cleaning process and repatriate the remaining seeds back to the collection site. Approval of this collecting technique should be received from the owner of the site before proceeding.

If you make a maternal line collection or equalizing representation collection, please note it somewhere on the datasheet. Also, for equalizing representation collections make a note of approximately how many seeds were removed from each plant.

If you are not sure what collecting strategy is best for a potential collection, please contact seed bank staff to assist you in making that decision.

Assessing Populations and Selecting a Collecting Strategy



7. IDENTIFICATION AND HERBARIUM SPECIMENS

It is critical to the value of the seed collections that the species is accurately identified. **A collection is only as good as the information that accompanies it.** Voucher material is essential to enable the accurate identification of seed collections. Collectors are required to collect 2 herbarium voucher specimens for all *Seeds of Success* seed collections and to enter comprehensive identification notes on the field data form. These specimens will be held at Chicago Botanic Garden and the United States National Herbarium where they will be available

for study or for classification by visiting taxonomists. A DNA voucher is also required (see the DNA Collection Protocol for details). Digital photographs, especially of flowers or organs that may be damaged by pressing and drying, are also required and should be sent to dsollenberger@chicagobotanic.org. Electronic copies of the field form should be saved under the correct Latin name, and photo files should use the photo naming convention outlined in Section 9. Do not mount the voucher materials to a herbarium sheet or make a herbarium label for the collection.

Collectors wishing to learn the correct technique for herbarium specimen preparation should accompany an experienced botanist taking specimens in the field. Literature available to consult includes: Bridson and Forman (1992), Radford, Dickison, Massey and Bell, 1974, and Tim Ross (1994).

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. Herbarium specimens must be taken from the exact population earlier in the season (e.g. for the purposes of identification and population monitoring). The herbarium material must truly represent the individuals from which seeds were collected. If a preliminary trip is not made and material for a herbarium voucher specimen is inadequate at seed collection time, collectors need to follow one of the options below:

- Identification is carried out in the field by an acknowledged expert familiar with the species.
- Representative individual(s) of the population are tagged and recorded with GPS so that herbarium specimens can be taken from these individuals in the following season when vegetative and fertile material would be available.

If you need help verifying your specimen, please have colleagues at local or regional herbaria assist you. Please indicate on the field data form that you intend to pass a set of herbarium duplicate specimens to a local taxonomist (together with a copy of the field data form) for verification. Do not assume that all herbaria are willing to provide this service.

Nomenclature will follow Kartesz and Meacham (1999), Synthesis of the North America Flora (<http://www.bonap.org/synth.html>). This is the standard taxonomy used in the USDA PLANTS Database (<http://plants.usda.gov>) and other national databases. Only Kartesz scientific names will be used on the species tracking lists and only Kartesz scientific names should be used on the field data forms. Where subspecies and/or varieties are listed in Kartesz and Meacham, identification should be made 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.

8. SEED COLLECTION TECHNIQUES

Seed collection should follow the outline in the table below.

	Method	Rationale
1.	Assess the target population and confirm that a sufficient number of individual plants (usually 50) have seeds at natural dispersal stage.	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.
2.	Carefully examine a small, representative sample of seeds using a cut test and for smaller seeds a hand lens.	Estimate the frequency of empty or damaged seeds and confirm that the majority of seeds are mature and fully formed.
3.	Collect mature, dry seeds into double-bagged brown paper bags. Large collections can be made using plastic buckets and then transferred into bags.	Ensure the highest possible viability at collection and maximize the potential storage life at the Seed Bank.
4.	In general, cleaning should be left to the Seed Bank staff. If seeds can be liberated from their fruits quickly and easily, by shaking the open fruits over a container, carry this out and note it on the field data form.	Maximize the use of available field time and clean and prepare seeds in controlled laboratory conditions.
5.	Fleshy fruits should be collected directly into plastic bags and allowed to aerate.	Fleshy fruits decompose rapidly and poor storage can lead to mold infested seed collections.
6.	Sample equally and randomly across the extent of the population, maintaining a record of the number of individuals sampled.	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.
7.	Collect no more than 20% of the viable seed available on the day of collection.	Ensure that the sampled population is not over collected and is maintainable.
8.	Collect 10,000 to 20,000 viable seeds.	Enable maximum use and study of the collection.
9.	Collections between 5,000 and 10,000 viable seeds are welcome.	Less use will be made of these collections.
10.	Collections between 3,000 and 5,000 viable seeds are welcome, but distribution opportunities are limited.	These collections will be stored for long-term conservation, but will probably not be available for distribution.
11.	If a population is very small, (less than 20 individuals) harvest and collect from each mother plant separately. Label each sample with a suffix e.g. a, b, c, to the collection number. These will not normally be collected for <i>Seeds of Success</i> , but are acceptable for research and conservation purposes. Collections of this type made on behalf of the U.S. Forest Service Region 9 are highly sought.	Ensure that the full genetic diversity of particularly vulnerable plant populations can be successfully released at a later date. This is useful for plants that are widespread within an ecoregion or habitat, but never occur in large populations.

12.	For each collection, estimate the viable seed production per fruit, per individual and per population, and note these on the field data form.	Document species seed biology and better assess the influence of collecting on the population.
13.	Clearly label all bags with your name, date and latin name. If there are multiple bags, label 1 of 3, 2 of 3, etc.	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.

Some additional information can be found online under Frequently Asked Questions.

9. FIELD DOCUMENTATION

Record information for the seed collection using copied field data forms. Fill out all fields that are in **bold**. Both the form and an explanation of how to complete many of the fields on the collection form can be found on our website. Either email the completed data forms or send them along with the seed collections to the Seed Bank Manager, David Sollenberger.

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:

1. Landscape Level/Population
2. Individual Plant
3. Material Collected (seed)

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):

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, etc.

Send images to the Seed Bank Manager, David Sollenberger, on CD or DVD via FedEx.

10. CARE OF SEED COLLECTIONS AFTER HARVEST

In general, **keep the seed collections in a cool, dry place** (e.g. air conditioned room) prior to sending to the seed bank, but do not freeze them. Take care that seed collections do not overheat, for example by being left in a locked vehicle in full sun. Exposure to such sustained high temperatures can badly damage the seed collections. Try to 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.

If the collection is damp, as soon as possible spread the seeds out on newspaper to dry before dispatching material to CBG. Either dry them outside in the shade or in a well-ventilated room or use a fan to gently blow over the seeds.

In a few cases, where, for example, seeds have been collected fully mature within dry, bulky fruits or capsules, it may be prudent to open the fruits carefully and separate the seed by hand in preparation for shipping. In most cases, it is best to leave the task of cleaning the collections to processing staff that have a range of facilities to carry out this task once the collections arrive at CBG.

Fleshy fruits will require careful handling and rapid dispatch to the seed bank; they may also require partial cleaning: contact the Seed Bank Manager as soon as possible for advice.

11. SHIPPING COLLECTIONS TO CBG

In general, **it is critical to the successful conservation of the seed that it is dispatched to the seed bank within a few days of collection**, together with the completed field data forms, using the FedEx account number provided on the collector's signed contract or by arrangement with the Seed Bank Manager. Voucher photos, DNA specimens and herbarium specimens may be forwarded at a later date, quoting the collectors name and the reference number given to the seed collection, or can be included with the seed collections.

Clearly label seed bags and then securely package them for shipping to CBG. We recommend the box selected for shipping be an adequate size to prevent collections from shifting in transit. Use packing material to prevent this, if necessary. Do not ship seeds in plastic or other non-breathable containers.

Ship to David Sollenberger, Seed Bank Manager at:
Plant Conservation Science Center
Chicago Botanic Garden
1000 Lake Cook Road
Glencoe, IL 60022

11. PROGRAM CONTACTS

11a. Dixon National Tallgrass Prairie Seed Bank Staff

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11b. US National Program Contacts

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