CHICAGO BOTANIC GARDEN
TECHNICAL PROTOCOL
FOR THE COLLECTION, STUDY, AND CONSERVATION OF SEEDS FROM NATIVE PLANT SPECIES
for
SEEDS OF SUCCESS

1. INTRODUCTION

Seeds of Success (SOS) in the United States is a conservation project between the Chicago Botanic Garden (CBG), the Bureau of Land Management (BLM), the Royal Botanic Gardens, Kew (RBG, Kew) and the Plant Conservation Alliance. The Millennium Seed Bank Project (MSBP) is related to *Seeds of Success* in that the collections made for SOS contribute to the international conservation project (http://www.rbgkew.org.uk/msbp/index.html). Often, CBG staff will refer to *Seeds of Success* as the Millennium Seed Bank Project. The official Seeds of Success website can be found at http://www.nps.gov/plants/sos/.

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 Chicago Botanic Garden has agreed to collect seeds from 1,500 native species from 2003-2009. The Garden's 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 habitats are targeted now. By drying the seeds to low moisture content and freezing them in sealed containers, those seeds can remain viable an average of 200 years for many species. Half of each seed collection is sent to RBG, Kew where the seeds are stored in freezers in anticipation of a worst-case scenario from climate change, habitat destruction, etc. The remaining half is split between CBG and the USDA National Center for Genetic Resources Preservation and seeds are available for research and restoration.

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. Seeds from **one** spontaneous population for each species targeted for collection will be gathered from locations throughout the Midwest and northern Great Plains. Target species can be found at http://cbgseedbank.org/targetspecies.html. We are not limited to these species and we can request to make seed collections of species claimed by another U.S. collecting group. See our protocol on how to request a species. Species can only be collected once for the Millennium Seed Bank and only one sample of each species, regardless of variety or subspecies, can be sent to RBG, Kew.

3. SPECIES EXCLUDED FROM THIS PROJECT

The collecting focus of this project is on species needed for restoration and conservation of widespread native species. The species that will be 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 will try and obtain permits for sites that a collector requests. Depending on the land-owning institute, it may take up to 6 months to get permission, but usually it takes a 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.

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 ***in excess of 50 individuals, from within a single population,*** where available and to look for populations with larger numbers of plants.

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.

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.
- Small populations (less than 50 individuals) or those that will yield less than 3,000 viable seeds in a collection following the sampling strategy above should not 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.

7. IDENTIFICATION AND 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. Collectors are required to collect between 2 and 4 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, Royal Botanic Garden, Kew, and United States National Herbarium and other appropriate regional, herbaria where they will be available for study or for classification by visiting taxonomists. Close-up photographs, especially of flowers or organs that may be damaged by pressing and drying, are welcome and should be sent

to msb@chicagobotanic.org. Files should be saved under the correct latin name. 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 seed was 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.
- RBG, Kew may be able to prepare a cultivated voucher from some taxa, with the exception of large shrubs and trees.

If you need help verifying your specimen, please have colleagues at local or regional herbaria verify your specimens. 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 at RBG, Kew.	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 in *Seeds of Success*.	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 the information for the seed collection using copies field data form . 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 or mail the completed data form and send to the project coordinator at CBG as soon as possible to document collection of the species.

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 relatively straight-forward and rapid to open the fruits carefully and to separate the seed by hand ready for shipping. In most cases, it is best to leave the task of cleaning the collections to processing staff who 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 project coordinator 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 *2-day* FedEx or by arrangement with the program coordinator. Voucher photos and herbarium specimens may be forwarded to the appropriate authority at a later date, quoting the collectors name and the reference number given to the seed collection.

Seed bags should be clearly labeled (inside and out) and then securely packaged for shipping to CBG. The following packaging is recommended, either:

- a canvas or thick cotton sealable sack
- woven PVC or nylon air freight sack
- sturdy cardboard box (secured with string to permit customs inspection and resealing) into which cotton (not paper) seed bags have been placed.

Do **not** use the following for shipping to the CBG:

- any non-breathable bags or containers
- made from plastic
- made from PVC backed fabric

Ship to coordinators at:
Betsy Allen and Emily Yates
Institute for Plant Biology and Conservation
Chicago Botanic Garden
1000 Lake Cook Road
Glencoe, IL 60022

11. PROGRAM CONTACTS

11a. CBG Millennium Seed Bank Coordinators

Betsy Allen
Institute for Plant Biology and Conservation
Chicago Botanic Garden
1000 Lake Cook Road
Glencoe, IL 60022
tel: (847) 835-6957
e-mail: ballen@chicagobotanic.org

Emily Yates
Institute for Plant Biology and Conservation
Chicago Botanic Garden
1000 Lake Cook Road
Glencoe, IL 60022
tel: (847) 835-6861
e-mail: eyates@chicagobotanic.org

To contact us both by email please use: msb@chicagobotanic.org
www.cbgseedbank.org

11b. US National Program Contacts

SOS Webmaster
Olivia Kwong
Plant Conservation Alliance/Center for Plant Conservation
(use the same addresses as listed above for Peggy)
Tel: 202-452-0392
Fax: 202-452-7702
Email: plant@plantconservation.org or olivia_kwong@blm.gov

National Collections Data Manager
Mary Byrne
Bureau of Land Management
(use the same addresses as listed above for Peggy)
Tel: 202-452-7767
Fax: 202-452-7702
Email: mary_byrne@blm.gov

11 c. Coordinator for the Americas at RBG, Kew

Michael Way, BSc. MIEEM
Seed Conservation Department
Royal Botanic Gardens, Kew
Wakehurst Place, Ardingly, Haywards Heath
West Sussex, RH17 6TN, UK
Tel: 011+44 1444-894106
Fax: 011+44 1444-894110
Email: m.way@rbgkew.org.uk
http://www.rbgkew.org.uk/seedbank/msb.html