CPC Best Plant Conservation Practices to Support Species Survival in the Wild

Lilium occidentale

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About the Center for Plant Conservation

CPC's mission is to ensure stewardship of imperiled native plants. To do this, we implement the following tested and effective strategy: We advance science-based best practices in plant conservation through our network of conservation partners known as Participating Institutions. Our network actively applies these practices to save plants from extinction here in North America as part of the CPC National Collection of Endangered Plants. We share best practices with conservationists all over the world and advocate for plants and their value to humankind.

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Dedication

In gratitude to all of those working to save plants from extinction

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Acknowledgments

These best practices represent a compilation of previous CPC guidelines with updated recommendations from current research on rare plant conservation. We thank the authors and editors Don Falk, Kent Holsinger, Greg Wieland, Peggy Olwell, Constance Millar, Edward O. Guerrant, Jr., Kay Havens, Mike Maunder, and Kris Haskins for generating *Guidelines for the Management of Orthodox Seeds* (Wieland 1995), *Center for Plant Conservation Sampling Guidelines for Conservation of Endangered Plants* (Falk and Holsinger 1991), *Revised Sampling Guidelines for Conservation Collections of Rare and Endangered Plants* (Guerrant et al. 2004), *Guidelines for Developing a Rare Plant Reintroduction Plan* (CPC 1996), and *Center for Plant Conservation Best Reintroduction Practice Guidelines* (Maschinski et al. 2012). These are the foundation of these consolidated guidelines.

These best practices also incorporate protocols from FAO's *Genebank Standards for Agriculture* (FAO 2014) and *MSBP Seed Conservation Standards* (MSB 2015) and we gratefully acknowledge those contributions to our recommendations.

CPC board members, conservation officers, and guests at CPC National meetings in 2016, 2017, and 2018 met in small groups to discuss and revise components of these guidelines. Many contributed ideas for new considerations needed to conserve rare plants. We are especially indebted to Christina Walters for her insights about practices and possibilities for best storing seeds and Valerie Pence for sharing her expertise about tissue culture and cryopreservation. Sean Hoban and Seana Walsh provided practical advice about maintaining rare plants in living display collections when seed storage isn't possible. Discussions with plant population geneticists Kay Havens, Jeremie Fant, Andrea Kramer, Jennifer Ramp Neal, Christie Edwards, and Stephanie Steele improved and updated genetic guidelines. Armed with analysis of reintroduction meta-data, Matthew Albrecht helped update the reintroduction guidelines. Pati Vitt and Kay Havens made thoughtful recommendations about assisted migration, especially in the face of climate change. Undaunted by non-millennials, Katie Heineman cheerfully steered us into a new age of data management and sharing.

The polished version of these best practices would not have been possible without careful copyediting by Juliana Scott Fein. Robin Mouat infused exceptional TLC to do the format, layout, and illustrations. Thanks to her vast experience illustrating textbooks, she ably translated our ideas into sensible lovely illustrations. Several CPC network conservation officers contributed their beautiful photographs, enriching the aesthetics and sharing their plant conservation experiences.

Joyce Maschinski is grateful to those who have contributed much to her understanding of rare plants, especially current staff members: Stacy Anderson, Joe Davitt, Christa Horn, Katie Heineman, Stephanie Steele, and Tobin Weatherson, and those once in her nest, who have fledged to become amazing plant conservationists: Jan Busco, Joanne Baggs, Joie Goodman, Jimmy Lange, Sheila Murray, Jennifer Possley, Devon Powell, Julissa Roncal, Kristie Wendelberger, and Sam Wright.

Preamble

John R. Clark

Throughout much of human history, banking seeds has been important for human survival. While crops have been domesticated since at least 9500 BCE, humans have been collecting, processing, and storing plants for the winter months for much longer. By some estimates, humans have been harvesting and using seeds for over 20,000 years.

It is truly amazing then that what we do today in plant conservation—collecting and storing seeds for the future—has its basic beginnings in the foundation of humanity itself. It is the essence of life that plants bring to us, and how inextricably linked to plants we all are, that makes what we do in the Center for Plant Conservation (CPC) so vital.

Many challenges faced by our ancestors are faced today by those who work to Save Plants: Have we collected enough seed? Will these seeds survive long enough for us to use them? Have we protected the plants still out there so that more seeds will be available in years to come? These and many other considerations are addressed each year by the Center for Plant Conservation and its network of plant conservation professionals.

The CPC exists to serve these plant conservationists in the effort to Save Plants from extinction.

CPC was founded in 1984 with the guiding principal that the world's plant conservation experts must come together to make a meaningful difference in preserving plant diversity for future generations. A principal goal is to advance the science of plant conservation and to openly and effectively share best practices with as many conservation practitioners as possible. These guidelines are the most recent culmination of our collective efforts.

Beyond just banking seeds, plant conservation is multifaceted and runs the spectrum from monitoring populations in the wild to long term management of species in gardens and/or seed banks to reintroductions to the wild. CPC guidelines

cover this spectrum of efforts and include cutting-edge recommendations on collecting and storing seed, increasing seed from small samples, and also using alternative storage and propagation methods, such as tissue culture and cryopreservation.

Moreover, the current guidelines also include vital information about what a true conservation collection is and how to manage one. Recent advances in genetics now allow us unparalleled opportunities to truly understand genetic diversity and apply this knowledge to capture and store this diversity effectively for the future.

CPC is committed to supporting its Participating Institutions—collectively known as the "CPC Network" or "Network"—by providing technical and scientific expertise both directly and through other CPC Participating Institutions, by advocating for the National Collection and the organizations dedicated to the conservation of plants in the National Collection and through securing and distributing direct funding to support conservation efforts when possible. These guidelines offer a tangible and immensely useful tool for all plant conservation practitioners both in CPC as well as to anyone willing and able to work towards our shared mission to Save Plants.

I wish to thank CPC conservation officers for contributing their expertise to the updates of these guidelines. I know your dedication runs deep not only for the plants we work to save, but also for the people committed saving them. Your work is an inspiration to others.

Finally, thank you to all who will use these guidelines to Save Plants more effectively. Only through repeatable, science-based practice will we be able to Save Plants on a scale meaningful enough given the challenges we face.

Yours in our joint efforts to Save Plants,

John R. Clark
President and CEO
Center for Plant Conservation

A conservation collection *is an ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues, or whole plants of at least 50 unrelated mother plants, and breadth, meaning it consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.*

Preface

Joyce Maschinski and Christina Walters

One in five plant species are at risk of extinction worldwide. Growing concerns for the loss of plant genetic diversity and species' extinctions, as well as advancing know-how to make successful ***conservation collections***, motivates CPC Network scientists to collect seeds from ***wild populations*** and bank them.

The great diversity of plants throughout the world helps define our sense of place and our cultural heritage. Plants have great economic value—providing food, shelter, medicine, and the basis of our livelihoods.

Although humans have been storing seeds for millennia, most of our knowledge about the efficacy of storing seeds derives from plants that have food value. In contrast, we have less knowledge of wild species, and there are great differences in seed traits between crops and ***wild species***. Wild species that are rare and endangered pose challenges to seed banking.

Since 1984, the Center for Plant Conservation (CPC), composed of a network of world-class botanical institutions, has been conducting rare plant species conservation. By 2018, CPC Participating Institutions collectively held over one-third of the globally rare North American plant species (over 1400 taxa) in conservation collections within botanic gardens. In making and using our rare plant collections, CPC scientists have developed expertise and have experienced many triumphs and challenges. Our research on endangered plants has resulted in peer-reviewed publications covering pillars of plant conservation practice including seed storage behavior, germination, propagation, genetics, and reintroduction. For more than 30 years, CPC scientists have collaborated to generate CPC Best Practice Guidelines that form the basis of rigorous plant conservation practice worldwide. We understand nuances of making conservation collections from wild habitats; we document the species' ***germination*** and storage requirements; we adhere to genetic considerations while collecting, maintaining, and reintroducing the species to the wild; and we have expertise in ***reintroduction*** science.

Why Should We Make Conservation Collections of Rare Plant Species?

1. CPC values ***biological diversity*** and believes that preserving the diversity of species and biological communities is essential to human welfare.

2. Preventing untimely extinction of populations and species is possible through conservation actions and stewardship.

3. Rare plant species have special attributes that make them vulnerable to extinction. Our conservation collections help us better understand rare species and ultimately can help sustain species survival in natural settings.

If we are to curb plant extinction, there is an urgent need to involve more people in good conservation practice. Our updated CPC Best Practice Guidelines reflect updated knowledge about best scientific practice. We wish to overcome barriers for botanical institutions seeking to expand their conservation programs by providing the most up-to-date methodology needed to engage in important plant conservation work. The intention of the *CPC Best Plant Conservation Practices to Support Species Survival in the Wild* is to provide an active channel of communication and learning for emerging and seasoned rare plant curators that can lead to widespread understanding and adoption of best practices, as well as discourse when gaps are identified or changes are needed. Ultimately, we know that preventing plant extinction will require more institutions making high-quality rare plant conservation collections in support of species' survival in the wild.

CPC Best Plant Conservation Practices to Support Species Survival in the Wild offer all of us targets we strive to hit in our plant conservation practice. We welcome you to join the conversation and to contribute to the science and to the stories about how practice improves through experience. Please contact the Center for Plant Conservation at info@saveplants.org.

What You Can Find in Each Section

Part 1, Conventional Seed Banking to Support Species Survival in the Wild updates our previously published *CPC Guidelines for the Management of Orthodox Seeds* (Wieland 1995) and incorporates protocols from FAO's *Genebank Standards for Agriculture* (FAO 2014), *MSBP Seed Conservation Standards* (MSB 2015), findings from our research, and the published research from around the world. It provides CPC Participating Institutions and our plant conservation colleagues worldwide updated, practical advice tailored to rare plant seed banking.

Part 1A, Introduction presents a descriptive and graphic overview of steps in the process of conventional seed banking.

Part 1B, Collecting Seeds from Wild Rare Plant Populations describes how to prepare for and make seed collections to maximize the amount of genetic diversity held in the seed collection. Advice for numbers of seeds, numbers of mother plants, and numbers of populations is given.

Part 1C, Splitting Samples for Safety Duplication Storage and Testing describes recommendations for splitting the seed collection for purposes of testing seeds and storing in two locations. Splitting the collection provides a backup in case something like fire or flood destroys one of the portions of the collection.

Part 1D, Cleaning, Processing, Drying, and Storing Orthodox Seeds provides descriptions of how orthodox seeds should be treated before and during storage to maximize their longevity. Specific details on how to adjust and maintain seed moisture and temperature, characteristics of containers needed, and storage temperatures are given.

Part 1E, Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration provides recommendations for actions that can be taken with extremely small samples of fewer than 100 seeds. Often, for extremely rare plants, it will only be possible to collect a few seeds.

Part 2, Alternatives to Conventional Seed Banking. Because not all seeds tolerate freezing for 20 years or more, Part 2 presents guidelines for storing ***non-orthodox*** or ***exceptional species*** that have seeds that do not store well in the freezer. Exceptional species may have seeds that are also known as ***recalcitrant***, which die immediately when ice forms (Walters 2015), or ***intermediate***, which may be able to live for only short periods at freezing temperatures for reasons as yet unknown (Walters 2015). ***Tissue culture***, ***cryogenic storage***, and ***field genebanks*** are suitable alternative methods for ***ex situ*** conservation of exceptional species.

Part 2A, Introduction describes and presents a graphic overview of the three alternatives to conventional seed banking: tissue culture, cryopreservation, and field genebanks. Each technique requires different institutional infrastructure and staff expertise.

Part 2B, Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation offers recommendations for special needs of exceptional species while collecting and maintaining them. Tissue culture and cryogenic storage at temperatures < –150°C are generally more expensive than conventional seed storage and require specialized expertise and facilities. The expense and quantity of genetically diverse individuals that can be reasonably conserved by each method differ and will depend upon institutional capacity. Because little is known about rare species' storage needs using these practices, carefully documented experimentation is important.

Part 2C, Guidelines for Field Genebanks or Inter Situ Collection covers recommendations for whole plant living collections that can be maintained at a CPC institution or on a property that offers long-term security for the collection. Particularly appro-

priate for long-lived species, these provide exposure to natural climatic conditions with additional cultivation support, water, and fertilizer to ensure the collection's health and survival. Field genebanks require adequate space to grow specimens and long-term institutional commitment. Like other conservation collections field genebanks help preserve the genetic diversity of a species. Because small numbers may need to be maintained at any one institution, collaborations and cross-fertilization across sister institutions holding a species can facilitate healthy next generation seed production.

Part 3, Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection presents updated guidelines in a new format that incorporates research and experience of CPC conservation officers. In addition to previous CPC guidelines, *Center for Plant Conservation Sampling Guidelines for Conservation of Endangered Plants* (Falk and Holsinger 1991) and *Revised Sampling Guidelines for Conservation Collections of Rare and Endangered Plants* (Guerrant et al. 2004), new recommendations from current literature are provided.

Part 3A, Introduction presents a graphic overview and justification for gathering, maintaining, and using the maximum diversity possible when making, maintaining, or using a conservation collection.

Part 3B, Genetic Guidelines for Acquiring a Conservation Collection addresses how to approximate good genetic representation while collecting a wild species from a single population or across its range.

Part 3C, Genetic Guidelines for Maintaining a Conservation Collection raises awareness of genetic problems that may arise in an ex situ setting and ways that practitioners may counteract them.

Part 3D, Genetic Guidelines for Using Portions of the Conservation Collection for Reintroductions and Other Purposes presents recommendations to help approximate good genetic representation of plants or seeds used to create new populations. The total number used and the source of the plants or seeds should be maximized for best success.

Part 4, Rare Plant Reintroduction and Other Conservation Translocations provide updates to previous CPC reintroduction guidelines—*Guidelines for Developing a Rare Plant Reintroduction Plan* (CPC 1996)—and *Center for Plant Conservation Best Reintroduction Practice Guidelines* (Maschinski et al. 2012).

Part 4A, Introduction presents a descriptive and graphic overview of the updated guidelines.

Part 4B, Justifying and Deciding Whether to Conduct a Reintroduction or Other Conservation Translocation is fundamental. Whenever practitioners can involve all stakeholders in this process, the greater the chance that all parties will be supportive of the reintroduction and the greater the chance for project success.

Part 4C, Preparing the Reintroduction includes several components: "Making the Plan," "The Law, the Land, and Funding," "Understanding Species' Biology," "Site Selection," "Genetics Considerations," "Source Material and Horticulture," and "Planning for Population Growth." For each component, we provide checklists or questions that aim to help guide planning and improve reintroduction success.

Part 4D. Implementing the Reintroduction describes actions that can help practitioners on the day of the reintroduction.

Part 4E, After the Installation covers the topics of conducting aftercare and designing appropriate monitoring plans. A list of actions that are essential for monitoring is presented.

Part 5, CPC Best Practices for Documentation and Data Sharing provides the fundamentals needed for keeping and sharing information related to rare plant conservation collections.

Part 5A, Introduction describes documentation as the essential representation of the scientific and legal accuracy of our conservation collections. The information held by individuals will only be able to help the collective effort to Save Plants if the information is uploaded into a database where others can use it.

Part 5B, CPC Best Practice for Documentation reviews the documentation needed to retain the highest value of conservation collections. In order for information related to our rare plant collections to be available for internal and external use, it is important for collectors and researchers to document collection information at the time of collection, during care at facilities, and after the species returns to the wild.

Part 5C, "CPC Best Practice for Distributing Samples and Information describes legal and practical decisions related to distributing rare species' samples.

The Glossary provides definitions for the specialized terminology associated with seed science, seed banking, and conservation collections.

The Supplementary Materials section contains original publications and additional background relevant to the specific guidelines.

Where to Go for Additional Help

As you read the sections, you may find unfamiliar terms or concepts that stimulate further questions. ***Boldface italic words*** are defined in the glossary. We encourage newcomers to explore additional explanations we provide in Frequently Asked Questions and the Supplementary Materials section or contact us at info@saveplants.org for personalized assistance.

FAQ

Text in blue within the documents provides a hyperlink to the associated material.

Who Should Use These Guidelines?

Seed science is a rapidly growing field. To keep abreast of changes in technology and practice, the CPC Best Practices will be a "living" document that is reviewed and updated periodically. These guidelines will be helpful to experienced seed collectors, seed banking experts, and newcomers to rare plant conservation.

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Photo Credits

Cover: Edward O. Guerrant, Jr., Endangered *Lilium occidentale* flower. ***Parts 1–5:*** *vectorplusb*, iStock Getty Images, International Standards world map illustration. *Michael Burrell*, Equipment and Sources box, loupe.

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Maureen Wilmot, Vice President, Operations and Advancement of the Center for Plant Conservation, oversees the operations and development activities of CPC. She brings more than 25 years of experience in government and non-profit management, fundraising, communication and outreach, marine science, and environmental policy to the organization. Her goal is help organizations be resilient, sustainable, and effective. She holds a B.S. in Biology from UC Santa Cruz and a M.A. in Marine Policy from University of Rhode Island.

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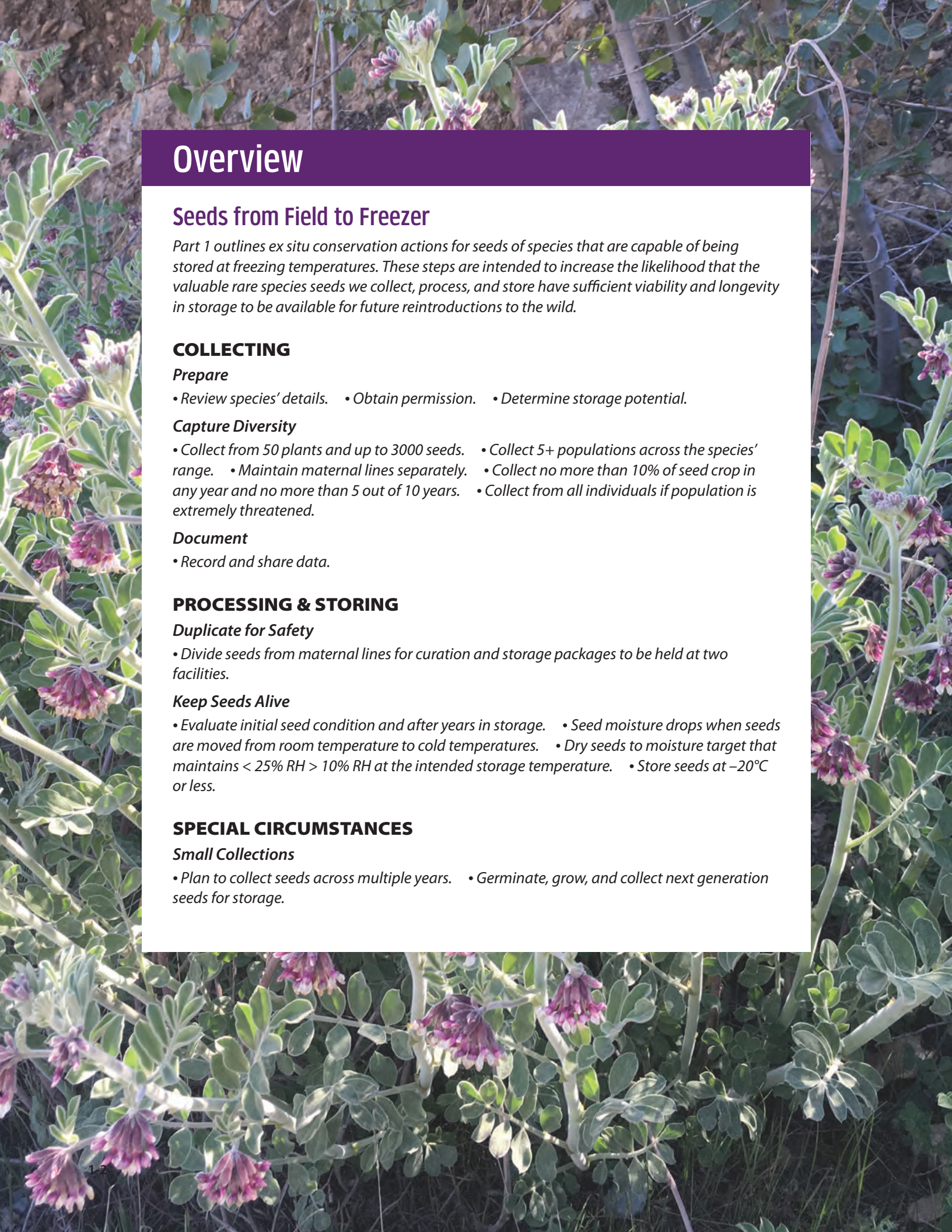
1 Conventional Seed Banking

Conventional Seed Banking to Support Species Survival in the Wild

Center for Plant Conservation Best Practices



Overview

Seeds from Field to Freezer

Part 1 outlines ex situ conservation actions for seeds of species that are capable of being stored at freezing temperatures. These steps are intended to increase the likelihood that the valuable rare species seeds we collect, process, and store have sufficient viability and longevity in storage to be available for future reintroductions to the wild.

COLLECTING

Prepare

• Review species' details. • Obtain permission. • Determine storage potential.

Capture Diversity

• Collect from 50 plants and up to 3000 seeds. • Collect 5+ populations across the species' range. • Maintain maternal lines separately. • Collect no more than 10% of seed crop in any year and no more than 5 out of 10 years. • Collect from all individuals if population is extremely threatened.

Document

• Record and share data.

PROCESSING & STORING

Duplicate for Safety

• Divide seeds from maternal lines for curation and storage packages to be held at two facilities.

Keep Seeds Alive

• Evaluate initial seed condition and after years in storage. • Seed moisture drops when seeds are moved from room temperature to cold temperatures. • Dry seeds to moisture target that maintains < 25% RH > 10% RH at the intended storage temperature. • Store seeds at –20°C or less.

SPECIAL CIRCUMSTANCES

Small Collections

• Plan to collect seeds across multiple years. • Germinate, grow, and collect next generation seeds for storage.

A Introduction

Conventional seed banking is a fundamental plant conservation practice within the CPC network and around the world. In 1995, CPC published *Guidelines for the Management of Orthodox Seeds* (Wieland 1995). These guidelines not only presented practical advice for preserving the genetic diversity of seeds of the rarest plant species in North America based upon the best science of the period, they blossomed from a partnership with the ARS-USDA National Laboratory for Genetic Resources Preservation (formerly the National Seed Storage Laboratory) in Fort Collins, Colorado. The dedicated NLGRP staff, their excellent facilities, and collaborative research with CPC conservation officers have been central to our growing understanding of how to store seed while maintaining ***viability***. While much has stayed the same, this updated version details some of the technological advances that have emerged over the last 25 years.

In addition to using recommendations from our previously published guidelines (Wieland 1995), these updated guidelines incorporate protocols from FAO's *Genebank Standards* for agriculture (FAO 2014), *MSBP Seed Conservation Standards* (MSB 2015), our research, as well as published research from around the world. Note that the international guidelines from FAO and MSBP are broad, encompassing advice for economically important and common species, while Part 1, "Best Practices for Conventional Seed Banking to Support Species Survival in the Wild" pertain to collections of rare wild species and assuring that seeds can live long enough in storage to support a conservation mandate.

Prior to making seed collections, there are several considerations a practitioner can make depending upon the type of plant material that is available. (See "Questions to Ask before Acquiring a Conservation Collection" and "Questions to Ask to Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term.") In some cases, you may need to do a reconnaissance trip to a ***population*** before you will be able to answer the questions about whether seeds are present or not. In other cases, you may need to conduct preliminary laboratory trials to determine whether seeds are viable or not, or desiccation tolerant or not.

Seeds capable of conventional freezer storage (temperatures –18°C + 3°C) are called ***orthodox*** (see "What Is an "Orthodox Seed?"). Conventional freezer storage is relatively inexpensive and highly accessible to many institutions. In most cases, the aim is to store long-term (> 20 years). If seeds are not desiccation tolerant (also known as ***exceptional species, recalcitrant, or intermediate***; see "What Is a 'Recalcitrant' Seed?" and "What Is an 'Intermediate Seed?") or you are only able to collect shoots, rather than seeds, see Part 2, "Alternatives to Conventional Seed Banking."

Several steps are required to bring seeds from the wild into the seed laboratory for processing before placing into cold storage (see Overview). Each are covered in Part 1, "Conventional Seed Banking to Support Species Survival in the Wild," and are intended to help practitioners keep orthodox seeds alive for as long as possible so that the seeds may be used for future ***reintroductions*** to the wild. *We urge practitioners to follow the NEW practices to increase the longevity of seeds in storage.*

What Is an "Orthodox" Seed?

A seed's physical and physiological states determine whether it can be stored by conventional means or not. ***Seed water content***, relative humidity and temperature are key interacting factors contributing to ***longevity*** (Figure 1.1). In order for a seed to be stored and survive freezing temperatures, a prerequisite is that it is capable of surviving ***desiccation*** or removal of most of the water in its cells.

Research from the 1950s and 1960s demonstrated that drying and cooling crop seeds increased survival time (i.e., longevity). This response became known as "orthodox seed behavior" and is described by Harrington's *Thumb Rules*, which state: (1) For each 1% decrease in ***moisture content***, the storage life of the seed is doubled and 2) For each 10°F (5.6 °C) decrease in storage temperature, the storage life of a seed is doubled. Harrington also coined the *Hundred Rule*, which indicates that the storage temperature (°F) and Relative Humidity (%) should add up to less than 100 to achieve safe storage (Harrington 1960). Later Ellis and Roberts (1980) developed ***viability*** equations that predict the proportion of seeds that are viable after storage under a variety of conditions. These have been fundamental to seed conservation predictions for orthodox seeds.

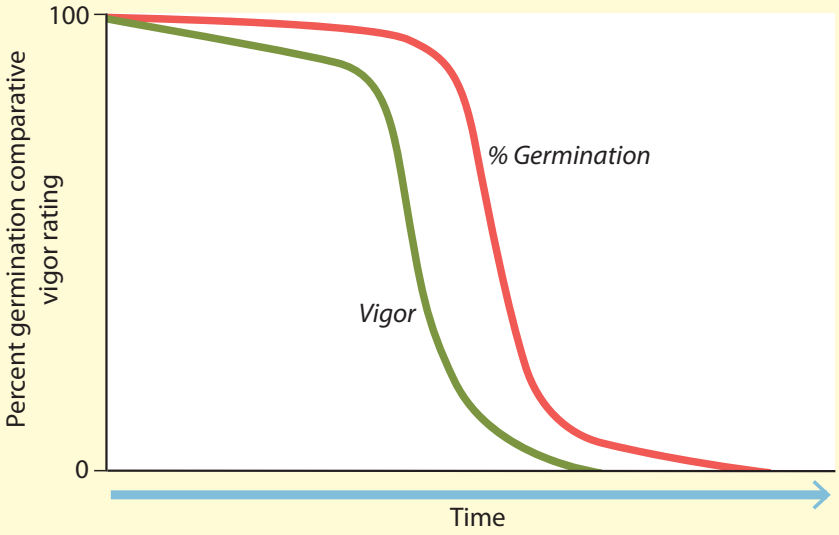


FIGURE 1.1 The decline in vigor and germination of a seed accession over time. The amount of time a seed is viable in storage will be a function of seed characteristics, storage temperature and seed moisture. (*Redrawn from Harrington 1960.*)

What Is a "Recalcitrant" Seed?

Eric Roberts (1973) coined the word 'recalcitrant' to refer to seeds that are not ***orthodox***. While orthodox seeds can be stored conventionally, ***recalcitrant*** seeds cannot be stored conventionally.

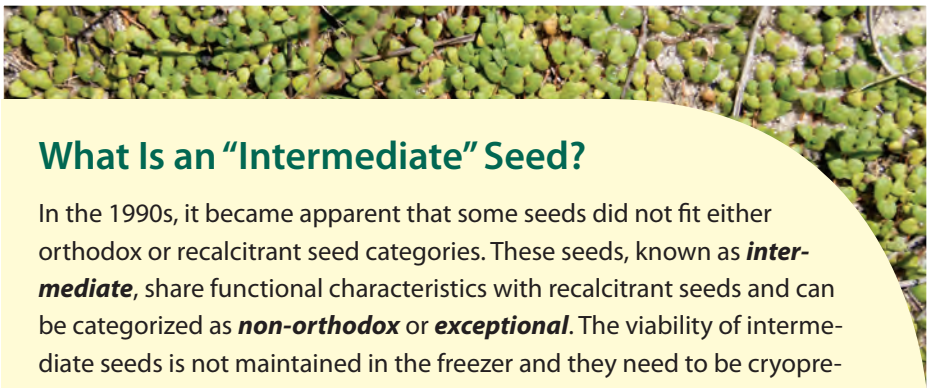
The term "recalcitrant" anthropomorphizes seed responses to water loss. A recalcitrant seed tolerates some water loss, but not the extreme level survived by orthodox seed. Water remaining in recalcitrant seeds forms lethal ice crystal during conventional storage.

Removing embryos from recalcitrant seeds and storing them at liquid nitrogen temperatures is a possible solution to the problem (Al-Zoubi and Normah 2015). Protocols developed for several North American species are labor-intensive but give high survival (Reed 2008). Examples of North American recalcitrant seeds that survive cryopreservation are: *Zizania texana*, *Howellia aquatilis*, *Acer saccharinum*, and *Quercus* sp. (although species native to Mexico have not yet been tested). Hawai'i and Florida may be "hotspots" within the US for seed recalcitrance, and yet we see a low incidence (<5% of species) (for example, Salazar et al. 2017).

"Recalcitrant" is also used to describe a seed that is difficult to germinate, which happens when seeds lack embryos (that is, "empty" seeds), have fastidious germination requirements (that is, ***dormant*** seeds or those with rudimentary embryos), or age quickly (possibly ***intermediate***-type seeds). Some examples of U.S. natives that have been called recalcitrant but survive considerable desiccation are *Helonias bullata*, *Actaea racemosa*, *Asimina tetramera*, *Castanea ozarkensis*, and *Magnolia ashei*.

It is helpful to look at the categories of seed storage physiology for orthodox, recalcitrant, and intermediate seeds in the context of water content and temperature. The interaction between water content and temperature that affects the physical structure of cytoplasm is portrayed in a phase diagram, which includes a vitrification-plasticization curve (Figure 1.2). All seeds have a threshold water content, below which they are damaged by further drying (Walters 2015). At room temperature, orthodox seeds can be dried safely to water contents between 0.03 and 0.08 g H_2O/g total mass (3 to 7%), while safe water content for recalcitrant seeds is above 0.20 g H_2O/ g total mass (> 20%). Intermediate seeds lie in between these two levels (Figure 1.2). Below the lower thresholds, some seeds may experience increased ageing and die (Walters 2015).

Examining seeds in the context of how much cell volume is occupied by water, dry matter and void space is a promising direction for research. Consider the contents of cells within a seed. As seeds mature they accumulate food reserves which replace



What Is an "Intermediate" Seed?

In the 1990s, it became apparent that some seeds did not fit either orthodox or recalcitrant seed categories. These seeds, known as ***intermediate***, share functional characteristics with recalcitrant seeds and can be categorized as ***non-orthodox*** or ***exceptional***. The viability of intermediate seeds is not maintained in the freezer and they need to be cryopreserved in liquid nitrogen. Intermediate seeds can be dried to lower water contents than recalcitrant seeds, and this means that they can be cooled at 30° to 100°C/min (compared to hundreds of degrees per sec for recalcitrant seed tissues) and still avoid lethal ice formation. Unlike recalcitrant seeds where the embryonic axis must be surgically excised to achieve required cooling rates, intermediate seeds can be placed whole into liquid nitrogen, and this process requires less labor and there is usually no impact on survival from the initial exposure to liquid nitrogen.

An early estimate of the incidence of intermediate behavior, about 10%–15% of angiosperms world-wide (Dickie and Pritchard 2002), was based on only one of several syndromes now recognized for the category (Walters 2015). Now we believe there are many more seeds that could be classified as intermediate. Moreover, the tendency to produce intermediate seeds might be a characteristic of a population and its ecology, rather than a species. Intermediate seeds were originally characterized in crop seeds—papaya (Ellis et al. 1991) and coffee (Eira et al. 2006)—but U.S. natives such as several *Cuphea* and *Salix* species also exhibit intermediate traits (Crane et al. 2006; Ballesteros and Pence 2017). We believe between 25% and 50% of endangered species from Hawai'i, especially in *Campanulaceae* and *Gesneraceae*, produce intermediate-type seeds (Walters, Weisenberger and colleagues, unpublished data).

Intermediate seeds are characterized by at least one of the following symptoms:

- Longevity is highest if seeds are dried to between 45 and 65% RH compared to the 15 to 20% RH optimum observed for orthodox seeds.
- Seeds age faster when stored at conventional freezer temperatures compared to refrigerated temperatures. Faster aging might be detected within days, months or years, making it difficult to identify which species' seeds are intermediate.
- Longevity of seeds increases with drying and cooling (as with orthodox seeds), but seeds still age rapidly during conventional storage and will die within about 5 years.

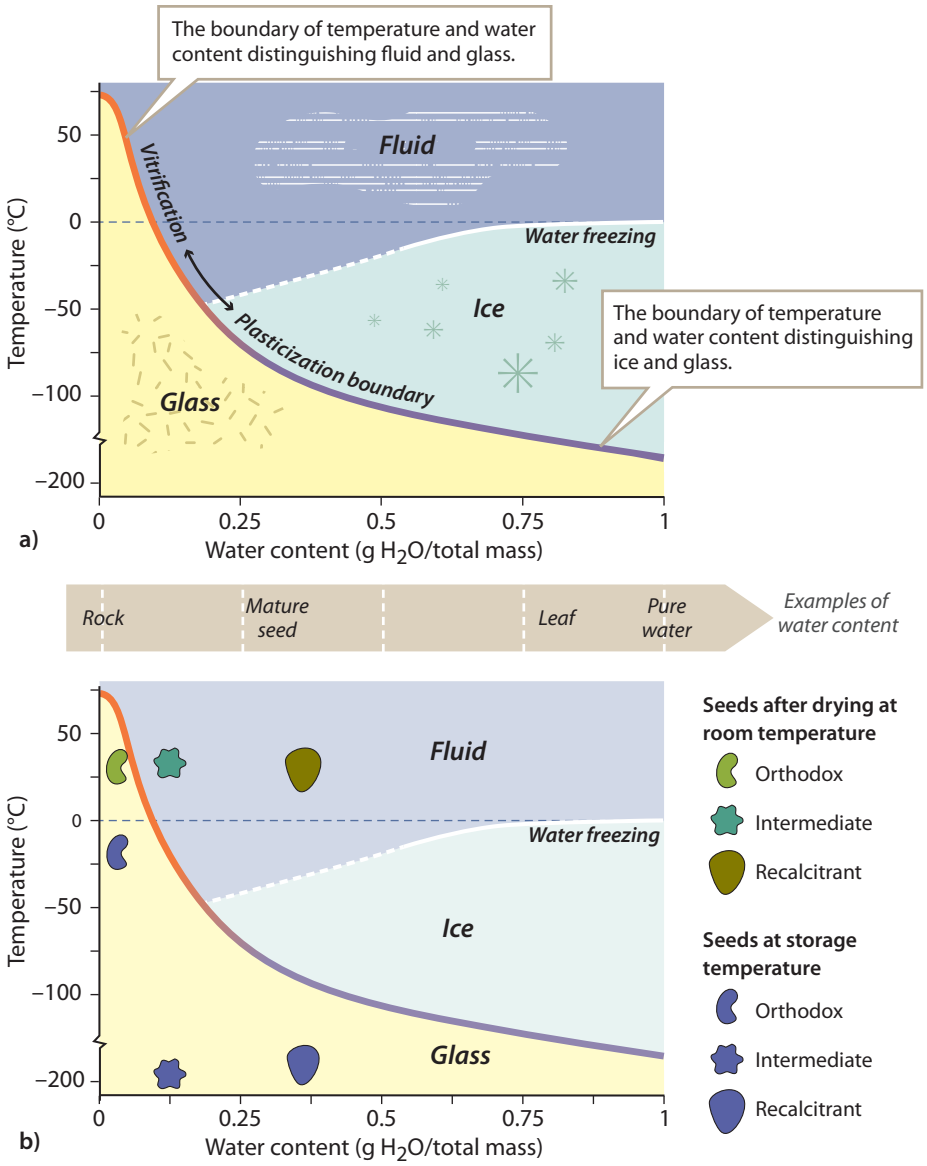


FIGURE 1.2

Orthodox, recalcitrant and intermediate seeds and the vitrification-plasticization curve.

a) Phase diagram showing boundaries between fluid, ice and glass in relation to temperature and water content.

b) Seeds categorized as orthodox, intermediate, or recalcitrant are dried to different water contents. To preserve viability, seeds must be dried and cooled. Note that at conventional freezer temperatures (–20° C), orthodox seeds are in glass, however intermediate and recalcitrant seeds must be cooled rapidly to liquid nitrogen temperatures to be in glass and avoid ice formation.

water and reduce the water content. However the water potential (availability of water to participate in reactions) stays about the same until seeds mature and separate from the maternal plant. No longer having a supply of water, orthodox seeds dry, causing cells to shrink and molecules within the cells to compress. The proportion of cell volume occupied by dry matter at the onset of dehydration can help predict the amount of water loss that can be tolerated by a seed.

To preserve viability during storage, the molecules within a cell must compress until a glass forms (also called ***vitrification***). Orthodox seeds survive to very low water contents and therefore tolerate glass formation; hence, they can be safely preserved in the freezer under conventional storage conditions. In contrast, threshold water contents for recalcitrant and intermediate seeds are higher than the water content required to form a glass. The only way to achieve a glassy state is to cool the cytoplasm profoundly. But, cooling cells containing water is dangerous because lethal ice crystals will likely form. Therefore, preservation methods must cool seeds rapidly to avoid ice formation. For recalcitrant seeds, this can be on the order of hundreds

of degrees per second, and it may require excising the ***embryo*** to make the sample small enough to cool quickly enough. Once the glass forms, the material must be stored at liquid nitrogen temperatures to avoid warming and inducing ice formation. Because rare species' seeds come in all shapes, sizes, and physiologies, we present this physical chemistry perspective to help practitioners understand challenges to preservation and make wise decisions for their seeds.

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B Collecting Seeds from Wild Rare Plant Populations

Center for Plant Conservation Best Practices

Summary

- Species characteristics, legal parameters, and the purpose of the collection influence decisions about timing, locations, and numbers of seeds (or other tissues) that will need to be collected.
- Ethics of doing no harm to the wild rare plant population guide actions in the field.
- The intention of capturing representative genetic diversity guides the number of individuals and number of populations to target for collection.
- Careful documentation is essential for maximizing the value of the conservation collection.

The primary purpose of a conservation collection is to support species' survival and reduce the extinction risk of globally and/or regionally rare species. A ***conservation collection*** is an ***ex situ*** (off-site) collection of seeds, plant tissues, or whole plants that has accurate records of ***provenance***, differentiated maternal lines, and diverse genetic representation of a species' ***wild populations***. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues, or whole plants of at least 50 unrelated mother plants from each population, and *breadth*, meaning that it consists of ***accessions*** from multiple populations across the range of the species. Conservation collections of seeds should have initial ***germination*** and ***viability testing***, developed cultivation protocols, and periodic long-term viability testing.

Make preparations before making collections.

- See "Questions to Ask before Acquiring a Conservation Collection" and "Questions to Ask to Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term."

- Know how to identify the species and know its natural and cultural history. Consult with local botanical experts and agency ***recovery staff.*** Work with in-country partners to make collections. By acquiring data on the species' ***phenology*** at the target ***population*** site, you may streamline your collection trips. Visit publicly available plant collections databases, such as the Global Biodiversity Information Facility (GBIF), to determine the time of year a species typically produces flowers or fruits. Explore citizen science projects, such as iNaturalist, to view ***georeferenced*** photos of plant observations in real time. Take a reconnaissance trip to verify the actual timing of flowering and fruit set so that you can capture seeds when they are ripe. Recording flowering date and maturity of seeds in the population can aid future collections and can be reported with accession information. (See Example Monitoring Form.)

Questions to Ask

Before Acquiring a Conservation Collection

____ Does collecting pose a threat to the wild population?

____ What is the purpose of the collection? Note: These guidelines pertain to conservation collections. Depending on the purpose of the collection, sampling strategy and numbers can vary (Guerrant and Fiedler 2004; Guerrant et al. 2004).

____ Can the ex situ collection be made such that it benefits the species' survival and reduces extinction risk?

____ How many estimated or known numbers of individuals and populations exist? (The sampling universe is known.)

____ What is the breeding system?

____ Is the taxon monoecious or dioecious?

____ Is it self-compatible or self-incompatible?

____ What is the propagule dispersal mechanism?

____ In what types of habitats does the species grow?

____ Should seeds or other tissues be collected? (See "Questions to Ask to Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term.")

____ What is the storage capability of the taxon? Can the seeds be stored in a seed bank or will the other forms of ex situ specialized propagation and care be required?

____ How long will material be stored?

____ How can the plant material be propagated? Do you know the horticulture requirements for growing plants from seeds or cuttings?

____ What level of attrition or mortality of collected material is expected in storage and regeneration? (See Guerrant and Fiedler 2004).

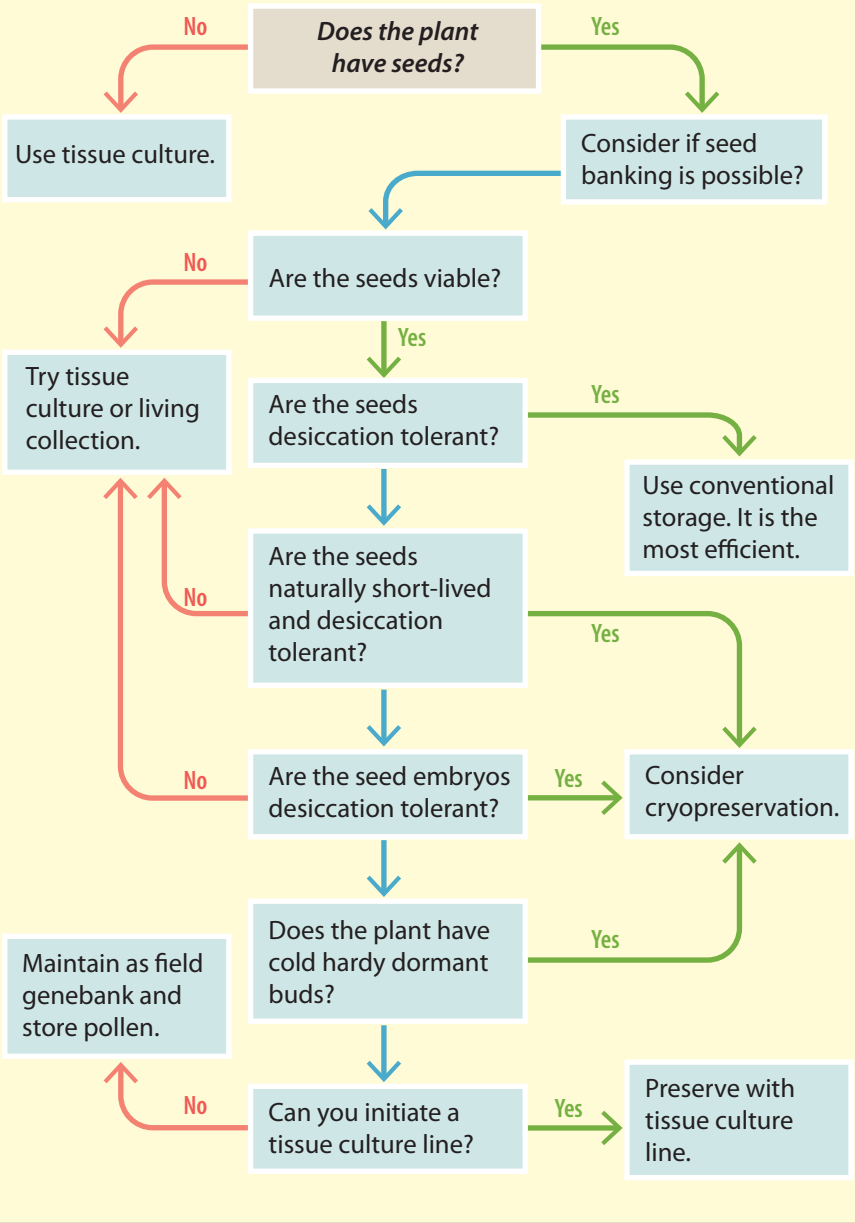
____ Will the material be used for a reintroduction or conservation translocation?

◗ Understand legal obligations for collection, transport, and propagation. Obtain permission from landowners to make seed collections and report permit numbers in accession records. Realize that obtaining permits for listed species may require obtaining permits from landowners and regulatory state and federal agencies; the process may take as long as 6 months to 1 year, so it is important to begin the process well in advance of your collecting season.

FAQ How can I obtain permits to make seed collections?

Questions to Ask

To Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term



- Know sources for gathering reliable information about species' conservation status.

FAQ How can I find out a species conservation status?

- Research the seed storage requirements of a ***taxon***, as this will determine how the seed will need to be processed after collection.

FAQ How do I know my species storage requirements?

- To avoid over collection and/or to learn from other collectors, survey other ***ex situ*** collections to determine what species and populations have already been collected. For example, check records in Plant Search for Botanic Gardens Conservation International (https://www.bgci.org/plant_search.php) or California Plant Rescue (https://www.caplantrescue.org/).

- Participating Institutions can search for priority species for their region in the CPC PI portal (www.saveplants.org/login) on the "Rare Plant Finder" tab.

- Become familiar with ***Sentinel Plant Network*** (http://www.sentinelplantnetwork.org/) and ***Weed Risk Assessments***. Evaluate potential pest/pathogen issues and invasive behavior of the species you are collecting (Gordon and Gantz 2008; Gordon et al. 2008a and 2008b; Reichard et al. 2012).

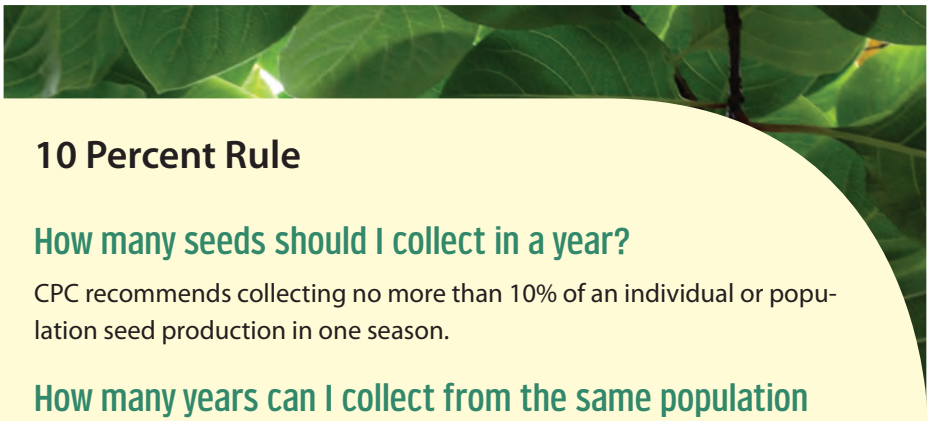
While collecting, do no harm to collecting site or the rare plant population.

- If no previous specimen exists for the species at your collecting site or if the last known specimen is more than 10 years old and the population is large enough to accommodate removing one plant or plant part, document the identification of the species with a ***voucher specimen***. If the population is not large enough, take good photographs. Note that permission to collect the voucher may be required prior to the collection.

- Collect within permit guidelines. To minimize impact on the wild population, collect no more than 10% (or the maximum allowed by permits) of an individual plant's reproductive output and/or no more than 10% of the ***population reproductive output*** in a season (Menges et al. 2004). For many species making collections at this intensity can be sustainable over multiple years, but the intensity and frequency of safe collection is influenced by population and climate specifics (See the "10% Rule" box and Part 3B, "Acquiring a Conservation Collection: Center for Plant Conservation Best Practices").

- Adhere to highest outdoor standards. Leave only footprints. Some habitats are extremely fragile. Adjust actions accordingly, including being mindful of habitats that are particularly sensitive to trampling and erosion.

- Be aware of any ***sensitive animal species*** at your sites. Access may require permits, training, or adjusted timelines if protected animal species co-occur with or near your species of interest.



10 Percent Rule

How many seeds should I collect in a year?

CPC recommends collecting no more than 10% of an individual or population seed production in one season.

How many years can I collect from the same population without doing harm?

CPC recommends collecting no more than 10% of an individual or population seed production in one season and no more than 10 out of 90 years.

The research that supports this recommendation is derived from Menges et al. (2004). Note that it is important to know some aspects of the species population demography, life history, and the initial population size to determine whether your population could be impacted by harvesting 10% of its seed crop in multiple years. If you have enough data, it is possible to generate models to examine the sensitivity of population growth to reduction in fecundity caused by seed harvest. In the absence of this data, realize that generally a population with fewer than 50 individuals will have a higher extinction risk than larger populations. Species that depend on annual fecundity would be most sensitive to harvest. These would be short-lived species (especially annuals) that don't store seeds in a persistent seed bank (Figure 1.3).

Menges et al. (2004) used theoretical modeling in which they categorized 22 species (25 populations with published demographic data) into three types: Extinction Prone, Sensitive I (high initial extinction risk), Sensitive II (low initial extinction risk), and Insensitive. Insensitive species, nine species with populations with 50 or more individuals, could withstand any intensity of harvest over 100 years and had no extinction risk. The insensitive species they modeled were: *Ardisia escallonioides, Calochortus obispoensis, Erythronium elegans, Neodypsis decaryi, Pedicularis furbishiae* at Hamlin, *Primula vulgaris, Themeda triandra*, and *Thrinax radiata*. Note that these species are trees, shrubs, and iteroparous herbaceous perennials. Species categorized as extinction prone had 100% extinction probability with or without seed harvests. They included: *Arabis fecunda, Ariseaema triphyllum, Eupatorium perfoliatum*, and *Pedicularis furbishiae* at St. Francis. The Sensitive I species (*Danthonia sericea* and *Eupatorium resinosum*) were iteroparous herbs with clonal growth that had high extinction risk >40% without seed harvest and increased extinction risk with seed harvest above 10% in 50% of the years, while Sensitive II species (*Arabis fecunda, Astragalus scaphoides, Calathea ovandensis, Dipsacus sylvestris, Fumana procumbens, Heteropogon contortus,*

(The 10 Percent Rule cont.)

Horkelia congesta, Pana quinquefolium, Pedicularis furbishiae, and *Silene regia*) had initially low extinction risk that increased with seed harvest frequency and intensity at levels above 10% harvest in over 10% of years. Frequent low-intensity harvests produced models with lower extinction risk than infrequent high-intensity harvests.

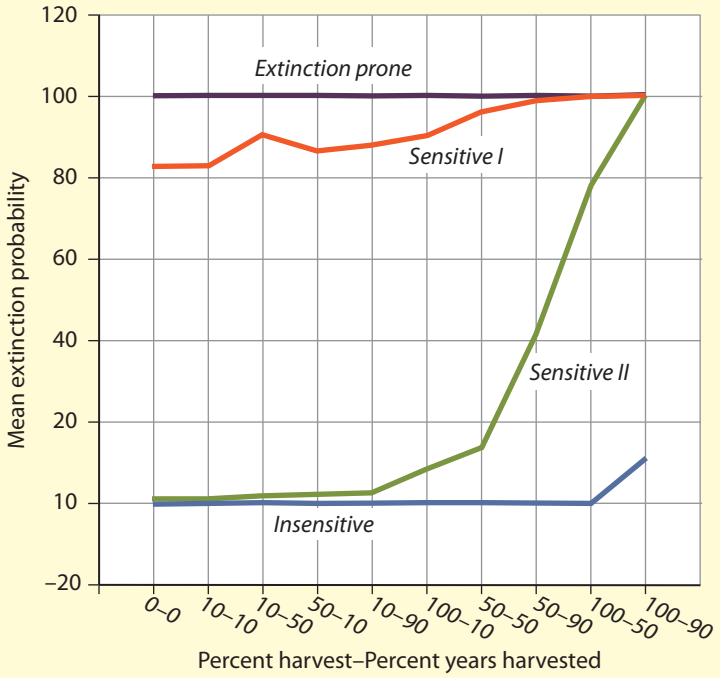


FIGURE 1.3 Extinction percentages by sensitivity class for initial population size of 50. Models conducted with 25 populations of 22 total species. Note 19 of 25 populations can withstand harvest of 10% of seeds in 10 of 90 years.

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Capture representative genetic diversity.

- Capture ***representative genetic diversity*** across the population's spatial expanse and diversity of ***morphology*** and seed appearance. Include seeds from large and small maternal plants, along the edge and from the center of the population. It is also good to sample across years to capture diversity.

- Strive to collect mature seeds.
  - Wild populations will almost invariably have seeds at different stages of maturity. If possible, visit a single site multiple times to collect mature seeds on several dates during the fruiting period. However, if only one harvest is possible (perhaps because a site is remote), collectors should sample representatively. A ***sample*** containing more than 10% ***immature seed*** must be processed expeditiously and should be targeted for cryogenic storage.
  - If possible, collect parallel leaf samples for DNA banking while making seed collections.

Plan your sampling strategy for the collection.

- Use ***population size*** and ***fecundity*** to plan your sampling strategy for the collection.

- An ideal collection would have 3000 seeds from 50 maternal plants for each ***accession***, where an accession is defined as a collection occurring within one plant population, which can be collected over several consecutive days.

Why should I try to collect 3000 seeds? FAQ

- *Ideal is not always reality.* Some populations are simply too small to produce 3000 seeds in a season—or even across multiple years. For the rarest taxa, collecting fewer than 100 seeds may be the only option. If you cannot collect 3000 seeds or seeds from 50 maternal plants, collect no more than 10% of the seed output of a population in a season. Do the best you can.

- Realize that extremely small seed collections (<100 seeds) will require making additional collections in the future. Plan to make additional collections. If more than 300 seeds cannot be collected within 5 years, to increase quantities of seeds in storage, try a seed increase rather than wild collection. See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration" if it is unlikely that a collection can ever surpass 100 seeds or 300 seeds in 5 years.

- Strive to collect from 50 maternal plants (see Part 3, "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection;" Bureau of Land Management 2016).

Why collect from 50 maternal plants? FAQ

- If a population has fewer than 100 individuals and maternal plants produce small numbers of seed, attempt to capture up to 10% of the seeds from each of the reproductive individuals. For larger populations, subsamples are sufficient (see Figure 3.1).

- It is always better to collect and maintain maternal lines because it gives options to equalize family lines in ***reintroductions*** and may add value to potential projects down the line or a use unanticipated at the time of collection. If your species has in-


THE CHEVY BLACK FRIDAY SALE

dividuals that produce fewer than 20 seeds, so that collecting 10% of a maternal line equals one or two seeds, it may be appropriate to bulk the collection, maintaining an equal number of seeds collected from each mother plant.

Seek to capture at least five populations.

◗ If they exist, seek to capture at least five populations of a species, across space and time (Falk and Holsinger 1991). (See Part 3, "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection.")

◗ If it is a widespread species, collect from populations across the distribution and capture each ***ecoregion***.

◗ If it is narrowly distributed, collect from as many populations as possible.

Document the collection appropriately.

◗ Essential accession information includes: institution name, accession number, collector, collection date, species name, family, locality information, georeferenced latitude and longitude, site ownership, permit documentation, and population information (the total number of individuals in the population, number of reproductive individuals, and number of individuals sampled for seeds that were harvested). (See CPC Field Collection Form.)

- Providing habitat information may provide clues to germination or tissue culture requirements of the species. Recommended fields include light and moisture conditions, soil type, slope orientation, and associated species. Provide photos of the habitat and the plant in its habitat.
- Be sure to document any associated collections (for example, leaf litter, soil, mycorrhizal fungi) and maintain the link through processing of samples.
- Gather and report additional accession data according to their institutional protocols. Complying with International Transfer Format for Botanic Garden Plant Records (https://www.biodiversitylibrary.org/bibliography/45427#/summary) and/or Darwin Core standards (http://rs.tdwg.org/dwc/) will allow easy transfer of information to partners.
- Complete one field form per accession. Multiple accession numbers and field forms only need to be created for collections made from populations, which are separated by at least 1 kilometer.
- Transmit accession data to CPC and ARS-USDA National Laboratory for Genetic Resources Preservation (NLGRP) via online form provided to Participating Institutions through the CPC PI portal, which can be accessed at www.saveplants.org/login. Once inside the PI portal, the electronic accession form can be found under the "NLGRP" tab.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Acquisition of Germplasm

4.1.1 All seed samples added to the genebank collection have been acquired legally with relevant technical documentation.

4.1.2 Seed collecting should be made as close as possible to the time of maturation and prior to natural seed dispersal, avoiding potential genetic contamination, to ensure maximum seed quality.

4.1.3 To maximize seed quality, the period between seed collecting and transfer to a controlled drying environment should be within 3 to 5 days or as short as possible, bearing in mind that seeds should not be exposed to high temperatures and intense light and that some species may have immature seeds that require time after harvest to achieve embryo maturation.

4.1.4 All seed samples should be accompanied by at least a minimum of associated data as detailed in the FAO/Bioversity multi-crop passport descriptors.

4.1.5 The minimum number of plants from which seeds should be collected is between 30-60 plants, depending on the breeding system of the target species.

MSB Partnership Collections *(Millennium Seed Bank Partnership 2015)*

Collecting

Seed, herbarium vouchers, and data are collected to recognised protocols or guidelines:

1.1 Genetic materials, including traditional knowledge, are legally collected and conserved.

1.2 Collection names are verified (ideally by reference to herbarium voucher specimen).

1.3 Genetic diversity of sampled population is adequately represented.

1.4 Essential field data is recorded.

1.5 Survival of source population is not compromised.

FAQ Frequently Asked Questions

How can I obtain permits to make seed collections? The first step is to check the ***species conservation status*** and the ownership of the lands where the species occurs. If your target species is listed under the Endangered Species Act, check with your local U.S. Fish and Wildlife Service (USFWS) office. Depending upon the land ownership where the species occurs, you may or may not need to have a USFWS permit, but it is always good to have a discussion with the recovery staff before you make a collection. If the species is not federally listed but is protected by your state, contact the state agency that issues collecting permits. Google "collecting permit rare species STATE NAME." If your target species grows on private land, it is also good to get written permission from the landowner before making the collection.

How can I find out a species conservation status? Reliable sources for determining a species conservation status include: U.S. Fish and Wildlife Service (www.fws.gov), Nature Serve (www.natureserve.org/), The Institute for Regional Conservation (http://regionalconservation.org/), California Native Plant Society (http://www.cnps.org/cnps/rareplants/rareplantdata.php), and state natural heritage programs.

How do I know my species storage requirements? See "Determining Storage Requirements" box.

Why should I try to collect 3000 seeds? Collections of 3000 seeds 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; and a substantial amount of seed can be conserved as a long-term safeguard against loss of the wild population (Bureau of Land Management 2016).

Why should I try to sample from 50 maternal plants? The 50 maternal lines recommendation is supported by the sampling strategy from the Bureau of Land Management Technical Protocol for the Collection, Study, and Conservation of Seeds from Native Plant Species for Seeds of Success (2016).

For many potential users of and uses for the collection, it is important to maximize the number of ***alleles*** (variants of genes) present within the sample by capturing the greatest proportion of those alleles represented in the field population. The number of different alleles in a population reflects its genetic diversity. Sampling from (1) 30 randomly chosen individuals in a fully ***outcrossing***, or ***outbreeding***, sexual species, or (2) 59 randomly chosen individuals in a ***self-fertilizing*** species will capture at least one copy of 95% of the population's alleles which have frequencies of at least 0.05 (Brown and Marshall 1995).

Determining Storage Requirements

Several authors have examined patterns in seed storage behavior (see references) that can help collectors. Begin with a literature review to check if any previous research has been done on your taxon. You can check congeners, but beware that this is not always reliable or conclusive. Our Hawaiian colleagues have found quite varying storage behavior within a single genus (Walters, Weisenberger and Clark, personal communications). Many factors determine variation in seed tolerance to desiccation or freezing. The following are some general patterns observed in seeds that tend to withstand orthodox storage or not.

Trait	Likely to Be Orthodox (Desiccation and Freezing Tolerant)	Questionable Tolerance to Orthodox Storage
Habitat	Arid is especially likely; If it is not growing in a wetland, it is is likely	Wetland, riparian
Conditions in nature	Seeds normally experience dry down and/or hard freezes	Seeds normally remain moist and do not experience hard freezes.
Season of seed production	Not spring	Spring
Life form	Not tree	Trees
Seed bank	Persistent	Not persistent
Dormancy	With dormancy	No dormancy
Seed moisture content at time of maturation	Dry when it is naturally shed from plant	High (30%–70%)
Seed size		Very large (avocado seeds aren't desiccation tolerant) or very small (orchid seeds and fern spores require storage in liquid nitrogen)

Plant Groups with High Proportion of Desiccation Sensitive Seeds		Plant Groups with Predominantly Orthodox Seeds
ANITAGrade	*Malpighiales*	*Solanaceae*
Arecales	*Myrtales*	*Poaceae*
Ericales	*Orchidaceae*	*Asteraceae*
Fagales	*Oxalidales*	*Brassicaceae*
Icacinales	*Santalales*	
Laurales	*Salicaceae*	
Magnoliales	*Sapindale*	

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 very rare 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.

For research purposes, and for the conservation of rare species that occur in populations of fewer than 50 individuals, as well as for less fecund common species, where collections will result in fewer than 3000 seeds, we recommend collecting seeds along maternal lines. In a maternal line collection, seeds from each individual plant (maternal line) are collected and bagged separately (as opposed to bulking the seed collection of multiple plants into one or two bags as we do for regular seed bank collections). This ensures the greatest genetic diversity is available in a small collection. When we combine seeds in a bulk collection, there is only a small chance any particular parent's offspring will be represented when a portion of the collection is removed to restore a new population. For small populations or small seed collections, collecting along maternal lines allows equal representation of all parent's offspring (seeds) in a newly restored population, provided a portion of the seeds are distributed equally from each maternal line (Bureau of Land Management 2016).

What should I do if I was unable to collect more than 100 seeds?
See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration."

Will it be damaging to collect 10% of the seed production in sequential years? See the "10% Rule."

Equipment List

- GPS
- Clippers
- Marker
- Notebook
- Tweezers
- Collecting envelopes (coin envelopes or glassine envelopes)
- Paper bags
- Camera
- Glue sticks or tape to seal envelopes
- Flagging to mark individuals in the field

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C Splitting Samples for Safety Duplication Storage and Testing

Center for Plant Conservation Best Practices

Summary

- The old adage "Don't put all your eggs in one basket" applies to seed banking. Divide each accession and store each half at a different safe seed banking facility.
- Create curation packages to place inside storage packages. Seeds in curation packages can be used for testing initial and long-term viability. Seeds in storage packages are intended to be stored long-term.
- Understand the rules of your storage facility and storage agreement.
- Make a collection that is as large as is feasible to account for testing needs and future conservation actions.
- For small seed collections, it may be necessary to grow plants and collect next generation seeds for testing and storing.

CPC seed collections are valuable for the conservation of rare plants. CPC recommends dividing collections to ensure that the representative samples of seeds will be safely duplicated to mitigate for loss caused by natural or human-caused catastrophes. Divisions into ***curation packages*** enable us to protect the ***long-term storage packages*** while we gain information about seed characters, their cultivation, and storage capacity.

Determine storage facilities.

- For each ***accession***, determine the ***primary*** and ***backup facilities*** for long-term storage. The backup facility should be geographically separate, politically stable, and relatively safe from natural catastrophe with the same or better conditions as those of the primary seed bank.

Plan for safety duplication agreements.

- Any ***safety duplication*** arrangement requires a clearly signed, legal agreement between the depositor and backup institution.

- The agreement should clearly detail the responsibilities of the parties and terms and conditions under which the material is maintained.

- The primary site long-term storage institution may be a CPC institution and the backup institution can be the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado. CPC has a ***Material Transfer Research Agreement (MTRA)***. Online form is available at www.saveplants.org/login to CPC Partici-

pating Institutions. The MTRA covers CPC Participating Institution seed accessions transferred to and stored for research purposes at NLGRP. Each Participating Institution may also develop a separate ***black-box storage*** agreement with NLGRP. See "CPC Participating Institution Agreement for Seed Banking and Data Sharing at National Laboratory for Genetic Resources Preservation (NLGRP)," page S3.

A portion of each accession will be used to gather information about percent germination, propagation, and longevity in storage.

- This is one of the reasons for making a collection that is as large as is feasible (ideally 3000 seeds).

For collections with fewer than 100 seeds, there are special considerations.

- Recognize that a small collection will require more work to have the best conservation value. Attrition or mortality of a portion of any seed collection should be expected after seeds are stored, germinated, or grown in a greenhouse (Guerrant and Fiedler 2004, Guerrant et al. 2004). Starting a ***conservation collection*** with as many seeds as is feasible helps ensure that adequate genetic diversity remains for conservation actions.

- Use the seeds for propagation trials and seed bulking. Collect and store the first generation offspring (***F_1 generation***). Note that this will require meticulous conservation horticultural care and require high maintenance. At your institution, assess your ability to maintain meticulous labels and records of ***maternal lines***. Engage another CPC Participating Institution or partner willing to house and manage a nursery collection if necessary. See Part1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration."

If I have maternal line samples with fewer than 50 seeds, should I divide these low-producing lines? FAQ

- Schedule additional seed collections from the wild population to increase the total number of seeds that can be used for storage and testing.

- Consider collecting tissue for tissue culture. Engage a partner with expertise in tissue culture if necessary.

For collections with greater than 100 seeds, divide the sample.

- Divide to accommodate long-term storage, testing, and ***sample*** maintenance.

- Divide each maternal line into packages for storage and curation: (1) primary institution long-term storage, (2) backup institution long-term storage, (3) curation package for primary institution, and (4) curation package for backup institution (Figure1.4).

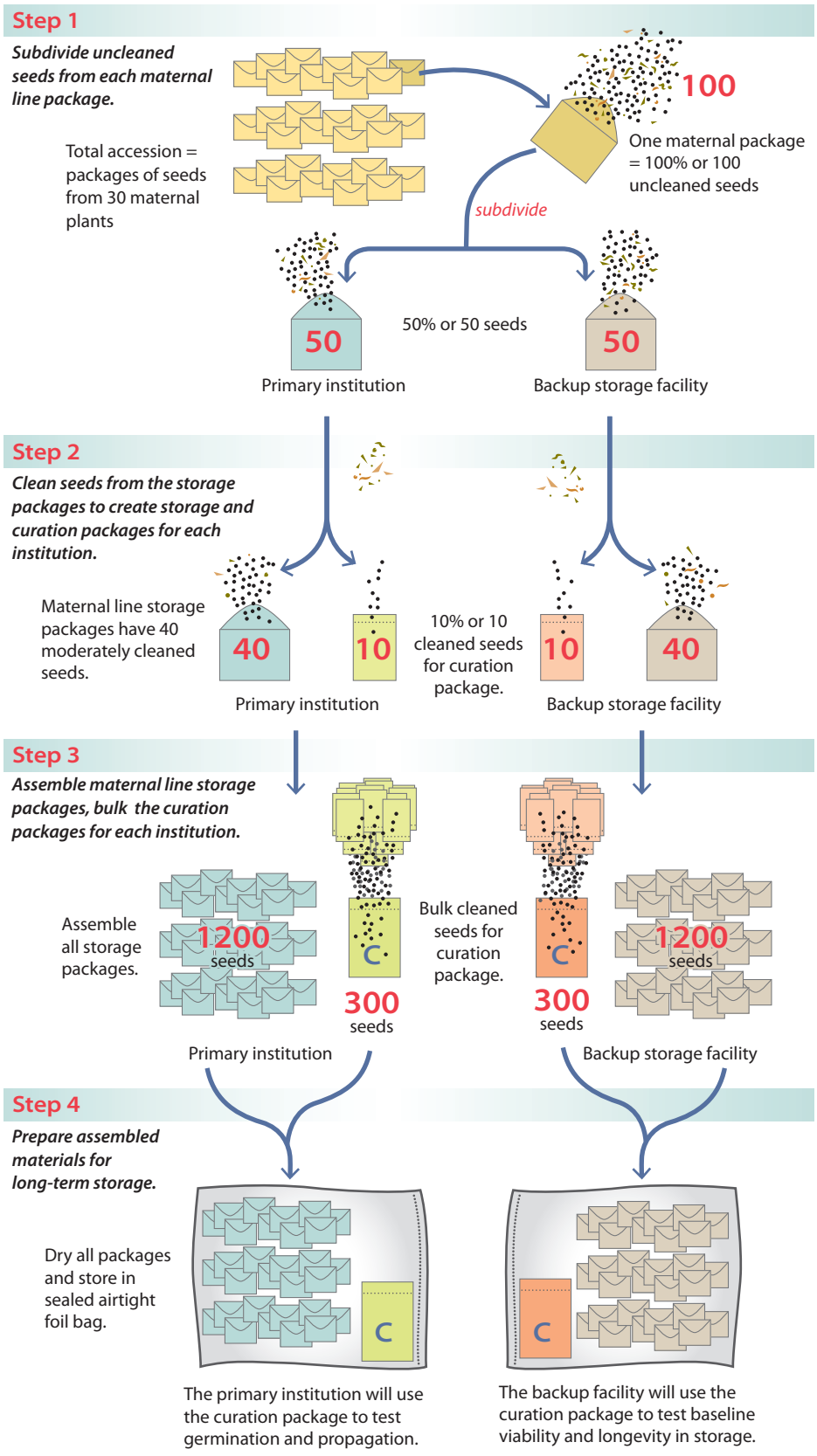

FIGURE 1.4

Steps for splitting accessions by maternal line for duplicate storage and testing.

◗ For long-term storage, place approximately 40% of the seeds of each maternal line into a package for primary and backup institution (see steps 1 and 2 in Figure 1.4). Seeds may be moderately cleaned to reduce processing time. All separate maternal line packages should be placed into a large package that will contain all maternal lines of an accession. Each envelope should be marked clearly and neatly on the lower portion of the envelope with the accession number and maternal line number (for example, 2018-0075, #32).

◗ Curation packages of cleaned seeds containing approximately 10% of total accession are needed for primary and backup institutions (see steps 3 and 4 in Figure 1.4). The seeds from maternal lines should be bulked in the curation package for NLGRP with even representation across total maternal lines collected. The primary institution may use the curation package to test ***germination*** of the accession and develop propagation protocols. Some tests may best be done with maternal lines bulked, but if using the seeds for reintroductions, keep maternal lines separated (see Part 3, "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection"). The backup institution may use the curation package to gather information about seed characteristics and longevity in storage. Clearly mark the curation package "Curation" and place inside the large storage package (see steps 3 and 4 in Figure 1.4). Curation packages can be easily removed from the main storage package, thus avoiding the problem of exposing the entire collection of stored seeds to changing temperatures.

◗ If a single maternal line has less than 20 seeds, but the whole sample has at least 300 seeds, don't divide the single maternal line. Rather, delegate it to one of the long-term storage groups. It is possible to use only fecund maternal lines with abundant seeds for trials to learn how to propagate the species.

- If there is a particular interest in evaluating differences across maternal lines, then *curation packages would need to be separated by maternal line*. Prior arrangements would need to be made with the backup institution to test by maternal line and would require a research proposal and funding support.

Send relevant accession data to the backup institution.

◗ Information should be safely duplicated in a database at the backup institution.

◗ Tracking the destination of maternal lines can be done by accessioning each maternal line or by notation in the comments fields of database.

◗ For the rarest species, it is advisable to track maternal lines separately in the accession, seed germination, and propagation databases.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Safety Duplication

4.9.1 A safety duplicate sample for every original accession should be stored in a geographically distant area, under the same or better conditions than those in the original genebank.

4.9.2 Each safety duplicate sample should be accompanied by relevant associated information.

MSB Partnership Collections *(Millennium Seed Bank Partnership 2015)*

Storage and duplication

3.5 Collections are duplicated at –20°C ± 3 °C and 15% eRH ± 3% at a second, geographically-separate, facility or reason for non-duplication recorded (reasons include: low seed number, accession being regenerated and/or on priority list for recollection).

FAQ Frequently Asked Questions

If I have maternal line samples with fewer than 50 seeds, should I divide these low-producing lines? If a maternal collection has 50 seeds, the groups for long-term storage would have 15 seeds to the primary facility, 15 seeds to secondary facility, and 10 seeds for each of the two testing packages. We recommend not splitting a maternal line package if it has 20 seeds or less.

Is there a minimum number of seeds in one maternal line sample that would preclude dividing into the four subgroups? If a single maternal line has 20 seeds or less, don't divide the single maternal line. Delegate it to one long-term storage group.

Why are four groups necessary? Duplicate long-term storage packages safeguard against anything happening to an accession housed within a single facility. Retrieving seeds from sealed ***foil envelopes*** to maintain temperature and humidity levels requires opening packages, removing seeds, and resealing packages within the cold storage room or freezer. It is most expeditious for researchers to have test packages with mixed genetic lines so that they can easily gather the number of seeds they need for germination and ***viability*** trials.

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D Cleaning, Processing, Drying, and Storing Orthodox Seeds

Center for Plant Conservation Best Practices

Summary

- Careful documentation and accession information will ensure the value of the conservation collection.
- Evaluate initial quality of seeds and keep record of the number of apparently good seeds in the accession. These data can be used to evaluate if the seed health changes after storage.
- For seeds that are tolerant of drying and freezing, dry to appropriate moisture targets to maintain appropriate relative humidity during storage. Store seeds at subfreezing temperatures.
- Monitor storage conditions and seed viability periodically.

Maintaining a ***conservation collection*** requires an institutional commitment for the benefit of plant conservation. To maintain the highest conservation value, awareness of accurate recordkeeping, conditions needed to ensure high seed quality, and monitoring seeds to determine seed survival are necessary. New research has shed light on practices that can improve seed longevity in storage. Keeping ***orthodox*** seeds alive for as long as possible will provide many options for using the seeds for future reintroductions. *We urge practitioners to follow the NEW practices to increase the longevity of seeds in storage.*

Accession your seed collection and submit an accession form with the seed shipment.

- Assign a unique identifying number to each incoming ***sample*** to link the collection to the associated data and ensure identification of specific sample records across institutions.

- Key information needed with the accession includes: collection date, location, collector, institution, habitat type, and number of ***maternal lines*** collected. Additional information needed by the seed bank includes: date the seeds arrive to the storage facility, description of seed treatment prior to arrival, and RH and temperature to which seeds were exposed and length of time. These factors help researchers troubleshoot and assess processing speed.

- An online accession form is available to facilitate data sharing between collectors and the seed bank facilities. Germplasm sent to the National Laboratory for Genetic Resources Preservation (NLGRP) research should be accompanied by the submission of the electronic accession form available through the CPC PI portal (see http://saveplants.org/login/pi-portal/).

Clean seeds to reduce bulk and remove diseased tissues, bugs, or non-target species' seeds.

- During cleaning, remove and discard chaff, seeds with insect damage, ***immature seeds***, or seeds obviously lacking an embryo (see Figure 1.4).

- If there is insect damage, consider putting no-pest strips into collection bags overnight. Don't spray seeds with insecticides directly as the chemical residue might affect seed longevity or jeopardize the health of a person working with the seeds.

- Save small and large seeds to capture genetic diversity (Basey et al. 2015).

- Realize that the longer the seeds remain in ***ambient conditions***, the more likely they are to lose ***viability***.

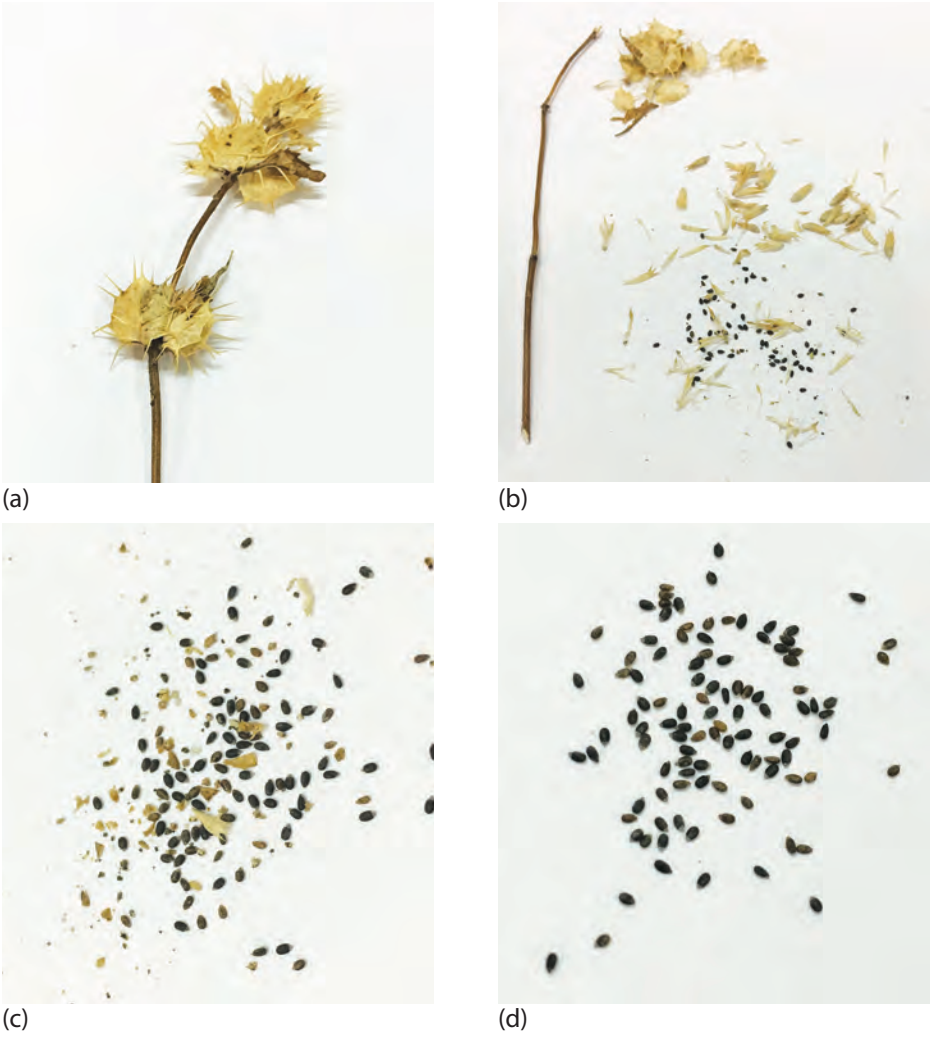

FIGURE 1.5

Stages in seed cleaning process. (a) inflorescence of San Diego thornmint; (b) large chaff separated from seeds; (c) moderately cleaned seed with large chaff removed. This stage is appropriate for ***long-term storage***. Moderate cleaning can save time so that seeds can be processed quickly, dried, and placed into cold storage; (d) meticulously cleaned seed. This level is appropriate for ***curation packages*** that require seed testing.

Evaluate the quality of seeds.

◗ Determining viability of the collection can begin with visual assessment. If you have fewer than 100 seeds, examine seeds under a microscope to estimate whether they are empty or filled. With such a small sample, it is best not to destructively sample any seeds.

◗ If you have an adequate seed accession (500 seeds or more), dissect 5–10 seeds under a dissecting microscope and assess percentage of the sample with filled, intact embryos. Extrapolate the percentage of the accession that is likely to be filled.

◗ If more than 50% of your seeds are hollow, this may indicate herbivory or low reproductive viability. Consider making another collection of this ***population***.

◗ Report percent viability and how it was determined with accession records sent to the seed bank.

Divide seeds for storage.

◗ Divide seeds according to Part 1C "Splitting Samples for Safety Duplication Storage and Testing." Recall that your accession is subdivided into two ***long-term storage packages*** with maternal lines separated (~40% of accession to each ***primary*** and ***backup facility***) and two curation packages (~10% of accession to each primary and backup facility).

◗ Recall that the precision of cleaning differs between the long-term storage packages and the curation packages. Recall that the precision of cleaning differs between the long-term storage packages and the curation packages (Figure 1.5). Stored seed may be moderately cleaned, while seeds in the curation packages require thorough cleaning and each should contain a total of at least 50 seeds from all maternal lines or approximately 10% of the accession. These are needed for testing seed viability, ***germination***, and/or longevity in storage. Place the curation package labeled "Curation" into the large envelope used for long-term storage sample (see step 4 in Figure 1.4).

Assemble the storage package.

◗ To estimate the total number of seeds in the storage packages, clean, count and weigh up to 25 maternal lines (a subset of the total in your accession).

- Provide a seed count on maternal line packages and record seed counts and weights on the Maternal Line Count Example.xls spreadsheet.
- Measure the mass of the cleaned maternal line with the discarded chaff.

◗ Extrapolate to other maternal lines and to total accession seed count using the Seed Count Conversion Worksheet (See www.saveplants.org/login, on the forms tab.) For example, seed counts for maternal lines with more than 100 seeds can be calculated by multiplying the weight by 100 and dividing this number by the weight of 100 seeds.

◗ Indicate on seed packages actual counts like this: "100 seeds." Indicate estimated counts on seed packages like this: "~ 1000 seeds."

Assemble the curation package.

◗ The curation package allows for easy, quick removal from a large storage package that remains in a freezer. Seeds in the curation package can be easily used to test seed viability after storage – thus validating the health of the whole accession. Without a curation package, the entire storage package and each maternal line envelope would need to be retrieved from the freezer for seed testing. In the time required to remove seeds from each maternal line envelope, the whole accession is subject to warming, which may reduce its longevity.

◗ Count and report seeds in the curation packages.

◗ Place counted, clean seeds into Uline S-11591 2 x 3.5" flat, glassine bags.

◗ NLGRP will measure mass of 10 individual seeds. Estimate mass and seed numbers of the storage samples.

- Knowing seed mass can provide good diagnostic information that can allow estimates of the number of seeds in a whole sample, establish the appropriate medium for germination testing, indicate differences among populations, or indicate maturity or seed fill (that is, if the seed does have an embryo).
- Measurements of individual seeds are much more useful than a single measurement of a bulked sample because it provides a good characterization of the variation within the sample. Individual mass measurements require a precision balance. An electronic balance has a 0.1 µg resolution, which is almost sufficient for dust-sized seeds. NLGRP will provide seed mass in *milligram* units (grams multiplied by 1000).

◗ The Primary Institution may elect to keep curation packages separated by maternal lines. This can increase opportunities for equalizing family lines for restoration.

Process seed as quickly as possible.

◗ Maintain seeds while processing in a cool, dry location. Avoid exposure to high humidity, heat, or direct light. Periodically check the seeds for insect damage.

Dry to appropriate moisture targets to maintain appropriate relative humidity during storage.

◗ Moisture target is set by storage temperature and risk of failure of storage temperature. Target should be no more than 25% RH (lower risk of failure) and no less than 10% RH (higher risk of failure) *at the intended storage temperature*. See the box "Recommended RH and Temperatures for Drying Seeds in Seed Lab" (Walters 2004) and "Calculating Storage RH Using Kew SID Modules," (http://data.kew.org/sid/).

◗ Keep all maternal lines of an accession together for drying. Spread them out so that drying is effective.

◗ Conditions needed to dry to the moisture target are constrained by drying time and ***drying temperature***.

FIGURE 1.6 Silica gel is orange when activated and turns green when deactivated

- Drying time should be commensurate with drying temperature, ranging from less than 1 week for drying at 25°C and less than 1 month for drying at 5°C.

Maintain seeds at temperatures below 25°C.

- Drying temperatures that exceed 25°C can damage seeds.

Continuously monitor relative humidity and temperature.

- Continuously monitor RH and temperature of drying conditions. (See recommended equipment list and sources below.)

Dry seeds using desiccators and drying agent.

- The simplest way to dry seeds is to place seeds in a ***desiccator*** with ***silica ge***l. Monitor the RH regularly. Replace silica gel as needed.

- A more precise method for drying seeds is to use salts in a desiccator. This will allow drying to a particular RH percentage.

Relative humidity will drop as temperatures chill.

- Relative humidity influences ***internal seed moisture***.

- Recall that ***target storage RH*** should be no more than 25% RH (lower risk of failure) and no less than 10% RH (higher risk of failure) at the intended storage temperature. Drying RH to achieve target storage RH is approximately 25%-35% RH if drying at 25°C and 15%-25% RH if drying at 5°C (Walters 2004, unpublished 2019). See "Recommended Relative Humidity and Temperatures for Drying Seeds in the Seed Lab. Table 1.1"

- If more precise guidelines are desired, use Kew SID isotherm module. See "Calculating Storage RH Using Kew SID Modules" at (http://data.kew.org/sid/).

- It is possible to use multiple steps to ensure rapid drying and desired accuracy of target RH. For example, drying over silica gel to achieve very low RH with a final overnight adjustment at room temperature over a saturated potassium acetate or calcium chloride solution would be fine.

- It is more important to get seeds dried and placed at storage temperature than it is to achieve precise drying RH.

Quickly transfer seeds to storage envelopes.

- Quickly transfer seeds from desiccators to ***foil envelopes***, and seal and move them to subfreezing temperatures.

- Maintain target moisture using suitable, moisture-proof containers. CPC recommends using sealable foil envelopes (see Equipment List and Sources).

- Seed storage containers must be resistant to breaks, tears, or punctures from sharp seed parts.

Recommended Relative Humidity and Temperatures for Drying Seeds in the Seed Lab

Orthodox seeds should be dried quickly so that they will have approximately 12% water content at the intended storage temperature.

The RH % target should be no more than 25% RH (lower risk of failure) and no less than 10% RH (higher risk of failure) *at the intended storage temperature.*

TABLE 1.1 Recommended drying conditions for seeds stored in moisture-proof containers at various temperatures. The given drying temperature and RH combinations give a storage RH of 20% at the indicated storage temperature.

Drying Temperature (°C)	Drying Relative Humidity for Storage at 15°C	Drying Relative Humidity for Storage at 5°C	Drying Relative Humidity for Storage at –20°C
25	28%	33%	35%
15	20%	26%	38%
5	14%	20%	32%

Drying seeds at temperatures less than the storage temperature is not cost-effective and therefore is strongly discouraged.

Note that practitioners should feel comfortable achieving RH targets ± 3% while drying seeds.

Walters (2004, unpublished 2019)

- Storage containers must have a maximum ***water vapor transmission rate (WVTR)*** of 0.005 g H_2O/m^2/day, measured directly or reported by manufacturer using standard conditions. Lower WVTR is recommended for locations at sea level with high ambient RH.

- Clearly label storage envelopes with the name of the plant, the accession number, collection date, the date placed into storage, the number of maternal lines, and the estimated number of seeds.

Mail duplicate seed accession to backup facility.

- Contact NLGRP prior to mailing the duplicate processed, dried, and packaged seed accession.

- Mail seeds in container that can protect them from compression and temperature fluctuations. DO NOT FREEZE or place cold packs into seed container prior to shipping unless NLGRP recommends for your specific accession.

Store orthodox seeds at subfreezing temperatures.

- Hold samples at < –20°C ±2°C.

- Samples may be warmed periodically for viability monitoring or distribution. Sample exposure to room temperature should be kept to a minimum, be recorded, and not exceed 5 days in any year.

- There should be concerted attempts to avoid warming the entire sample when only a small subsample is needed. Warming may decrease the lifespan of the seeds.

- For walk-in freezers, observe human safety requirements by OSHA. Don't stay in the freezer longer than 5 minutes.

Monitor storage temperatures continuously.

- Monitor storage temperature continuously with equipment that will send automatic alarms when temperatures are out of range.

- Maintain incident records when storage temperature warms above –15°C (for freezer storage), –60°C (for –80°C storage) and –150°C (for cryostorage).

- Determine whether or not a management action is required (for example, replacing equipment, checking power sources, etc.).

- Place contact numbers for service onto front of freezer.

Test seed viability after several years in storage.

- When you test seed viability after several years in storage, check the RH of bags under storage conditions. (See equipment suggestions below.)

- To confirm RH is holding steady, spot-check the RH in a subset of newly sealed bags that contain seeds held at storage temperature (–20°C). Spot-check the RH of some bags after they are stored for 5, 10, 15, and 20 years.

- Replace storage containers within 30 years of banking.

- See Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation" for recommendations for storing seeds that are intolerant of desiccation and/or freezing.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Drying and Storage

4.2.1 All seed samples should be dried to equilibrium in a controlled environment of 5–20°C and 10–25 percent of relative humidity, depending upon species.

4.2.2 After drying, all seed samples need to be sealed in a suitable airtight container for long-term storage; in some instances where collections that need frequent access to seeds or likely to be depleted well before the predicted time for loss in viability, it is then possible to store seeds in non–airtight containers.

4.2.3 Most-original-samples and safety duplicate samples should be stored under long-term conditions (base collections) at a temperature of –18 ± 3°C and relative humidity of 15 ± 3 percent.

4.2.4 For medium-term conditions (active collection), samples should be stored under refrigeration at 5–10°C and relative humidity of 15 ± 3 percent.

MSB Partnership Collections *(Millennium Seed Bank Partnership 2015)*

Processing

Seed collections are accessioned, dried and processed according to recognised protocols or guidelines:

2.1 Unique accession reference number is assigned to all incoming material.

2.2 Collections are placed in cool/ambient drying conditions of 15% RH ± 3% within 4 weeks of collection (Immature seeds are ripened before drying; microscopic seeds (e.g. orchids) are dried for a maximum of 1 week).

2.3 Collections are cleaned to remove empty, poorly developed and insect-infested seeds and debris.

2.4 Purity is assessed by X-ray and/or cut test.

Storage and Duplication

3.1 Seed collections are banked as soon as possible after drying to equilibrium with 15% RH ± 3% (cool/ambient temperature), and within 6 months of collection (microscopic seeds are banked within 1 week of drying).

3.2 Collections are held in air-tight (hermetic) containers.

3.3 Collections are stored at –20°C ± 3°C.

3.4 Collection size is monitored to ensure that sufficient potentially viable seeds are available for effective management and distribution to users.

FAQ Frequently Asked Questions

How do I know what the appropriate moisture target for my species is? Review recommended drying conditions for seeds stored in moisture-proof containers at various temperatures. The given drying temperature and RH combinations give a storage RH of 20% at the indicated storage temperature (Walters 2004).

Drying Temperature (°C)	Drying Relative Humidity for Storage at 15°C	Drying Relative Humidity for Storage at 5°C	Drying Relative Humidity for Storage at –20°C
25	28%	33%	35%
15	20%	26%	38%
5	14%	20%	32%

How many incidents in which temperature or humidity is recorded out of range should trigger a management action? Any incident should trigger a review of equipment, power supply, and sensor function.

Equipment List & Sources

Blotter Paper and Germination Paper

Anchor Steel Blue Seed Germination Blotter: Anchor Paper Co., http://www.anchorpaper.com/index.php/seed-solutions/germination-papers/#blueblotterpaper (800) 652-9755

Seed Separation Devices

Agriculex Column blower (to separate seeds from chaff quite extensively Agriculex, agriculex.guelph.org, (519) 837-0871

Desiccators

SP Scienceware stackable desiccator chambers by Bel-Art are available from Cole-Parmer (www.coleparmer.com), Zoro (www.zoro.com), and others.

Temperature and Relative Humidity Sensors

Wireless (real-time, live)

OmniSense, www.omnisense.com, (843) 522-0350

You will need to purchase a gateway and sensors individually or you may purchase together in the Drifi Restoration Moditoring Kit. There is a US $20 monthly data access fee.

Data Loggers

HOBO Temp/RH 2.5% Data Logger (most accurate and longest lasting)

Onset, www.onsetcomp.com, (800) LOGGERS.

iButtons (small, about the size of a quarter)

Maxim Integrated, www.maximintegrated.com

iButtonLink Technology, www.ibuttonlink.com, (262) 662-4029

Humidity/temperature pen (inexpensive, with a digital display)

Fisher Scientific, www.fishersci.com, (800) 766-7000

Foil Laminate Bags

Protective Packaging Ltd. has foil laminate bags of different sizes. One popular size is PP01625. Protective Packaging Ltd., www.protpak.com,
+44 (0) 161 976 2006

Glenroy, www.glenroy.com,
(262) 255-4422. Must be a USA company for orders over 30,000.

Flex-Pak Packaging Products, Inc., www.flex-pak.biz, (630) 761-3335

Sealer for Bags

Rennco heat sealer (recommended model is LS-18-120) to seal barrier foil envelopes, rather than using an iron, to ensure a fully sealed package. The optimal temperature is 177°C, according to the engineer who tested the specifications on CPC packaging material. Relatively inexpensive, used heat sealers can be found online.

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E Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration

Center for Plant Conservation Best Practices

Summary

- Some species produce so few seeds in the wild that collections of 100 seeds or less are expected. These require additional care.
- For best conservation value, increase seeds before storage by taking steps to grow to maturity, collect next generation seeds, and store.
- Alternatively, plan to collect seeds across multiple years to build the total number held in storage.
- Preferentially use stored material for research or ***reintroduction*** rather than wild collected seed.

CPC encourages practitioners to collect from the rarest plant populations as these have high extinction risk. Some of the rarest species may not have ***populations*** large enough to support a collection of 3000 seeds, the number of seeds for a collection suggested by National Laboratory for Genetic Resources Preservation (NLGRP). Note that 3000 seeds is a target, not a dictum. If you are lucky enough to make a collection of more than 3000 seeds, it will mean more seeds will be available for future uses. For collections of 100 to 3000+ seeds, follow the practices outlined in Part 1D, "Cleaning, Processing, Drying, and Packaging Seeds for Conventional Storage." This section pertains to or those collections that have fewer than 100 seeds.

Small seed collections present a challenge for seed banking and may require additional actions.

- If a collection has fewer than 100 seeds, the following steps should be taken:
  - Collect species information on ***breeding system***, ecology, and biology.
  - Plan to make additional collections from the population in different years to increase the total number of seeds in storage.
  - Collecting from extremely small populations with sporadically reproducing individuals may require that you tag individuals so that you can be assured of the genetic representation in your collection across years.
  - Try growing seed. Particularly small seed accessions may require advice from experts.
  - Consider making plans to collect tissues for tissue culture. Develop a plan with an expert prior to collection. See Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation."

Plan to increase the number of seeds for storage or reintroduction use.

- Because ***genetic drift*** and artificial selection are possible in a cultivated setting, strive to have at least 30 ***randomly chosen*** individuals of a fully ***outbreeding*** sexual species (***outcrossing***) or 59 randomly chosen individuals of a self-fertilizing species as the seed-bearing plants as source material (Brown and Marshall 1995).

- Because genetic drift and artificial selection are possible with every generation, use the most original ***sample*** to regenerate accessions (prioritize wild first, then the first generation (***F_1***), then second generation (***F_2***) to grow for increasing the total number of seeds.

- When you regenerate a seed collection, it is advisable to ***immigrate*** genes (via pollen or new seeds) from the wild to help add diversity if the ***wild population*** still exists.

- For very small seed collections, keep in mind that it may not be possible to produce 30 seed-bearing plants. Research the best ***germination***/propagation protocols to ensure that enough source plants can be propagated (Deno 1993; Cullina 2000, 2002; Baskin and Baskin 2003, 2014; U.S. Department of Agriculture, Forest Service 2008; Native Plant Network, https://npn.rngr.net/propagation).

- Store wild and next generation material (if the species conservation status allows). Be sure to document whether seed is wild collected or next generation (for example, F_1, F_2, Backcross, or other) in the database. Note the growing conditions of the F_1 generation as well.

How can I prevent cross pollination in my nursery? FAQ

- Take care to prevent ***cross-pollination*** from non-target pollen sources (with ***congeners*** or from other populations).

- Facilitate outcrossing by ***hand pollination***. If feasible, do experimental hand-pollinations to maximize opportunities for setting fruit and understanding mating system. Label flowers to track ***open pollinated*** versus ***hand pollinated*** from known pollen and maternal sources.

- For reintroductions, it is important to equalize the number of plants across the ***maternal lines*** represented in the ***outplanting***. Therefore, track maternal lines as you conduct seed increases.

Gather baseline data on seed viability.

- Gather baseline data on seed viability of every accession prior to placing into cold storage and periodically test stored material for ***viability***.

- Remember that the lifespan of seed in storage is not infinite.

- The periodicity of monitoring should vary with any known ecological and storage characteristics. Seeds with tropical oils, from aquatic habitats, or from spring fruiting parents may be short-lived, therefore they should be tested accordingly.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Regeneration

4.4.1 Regeneration should be conducted when the viability drops below 85 percent of the initial viability or when the remaining seed quantity is less than what is required for three sowings of a representative population of the accession. The most-original-sample should be used to regenerate those accessions.

4.4.2 The regeneration should be carried out in such a manner that the genetic integrity of a given accession is maintained. Species-specific regeneration measures should be taken to prevent admixtures or genetic contamination arising from pollen geneflow that originated from other accessions of the same species or from other species around the regeneration fields.

4.4.3 If possible at least 50 seeds of the original and the subsequent most-original samples should be archived in long-term storage for reference purposes.

◗ Periodic ***viability testing*** eventually will reveal a decline in sample quality prompting a management decision about the value of the sample and the need for replenishment. When possible, use ways to sample very few seeds or non-destructive viability testing such as ***differential scanning calorimetry (DSC)*** or RNA (Walters, personal communication).

- If you can detect a change in the viability of a stored accession, it is time to regenerate it.
- Any resulting rare seedlings should be raised to maturity, and any germination/ propagation protocols should be recorded to inform future reintroduction efforts.
- When refreshing stock, follow guidelines Part 3C, "Genetic Guidelines for Maintaining a Conservation Collection."
- Realize that seed that is no longer capable of germinating may still be valuable for genetic research.

Use stored material for reintroductions.

◗ When practitioner is ready to do reintroductions, preferentially use stored material rather than wild collected seed.

◗ If populations are adequate in the wild, add wild genes (via seed or pollen) to the reintroduction.

FAQ Frequently Asked Questions

How can I prevent cross-pollination in my nursery? Bagging the flowers of your target species will prevent or minimize cross-pollination. You can physically separate congeners in different locations where there are barriers that pollinators cannot cross. See the management guidelines in Maunder et al. (2004).

Why should I strive to collect 3000 seeds? See Part 1C, "Splitting Samples for Safety Duplication Storage and Testing." A collection of 3000 seeds will have 1200 placed into storage at ***primary*** institution and 1200 seeds placed into storage at ***backup*** seed bank facility. The curation samples will contain 300 seeds for each institution. When a sample is tested initially for mass, RNA quality, and viability, this will deplete 50 seeds. Subsequent viability tests of 50 seeds will be required—250 seeds enable viability tests for 5 more periods (for example, at 1, 5, 10, 15, and 20 years).

Why should or shouldn't I place a small seed sample into a seed bank for long-term storage? You should place a small seed sample in storage if you have a plan for collecting more seed from the wild or for regenerating more seeds in the near future. Seed banking may buy time provided the seeds you are attempting to store are tolerant of drying and freezing. Think of a rare plant population that produces a small number of seeds as a cry for help. More conservation actions will be needed to bring back the species from the brink of extinction.

If I only have a few seeds, what is the safest way to propagate them? How can these protocols be shared? Begin by reviewing the literature and discussing the possible germination requirements with experts. Unfortunately, congeners may not always help you determine the germination or storage potential of a rare species, but congeners may give you some good ideas if no other protocols are known. Conducting trials as experiments will contribute to knowledge of the species biology. Good resources for germination requirements include Baskin and Baskin (2014) and Deno (1993).

If a rare population has few individuals or little seed production, it may require ***clonal*** techniques to generate enough numbers for conservation action. Consider collecting tissues for tissue culture or collecting pollen for storage in liquid nitrogen. Some species may require maintenance in a living collection in a botanical garden or protected natural area.

Who makes the seed banking decisions at your institution? Is this the correct person? We recommend that seed banking decisions are done by a committee that would include a colleague with restoration expertise and one with horticultural expertise. It will be necessary to determine whether the seeds can be replenished and whether there is a good plan for outplanting that will secure the species. Often, we cannot easily return the species to the wild safely or we cannot produce viable seed for various reasons. Decisions to germinate seeds from a very small accession should be made in consultation with several botanical garden staff and the species ***recovery staff.***

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Glossary Terms

accession—A collection occurring within one plant population at one location that may be collected over several consecutive days. In botanical garden databases, an accession is given a unique number that can be tracked through time.

allele—DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to- many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity.

ambient conditions—The relative humidity and temperature of the room. When processing seeds for long-term storage, it is a good idea to check the room temperature and humidity. Seeds will have best chance for long-term survival if processed at temperatures below 25°C. Humidity levels can be taken below ambient levels when using a desiccator.

backup facility—A second seed bank or nursery where a representative portion of an accession can be stored.

black-box storage—The seeds in the seed bank are stored under "black box" arrangements, meaning that overseers of the seed bank will never open or test any of the seed packages.

breeding system—The method by which a plant can successfully produce seeds. Plants have three basic breeding systems: outbreeding or outcrossing, where pollen from a different individual is needed to fertilize the egg of the maternal plant to produce seeds successfully; selfing or self-fertilization, where pollen from the same individual can fertilize the egg and produce seeds; or apomixis, where seeds can be set without fusion of gametes.

clonal—Type of asexual reproduction in plants that produces new individuals with the same genetic makeup as the mother plant (unless unusual mutations occur). Examples include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

congeners (congenerics) —Members of the same genus.

conservation collection—An ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues or whole plants of at least 50 unrelated mother plants, and breadth, meaning it consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.

conventional storage—Storage at freezer temperatures (–18°C ± 3°C or 0°F ± 5°F).

cross-pollination—The transfer of pollen from the anther of a flower of one plant to the stigma of the flower of another plant of the same species.

cryopreservation—Storing at liquid nitrogen temperatures, usually at –170°C to –180°C (vapor above liquid nitrogen) or at –196°C (in liquid nitrogen) to maintain viability.

curation package—A separate envelope containing one to a few seeds of each maternal line in an accession that can be used to test germination and longevity of viability after storage over time.

desiccators—Sealable enclosures containing desiccants used to equilibrate the moisture of seeds prior to long-term storage. Desiccators create and maintain dry environments to ensure stable moisture content.

differential scanning calorimetry (DSC) —A technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. This is relevant to seed storage in that lipid composition within a seed can be measured using this technique without harming the seed.

dioecy—Having male and female flowers on different individuals.

dormant—a state of slowed activity, in seeds, alive, but not actively growing

drying agent—Any substance that can remove moisture from seeds. Examples include salts and silica gel.

drying temperatures—The temperature at which seeds should be held while drying to the target moisture content. Safe drying temperatures to maximize seed longevity are below 25°C.

ecoregion—A geographically defined area with distinctive ecology that may change with changing global conditions.

embryo—the part of a seed, consisting of one or more cotyledons and precursor tissues for the leaves, stems, and roots.

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

exceptional species—Non-orthodox species that cannot be conserved long-term using conventional seed banking methods. This includes species with few or no seeds available for banking, species with seeds that are intolerant of desiccation and freezing, or seeds that can tolerate drying, but not freezing, or species that may only tolerate storage at –20°C for less than 10 years.

F_1 generation—First generation.

F_2 generation—Second generation.

foil envelopes—Protective packaging that maintains stable moisture levels within the package and is recommended for long-term seed storage.

genetic drift—Variation in the relative frequency of different genotypes in a small population, owing to the chance disappearance of particular genes as individuals die or do not reproduce.

georeferenced—Points taken with GPS that can be used to locate a plant again. Herbarium specimens and seed collections when georeferenced can contribute greatly to our understanding of how plant populations may change in the future.

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

hand-pollination (hand pollinated) —A process where pollen is manually and deliberately transferred to the receptive portion of a flower (the stigma). This technique is often used to control the parentage and to maximize pollen transfer in hopes of achieving fertilization and good seed set.

immature seeds—Seeds with underdeveloped embryos. To determine whether a seed is fully developed may require cutting it open and looking under a microscope.

immigrate—To move into an area. In a context of conservation collection health, it is sometimes necessary to allow genes or seeds from a wild population to move into an ex situ population.

intermediate (seed) —Share functional characteristics with recalcitrant seeds and should be stored in liquid nitrogen. When dried to 50%–75% RH, they have a longer shelf-life than storage at 15%– –35% RH levels recommended for orthodox seeds. Seeds age faster when stored at conventional freezer temperatures compared to refrigerated temperatures. Faster aging might be detected within days, months or years, making it difficult to identify which species' seeds are intermediate. Longevity of seeds increases with drying and cooling (as with orthodox seeds), but seeds still age rapidly during conventional storage and will die within about 5 years.

internal seed moisture—Water content of a seed. This can be measured using a process that will destroy the seed: weighing a seed, drying it until there is no change in its mass, and calculating water content mathematically. Obtaining individual seed moisture content is recommended. For very small seeds, this requires a very precise balance.

long-term storage—At least 5 years. Note that the length of time a species' seed can stay alive in dry, cold storage will vary across species.

long-term storage packages—Foil package containing CPC seed collections with maternal lines separated for a single accession (collection of seeds from a single wild population on a single date) that is intended to stay in storage for at least 5 years.

longevity—how long a seed remains viable.

maternal lines—The offspring (seeds or plants) from a single mother plant are distinguished with unique identifying number, stored in a separate package, and labeled if grown in a nursery. Knowing the number of maternal lines in a conservation collection is an estimate of the genetic diversity represented.

maternal plants—Individual plants producing seeds. Keeping track of seeds from each maternal plant allows for estimates of genetic diversity in a collection and allows for maintaining even representation of maternal lines while growing the accession in the garden's nursery or reintroducing to the wild.

Material Transfer Agreement—The agreement that CPC has with the Resources Preservation Unit - Seeds of the National Laboratory for Genetic Resources Preservation, Fort Collins, Colorado, to store national collection rare plant seed accessions in long-term black-box storage.

Material Transfer Research Agreement—The agreement that CPC has with the Plant Germplasm Preservation Research Unit of the National Laboratory for Genetic Resources Preservation, Fort Collins, Colorado, to conduct research on and store national collection rare plant seed accessions long-term.

monoecy—Having flowers with only one sex (male or female) or flowers of both sexes carried on a single plant.

morphological—Pertaining to the form or structure.

non-orthodox species—See exceptional species.

open pollination (open pollinated)—Allowing flowers to be pollinated naturally by wind, insects, or birds. In studies, open pollination is used as a comparison to hand pollinated trials to determine the highest seed set that could be expected in a flower.

orthodox seed—Seeds that survive with very little water in their cells and also survive prolonged storage at –20°C. Orthodox seeds survive drying at 15% relative humidity which translates to water contents less than 0.08 g H_2O/g total mass (<8%).

outbreeding—A condition where flowers of one plant receives pollen from another plant of the same species.

outcrossing—The form of plant reproduction that requires pollen from another plant of the same species to form seeds.

outplanting—Transplanting from a botanical garden nursery (ex situ setting) to a wild setting for purposes of reducing the extinction risk of a species and allowing persistence in a natural setting.

phenology—The timing of key life history events in a plant's life, such as flowering or fruiting.

population—A group of potentially interbreeding individuals that share a common ancestry or gene pool.

population reproductive output—Total seeds produced in a population within one growing season.

population size—The number of individual plants of all ages in the population.

primary facility—The seed bank or botanical garden where the accession is stored. A duplicate of the same accession is stored at a backup facility.

provenance—The place of origin.

randomly chosen—A formal process for selecting an unbiased sample.

recalcitrant seed—Seed incapable of conventional storage due to desiccation intolerance and/or freezing intolerance.

recovery staff—Personnel of U.S. Fish and Wildlife Service working toward ending extinction of federally listed species.

reintroduction(s)—intentional movement of species into habitat it previously occupied.

representative genetic diversity—Best captured by making seed collections across the spatial extent of the population, from plants that are not physically close to one another, and from plants of all sizes and levels of seed output.

safety duplication—A half of a collection that may be stored in a second location as a precaution against losing the accession due to natural catastrophe.

sample—A portion of the population that is collected at one time. CPC recommends collecting a sample of no more than 10% of the seeds produced within a population in a growing season.

sampling—Strategy to use to collect the representative genetic diversity in a population.

seed water content—Water in seeds that is measured from the fresh and dry mass of the seed, fresh mass being at ambient conditions and dry mass being measured after placing the seed in a drying oven at 95 to 100°C. Water content is calculated by the ratio of the fresh minus dry mass to either the fresh (i.e., total mass) or dry mass. Different labs may prefer expressing water content on a total or dry mass basis, and it's a minor difference when water content is less than 0.15 g/g (15%). Putting seeds at 95-100°C is lethal and so measuring water content is a destructive test.

self-compatibility—A condition where a flower of an individual can receive pollen from itself and set good seeds.

self-fertilizing—Pollen from one plant fertilizes a flower of the same plant such that good, viable seed results. This technique is often used experimentally to determine whether this is possible for a species.

self-incompatibility—A condition in flowering plants that prevents self-fertilization and thus encourages seeds to be set when pollen from a different unrelated individual fertilizes the eggs of the mother plant. One of the mechanisms that causes this is a special allele (SI) that prevents pollen from germination on the receptive surface of the flower called the stigma.

sensitive animal species—Those with protected legal status by state or federal agency.

Sentinel Plant Network—A collaboration between the National Plant Diagnostic Network and the American Public Gardens Association to improve the ability to detect and respond quickly to serious plant pests and diseases.

silica gel—A granular, vitreous, porous form of silicon dioxide made synthetically from sodium silicate that can be used to dry seeds.

species conservation status— the designated level of endangerment of a species as determined by the U.S. Fish and Wildlife Service, NatureServe, IUCN or state governmental agency.

target storage RH—The relative humidity sought to maximize seed longevity while seeds are held in storage at a particular temperature.

taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

Thumb Rules or Hundred Rule—Developed by J. F. Harrington (1916–2002) at UC Davis in the 1950s to guide storage conditions for maintaining seed viability, states: "Seed lifespans double for every 1% decrease in water content or 10°F decrease in temperature." According to Harrington's "Hundred Rule," seed viability can be maintained (for 5 to 10 years) if the sum of the relative humidity and temperature (in °F) is less than 100.

viability—Ability to live and survive successfully.

viability testing—Systematically checking and counting the number of live seeds in a sample.

voucher specimen—A pressed plant sample deposited in an herbarium for future reference.

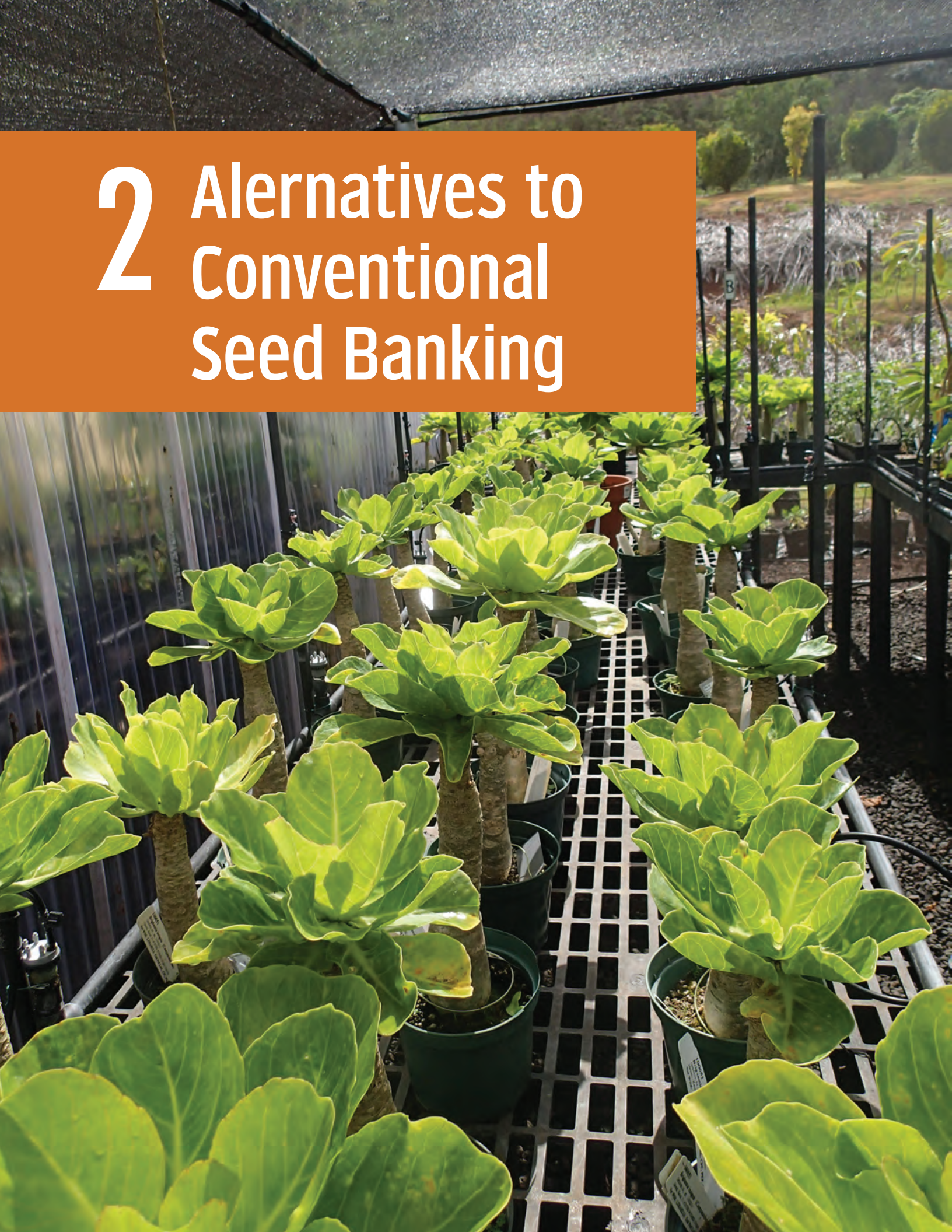
water vapor transmission rate—The amount of water lost or gained across a storage container membrane in a given period of time.

Weed Risk Assessments—A science-based evaluation of the potential of a plant species to establish, spread, and cause harm in a region. Several weed risk assessments exist for different regions of the U.S. (for example, Hawai'i; https://sites.google.com/site/weedriskassessment/home, California: http://www.cal-ipc.org/solutions/research/riskassessment/, and Florida: http://edis.ifas.ufl.edu/ag376).

wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.

wild species—a species that is usually found growing in the wild without human intervention (i.e., in situ). We might also say they are from 'natural populations.' Usually we distinguish wild and domesticated species, the latter has been changed by humans usually for the purposes of cultivation. Arboreta and botanical gardens often grow or store individuals collected in the wild. We would call this "ex situ."

2 Alernatives to Conventional Seed Banking



Alternatives to Conventional Seed Banking

Center for Plant Conservation Best Practices

Overview

Alternatives to Conventional Freezer Storage

Part 2 describes the special handling and options available for species that have seeds that cannot withstand desiccation and freezing.

TISSUE CULTURE

Prior to Collection

• Review species' biology and knowledge of seeds.

While Collecting

• Collect both shoots and seeds. • Balance capturing diversity of 50 maternal lines with institutional capacity. • Transport material quickly to lab.

Seeds

• Test viability and desiccation tolerance.

In Vitro

• Test viability and desiccation tolerance. • Prepare shoot tips for cryopreservation or acclimatize to grow in natural environment.

CRYOPRESERVATION

Seeds

• Place in appropriate airtight container.

Shoots

• Isolate tips. • Follow protocols, documenting each step. • Modify protocols as necessary and document. • Strive for 10 shoot tips per maternal line.

Recover

• After cryopreservation, warm material. • Place onto nutrient medium.
• Take steps to avoid contamination. • Acclimatize.

FIELD GENEBANKS

Prior to Collection

• Review species' biology and institutional capacity. • If possible, use genetic studies to guide collection targets.

While Collecting

• Follow standard protocols.

Maintaining the Collection

• Use good horticulture. • Monitor genetic diversity, propagating under-represented maternal lines. • Minimize artificial selection and genetic drift. Immigrate 5 new individuals from wild or sister institution collections periodically.

A Introduction

For ***exceptional*** (or ***non-orthodox***) plants that cannot be conserved long-term using conventional seed banking methods, three alternatives traditionally used by botanical gardens are ***tissue culture***, ***cryogenic storage***, and ***field genebanks.*** (See Overview.) Each requires different institutional commitment to space, resources, staff time, and expertise.

Considering the broad diversity of plant groups, like orchids, that require cryogenic storage, many of the world's plant species may require alternatives to ***conventional storage*** (e.g., Seaton et al. 2018). Because most tissue culture and cryopreservation standards have been developed with commercially important species, we have relatively little information about the intricate needs of wild rare species' storage needs.

Tissue culture and cryogenic storage are integral processes to long-term secured conservation collections. Growing seeds or plant shoots on ***in vitro*** medium is the most delicate horticultural treatment available. Though standard protocols exist, as is true for conventional seed banking, most protocols have been developed for commercially important species. Wild rare plants have unique needs. This means that carefully documented research by CPC practitioners can contribute importantly to the growing science. Collaborative work across CPC Network partners may help spread costs for efficient ***ex situ*** (off-site) conservation and may certainly aid our collective understanding of best practices for rare plant species' conservation. For example, some species have seeds that are short-lived even in cryogenic storage, while other species have embryos that can withstand cryogenic freezing, but they are difficult to regenerate back to rooted whole plants (Xia et al. 2014). Because the longevity of wild rare species' seeds stored cryogenically is largely unknown, cryogenic storage provides a ripe opportunity for experimentation and scientific advancement.

Field genebanks and ***inter situ*** collections are increasingly recognized as important stopgaps to plant extinction. New genetic research is enabling practitioners to evaluate the size of collection needed to capture a good representation of the genetic diversity of the species. Due to space constraints, collaborations across institutions are necessary. Planned fertilizations across sister institutions or from ***wild populations*** can help maintain the genetic health of the collection and foster valuable next generation reproduction.

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B Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation

Center for Plant Conservation Best Practices

Summary

- Tissue culture and cryopreservation are alternative storage methods for exceptional species that produce few seeds or seed that are intolerant to drying or freezing.
- Adequately storing exceptional species requires specialized expertise, infrastructure, and greater resources than conventional seed storage.
- Because species-specific protocols are unknown for many rare plant species, this area of plant conservation is a pioneering investigative field. CPC practitioners have opportunities to contribute significantly to the field.

Exceptional plants are those that cannot be conserved long-term using conventional seed banking methods. This includes species with few or no seeds available for banking, species with seeds that are intolerant of ***desiccation*** and freezing, or seeds that can tolerate drying, but not freezing, or species that may only tolerate storage at –20°C for less than 10 years. For ***ex situ*** conservation, such species require methods alternative to ***conventional storage***, such as ***cryopreservation*** and ***in vitro*** methods. Many of the world's plant species may fit these storage categories (see Seaton et al. 2018). The primary purpose of a ***conservation collection*** of an exceptional species is to support the species' survival and reduce its extinction risk, therefore accurate records of provenance, differentiated ***maternal lines***, and diverse genetic representation are prerequisites.

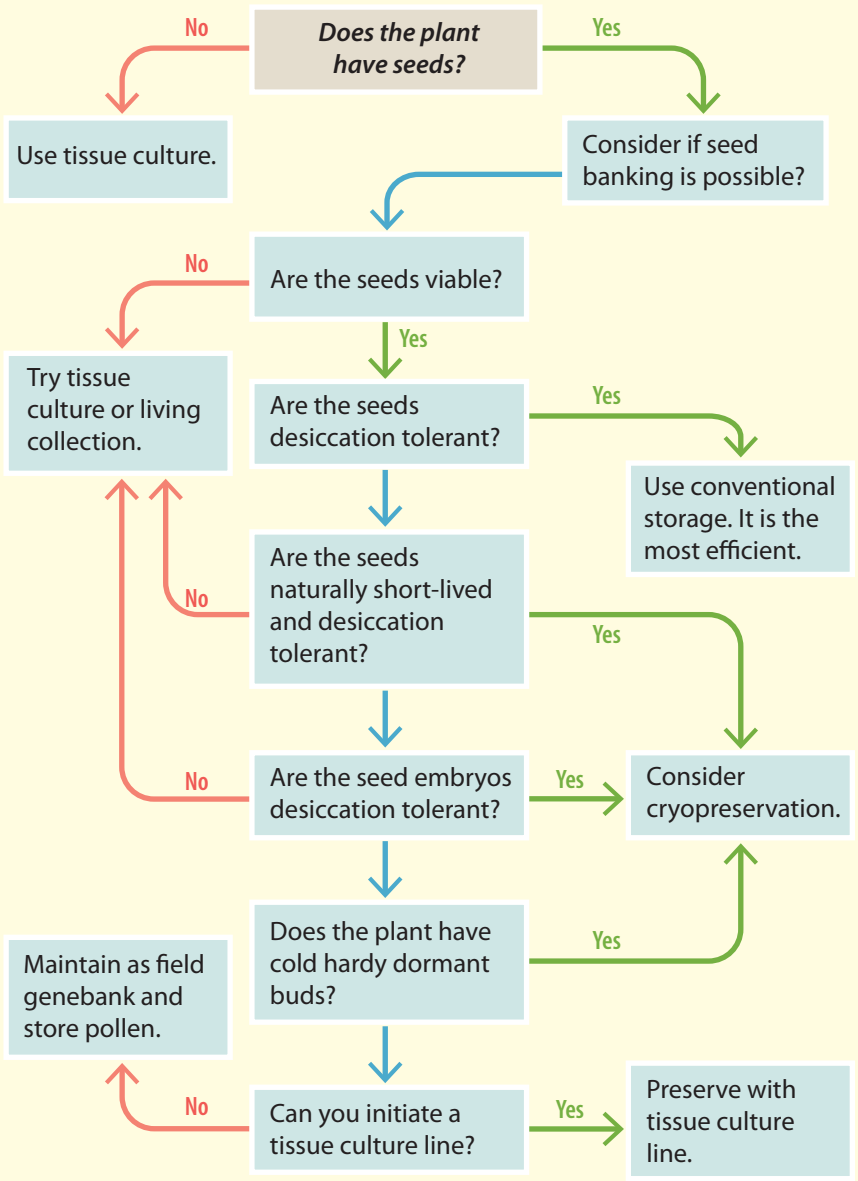
Cryopreservation or ***tissue culture*** are alternative storage processes to conventional storage, but these alternatives are only real conservation solutions if the practitioner is able to restore a tissue cultured or cryopreserved plant part back to a rooted plant that is capable of being transplanted into the wild. As is true for seed research, much more has been done on agricultural or commercially important species than for rare wild species. As more CPC practitioners conduct tissue culture and cryopreservation research, the more will be known about the specific needs of rare species and the more possible it will be to examine patterns. At this stage in the development of the science, we provide recommendations based upon FAO guidelines and recent research noting that a large difference between these bodies of research stems from the fact that because our species are rare, we usually have few ***propagules*** to initiate studies, and we are thrilled to have any survival!

Space and staffing capacity will always impact an institution's conservation program. Exceptional species require a greater commitment of staff time, specialized expertise

of staff, and greater infrastructure than conventional seed banking. In this light, we encourage practitioners to reach out to the experienced in vitro (tissue culture) and cryopreservation specialists in the CPC network if they are planning to create internal programs or if they desire to collaborate on research projects for exceptional species.

Questions to Ask

To Determine the Most Efficient Way to Preserve the Plant Tissue Long-Term



Determining Storage Requirements

Several authors have examined patterns in seed storage behavior (see references) that can help collectors. Begin with a literature review to check if any previous research has been done on your taxon. You can check congeners, but beware that this is not always reliable or conclusive. Our Hawaiian colleagues have found quite varying storage behavior within a single genus (Walters, Weisenberger and Clark, personal communications). Many factors determine variation in seed tolerance to desiccation or freezing. The following are some general patterns observed in seeds that tend to withstand orthodox storage or not.

Trait	Likely to Be Orthodox (Desiccation and Freezing Tolerant)	Questionable Tolerance to Orthodox Storage
Habitat	Arid is especially likely; If it is not growing in a wetland, it is is likely	Wetland, riparian
Conditions in nature	Seeds normally experience dry down and/or hard freezes	Seeds normally remain moist and do not experience hard freezes.
Season of seed production	Not spring	Spring
Life form	Not tree	Trees
Seed bank	Persistent	Not persistent
Dormancy	With dormancy	No dormancy
Seed moisture content at time of maturation	Dry when it is naturally shed from plant	High (30%–70%)
Seed size		Very large (avocado seeds aren't desiccation tolerant) or very small (orchid seeds and fern spores require storage in liquid nitrogen)

Plant Groups with High Proportion of Desiccation Sensitive Seeds		Plant Groups with Predominantly Orthodox Seeds
ANITAGrade	*Malpighiales*	*Solanaceae*
Arecales	*Myrtales*	*Poaceae*
Ericales	*Orchidaceae*	*Asteraceae*
Fagales	*Oxalidales*	*Brassicaceae*
Icacinales	*Santalales*	
Laurales	*Salicaceae*	
Magnoliales	*Sapindale*	

Make preparations before making collections.

- Acquire legal permission to make collections.

- Research the seed storage requirements of a ***taxon***, as this will determine how rapidly the seed or tissue will need to be processed after collection and the tools and containers needed for collection. (See "Determining Storage Requirements" box.)

FAQ How do I know my species storage requirements?

- To avoid over-collection and/or to learn from other collectors, survey other ex situ collections to determine what related species and/or populations have already been collected. You can check the database on the Botanic Gardens Conservation International website at https://www.bgci.org/plant_search.php.

- You can search for priority species for your region in the CPC PI portal (www.saveplants.org/login) on the "Rare Plant Finder" tab.

While collecting, do no harm to the collecting site or the rare population.

Strive to collect plant material at the appropriate stage of maturity.

- Note maturity of seed in your accession. In general, it is best to collect mature spores and seeds. However, immature seeds may be able to be stored in liquid nitrogen or have embryos extracted for in vitro culture.

- Collect tissue that is in condition appropriate for your method. (See Figure 2.1.)

- For shoot collection, collect young growing or newly grown tissue. Generally, tissue that is too old will not take to traditional propagation by cuttings or tissue culture.

- If tissues cannot be transported quickly to a lab, consider making in vitro field collections. (See Pence et al. 2002.) This will require collecting tissue with sterile instruments with 70% ethanol.

- For stem tissue, document the maturity or developmental state of the shoot (soft/hardened, leaf expansion stage, degree of expansion, color, etc.)

- Take photos of the seed pods or shoots to document aspects of maturity.

When appropriate, collect associated soil mutualists.

- For terrestrial orchids, gather soil close to the plant to capture mycorrhizae (Batty et al. 2002).

- Mycorrhizae may be important for germination and cultivation of some species.

Transport to seed banking or tissue culture facility in the shortest time possible and in the best condition possible.

◗ The more rapid the processing, the better chance of survival. Before shipping seeds, converse with the recipient scientist at the seed bank or propagation facility to be sure that someone will be present to receive and process the accession promptly.

◗ Ship seeds or tissues overnight to seed bank or propagation facility. Provide special care to specimens while transporting to banking or culture facility. Wrap stem cuttings in barely moist (not sopping wet) paper towels and enclose each maternal line in a labeled Ziploc™ bag. Use a Styrofoam container to minimize temperature fluctuation during transport. Don't ship with ice or dry ice. If overnight shipping is not possible, consider in vitro field collection.

Capture representative genetic diversity.

◗ In the absence of genetic data, capture the breadth of diversity of the species by collecting seeds or tissues from spatially separated individuals with different appearances, collecting from multiple populations, and keeping all accessions separated by maternal line.

- Consider the ***genetic diversity*** represented by the propagule you are collecting/preserving. Vegetative material (roots or shoots) represents the same ***genotype*** as the parent. Seeds or spores are the product of fertilization potentially from multiple parents, thereby they have potentially greater genetic diversity than vegetative material. (See Figure 2.2.)
- For some exceptional species (ferns and orchids), it is very easy to obtain hundreds of genetically related seeds or spores from a single ***maternal plant***. Take care to collect seeds or spores from many individuals to increase the potential

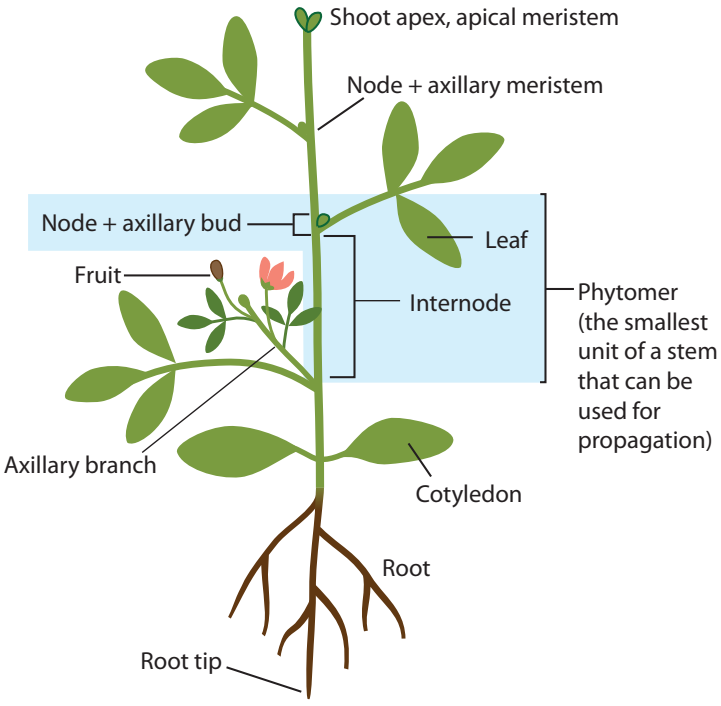


FIGURE 2.1

Meristem architecture. Though meristem architecture varies across species, understanding it for the target rare species will inform what propagation options are available.

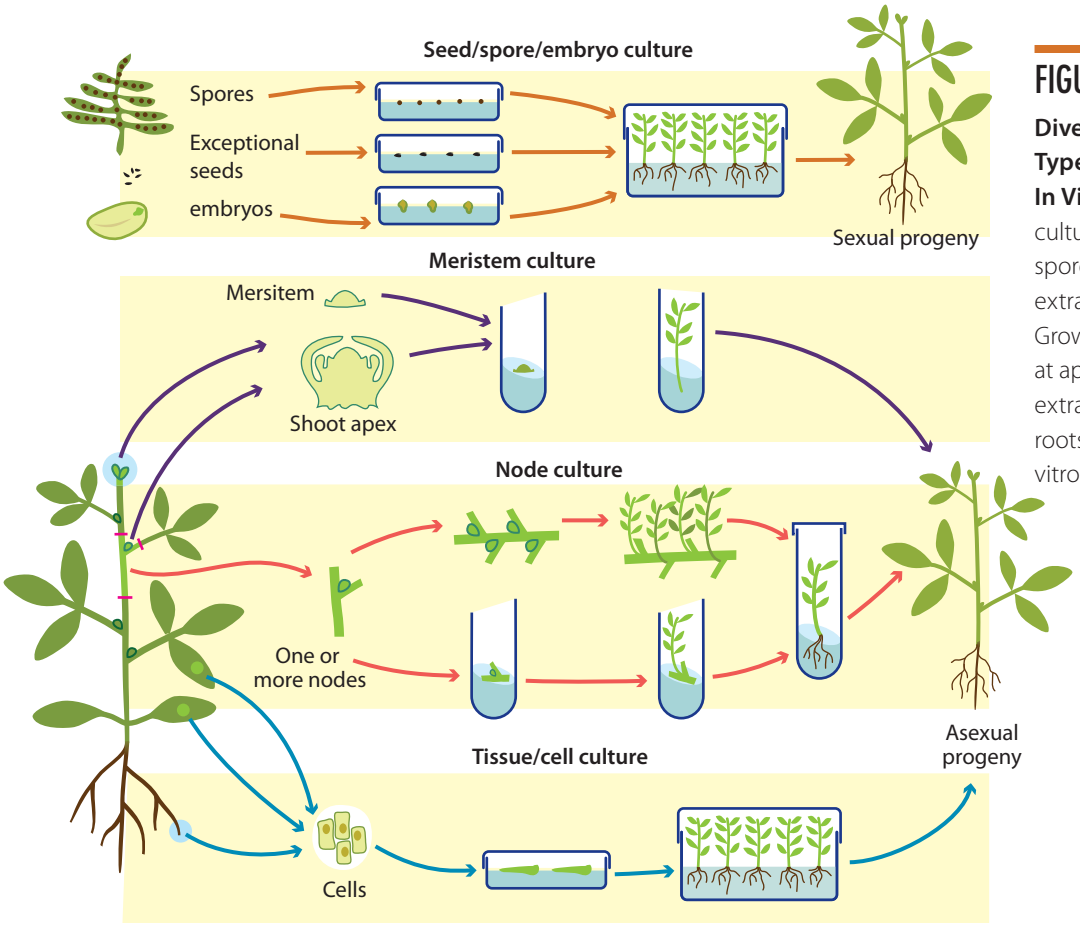


FIGURE 2.2

Diversity of Propagule Types and Options for In Vitro Culture. In vitro culture may be initiated with spores, seeds or embryos extracted from seeds. Growing tips from meristems at apex or nodes or cells extracted from leaves or roots can be sources for in vitro culture.

diversity represented in your accession. (See Part 1B, "Collecting Seeds from Wild Rare Plant Populations").

- If possible, collect parallel leaf samples for ***DNA banking*** while making seed collections.

Plan your sampling strategy for the collection.

◗ Use ***population size*** and ***fecundity*** to plan your ***sampling*** strategy for the collection.

◗ The ultimate number targeted for collection will likely depend on an institution's capacity to process and maintain the stored and living plants. Take into account labor, space, and facilities capacity as you make your collection (Pence 2011).

◗ Attempt to capture as many unrelated individuals from each population as possible. Strive to collect from 50 maternal plants (see Part 3 "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection;" and maintain maternal lines separately.

FAQ Why collect from 50 maternal plants?

- If collecting seeds from a population with less than 100 individuals, attempt to capture seeds from all reproductive individuals whether you are storing seeds or embryos cryogenically. For larger populations, subsamples from 50 maternal plants are sufficient.
- If collecting shoots for tissue culture, base numbers to collect on the architecture of the plant and the number of ***axillary buds*** present at ***nodes***. For spe-

cies with multiple branches, collect one to five stem cuttings from each mother plant. For species with a single ***meristem***, be sure the plant architecture supports axillary buds before removing the ***apical meristem***. If buds are taken from the base of the plant or at soil level, they will likely require stringent decontamination with surface disinfectant agent and antimicrobial agents.

- Genetic studies can help determine the representation of the wild genes in the ex situ conservation collection (Griffith et al. 2015).

Seek to capture at least five populations of a species.

◗ If they exist, seek to capture at least five populations of a species across space and time. See Part 1B, "Collecting Seeds from Wild Rare Plant Populations."

Document the collection appropriately.

◗ Essential accession information includes: institution name, accession number, collector, collection date, species name, family, locality information, georeferenced latitude and longitude, site ownership, permit documentation, and population information (the total number of individuals in the population, number of reproductive individuals, and number of individuals sampled for seeds that were harvested). (See CPC Field Collection Form.)

- Providing habitat information may provide clues to ***germination*** or tissue culture requirements of the species. Recommended fields include light and moisture conditions, soil type, slope orientation, and associated species. Provide photos of habitat and plant in its habitat.
- Be sure to document any associated collections (for example, leaf litter, soil, mycorrhizal fungi) and maintain the link through processing of samples.
- Gather and report additional accession data according to your institutional protocols. Complying with International Transfer Format for Botanic Garden Plant Records (https://www.biodiversitylibrary.org/bibliography/45427#/summary) and/or ***Darwin Core*** standards (http://rs.tdwg.org/dwc/) will allow easy transfer of information to partners.

◗ Complete one field form per accession. Multiple accession numbers and field forms only need to be created for collections made from populations that are differentiated by at least 1 kilometer.

◗ Transmit accession data to CPC and NLGRP via online form provided to Participating Institutions through the CPC PI portal, which can be accessed at www.saveplants.org/login. Once inside the PI portal, the accessions submission form can be found on the "NLGRP" tab.

At the seed bank or cryopreservation laboratory, follow steps for material type to maximize its survival.

◗ Maintain maternal lines for any of these procedures. For highest conservation value, maintain material in a form that will allow for recovery of whole plants that can be used for reintroduction to the wild.

For Seeds

- Test the viability of fresh seeds by conducting a germination trial. For example, conduct an experiment with 10 replicates of 10 seeds. Reduce sample size of your test based upon how many seeds you have and how many you would ultimately like to store. Use seedlings for in vitro culture.

- Determine whether seeds fit categories for conventional storage. See "Determining Storage Requirements" box.

- If they fit orthodox categories, see Part 1, "Conventional Seed Banking to Support Species Survival in the Wild."

- If seeds are suspected to be exceptional and you have enough seeds, test five or more seeds to determine whether seeds can withstand desiccation. Desiccate seeds over salt solutions or ***silica*** using methods described in Part 1D, "Cleaning, Processing, Drying, and Storing Orthodox Seeds."

- If you have a precise scale, test individual seed water content of five to 10 seeds. If you do not have a scale of the precision needed for the seed size, this test can be done at NLGRP. Weigh, dry in oven to drive off water until there is no weight change, and reweigh. You can calculate ***moisture content***. If your seed has high moisture content when it is shed, then it is likely to be exceptional. (See Kew Information Sheet, Measuring Seed Moisture Status, http://data.kew.org/sid/viability/.)

- Once you have determined that seeds are exceptional, see the steps below for in vitro and cryopreservation.

FAQ Do I need to go through the in vitro step if I think long-term cryopreservation is necessary?

In Vitro Tissue Culture

- Review any previous in vitro studies on your species or genus to guide details of your procedures.

- If no other work has been done on your species or genus, conduct basic protocols for in vitro culture. Document steps at each stage. If tissues do not respond to the basic protocol, modify the protocol and test again. (See Figure 2.2 and Table 2.1.)

- Take steps to minimize contamination.

- Treat incoming material with surface disinfectant agent under ventilation hood.

- Include anti-microbial agents (for example, fungicide, PPM, etc.) in medium especially for material received from the wild.

- Monitor regularly for growth and contamination. Refresh medium as needed.

- Multiply the number of shoots in cultivation.

- Maintain enough material from each maternal line to guard against attrition of the line over time.

FAQ For Stage one of micropropagation, surface sterilization, how do I know what disinfectants and concentrations will be effective without damaging my material?

TABLE 2.1 Stages of ***Micropropagation*** (Adapted from Bunn and Tan 2002)

Stage	Operations	Comments
1. Establish and stabilize	Handle and pre-select target plants to reduce the potential of microbial contamination.	Pretreat explants to prevent browning.
	Choose ***explant*** type: shoot tips, flower buds, axillary buds, leaves, or embryos.	
	Stimulate ***axillary*** or ***adventitious shoot*** formation on appropriate medium.	Examine microbial colonies to determine if contamination is coming from fungi or bacteria living within plant tissues or from agar surface that was not effectively sterilized.
2. Multiply shoots	Divide the multi-branched explant. Place small shoots onto new media with hormones to stimulate more shoot development.	Check for contamination.
3. Roots form	Induce rooting by transferring well-developed shoots to media containing ***auxins***.	Plantlets are less susceptible to accidental microbial contamination at this stage.
4. Acclimate	Deflask rooted plantlets and acclimatize to greenhouse conditions.	Remove agar from roots. Use pasturized potting mix to remove a potential source of contamination.

- ***Acclimatize*** plants away from direct sunlight slowly.
  - Remove from culture, transfer to appropriate soil mixture, and maintain in high humidity either under plastic bags or glass. Slowly add ventilation. If plant tissue wilts, replace protective cover and proceed with hardening at a slower pace. This process may take months.
  - Arid-adapted plants, such as those from deserts, tend to have more difficulty acclimatizing from tissue culture to ***ambient conditions*** than species from wet or humid ecosystems. While still in culture, some pre-acclimatization hardening can be considered, such as providing greater aeration to the cultures, higher light, or lower nutrients, as appropriate for the species. Replace sealed cap with vented cap to harden the ***plantlet*** in the tube. Remove from culture, transfer to an appropriate soil mixture, and maintain in high humidity.

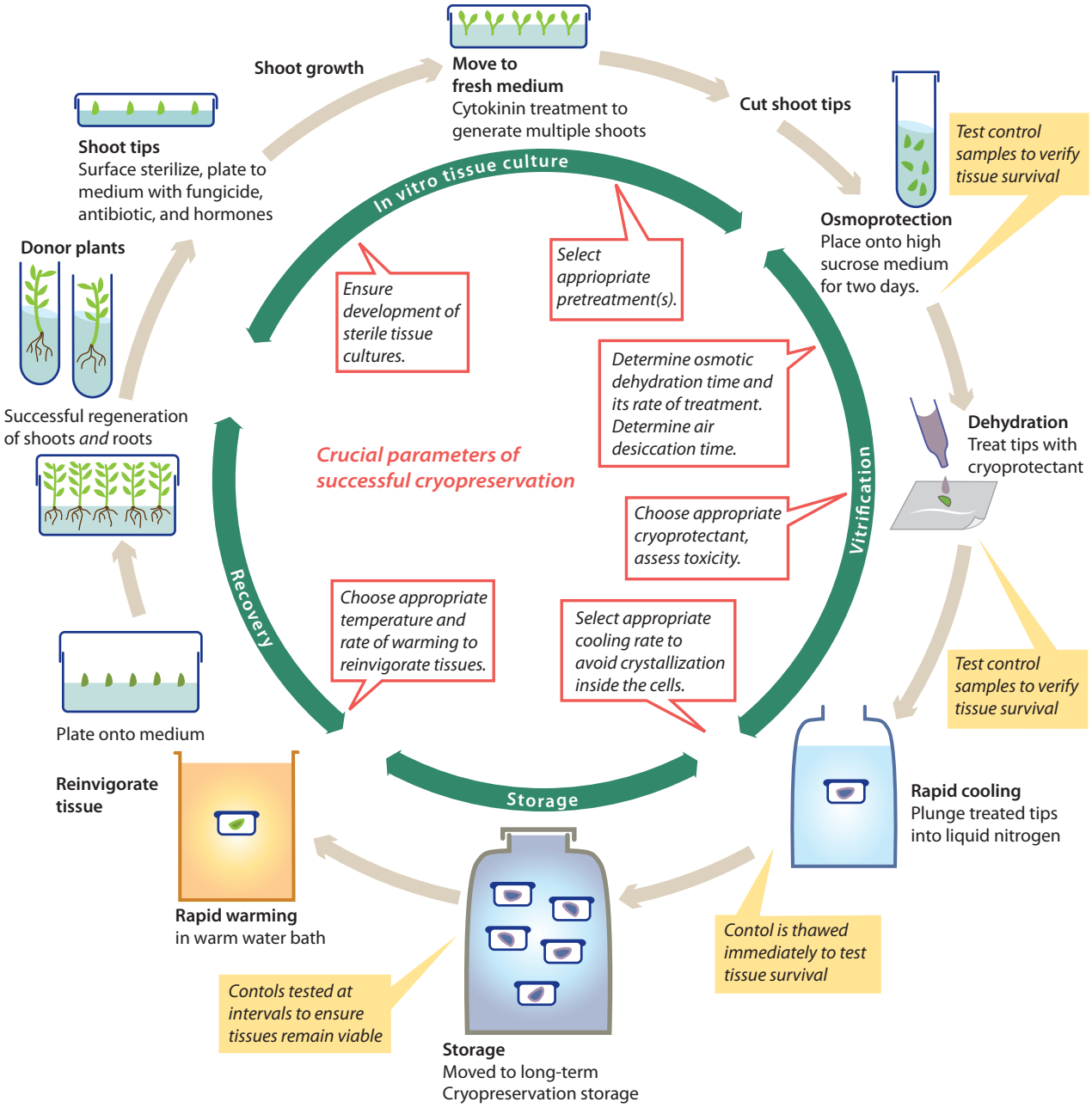


FIGURE 2.3

In vitro and Cryopreservation. From in vitro donor plants, shoot tips can be excised, sterilized, and placed onto a sterile nutrient medium. Application of cytokinin will encourage multiple shoots to grow. With the proliferated shoots, tests of cryopreservation options can ensue. Shoot tips are placed onto a high sucrose solution for osmoprotection, followed by immersion, incubation, or exposure to a cryoprotectant solution (depicted is encapsulation dehydration), exposed to liquid nitrogen, and then stored. Experimental steps along the process are advised for species with unknown cryopreservation tolerance. Experimental components may include molarity of osmoprotection solution, type of and duration of exposure to the cryoprotectant, the rate and duration of exposure to liquid nitrogen, and the duration of long-term storage. After storage, tips are warmed and recovered using in vitro methods.

Cryopreservation

- After seed tests (above) have been conducted, place into appropriate airtight container for storage in liquid nitrogen.

- For shoots that have been growing in vitro or for seed embryos, isolate shoot tips or embryos. This may require a dissecting microscope. Best cryopreservation usually occurs on small materials. For example, tips with size less than 2 mm × 2 mm are best to use. For shoots that have been growing in vitro or for seed embryos, isolate tips or embryos (Figure 2.2 and 2.3).

- Surface sterilize isolated seed embryos either before or after cryopreservation.

- Follow cryopreservation protocols . For each step in the cryopreservation protocol, test a control group to determine if the tissue has survived that step.

- Strive for 40% survival after liquid nitrogen exposure. If survival is lower than 40%, conduct more experiments with cryopreservation to improve survival, or bank more material.

- Strive to have replicate vials of at least 10 shoot tips per vial of each maternal line preserved.

- Recover material by warming in a 40°C water bath.

- Plate onto in vitro medium (see Saad and Elshahed (2012) for media descriptions). Use antioxidants to minimize tissue browning. The kind of antioxidant may vary across species.

- Take steps to avoid contamination.

- Document all steps in process.

- Once roots have formed, acclimate as above.

Duplicate accessions to maintain in one or more than one living collection (Fant et al. 2016).

Consider all attempts to store exceptional species as experimental.

- Because we have much to learn about the optimal ways to store exceptional species, all trials should be recorded using best scientific method.

- To maximize our ability to learn the best way to keep exceptional species alive in storage, do not store exceptional species in ***black-box storage***.

Monitor the viability of the accession after 5, 10, 15, and 20 years in cryostorage if enough material is available.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Acquisition of Germplasm

6.1.1 All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.

6.1.2 All material should be accompanied by at least a minimum of associated data as detailed in the FAO/ Bioversity multi-crop passport descriptors.

6.1.3 Only material in good condition and of consistent maturity status should be collected, and the sample size should be large enough to make genebanking a viable proposition.

6.1.4 The material should be transported to the genebank in the shortest possible time and in the best possible conditions.

6.1.5 All incoming material should be treated by a surface disinfectant agent to remove all adherent microorganisms and handled so that its physiological status is not altered, in a designated area for reception.

Standards for Testing for Non-orthodox Behaviour and Assessment of Water Content, Vigour and Viability

6.2.1 The storage category of the seed should be determined immediately by assessing its response to dehydration.

6.2.2 The water content should be determined individually, on separate components of the propagule, and in a sufficient number of plants.

6.2.3 The vigour and viability should be assessed by means of germination tests and in a sufficient number of individuals.

6.2.4 During experimentation, cleaned seed samples should be stored under conditions that do not allow any dehydration or hydration.

Standards for Hydrated Storage of Recalcitrant Seeds

6.3.1 Hydrated storage should be carried out under saturated RH conditions, and seeds should be maintained in airtight containers, at the lowest temperature that they will tolerate without damage.

6.3.2 All seeds should be disinfected prior to hydrated storage and infected material should be eliminated.

6.3.3 Stored seeds must be inspected and sampled periodically to check if any fungal or bacterial contamination has occurred, and whether there has been any decline in water content and/or vigour and viability.

Standards for In Vitro Culture and Slow Growth Storage

6.4.1 Identification of optimal storage conditions for in vitro cultures must be determined according to the species.

6.4.2 Material for in vitro conservation should be maintained as whole plantlets or shoots, or storage organs for species where these are naturally formed.

6.4.3 A regular monitoring system for checking the quality of the in vitro culture in slow-growth storage, and possible contamination, should be in place.

Standards for Cryopreservation

6.5.1 The explants selected for cryopreservation should be of highest possible quality, and allow onward development after excision and cryopreservation.

6.5.2 Each step in the cryo-protocol should be tested individually and optimized in terms of vigour and viability in retention of explants.

6.5.3 Means should be developed to counteract damaging effects of reactive oxygen species (ROS) at excision and all subsequent manipulations.

6.5.4 Following retrieval, explants should be disinfected using standard sterile procedures.

Document the experimental protocols carefully.

◗ Whenever steps of protocol are compared to controls, report survival of controls and treated groups. Remember that steps that are NOT successful will help future practitioners.

◗ Note the age of the material used.

◗ Take photos of your shoot tips in vitro culture.

◗ Note average shoot tip size and photograph this.

◗ Note condition of tips and any appearance differences across treatments (for example, hairy, shape, leaves present or not, etc.). Note survival of phenotypes.

◗ Track the type of medium used for pre-culture, stock, and recovery culture; note any additives used (for example, ABA, antibiotic, etc.); ***cryoprotectant*** used; cold hardening treatment; cooling rate and ***vitrification*** method used; and any modifications in standard procedures.

FAQ Frequently Asked Questions

How do I know my species storage requirements? See the "Determining Storage Requirements" box.

Why should I try to sample from 50 maternal plants? The 50 maternal lines recommendation is supported by the sampling strategy from the Bureau of Land Management Technical Protocol for the Collection, Study, and Conservation of Seeds from Native Plant Species for Seeds of Success (2016).

For many potential users of and uses for the collection, it is important to maximize the number of ***alleles*** (variants of genes) present within the sample by capturing the greatest proportion of those alleles represented in the field population. The number of different alleles in a population reflects its genetic diversity. Sampling from (1) 30 randomly chosen individuals in a fully ***outcrossing***, or ***outbreeding***, sexual species, or (2) 59 randomly chosen individuals in a ***self-fertilizing*** species will capture at least one copy of 95% of the population's alleles which have frequencies of at least 0.05 (Brown and Marshall 1995).

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 very rare 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.

For research purposes, and for the conservation of rare species that occur in populations of fewer than 50 individuals, as well as for less fecund common species, where collections will result in fewer than 3000 seeds, we recommend collecting seeds along maternal lines. In a maternal line collection, seeds from each individual plant (maternal line) are collected and bagged separately (as opposed to bulking the seed collection of multiple plants into one or two bags as we do for regular seed bank collections). This ensures the greatest genetic diversity is available in a small collection. When we combine seeds in a bulk collection, there is only a small chance any particular parent's offspring will be represented when a portion of the collection is removed to restore a new population. For small populations or small seed collections, collecting along maternal lines allows equal representation of all parent's offspring (seeds) in a newly restored population, provided a portion of the seeds are distributed equally from each maternal line (Bureau of Land Management 2016).

Do I need to go through the in vitro step if I think long-term cryopreservation is necessary? The necessity of tissue culture depends on the propagule that is available to you. Pollen and whole seeds may sometimes be preserved and recovered without ***in vitro***. Vegetative structures (such as meristem, shoots, and

buds) need to be cultivated and recovered using micropropagation. Some seeds may need to have the embryo removed to cryopreserve and recovered in vitro.

I have been successful with micropropagation and I think I am ready to cryopreserve. What is my next step to prepare my plants? If you are working with collaborators to do your cryopreservation, you need to be in touch with them on how they want to receive your material. If you will be doing the cryopreservation yourself, your next step is to test how your plants react to cryoprotectants.

What do I need to get started with tissue culture? Tissue culture techniques require special expertise, staff time, and additional infrastructure. A sterile location is recommended for ensuring that the plants you are growing in vitro are free of contaminants. A laminar flow hood will help keep contaminants away from the plants as you plant them in the media. You will also need to be able to control temperature and light intensity in your sterile space. Sterile containers are needed (and these can be newly purchased or autoclaved) as well as a variety of other tools, planting media, and hormones. And of course, your organization will need staff trained in using the equipment. Current staff can be trained through a variety of avenues. Working with partners will allow you to learn about the processes and/or allow you to address intermittent needs without starting your own program. Hiring commercial contractors for in vitro propagation of rare plants presents another option.

For Stage one of micropropagation, surface sterilization, how do I know what disinfectants and concentrations will be effective without damaging my material? How to sterilize your plant material will depend on the type of propagule you have as well as its characteristics (size, durability, maturity). There are common practices for plant tissue sterilization (see George 1996) with minimum exposure times varying with the disinfectant. Orchids present a special case, with their very tiny seed requiring a gentler touch. However, it may also be possible to sterilize their immature seed pods instead.

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C Guidelines for Field Genebanks or Inter Situ Collections

Summary

- Maintaining small populations of plants in protected places may be a necessary conservation strategy for some species; these are known as field genebanks. At botanical gardens, these are often part of living display collections.
- The genetic diversity of the field genebank requires awareness of maternal line health and representation over years in cultivation and may require intentional gene flow via cross-fertilization from wild populations or from sister institutions housing the species.
- Institutional collaborations and careful georeferenced record keeping and sharing will be essential for maximizing conservation value of field genebanks.

Some rare species whose seeds cannot be stored by conventional means or do not produce seeds (***exceptional species***; Pence 2014) will need to be maintained as whole plants growing in a group or ***population*** on property that can offer long-term security. ***Field genebanks*** have been traditionally developed for ***cultivars*** of commercially valuable plants that must be maintained as living clones (FAO 2013). Within the CPC network, field genebanks, sometimes called living collections, are grown at our botanical gardens or on properties where landowners have agreed to conserve and maintain living populations of the species for many years. When the field genebank is located near a water source and fenced so that plants are protected from herbivory, it improves the ability to care for the collection and the chances of the collection's long-term survival. Most CPC field genebanks support long-lived tree species with five to 20 unrelated individual plants per conservation collection. Most require collaborations across institutions, because it is unlikely that a single institution will have enough space available to house the 100 or more individuals needed to capture the desired genetic diversity of a species.

In Hawai'i, special field genebanks have been established and are known as ***inter situ*** collections. They are, in a sense, half-way houses between the full care facilities offered at a botanical garden and the "fend for oneself" life in the wild. Often located at mid-elevation between the plant's wild habitat (which in many cases can no longer support the species) and the practitioner's botanical garden, these semi-protected settings provide a place where plants can experience some natural climatic variation, while still receiving supplemental care when necessary.

Field genebanks require very long-term planning and commitment by property owners. Plants have the best of both worlds: exposure to some natural climatic conditions and extra help (usually in the form of water, fertilizer or hand-pollination) when necessary. This can help ensure maintenance of adaptive traits; support of mutualists like pollinators, seed dispersers, and soil microorganisms; and easy access to ***propagules*** for restoration.

Acquiring a Conservation Collection for Field Genebanks or Inter Situ Collection

Make preparations before making collections.

- Plan the conservation collection considering species' attributes and institutional capacity. (See "Questions to Ask before Acquiring a Conservation Collection" box and "Hierarchy of Questions to Ask to Determine the Most Efficient Way to Preserve the Plant Tissue Long- Term" box).

- Know how to identify the species and know its natural and cultural history. Consult with local botanical experts and agency ***recovery staff***. Work with in-country partners to make collections. By acquiring data on the species' ***phenology*** at the target population site, you may streamline your collection trips. Visit publicly available plant collections databases, such as the Global Biodiversity Information Facility (GBIF), to determine the time of year a species typically produces flowers or fruits. Explore citizen science projects, such as iNaturalist, to view ***georeferenced*** photos of plant observations in real time. Take a reconnaissance trip to verify the actual timing of flowering and fruit set so that you can capture seeds when they are ripe. Recording flowering date and maturity of seeds in the population can aid future collections and can be reported with accession information. (See Example Monitoring Form.)

- Understand legal obligations for collection, transport, and propagation. Obtain permission from landowners to make seed collections and report permit numbers in accession records. Realize that obtaining permits for listed species may require obtaining permits from landowners and regulatory state and federal agencies; the process may take as long as 6 months to 1 year, so it is important to begin the process well in advance of your collecting season.

FAQ How can I obtain permits to make seed collections?

- Know sources for gathering reliable information about species' conservation status.

FAQ How can I find out a species conservation status?

- Research the seed storage requirements of a ***taxon***, as this will determine how the seed will need to be processed after collection.

FAQ How do I know my species storage requirements?

- To avoid over collection and/or to learn from other collectors, survey other ***ex situ*** collections to determine what species and populations have already been collected. For example, check records in Plant Search for Botanic Gardens Conservation International (https://www.bgci.org/plant_search.php) or California Plant Rescue (https://www.caplantrescue.org/).

- Participating Institutions can search for priority species for their region in the CPC PI portal (www.saveplants.org/login) on the "Rare Plant Finder" tab.

- Become familiar with ***Sentinel Plant Network*** (http://www.sentinelplantnetwork.org/) and ***Weed Risk Assessments***. Evaluate potential pest/pathogen issues and invasive behavior of the species you are collecting (Gordon and Gantz 2008; Gordon et al. 2008a and 2008b; Reichard et al. 2012).

While collecting, do no harm to collecting site or the rare plant population.

- If no previous specimen exists for the species at your collecting site or if the last known specimen is more than 10 years old and the population is large enough to accommodate removing one plant or plant part, document the identification of the species with a ***voucher specimen***. If the population is not large enough, take good photographs. Note that permission to collect the voucher may be required prior to the collection.

- Collect within permit guidelines. To minimize impact on the ***wild population***, collect no more than 10% (or the maximum allowed by permits) of an individual plant's reproductive output and/or no more than 10% of the ***population reproductive output*** in a season (Menges et al. 2004). For many species making collections at this intensity can be sustainable over multiple years, but the intensity and frequency of safe collection is influenced by population and climate specifics. For species that will need to be maintained in a field gene bank, consider the total number of plants your institution and partner institutions could maintain so that you avoid over-collecting. (See Part 1B, "Collecting Seeds from Wild Rare Plant Populations" and Part 3B, "CPC Genetic Guidelines for Acquiring a Conservation Collection.")

- Adhere to highest outdoor standards. Leave only footprints. Some habitats are extremely fragile. Adjust actions accordingly, including being mindful of habitats that are particularly sensitive to trampling and erosion.

- Be aware of any ***sensitive animal species*** at your sites. Access may require permits, training, or adjusted timelines if protected animal species co-occur with or near your species of interest.

Capture representative genetic diversity.

- Collect within permit and institutional space constraints.

- Capture ***representative genetic diversity*** across the population's spatial expanse, ***morphological maternal plants*** and range of seed appearance. Include seeds or tissues from large and small maternal plants, along the edge and from the center of the population. It is also good to sample across years to capture diversity. Some populations have individuals that flower sporadically due to varying environmental conditions (Namoff et al. 2010, Griffith et al. 2015). Collecting in a single year will not capture total population genetic diversity. For the same reason, it is important to collect from early seeding, mid-season, and late seeding individuals within a year. This requires returning at multiple times to the population.

- For seeds, strive to collect mature seeds. If you detect immature seeds in your collection, attempt to germinate immediately.

- For stem cuttings, attempt to collect multiple replicates per maternal line of tissues at appropriate developmental stage for traditional vegetative propagation and tissue culture (that is, not excessively woody).

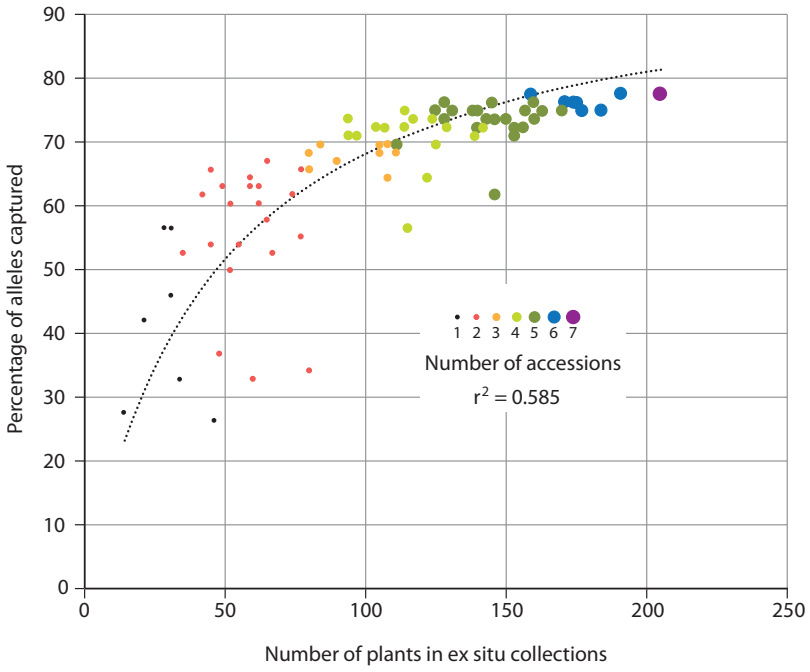

FIGURE 2.4

Genetic analysis can help quantify whether a conservation collection holds the majority of genetic diversity of a wild population. Griffith et al. (2015) compared the number of alleles measured in 10 ***microsatellite markers*** of the cycad *Zamia decumbens* to the numbers captured in 205 ***ex situ*** plants held in botanical garden collections. A single-accession collection (smallest points) would capture between 27% and 57% of ***in situ*** alleles, while the entire ex situ collection (7 accessions, 205 plants) captures 78% of wild population alleles.

- If possible, pair the collection with parallel leaf samples for DNA banking. Within the database, maintain linked genetic information with the plant growing in the collection.

◗ Genetic studies can help determine the number of plants needed to capture the majority of a wild population's genetic diversity. (See Figure 2.4 /3.2 in Griffith et al. 2015; see Hoban and Strand 2015; Hoban and Schlarbaum 2014 and Kashimshetty et al. 2017 for simulation studies related to efficient sampling).

Transport to propagation facility in the shortest time possible and in the best condition possible.

◗ Document the collection appropriately.

◗ Essential accession information includes: institution name, accession number, collector, collection date, species name, family, locality information, georeferenced latitude and longitude, site ownership, permit documentation, and population information (the total number of individuals in the population, number of reproductive individuals, and number of individuals sampled for seeds that were harvested). (See CPC Field Collection Form.)

- Providing habitat information may provide clues to ***germination*** or tissue culture requirements of the species. Recommended fields include light and moisture conditions, soil type, slope orientation, and associated species. Provide photos of habitat and plant in its habitat.
- Be sure to document any associated collections (for example, leaf litter, soil, mycorrhizal fungi) and maintain the link through processing of samples.

- Gather and report additional accession data according to institutional protocols. Complying with International Transfer Format for Botanic Garden Plant Records (https://www.biodiversitylibrary.org/bibliography/45427#/summary) and/or ***Darwin Core*** standards (http://rs.tdwg.org/dwc/) will allow easy transfer of information to partners.

◗ Complete one field form per accession. Multiple accession numbers and field forms only need to be created for collections made from populations, which are differentiated by at least 1 kilometer.

◗ Transmit accession data to CPC.

Maintaining the Field Genebank or Inter Situ Conservation Collection

At the propagation facility, follow steps for material type to propagate and maximize its survival.

Optimize conservation value.

◗ For highest conservation value, maintain adequate numbers of plants to allow for long-term sustainability of the collection and ability for plants within the collection to reproduce to provide material for other conservation uses.

◗ Realize that adequate numbers of 100+ individuals may require collaborations with other institutions.

Georeference the collection and label plants clearly.

◗ Use ***georeferencing*** to label collections.

◗ Replace labels with accession numbers as the collection ages.

Use appropriate cultivation practices to provide optimal conditions for growth and reproduction.

Monitor collection health; minimize weeds and pests.

Minimize artificial selection and genetic drift.

◗ While growing the conservation collection in the nursery, garden, or inter situ setting, minimize ***artificial selection*** and ***genetic drift***.

◗ If an accession must be maintained as whole plants for a number of generations, maintain as large a population as possible and provide periodic immigration of ap-

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Acquisition of Germplasm

5.2.1 All germplasm accessions added to the genebank should be legally acquired, with relevant technical documentation.

5.2.2 All material should be accompanied by at least a minimum of associated data as detailed in the FAO/Bioversity multi-crop passport descriptors.

5.2.3 Propagating material should be collected from healthy growing plants whenever possible, and at an adequate maturity stage to be suitable for propagation.

5.2.4 The period between collecting, shipping and processing and then transferring to the field genebank should be as short as possible to prevent loss and deterioration of the material.

5.2.5 Samples acquired from other countries or regions within the country should pass through the relevant quarantine process and meet the associated requirements before being incorporated into the field collection.

Standards for Establishment of Field Collections

5.3.1 A sufficient number of plants should be maintained to capture the genetic diversity within the accession and to ensure the safety of the accession.

5.3.2 A field genebank should have a clear map showing the exact location of each accession in the plot.

5.3.3 The appropriate cultivation practices should be followed taking into account micro-environment, planting time, rootstock, watering regime, pest, disease and weed control.

Standards for Field Management

5.4.1 Plants and soil should be regularly monitored for pests and diseases.

5.4.2 Appropriate cultivation practices such as fertilization, irrigation, pruning, trellising, rootstock and weeding should be performed to ensure satisfactory plant growth.

5.4.3 The genetic identity of each accession should be monitored by ensuring proper isolation of accessions wherever appropriate, avoiding inter-growth of accessions, proper labelling and field maps and periodic assessment of identity using morphological or molecular techniques.

Standards for Regeneration and Propagation

5.5.1 Each accession in the field collection should be regenerated when the vigour and/or plant numbers have declined to critical levels in order to bring them to original levels and ensure the diversity and genetic integrity is maintained.

5.5.2 True-to-type healthy plant material should be used for propagation.

5.5.3 Information regarding plant regeneration cycles and procedures including the date, authenticity of accessions, labels and location maps should be properly documented and included in the genebank information system.

proximately five migrants per generation from a wild source population or a sister institution housing the species and increase (triple, if possible) the sample size each generation (Havens et al. 2004).

Consciously maintain high genetic diversity of the collection.

- Maintain identities and numbers of maternal lines to capture diverse growth rates, flower production, and presumably genetic diversity. If plants die, take care to propagate new plants to maintain the number of maternal lines represented in the conservation collection.

- Accurate documentation in the database and on individual plants is key to accomplish this.

Minimize unintended hybridization.

- Maintain accessions of ***conspecifics*** from different populations or ***congenerics*** separated at distances that will minimize unintended hybridization. If you plan to collect seed in a given year, bag flowers and hand-pollinate or clip flowers of conspecifics from different populations or congenetics that year, as gardens might not be big enough for spatial isolation.

Document the horticultural care and conservation management given the collection.

Maintain the value of the collections.

- Realize that institutional memory is key to maintaining the value of these collections, as the individual plant lives may be longer than the average tenure of personnel working at the institution. For any record, imagine that someone 50 years hence will need to read and understand exactly what you did.

Capturing Material for Future Conservation Uses

Collaborate with sister institutions.

- Collaborate with sister institutions housing the species to maximize next generation reproduction (Fant et al. 2016)

- Inventory ex situ collections of sister institutions holding the species.

- If possible, conduct genetic studies to compare captive to wild population genetic diversity.

- It may be necessary to transport pollen from wild population or a sister institution for hand-pollination trials. If genetic fingerprints or ancestries are known, then compatible matches can ensue to optimize genetic diversity of next generation as is done with endangered animals (Princée 2016).

Collect seeds of the next generation when they develop.

- If possible, store via cryopreservation or distribute seeds to sister institution with interest in long-term preservation of the species. Maintain records of maternal line, dates of seed production, and storage details in database. See Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation."

Use next generation material for reintroductions or conservation translocations.

- When opportunities arise, use next generation material for reintroductions or conservation translocations. See Part 4, "Rare Plant Reintroduction and other Conservation Translocations."

FAQ Frequently Asked Questions

How can I obtain permits to make seed collections? The first step is to check the ***species conservation status*** and the ownership of the lands where the species occurs. If your target species is listed under the Endangered Species Act, check with your local U.S. Fish and Wildlife Service (USFWS) office. Depending upon the land ownership where the species occurs, you may or may not need to have a USFWS permit, but it is always good to have a discussion with the recovery staff before you make a collection. If the species is not federally listed but is protected by your state, contact the state agency that issues collecting permits. Google "collecting permit rare species STATE NAME." If your target species grows on private land, it is also good to get written permission from the landowner before making the collection.

How can I find out a species conservation status? Reliable sources for determining a species conservation status include: U.S. Fish and Wildlife Service (www.fws.gov), Nature Serve (www.natureserve.org/), The Institute for Regional Conservation (http://regionalconservation.org/), *California Native Plant Society* (http://www.cnps.org/cnps/rareplants/rareplantdata.php), and state natural heritage programs.

How do I know the genetic representation of my ex situ collection? You can collect tissues for genetic analysis from individuals in the ex situ collection and wild population. This is most accurately done if you sample and include all individuals in both ex situ and wild populations. Usually this is warranted for only those wild populations that are extremely rare in the wild.

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Glossary Terms

acclimatize—Harden off a plant so that it can survive under low humidity conditions.

adventitious shoot—A shoot that arises from a point that is not the shoot tip (for example a bud at a leaf axil).

allele—DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to- many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity.

ambient conditions—The relative humidity and temperature of the room. When processing seeds for long-term storage, it is a good idea to check the room temperature and humidity. Seeds will have best chance for long-term survival if processed at temperatures below 25°C. Humidity levels can be taken below ambient levels when using a desiccator.

apical meristem—Growing tissues at the tip of a shoot.

artificial selection—The process of modifying organisms by selection in breeding controlled by humans (for example, choosing a plant with numerous fruits and removing low fruit-producing plants in a breeding program will artificially select for fruit production).

auxins—A plant hormone that causes the elongation of cells in shoots and is involved in regulating plant growth.

axillary bud—A bud that grows from the axil of a leaf or node and has the potential to form stems and branches with leaves or reproductive shoots with flowers.

axillary shoot—The stem that grows from an axillary bud at the axil or base of a leaf.

congeners (congenerics) —Members of the same genus.

conservation collection—An ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues or whole plants of at least 50 unrelated mother plants, and breadth, meaning it consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.

conservation translocation—A definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of indigenous range including conservation introductions, comprising assisted colonization and ecological replacement.

conspecifics—Individuals of the same species.

congeneric—see congeners

conventional storage—Storage at freezer temperatures (–18°C ± 3°C or 0°F ± 5°F).

cryogenic storage—Storage below –130°C in specialized containers holding liquid nitrogen.

cryopreservation—Storing at liquid nitrogen temperatures, usually at –170°C to –180°C (vapor above liquid nitrogen) or at –196°C (in liquid nitrogen) to maintain viability.

cryoprotectant— A substance that prevents tissues from freezing, or prevents damage to cells during freezing.

cultivars—A plant variety that has been produced in cultivation by selective breeding (for example, a "Champagne" mango).

Darwin Core—A body of standard terms intended to facilitate the sharing of information about biological diversity by providing reference definitions, examples, and commentaries (http://rs.tdwg.org/dwc/).

desiccation—The process of removing moisture for the purpose of preservation.

DNA banking—Long-term storage of an individual's genetic material. In plants, leaves are common tissue that can be stored.

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

exceptional species—Non-orthodox species that cannot be conserved long-term using conventional seed banking methods. This includes species with few or no seeds available for banking, species with seeds that are intolerant of desiccation and freezing, or seeds that can tolerate drying, but not freezing, or species that may only tolerate storage at –20°C for less than 10 years.

explant—Tissue transferred to or from a tissue culture medium.

fecundity—The number of seeds or asexual propagules produced by an individual plant or population.

field genebank—Plants grown in the ground for the purpose of conservating genes. In botanical gardens, display collections of trees, shrubs, herbs can be considered field genebanks.

genetic diversity—Variation in the DNA sequence between distinct individuals of a given species (or population). The degree of genetic diversity can be compared between individuals or populations. Capturing the breadth of existing genetic diversity is the goal of a conservation collection.

genetic drift—Variation in the relative frequency of different genotypes in a small population, owing to the chance disappearance of particular genes as individuals die or do not reproduce.

genotype—The genetic constitution of an individual (genotyping is determining the genetic constitution of an individual).

georeferenced—Points taken with GPS that can be used to locate a plant again. Herbarium specimens and seed collections when georeferenced can contribute greatly to our understanding of how plant populations may change in the future.

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

in situ—In wild habitat.

in vitro—Micropropagation; plant tissues grown on nutritional medium in a sterile humid glass or plastic container.

iinter situ—In between ex situ (offsite and fending for itself) and in situ (in wild habitat), the inter situ conservation collection has diverse genetic representation and supplemental care. The term was first used to refer to the restoration of declining species in areas that are outside their current range but within historical ranges, inferred from paleoecological studies, but is now used to describe a semi-wild setting for ensuring species' survival.

maternal lines—The offspring (seeds or plants) from a single mother plant are distinguished with unique identifying number, stored in a separate package, and labeled if grown in a nursery. Knowing the number of maternal lines in a conservation collection is an estimate of the genetic diversity represented.

maternal plants—Individual plants producing seeds. Keeping track of seeds from each maternal plant allows for estimates of genetic diversity in a collection and allows for maintaining even representation of maternal lines while growing the accession in the garden's nursery or reintroducing to the wild.

meristem—Growing tip of plant.

micropropagation—In vitro; plant tissues grown on nutritional medium in a sterile humid glass or plastic container.

moisture content—The percentage of water in seed. %Moisture content = ((Weight of fresh sample – Weight of dry sample)/ weight of fresh sample) x 100. The target moisture for storing seeds equals no more than 25% RH (lower risk of failure) and no less than 10% RH (higher risk of failure) at the intended storage temperature.

morphological—Pertaining to the form or structure.

morphological maternal plants—

microsatellite markers—Genetic markers consisting of a series of short repeating base pairs of DNA that are variable within populations and can be used to identify individuals or species, or evaluate structure and gene flow between populations.

mutualists—two organisms that exist in a relationship in which both benefit.

nodes—Intersection along a stem holding one or more leaves, as well as buds which can grow into branches which in turn may produce leaves, cones, or flowers.

non-orthodox species—See exceptional species.

outbreeding—A condition where flowers of one plant receives pollen from another plant of the same species.

outcrossing—The form of plant reproduction that requires pollen from another plant of the same species to form seeds.

phenology—The timing of key life history events in a plant's life, such as flowering or fruiting.

plantlet—Young or small plant.

population—A group of potentially interbreeding individuals that share a common ancestry or gene pool.

population reproductive output—Total seeds produced in a population within one growing season.

population size—The number of individual plants of all ages in the population.

propagule— A general term to describe any plant material that can function to propagate a new plant, including seeds, stems, corms, tubers, or spores.

recovery staff—Personnel of U.S. Fish and Wildlife Service working toward ending extinction of federally listed species.

representative genetic diversity—Best captured by making seed collections across the spatial extent of the population, from plants that are not physically close to one another, and from plants of all sizes and levels of seed output.

self-fertilizing—Pollen from one plant fertilizes a flower of the same plant such that good, viable seed results. This technique is often used experimentally to determine whether this is possible for a species.

sampling—Strategy to use to collect the representative genetic diversity in a population.

sensitive animal species—Those with protected legal status by state or federal agency.

Sentinel Plant Network—A collaboration between the National Plant Diagnostic Network and the American Public Gardens Association to improve the ability to detect and respond quickly to serious plant pests and diseases.

silica gel—A granular, vitreous, porous form of silicon dioxide made synthetically from sodium silicate that can be used to dry seeds.

species conservation status— the designated level of endangerment of a species as determined by the U.S. Fish and Wildlife Service, NatureServe, IUCN or state governmental agency.

taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

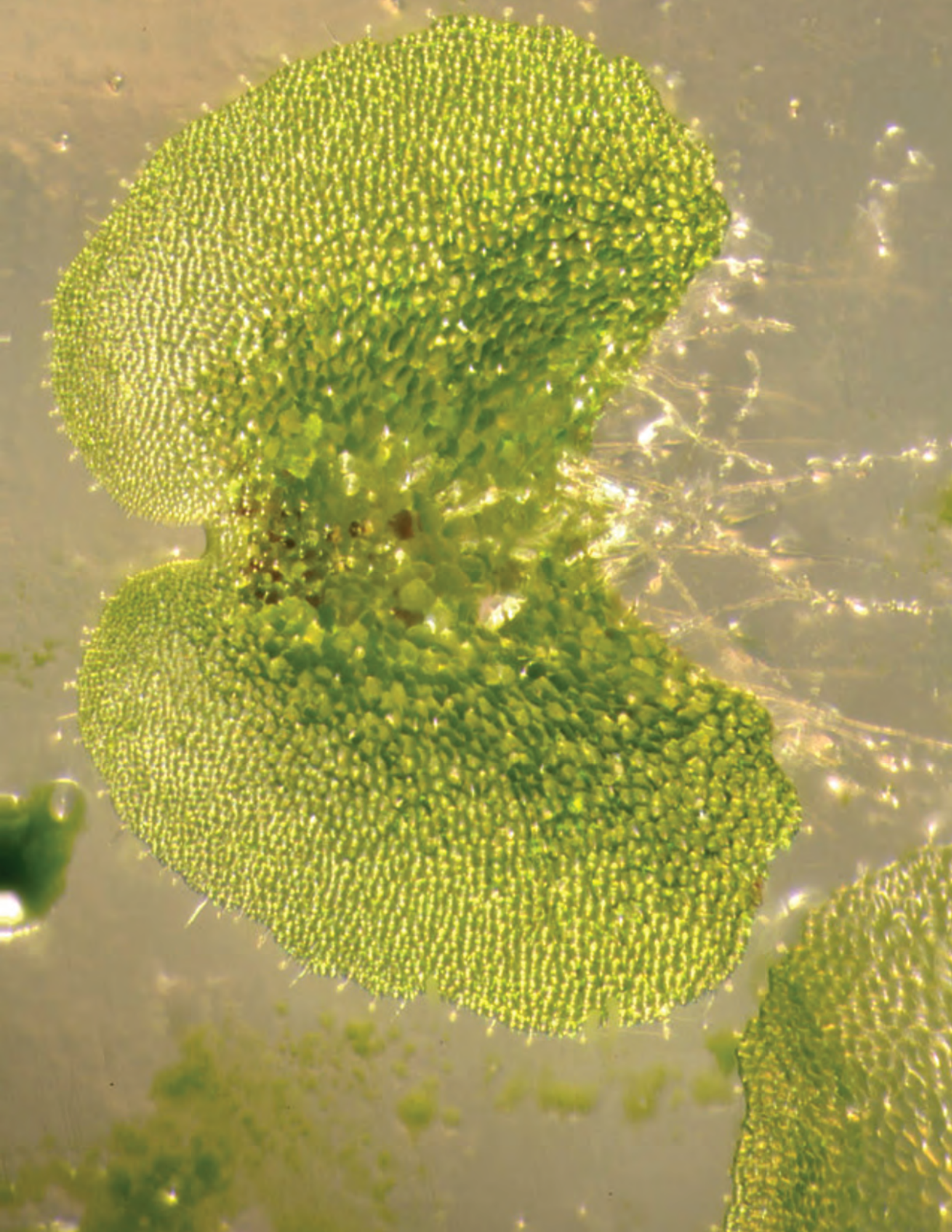
tissue culture—Growing cells in an artificial medium. In plant conservation, the cells may be whole seeds, spores, or meristems and the medium is usually some derivation of agar.

vitrification—To make liquids solid without forming crystals, a "glassy state." Forming ice crystals inside plant cells can be deadly. When a solid state can be achieved without ice, vitrification is the term used to describe ice free cryopreservation.

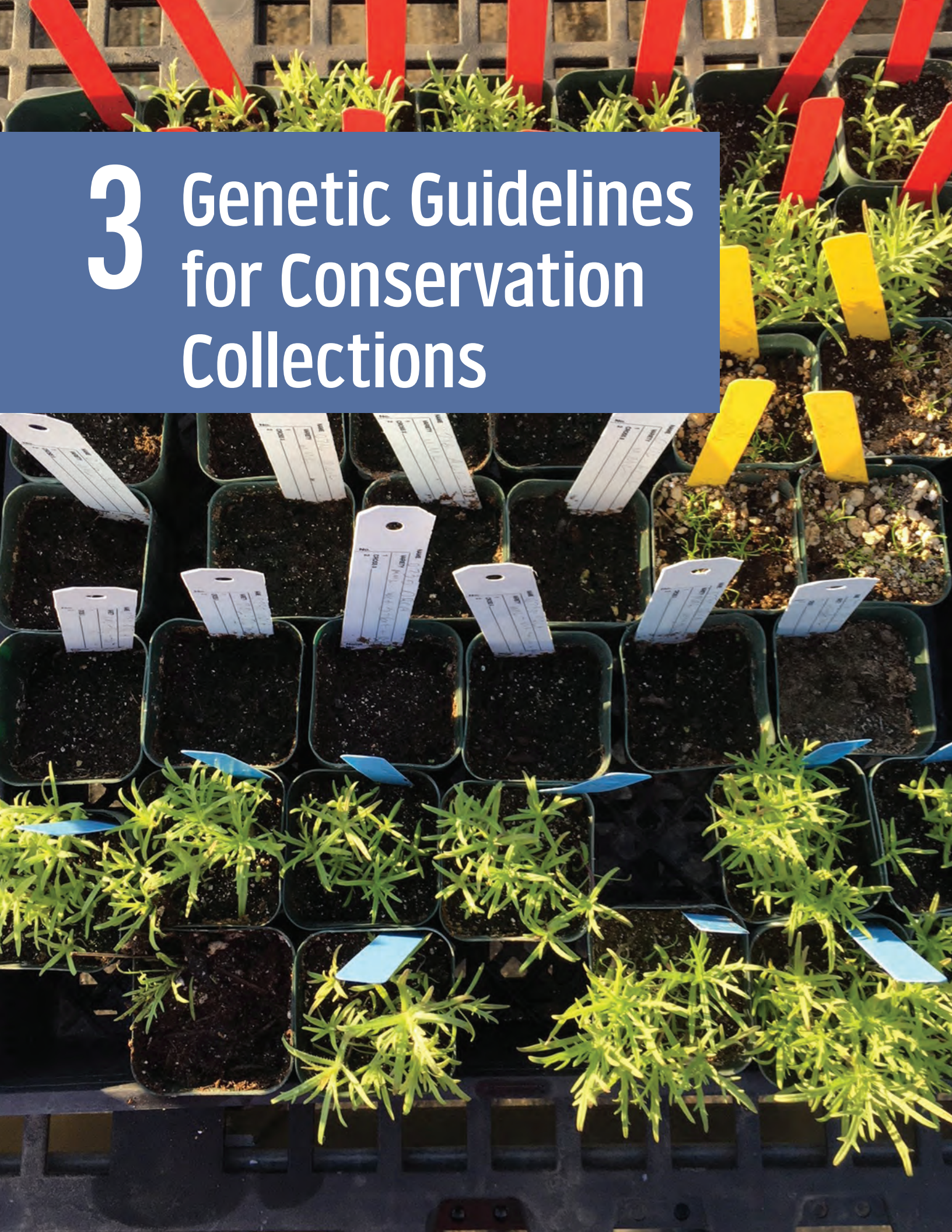
voucher specimen—A pressed plant sample deposited in an herbarium for future reference.

Weed Risk Assessments—A science-based evaluation of the potential of a plant species to establish, spread, and cause harm in a region. Several weed risk assessments exist for different regions of the U.S. (for example, Hawaii; https://sites.google.com/site/weedriskassessment/home, California: http://www.cal-ipc.org/solutions/research/riskassessment/, and Florida: http://edis.ifas.ufl.edu/ag376).

wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.

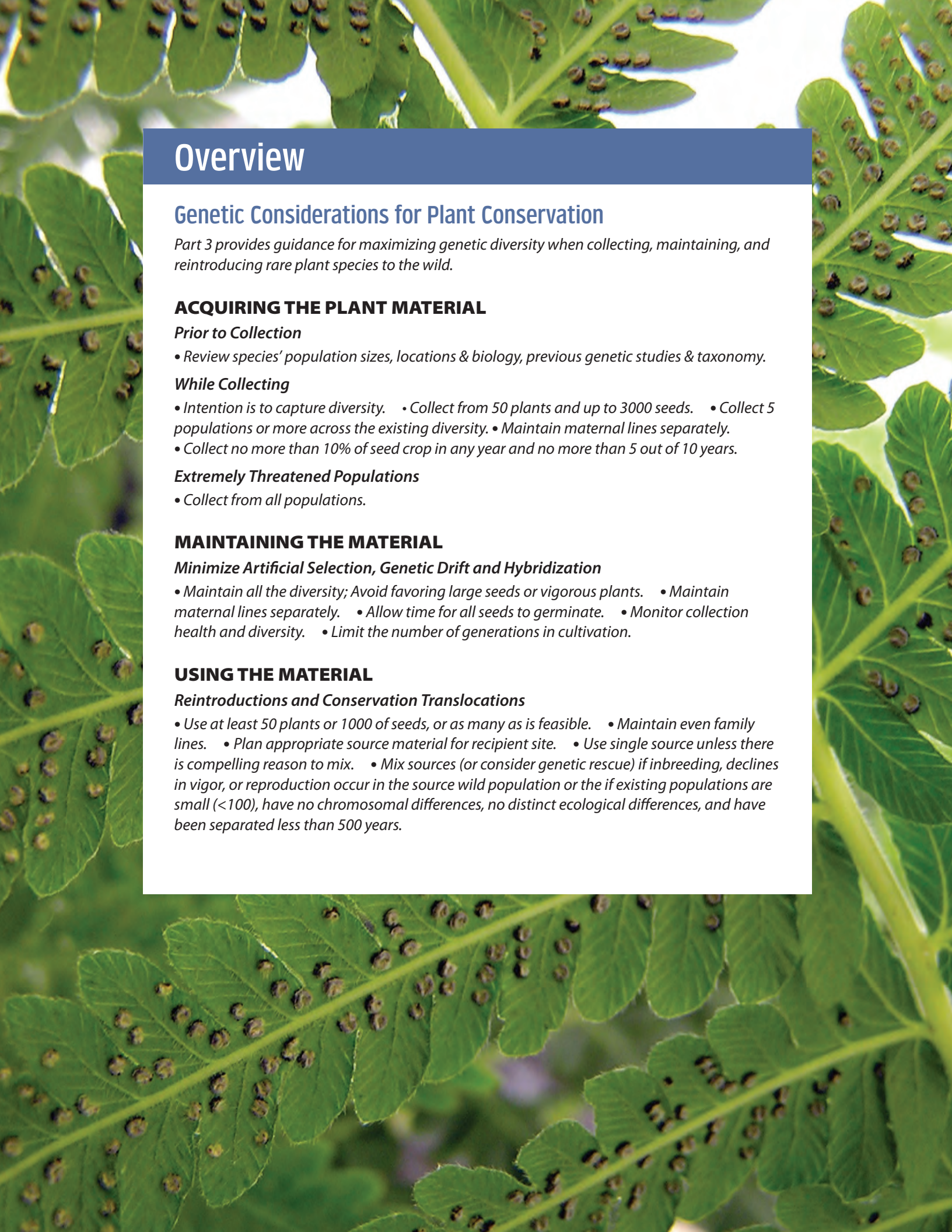


3 Genetic Guidelines for Conservation Collections



Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection

Center for Plant Conservation Best Practices



Overview

Genetic Considerations for Plant Conservation

Part 3 provides guidance for maximizing genetic diversity when collecting, maintaining, and reintroducing rare plant species to the wild.

ACQUIRING THE PLANT MATERIAL

Prior to Collection

• Review species' population sizes, locations & biology, previous genetic studies & taxonomy.

While Collecting

• Intention is to capture diversity. • Collect from 50 plants and up to 3000 seeds. • Collect 5 populations or more across the existing diversity. • Maintain maternal lines separately. • Collect no more than 10% of seed crop in any year and no more than 5 out of 10 years.

Extremely Threatened Populations

• Collect from all populations.

MAINTAINING THE MATERIAL

Minimize Artificial Selection, Genetic Drift and Hybridization

• Maintain all the diversity; Avoid favoring large seeds or vigorous plants. • Maintain maternal lines separately. • Allow time for all seeds to germinate. • Monitor collection health and diversity. • Limit the number of generations in cultivation.

USING THE MATERIAL

Reintroductions and Conservation Translocations

• Use at least 50 plants or 1000 of seeds, or as many as is feasible. • Maintain even family lines. • Plan appropriate source material for recipient site. • Use single source unless there is compelling reason to mix. • Mix sources (or consider genetic rescue) if inbreeding, declines in vigor, or reproduction occur in the source wild population or the if existing populations are small (<100), have no chromosomal differences, no distinct ecological differences, and have been separated less than 500 years.

A Introduction

Essential to plant conservation practice of the Center for Plant Conservation is taking action that will benefit species' survival and reduce the extinction risk of globally and/or regionally rare plant species. CPC participating institutions make conservation collections for this purpose. To maximize its value, a ***conservation collection*** has accurate records of provenance, differentiated ***maternal lines***, and diverse genetic representation of the species. But how do you know the best way to capture a diverse genetic representation when collecting? And how can you maintain high genetic diversity in an ***ex situ*** collection? What is the best genetic composition for creating a new population? Below we provide guidelines based upon best available scientific evidence.

Genetic diversity is the basis for any species' ability to cope with changes in its environment, including disease, stress, or extreme events. A mindset for gathering, maintaining, and using the maximum diversity possible drives the following recommendations (see Overview). Many recommendations ensure that in the absence of genetic data practitioners can collect and maintain the maximum level of genetic diversity. With more information obtained about a target species' biology, ecology, and genetics, the more accurate genetic sampling and management will be. It is our hope that these guidelines will help practitioners make good decisions. For particularly small populations, the existing genetic diversity may be limited, therefore maximizing the diversity of the conservation collection and of the reintroduction or ***conservation translocation*** is very important.

Many factors affect genetic integrity of a conservation collection. Collectors who attempt to capture the breadth of genetic diversity represented within a population and the genetic diversity across populations will improve the likelihood of success for all conservation steps that follow. Nevertheless, collectors should be aware of the possibility that ***genetic erosion*** may occur over time in their collections and take steps to minimize it. While in storage, some maternal lines may survive better than others, hence diversity may be reduced. Similarly, when regenerating the seeds or tissues from storage, differential ***germination*** and/or survival is likely, hence diversity may be reduced. With attrition at all steps along the way, the number of individuals available for a ***reintroduction*** will likely be less than the original wild collection. Planning for these losses is an essential component for genetic management of your conservation collection (see Overview).

These updated guidelines incorporate research and experience of CPC conservation officers, previous CPC guidelines, *Center for Plant Conservation Genetic Sampling Guidelines for Conservation Collections of Endangered Plants* (Center for Plant Conservation 1991), and *Revised Sampling Guidelines for Conservation Collections of Rare and Endangered Plants* (Guerrant et al. 2004b), as well as current literature. Key research on the threats of ***inbreeding*** versus ***outbreeding depression*** and concerns about ***genetic rescue*** informed these guidelines (Frankham et al. 2011; Frankham 2015), as well as serious thought about how changing climate will influence rare plant popula-

tions (Havens et al. 2015, Vitt et al. 2016). New genetic techniques are revolutionizing our abilities to examine genetic links to ecology and evolution of species (Ellegren and Galtier 2016). These in turn may offer solutions to difficult problems rare plants face.

Questions to Ask

Before Acquiring a Conservation Collection

____ Does collecting pose a threat to the wild population?

____ What is the purpose of the collection? Note: These guidelines pertain to conservation collections. Depending on the purpose of the collection, sampling strategy and numbers can vary (Guerrant and Fiedler 2004; Guerrant et al. 2004).

____ Can the ex situ collection be made such that it benefits the species' survival and reduces extinction risk?

____ How many estimated or known numbers of individuals and populations exist? (The sampling universe is known.)

____ What is the breeding system?

____ Is the taxon monoecious or dioecious?

____ Is it self-compatible or self-incompatible?

____ What is the propagule dispersal mechanism?

____ In what types of habitats does the species grow?

____ Should seeds or other tissues be collected?

____ What is the storage capability of the taxon? Can the seeds be stored in a seed bank or will the other forms of ex situ specialized propagation and care be required?

____ How long will material be stored?

____ How can the plant material be propagated? Do you know the horticulture requirements for growing plants from seeds or cuttings?

____ What level of attrition or mortality of collected material is expected in storage and regeneration? (See Guerrant and Fiedler 2004.)

____ Will the material be used for a reintroduction or conservation translocation?

B Acquiring a Conservation Collection

Center for Plant Conservation Genetic Guidelines

SUMMARY

- Planning the quantities to collect from one population and across the range of species will improve the chance of maximizing the genetic diversity of the conservation collection.
- Maintain maternal lines separately to help approximate potential genetic diversity and allow flexibility for use in future conservation translocations.
- Collect no more than 10% of a population's seed crop in a single year and no more than 5 years out of 10.

Prior to collection, ascertain what is known about current distribution, population sizes, and reproductive biology.

- See Part 1B, "Collecting Seeds from Wild Rare Plant Populations" and "Questions to Ask before Acquiring a Conservation Collection."

- Determine the locations and known (or estimated) sizes of populations.

- Research the reproductive biology including ***breeding system***, ***self-compatibility, monoecy*** versus ***dioecy***, and ***pollination mechanism***. Because all influence patterns of ***genetic diversity*** of the population, knowing this information will influence the collection protocol. (See Figure 3.2, "Summary of Collecting Recommendations for Numbers of Populations to Sample," and Figure 3.3, "Summary of Collecting Recommendations for Numbers of Individuals to Sample within a Population.")

- Check whether any previous genetic studies (***common garden*** or ***molecular***) have been done on the target species as these might inform collection priorities.

- If there known taxonomic issues in the species such as ***hybridization***, ***taxonomic ambiguity*** or ***polyploidy variation***, take ***herbarium vouchers*** and tissue samples for ***morphological*** and genetic studies that may be linked to your collection.

- If little is known about the species' breeding system or pollination mechanism, reviewing close relatives may give clues. Note that starting with close relatives may be helpful, but it is not always guaranteed that the biology will be exactly the same as the rare species.

Collect material to capture representative genetic diversity.

- Collect material (seeds, cuttings, spores, etc.) across the diverse spatial, ***ecological***, temporal and population sizes existing for the species to capture its representative genetic diversity.

- Collect from as many populations as resources allow, at least five populations that represent the range of variation in distribution, ecology, and population size (Falk and Holsinger 1991). The more populations collected, the greater the probability that the range of genetic variation will be captured. This is particularly true for any ***selfing*** or ***clonally*** reproducing species, where genetic diversity will be greatest between populations (Schoen and Brown 1991). The ideal would be to capture all populations especially for those species that have less than 20 populations in existence.

- Collect across the species' entire range, stratified across environmental features such as soil, elevation, and climate (Guerrant et al. 2014). Collect from both small and large populations, from edge and core parts of distribution.

- Generally, the amount of genetic variation captured increases with the total number of populations added. However, there is a point where the gain of unique ***alleles*** levels off and there is no gain in diversity when adding more individuals or populations. (See Figure 3.1; Griffith et al. 2015; Hoban and Strand 2015; and Hoban and Schlarbaum 2014 for simulation studies related to efficient sampling).

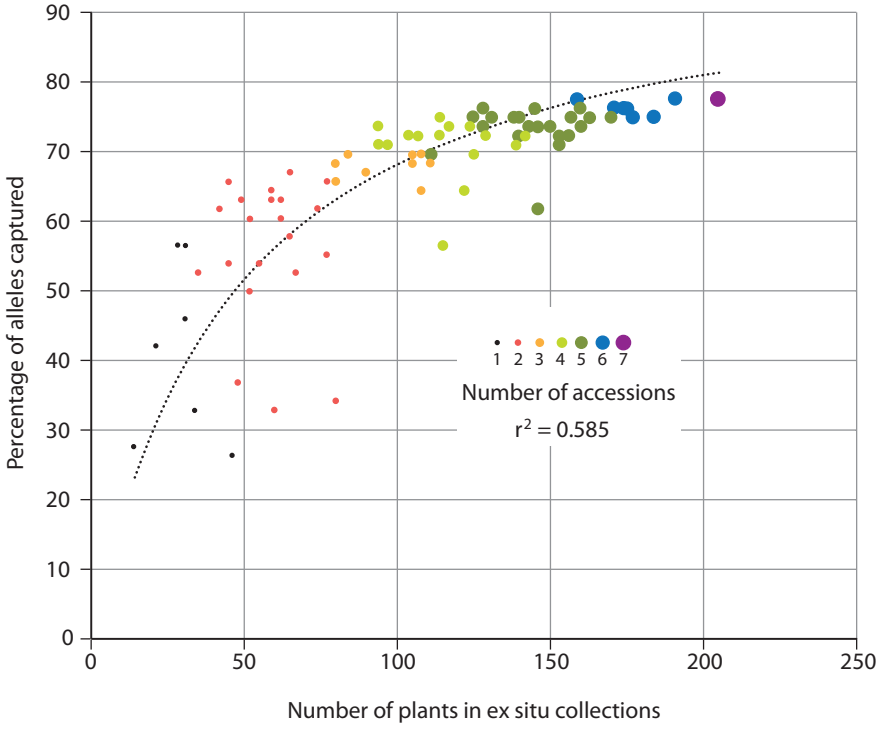


FIGURE 3.1

Genetic analysis can help quantify whether a conservation collection holds the majority of genetic diversity of a wild population. Griffith et al. (2015) compared the number of alleles measured in 10 ***microsatellite markers*** of the cycad *Zamia decumbens* to the numbers captured in 205 ***ex situ*** plants held in botanical garden collections. A single-accession collection (smallest points) would capture between 27% and 57% of ***in situ*** alleles, while the entire ex situ collection (7 accessions, 205 plants) captures 78% of wild population alleles.

- Basey et al. (2015) suggest this sampling strategy to maximize diversity capture:
  - Collect from plants that grow far apart as they are more likely to be unrelated.
  - Collect from large and small ***maternal plants***—robust and spindly, with abundant and few fruits. Collect seeds from very different-looking individuals.
  - Collect multiple times in a season to capture early- or late-flowering plants.
  - Collect from plants in all ***microhabitats*** at a site.
  - If collecting from multiple populations of the same species, use the same collecting strategy for all populations.

- Collect across years and multiple times in a season to capture early-, mid- or late-flowering plants as well as individuals adapted to variable climatic factors (Guerrant et al. 2014; Basey et al. 2015). For small populations with few reproductive individuals, it will be especially important to make collections across multiple years.

- For species that produce no seed, attempt to collect tissues from up to 50 unrelated individuals from multiple populations. The size of plants at maturity and space available will likely influence the number of plants that can be maintained long-term at any institution.

From how many populations should I collect?

The general rationale is that in highly ***outcrossing*** species with extensive ***gene flow*** (large ***neighborhood size***), populations are more similar (so one can collect from fewer populations). Whereas in a ***selfing*** species or one with limited gene flow (small neighborhood size), we expect populations to be very different, therefore we advise collecting from more populations.

From how many individuals should I collect within a population?

Outcrossing leads to higher diversity within a population than across populations, so focus collection efforts on collecting more individuals within a population. In a highly selfing species, you expect individuals to be similar, so you might be fine to collect from fewer individuals.

– Kay Havens

Plan your sampling strategy.

- Use population size and ***fecundity*** to plan your sampling strategy for the collection.

- An ideal collection would have a total of 3000 seeds from at least 50 unique unrelated maternal plants to maximize allelic frequencies represented in each accession (Brown and Marshall 1995; Guerrant et al. 2004; Guerrant et al. 2014). Note that the breeding system may adjust the representative number of maternal plants recommended. (See Figure 3.2, "Summary of Collecting Recommendations for Numbers of Populations to Sample," and Figure 3.3, "Summary of Collecting Recommendations for Numbers of Individuals to Sample within a Population.")

FAQ Why 3000 seeds?

FAQ Why 50 maternal lines?

- If maternal plants produce few seeds, it may be necessary to collect from more than 50 maternal plants to obtain a collection of 3000 seeds while collecting within permit restrictions.

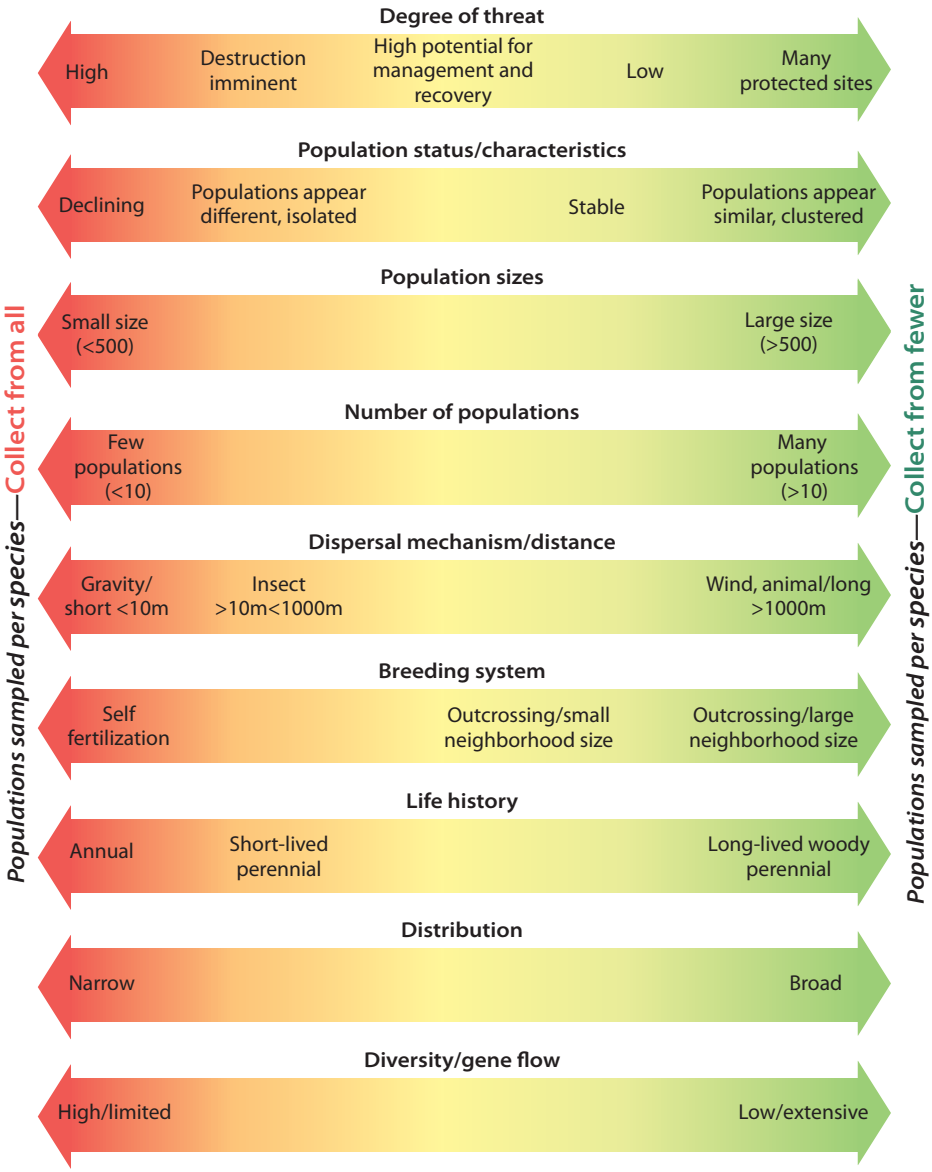


FIGURE 3.2

Summary of Collecting Recommendations for Numbers of Populations to Sample. CPC recommends collecting from at least 5 populations across the range of a species. You can use the row factors in this figure to refine your decision. If any of the row factors falls into the red zone, we recommend collecting from all populations. If factors fall into the yellow or green zone, then it is reasonable to collect from fewer populations. (Adapted from Falk and Holsinger 1991.)

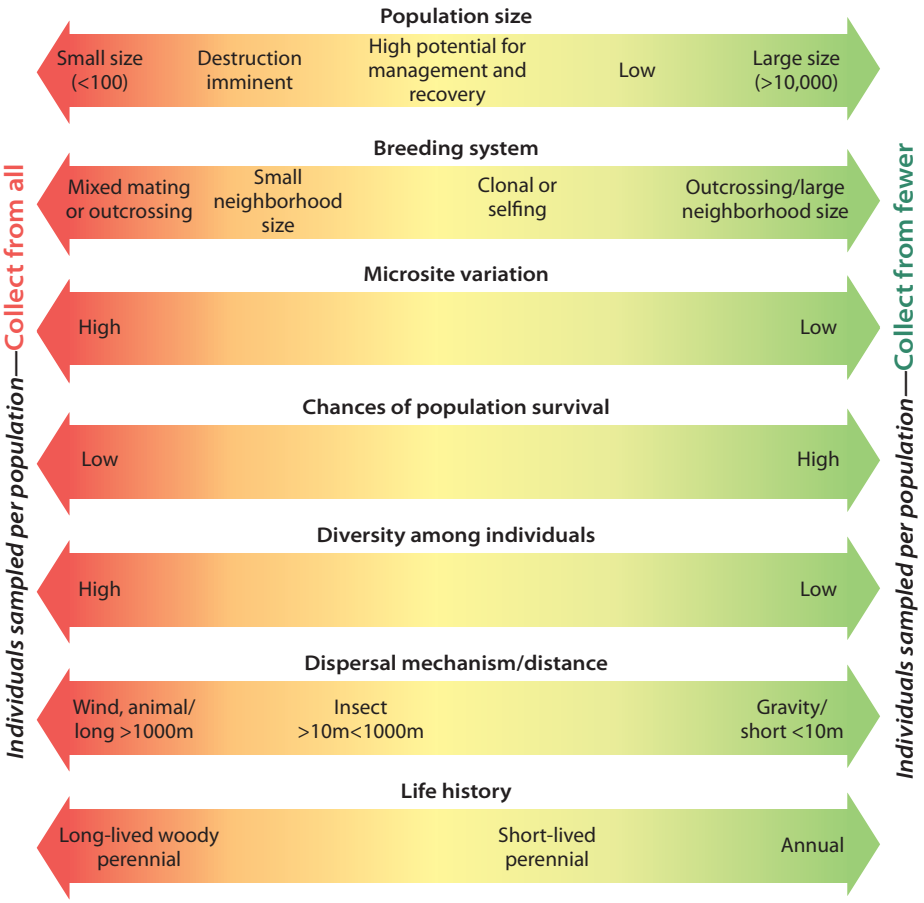


FIGURE 3.3

Summary of Collecting Recommendations for Numbers of Individuals to Sample within a Population. CPC recommends collecting from 50 unique maternal plants within a population. You can use the row factors in this figure to refine your decision. If any of the row factors falls into the red zone, we recommend collecting from all individuals. If factors fall into the yellow or green zone, then it is reasonable to collect from fewer individuals. (Adapted from Falk and Holsinger 1991.)

If populations are simply too small to produce 3000 seeds in a season, or even across multiple years, collecting a few seeds may be the only option. In these instances, attempt to capture up to 10% of the seeds from each of the reproductive individuals across multiple years. To avoid collecting from the same ***perennial*** individual across years, it may be necessary to place tags on individuals so that they can be identified in the future.

Realize that extremely small seed collections (<100 seeds) will require making additional collections in the future. If more than 300 seeds cannot be collected within 5 years, try a seed increase rather than wild collection to increase quantities of seeds in storage. (See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration" if it is unlikely that a collection can ever surpass 100 seeds or 300 seeds in 5 years.)

FAQ Will it be damaging to collect 10% of the seed production in sequential years?

Maintain accessions of each population separately with packages of maternal lines separated.

It is always better to collect and maintain maternal lines separately for several reasons. Separation allows flexibility to equalize family lines in reintroductions, may add value to potential projects down the line, or may be useful for a purpose unanticipated at the time of collection. Equalizing family size in a reintroduction maximizes

the effective population size and may help prevent genetic problems caused by inbreeding, genetic drift, and artificial selection (Havens et al. 2004).

- If your species has individuals that produce fewer than 20 seeds, so that collecting 10% of a maternal line equals 1 or 2 seeds, it may be appropriate to bulk the collection, maintaining an equal number of seeds collected from each mother plant. In this scenario, collecting from more than 50 maternal plants will be necessary to achieve a total collection of 3000 seeds.

Collect no more than 10% of the seed output of a population in a season for no more than 5 in 10 years.

- For many species, making collections at this recommended intensity can be sustainable over multiple years, but collectors should note that the intensity and frequency of safe collection is influenced by population and climate specifics. (See Part 1B, "Collecting Seeds from Wild Rare Plant Populations.")

FAQ

Will it be safe to collect 10% of seed production in sequential years?

- For small populations, collecting small percentages over multiple years is better than collecting more than 10% in one year. This will not only increase potential diversity represented in the sample but will minimize the impact on the wild population.

Drastic times may call for drastic measures.

- Extremely small populations or populations with high probability of ***extirpation*** in the foreseeable future may warrant ***rescue collections*** greater than 10%, up to 100%, to preserve the highest genetic diversity for the species possible (Guerrant et al. 2004).

- For populations of species with extremely low overall numbers, particularly those that have 10 or fewer reproductive individuals and a poor history of recruitment, or are known to be in precipitous decline, it may be necessary to rescue the population from whatever is threatening it in the wild population. Collect up to 100% of seed or whole plants at the discretion of the permitted collector. Such collection levels assume, of course, that adequate facilities, procedures, and resources are available to care for the material, and that such collections are part of a more inclusive strategy, which is endorsed by the appropriate regulatory agencies (Guerrant et al. 2004).

- If there are fewer than 50 individuals in the wild, or if viable seed is not available, ***tissue culture*** or vegetative reproduction may be required to maintain the species' genetic diversity. Collecting vegetative tissue should follow the same guidelines as for seed collections. Collect vegetative tissue from as many unrelated individuals from each population as possible. (See Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation.")

Collect herbarium vouchers, soil samples, and genetic samples (when possible) associated with the seed accession.

- Genetic samples can be collected and stored for future studies. (See "Guidelines for Tissue Collection and Storage Related to Genetic Studies.")

C Maintaining a Conservation Collection

Center for Plant Conservation Genetic Guidelines

Summary

- While the conservation collection grows in the nursery, take care to maintain genetic diversity.
- Keeping records and labels on maternal lines will help track the potential genetic diversity represented in the collection and can be used to equalize family lines for reintroductions or other conservation translocations.
- If maintaining more than one population or related species, take care to avoid unintended cross-pollination.

Minimize artificial selection and genetic drift.

- While maintaining seed or growing the accession in the nursery or garden, minimize artificial selection and genetic drift.

- Maintain ***ex situ*** collections as dormant seed if seeds are orthodox. This is a cost-effective method.

- During seed processing, take care to use cleaning equipment that allows for the greatest proportion of viable seeds to be processed (Basey et al. 2015).

- Seeds produced within a single population may vary in size. Take care to conserve seeds of varying size, rather than just large seeds (Basey et al. 2015).

- Seeds within the same capsule or pod may vary in maturity and require different storage protocols. (See Part 1B, "Collecting Seeds from Wild Rare Plant Populations.") Mature seeds may be able to be stored by conventional methods, whereas immature seeds may be able to be ***cryopreserved*** or grown as a display or nursery plant but would not survive freezing. (See Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation" and Part 2C, "Guidelines for Field Genebanks and Inter Situ Collections.") For particularly rare species, there may be value to conserving seeds of varying maturity to capture full ***genetic diversity***.

- Many seeds can be dried and maintained at cold temperatures for long periods. While in storage, they will be under reduced selection pressures, theoretically, although some genotypes may have lower survival when frozen (Crossa and Vencovsky 2011). Some species survive 5 to 10 years, but not longer, in cold, dry conditions. For these taxa, regenerating the seed collection may be necessary. (See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration.")

- For species that cannot be stored long-term as seeds, maintain multiple genetically unrelated plants in one or more living collections (Fant et al. 2016). The mini-

mum number of plants needed to represent the genetic diversity of a species in a ***conservation collection*** can be determined with genetic studies (Griffith et al. 2015).

- Be conscious of artificial selection while growing plants in the conservation collection. Attempt to mimic the water and nutrient regimes the species faces in the wild, especially for individuals that will be returned to the wild.

- For advice for plants held in tissue culture, see Part 2B, "Collecting and Maintaining Exceptional Species in Tissue Culture and Cryopreservation."

Maximizing germination of a seed accession may require using varying conditions and allowing enough time.

- Within a single accession, seeds may have variable ***germination*** rates or some seeds may have dormancy, while others do not. Planning to encompass this variation when doing germination trials will help ensure genetic diversity of seedlings.

- Labeling germination trials by ***maternal lines*** allows practitioner to track diversity and ensure equal representation in subsequent research or reintroduction trials.

Maintain material across maternal lines.

- Consciously maintain material across maternal lines to capture diverse growth rates, flower production, and presumably genetic diversity.

- Monitor the survivorship and health of the clearly labeled maternal lines represented in the accession.

- Maintain accurate records of the number of surviving individuals and total maternal lines to approximate the genetic representation of the collection. Note that genetic studies would be required to know the true genetic diversity in the collection.

- Avoid artificial selection. When trying to increase numbers of an accession, it is easy to choose the best and most fecund to replicate. It is important to have and maintain all maternal lines in the living collection whether they be on display in the garden, in a tissue culture lab, or in the nursery.

- If mortality occurs excessively for one maternal line, consider replanting to refresh the diversity represented.

Maintaining accessions offsite for generations requires periodic immigration from wild sources.

- If an accession must be maintained offsite as whole plants for a number of generations, maintain as large a population as possible and provide periodic immigration from a wild source population of approximately five migrants per generation and increase (triple, if possible) the sample size each generation (Havens et al. 2004).

Minimize unintended hybridization.

- Use knowledge of breeding system and wild population dynamics to inform these actions.

- Grow seeds from different populations at different times.

- If growing seeds from more than one population simultaneously, physically separate them or place them under netted cages to avoid pollen transfer.

- Note that there may be compelling reasons to cross-pollinate populations, such as one population consists of a single self-incompatible clone (Menges et al. 2016).

- Because genetic loss and change can occur during a single generation, best practice is to grow plants from wild-collected seeds (Basey et al. 2015) and preferably use F_1 or F_2 seeds for conservation introductions whenever possible. Seeds produced in a cultivated condition may have attributes that are disadvantageous in the wild, yet many ***conservation introductions*** must rely on seeds generated in a nursery in order to have adequate numbers to reintroduce to the wild. (See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration.")

D Using the Conservation Collection for Reintroductions and Other Purposes

Center for Plant Conservation Genetic Guidelines

SUMMARY

- Give your reintroduced population a good chance for survival by starting with adequate numbers of plants or seeds at the beginning.
- Determine the source of the plants or seeds.
- Determine whether the source should consist of seeds or plants from a single population or from mixed populations.

The larger the founding population the greater the chance of success in establishing self-sustaining populations.

In general, the larger the founding population, the greater the chance of it surviving to become an established, self-sustaining population will be (Guerrant 1996). Use as many individuals as is feasible (50 individuals or more) for a ***reintroduction*** or ***conservation translocation*** (Guerrant 1996, Albrecht and Maschinski 2012).

To compensate for propagule losses due to mortality during reintroduction, start with an estimate of desired numbers of individuals surviving to reproduction in a new founding population. Then, account for expected losses during establishment. Some of these calculated losses can be mitigated by maintaining backup clonal material. The greater number of individuals (from diverse ***maternal lines***), the greater chance that diverse genes are represented in the reintroduced population.

When growing the material for purposes of a reintroduction or other conservation translocations, keep in mind the reproductive biology of the species. For example, obtaining 10 female plants of a dioecious species may require planting twice as many seeds as the expected number of seeds that germinate if the sex ratio is 50:50.

Ascertain whether genetic studies are needed before conducting the reintroduction.

Decide whether genetic studies are needed before conducting a reintroduction and, if possible, conduct studies to measure genetic structure of the focal species (Neale 2012). (See "Are Genetic Studies Needed?" box, "What a Genetic Assessment Can Tell You and How That Information Can Be Applied" box, and Ottewell et al. 2016 for discussion of management options.)

Questions to Ask

When are Genetic Studies Needed?

Assessing the genetic diversity of wild populations can reveal insights about the biology of the species, however genetic studies can be expensive and may not always be necessary. They can include either molecular work (***genotyping, sequencing, genome*** or ***ploidy*** analysis) or common garden studies. These types of studies are advisable before collecting a rare species or before conducting a reintroduction if the wild populations have any of the following characteristics:

Within-population issues

____ Population has fewer than 50 individuals flowering and setting fruit.

____ The species is clonal.

____ Little or no viable seed is being set.

____ There are potential taxonomic concerns (taxonomic ambiguity, potential hybrids, or variation in ploidy).

Issues across the species' range

____ The species is declining and little is known about the biology or life history of the species.

____ The species has highly fragmented and isolated populations.

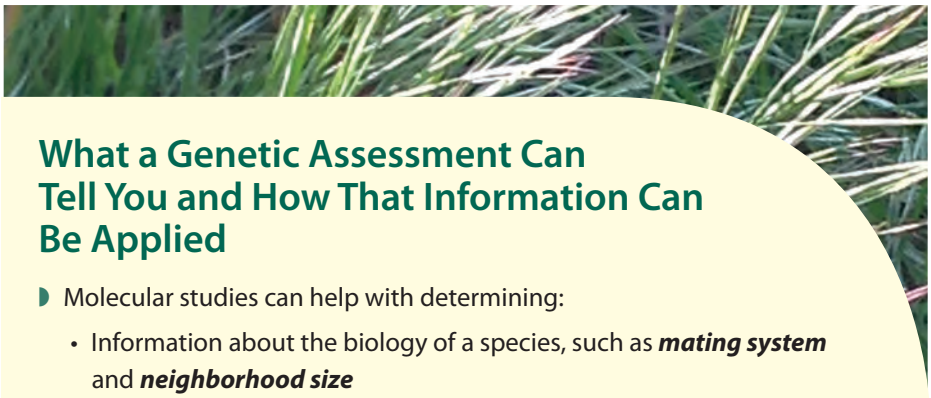
____ The species looks different in different locations.

____ One or more populations of the species has distinct ecology from the majority of populations.

Review and plan the source material that will be appropriate to introduce to a particular site (Basey et al. 2015).

◗ Identify the potential source material(s) available for conservation translocation. Note collection site ***ecological*** conditions, community structure, and proximity to the proposed recipient site (See Part 4C, "Preparing the Reintroduction; " Maschinski et al. 2012).

◗ Collect or retrieve from a seed bank the source material whose location has similar climatic and environmental conditions to the recipient site(s). Detailed information recorded on accession forms at time of the collection is essential for this evaluation.



What a Genetic Assessment Can Tell You and How That Information Can Be Applied

- Molecular studies can help with determining:
  - Information about the biology of a species, such as ***mating system*** and ***neighborhood size***
  - Levels of overall neutral diversity represented within the population. Although this does not always equate to amount of resilience a population has in response to stressors such as drought or disease, it can identify issues such as bottlenecks, genetic drift, or a limited number of ***founders.***
  - A quantified level of differentiation among populations, which is important for prioritizing populations for collection and identifying appropriate sources for reintroductions
  - ***Effective population size*** N_e. This helps identify how many unrelated individuals are reproducing in the population.
  - Degree of clonal/***asexual*** growth to ensure you collect the maximum number of unrelated individuals possible
  - Historic migration rates to identify natural levels of gene flow
  - Historic levels of inbreeding to minimize potential ***inbreeding depression*** in collections and reintroductions
- ***Genomic studies*** can aid in determination of:
  - Traditional measures listed for molecular studies above
  - Location of genes along chromosomes
  - Linking genetic fingerprint/genetic ***loci*** to ***ecological attributes***
  - Loci that may be subject to differential selection across populations, which can be used to identify locally adapted populations and prioritize populations for collection
- Common gardens can help:
  - Identify ***morphological*** or ***ecological*** differences between groups to ensure matching source to ***conservation translocation*** site
  - Quantify level of ***phenotypic*** differentiation among populations to identify potentially important adaptive differences
- Breeding studies can help:
  - Identify self-incompatibility ***alleles*** that might limit mating
- ***Flow cytometry*** can help:
  - Identify differences in ***ploidy/cytotypes*** that might lead to reproductive issues if mixed ploidy individuals are cross-pollinated

- The extent of gene flow between populations varies by species. Some may have isolated, locally adapted patches within a small area, whereas others may have great gene flow over great distances, therefore there isn't a simple relationship between distance and genetic relatedness (Richards et al. 2016).

- Use genetically heterogeneous ***founders*** to improve the ability to cope with varying conditions (Falk et al. 1996; Guerrant et al. 2004; Neale 2012). Theoretically, high levels of genetic diversity will equip the new population with adaptive potential needed to withstand ***stochastic and deterministic events*** including climate change and can defend against potential genetic pitfalls of small populations such as ***founder effect*** and ***inbreeding depression***.

Decide whether to use single source versus mixed populations.

- Sometimes it may be appropriate to use a single-source population, while other times it may be appropriate to mix populations.

- The decision of whether to mix source populations or keep separate should consider several factors: condition and context of the ***wild population(s)***, ***mating system***, dispersal mode, ploidy level, and genetic structure. (See Figure 3.2, "Summary of Collecting Recommendations for Numbers of Populations to Sample," and Figure 3.3, "Summary of Collecting Recommendations for Numbers of Individuals to Sample within a Population.")

- Traditionally, it is recommended to use founders from only a single wild population that is ecologically similar to the recipient site in order to preserve locally adapted genes. For example, if the species is an ***obligate outcrosser*** and is locally adapted to a site at very fine scale, then mixing populations may cause ***outbreeding depression*** (Neale 2012). This is especially true if there are known genetic differences between existing populations or if populations have more than 100 individuals, have distinct ecology, and have been separated for more than 20 generations (Frankham et al. 2011).

- Mixing source material in a restoration may be necessary if there is no appropriate ***ecological*** recipient site that matches the site where the population currently grows, if the available source material is limited, or if there is evidence of low genetic diversity or inbreeding depression in the source population (Dalrymple et al. 2012; Neale 2012). We recommend mixing source material if the ***taxon*** has extant populations of less than 100 individuals with no chromosomal differences, no distinct ***ecological*** differences, and if populations have been separated less than 500 years (Frankham et al. 2011).

- If mixing sources, keep track of each individual source through collection, production, and conservation translocation to allow for rapid response should any issues arise.

Use founders with evenly represented family lines.

- Collect and maintain seeds from each maternal line separately. In this way, it is possible to know and intentionally control even representation of the different founders.

- Minimize "unconscious" selection during seed increases or augmentation of natural populations. Note that variation in ***germination*** and growth of maternal lines should be expected. Resist the temptation to over-represent the winners—those abundantly available, vigorously growing maternal lines that may skew the diversity of the population—but rather consciously maintain even family line representation (Guerrant et al. 2004; McKay et al. 2005).

Consider genetic rescue when appropriate.

- When a wild or reintroduced population has low genetic diversity and signs of inbreeding depression, consider ***genetic rescue*** (Frankham 2015).

- Infusing new genetic stock into a wild or reintroduced population (genetic rescue) may be necessary to overcome detrimental effects of inbreeding (Frankham 2015). Introducing new individuals or genes (from pollen) could increase genetic diversity and fitness of a small, inbred population (DeMauro 1993; White et al. 2018).

- Aim to release equal numbers of individuals from each source population early in the reintroduction to promote balanced ***admixture*** in the descendant population (Havens et al. 2004; White et al. 2018).

- For species critically imperiled by threats that are genetically linked, genetic rescue may also comprise insertion of advantageous genes as is being done in crop development (Rinaldo and Ayliffe 2015).

Frequently Asked Questions

Why should I try to collect 3000 seeds? Collections of 3000 seeds 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; and a substantial amount of seed can be conserved as a long-term safeguard against loss of the wild population (Bureau of Land Management 2016).

Why should I try to sample from 50 maternal plants? The 50 maternal lines recommendation is supported by the sampling strategy from the Bureau of Land Management Technical Protocol for the Collection, Study, and Conservation of Seeds from Native Plant Species for Seeds of Success (2016).

For many potential users of and uses for the collection, it is important to maximize the number of ***alleles*** (variants of genes) present within the sample by capturing the greatest proportion of those alleles represented in the field population. The number of different alleles in a population reflects its genetic

diversity. Sampling from (1) 30 randomly chosen individuals in a fully ***outcrossing,*** or ***outbreeding***, sexual species, or (2) 59 randomly chosen individuals in a ***self-fertilizing*** species will capture at least one copy of 95% of the population's alleles which have frequencies of at least 0.05 (Brown and Marshall 1995).

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 very rare 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.

For research purposes, and for the conservation of rare species that occur in populations of fewer than 50 individuals, as well as for less fecund common species, where collections will result in fewer than 3000 seeds, we recommend collecting seeds along maternal lines. In a maternal line collection, seeds from each individual plant (maternal line) are collected and bagged separately (as opposed to bulking the seed collection of multiple plants into one or two bags as we do for regular seed bank collections). This ensures the greatest genetic diversity is available in a small collection. When we combine seeds in a bulk collection, there is only a small chance any particular parent's offspring will be represented when a portion of the collection is removed to restore a new population. For small populations or small seed collections, collecting along maternal lines allows equal representation of all parent's offspring (seeds) in a newly restored population, provided a portion of the seeds are distributed equally from each maternal line (Bureau of Land Management 2016).

Will it be safe collect 10% of the seed production in sequential years? See the "10% Rule" box.

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Glossary Terms

accession—A collection occurring within one plant population at one location that may be collected over several consecutive days. In botanical garden databases, an accession is given a unique number that can be tracked through time.

admixture—Interbreeding between two or more previously isolated populations, sometimes resulting in introducing foreign or unadapted genes.

allele—DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to- many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity.

artificial selection—The process of modifying organisms by selection in breeding controlled by humans (for example, choosing a plant with numerous fruits and removing low fruit-producing plants in a breeding program will artificially select for fruit production).

asexual—In plants a form of reproduction that does not involve pollen or flowers and therefore new individuals formed by this method have the same genetic makeup (unless unusual mutations occur). Types of asexual reproduction in plants include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

breeding system—The method by which a plant can successfully produce seeds. Plants have three basic breeding systems: outbreeding or outcrossing, where pollen from a different individual is needed to fertilize the egg of the maternal plant to produce seeds successfully; selfing or self-fertilization, where pollen from the same individual can fertilize the egg and produce seeds; or apomixis, where seeds can be set without fusion of gametes.

clonal—Type of asexual reproduction in plants that produces new individuals with the same genetic makeup as the mother plant (unless unusual mutations occur). Examples include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

common garden—An experimental technique wherein plants from more than one location are grown together in a single (hence common) garden. This method allows researchers to determine if differences observed in the geographically separated populations have a genetic basis.

conservation collection—An ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues or whole plants of at least 50 unrelated mother plants, and breadth, meaning it consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.

conservation introduction—Defined by IUCN as the intentional movement and release of an organism outside its indigenous range.

conservation translocation—A definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of indigenous range including conservation introductions, comprising assisted colonization and ecological replacement.

cryopreservation—Storing at liquid nitrogen temperatures, usually at –170°C to –180°C (vapor above liquid nitrogen) or at –196°C (in liquid nitrogen) to maintain viability.

cytotypes—A characteristic of a cell. Organisms of the same species with different cytotypes have different numbers of chromosomes.

deterministic event—A predictable or known event (for example, cold temperatures in winter north of the equator are predictable).

dioecy—Having male and female flowers on different individuals.

ecological—Pertaining to the ecology of an organism, the interactions of living organisms with each other and with their environment.

effective population size (N_e) —The number of individuals that contribute genes to succeeding generations. This number is typically less than the number of individuals in the whole population, because not all individuals may be reproductive.

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

extirpation—Local extinction, where a species ceases to exist in a chosen geographic area, while it still exists elsewhere.

F_1 generation—First generation.

F_2 generation—Second generation.

fecundity—The number of seeds or asexual propagules produced by an individual plant or population.

flow cytometry—A laser or impedence-based technology employed to count or sort cells. This can be used to analyze the ploidy levels or numbers of chromosome sets of individual plants in a population.

founder effect—The genetic composition of the individuals that create a new population.

founder(s)—The individual(s) that starts a new population.

gene flow—, or migration—Any movement of individuals, and/or the genes from one population to another. It can be described as limited, meaning that the individuals living near one another are closely related (for example, monkshood), or extensive, meaning that it is possible to find traces of genes in an individual that lives very far away (for example, wind-pollinated plants).

genetic diversity—Variation in the DNA sequence between distinct individuals of a given species (or population). The degree of genetic diversity can be compared between individuals or populations. Capturing the breadth of existing genetic diversity is the goal of a conservation collection.

genetic drift—Variation in the relative frequency of different genotypes in a small population, owing to the chance disappearance of particular genes as individuals die or do not reproduce.

genetic erosion—Loss of genetic diversity. For an endangered species with a limited gene pool loss of individuals equates to loss of genes and alleles represented for the species.

genetic rescue—Increasing genetic diversity from infusing new genes into a population. If population is inbred, this practice may increase chances that some plants will survive.

genome—A complete set of genes or genetic material present in a cell or organism.

genomic studies—evaluations of the specific arrangement of DNA on the chromosome.

genotype—The genetic constitution of an individual (genotyping is determining the genetic constitution of an individual).

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

herbarium vouchers—A specimen of a pressed plant that is deposited and catalogued for future reference.

hybridization—Cross species fertilization. In the context of conservation, it is often undesirable to have hybridization occur in a cultivated or wild setting, as the more common parent will likely swamp the genepool of the rare species.

in situ—In wild habitat.

inbreeding—Mating between closely related individuals. Over many generations, genetic disorders may arise.

inbreeding depression—Reduced fitness of progeny resulting from breeding of related individuals.

iloci—The location of a particular gene on a chromosome.

maternal lines—The offspring (seeds or plants) from a single mother plant are distinguished with unique identifying number, stored in a separate package, and labeled if grown in a nursery. Knowing the number of maternal lines in a conservation collection is an estimate of the genetic diversity represented.

maternal plants—Individual plants producing seeds. Keeping track of seeds from each maternal plant allows for estimates of genetic diversity in a collection and allows for maintaining even representation of maternal lines while growing the accession in the garden's nursery or reintroducing to the wild.

mating system—The way that plants produce seeds. Some plants can produce seeds in multiple ways, while others are restricted to a single mating system. The types of mating system include outcrossing or cross-pollination (a flower receives pollen from another plant of the same species), autogamy or self-fertilization (a flower receives pollen from the same plant) and apomixis (asexual reproduction without fertilization that is only possible with evolution of a modified flower). Mixed mating systems, in which plants use two or even all three mating systems, are not uncommon.

microhabitat(s) —Very localized abiotic (soil, light, and moisture) and biotic (associated plants, insects, and other animals). The nature of a microhabitat may greatly influence seedling and adult plant growth and survival. See also microsite(s).

microsatellite markers—Genetic markers consisting of a series of short repeating base pairs of DNA that are variable within populations and can be used to identify individuals or species, or evaluate structure and gene flow between populations.

molecular (studies) —Genetic studies.

monoecy—Having flowers with only one sex (male or female) or flowers of both sexes carried on a single plant.

morphological—Pertaining to the form or structure.

neighborhood size—The local area within which most matings occur.

obligate outcrosser—Pollen from a different plant (not self) is required for successful seed set.

outbreeding—A condition where flowers of one plant receives pollen from another plant of the same species.

outbreeding depression—Low fitness of progeny resulting from mating between two genetically distant (and usually physically distant) plants.

outcrossing—The form of plant reproduction that requires pollen from another plant of the same species to form seeds.

perennial—A plant that lives for many years.

phenotype—The measurable appearance of a trait.

ploidy—The number of sets of chromosomes in a cell.

pollination mechanism—The method in which pollen is transferred from anthers to the stigma of same or different flowers. Wind, insects, or birds are the most common pollinating mechanisms.

polyploidy variation—Differences in the number of sets of chromosomes present in a species or population.

population size—The number of individual plants of all ages in the population.

reintroduction(s)—intentional movement of species into habitat it previously occupied.

rescue collections—When an entire population is threatened, it may need to be removed from its location and brought to an ex situ or offsite facility for care.

self-compatibility—A condition where a flower of an individual can receive pollen from itself and set good seeds.

self-fertilizing—Pollen from one plant fertilizes a flower of the same plant such that good, viable seed results. This technique is often used experimentally to determine whether this is possible for a species.

selfing—Pollen from one flower fertilizes the same flower and successfully sets seed.

sequencing—Refers to genetic procedure to determine the composition and order of genes of an individual as in genotyping.

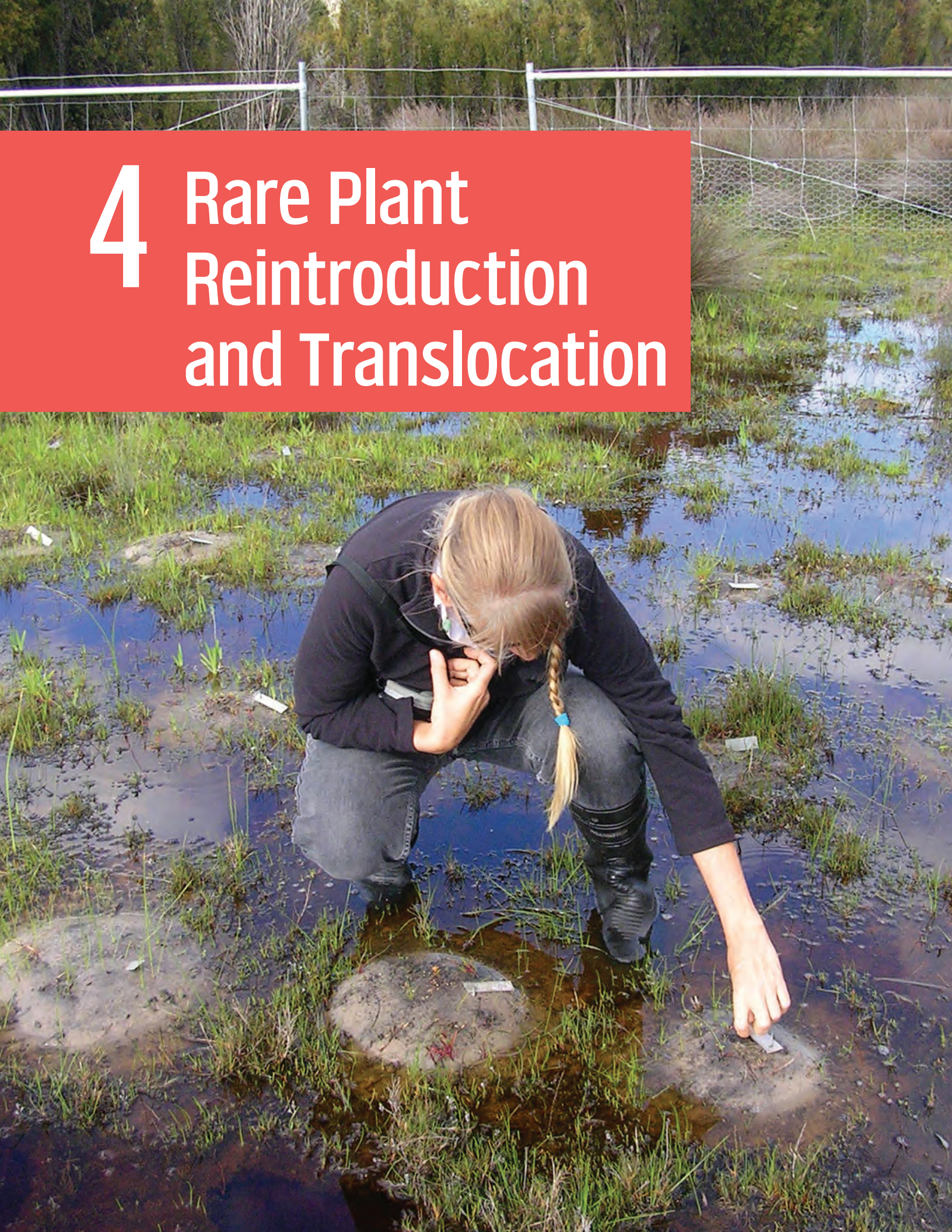
stochastic event—Unpredictable or chance event.

taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

taxonomic ambiguity—Unknown or undetermined genus and species designation of an organism.

tissue culture—Growing cells in an artificial medium. In plant conservation, the cells may be whole seeds, spores, or meristems and the medium is usually some derivation of agar.

wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.



4 Rare Plant Reintroduction and Translocation

Rare Plant Reintroduction and Other Conservation Translocations

Center for Plant Conservation Best Practices

Overview

Roadmap for Conducting a Reintroduction

Part 4 describes the framework for conservation actions that can improve the chance of successful rare plant reintroductions to the wild.

BEFORE YOU BEGIN

Justify the Need

- *Evaluate whether reintroduction is appropriate.*

Logistics

- *Make a plan.*
- *Review and follow laws.*
- *Collaborate with landowners.*
- *Procure funding.*

Know the Species

- *Gather information about the species' biology, ecology, & distribution.*
- *Consider genetics of existing and source populations.*
- *Assess a suitable recipient site.*
- *Select and match source material to site.*
- *Use good horticulture.*
- *Begin with large founder size.*
- *Plan for population growth.*

IMPLEMENTATION

Prepare the Site

- *Weed and thin canopy if necessary.*

Prepare the Plants

- *Label plants for long-term tracking.*
- *Plant in pattern and microsite conducive to good growth and pollination.*

Logistics

- *Choose best season for transplanting or seeding.*
- *Organize and bring all necessary materials and equipment to the site.*
- *Enlist enough people to help prepare and install the plants.*
- *Bring snacks and water.*
- *Limit the number of generations in cultivation.*

AFTERWARDS

Aftercare

- *Water, weed, and protect plants from herbivores and vandalism.*

Monitoring Plan

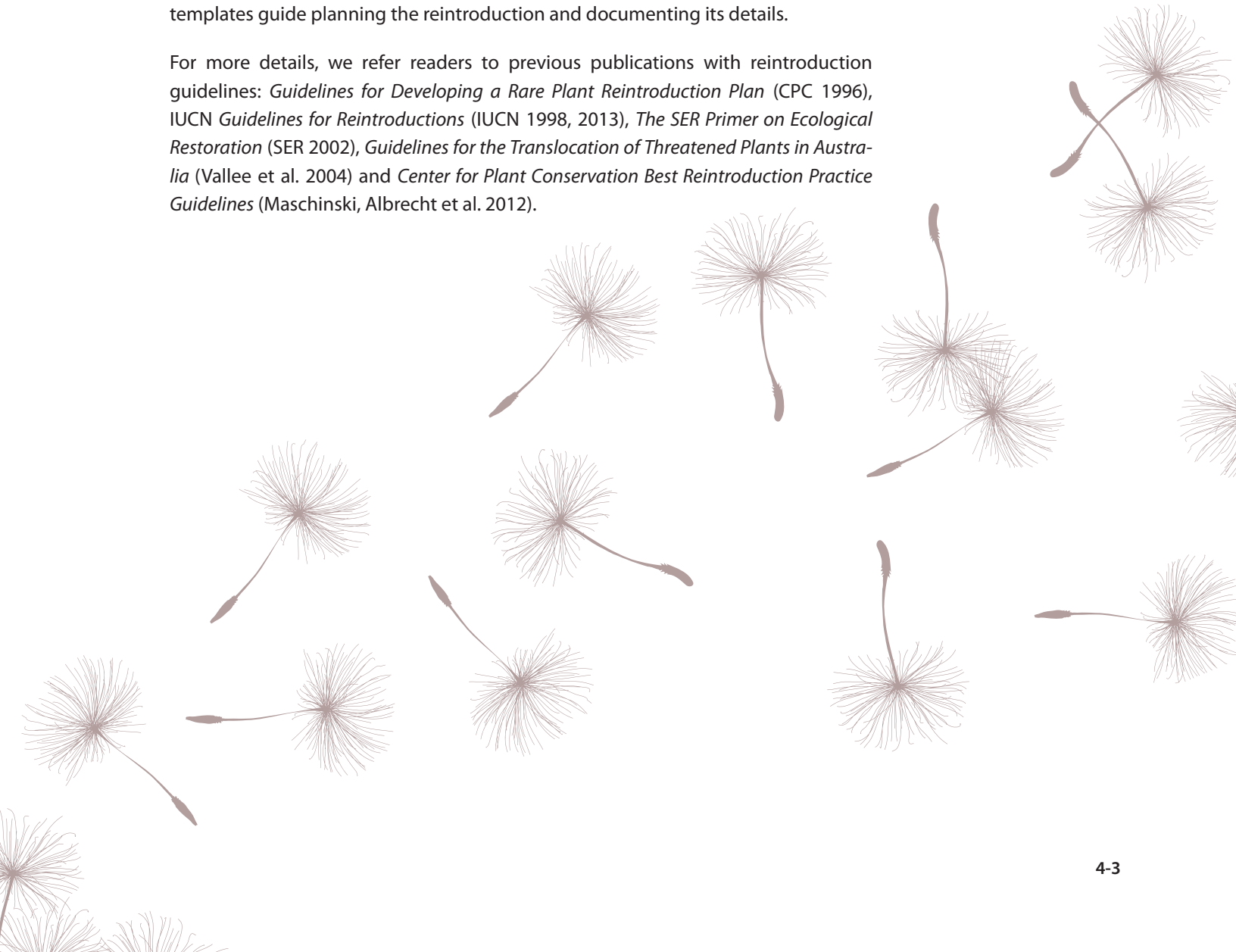
- *Managing long-term commitment.*
- *Analyze and report data.*
- *Document activities.*
- *Publish results.*

A Introduction

The ultimate goal of rare plant conservation is to ensure that unique taxa experience continued evolution within a natural context. The science of reintroduction is rapidly evolving. Over the past 30 years, conservation officers working with the Center for Plant Conservation (CPC) have conducted over 140 plant ***reintroductions*** and other ***conservation translocations*** of many species in many habitats. As we gather more information from our reintroductions, we have had an opportunity to modify our practice, incorporating the best of what experience has taught us. These updated *CPC Best Practices for Rare Plant Reintroduction and Other Conservation Translocations* reflect this collective experience and recent findings from peer-reviewed literature.

The updated *CPC Best Practices* provide a quick reference for practitioners to use when planning and executing rare plant reintroductions (see Overview). The new digital format aids accessibility while providing the most current information. The sections address frequently asked questions and provide supporting documents that provide further information about the basis for the guidelines. Checklists and templates guide planning the reintroduction and documenting its details.

For more details, we refer readers to previous publications with reintroduction guidelines: *Guidelines for Developing a Rare Plant Reintroduction Plan* (CPC 1996), IUCN *Guidelines for Reintroductions* (IUCN 1998, 2013), *The SER Primer on Ecological Restoration* (SER 2002), *Guidelines for the Translocation of Threatened Plants in Australia* (Vallee et al. 2004) and *Center for Plant Conservation Best Reintroduction Practice Guidelines* (Maschinski, Albrecht et al. 2012).



B Justifying a Reintroduction or Other Conservation Translocation

Summary

- Reintroduction is not the first step toward the conservation of a species, but rather follows a careful process of gathering information about the species, threats, alternative actions, and future needs.
- There are several considerations for justifying a reintroduction.
- Acknowledge that there are clear reasons to avoid reintroduction.

CPC does not support or promote ***reintroduction*** as an alternative to ***in situ*** ecosystem protection. All those working in plant conservation firmly agree that *the priority is to conserve species in situ and to preserve wild populations in natural habitats in as many locations as possible.* Reintroduction is never the first action to take for a critically endangered species, even when crisis is imminent. First steps for species in dire straits must be ***ex situ*** collection, threat control, and habitat management (Guerrant et al. 2004).

Prior to conducting any reintroduction, thorough status surveys and careful review of rarity status and threats should be undertaken. Reintroductions should only be considered if habitat protection is not possible or if the ***taxon*** is critically imperiled and appropriate sites and ***propagule*** source materials are available. CPC recognizes that reintroductions may need to be used as a tool to mitigate the impacts of climate change, because some in situ rare plant ***populations*** will be unsustainable within their current historical ranges.

To determine whether a species should be considered for reintroduction, it should meet the criteria described in the "Questions to Ask When Justifying a Reintroduction" box. If the species does not meet these criteria, a reintroduction should not be attempted at this time. If conditions should change in the future, a second evaluation could be done. For some taxa, it may *never* be appropriate to conduct reintroductions. For others, changed conditions and improved horticultural, genetic, and ecological knowledge may make it feasible to conduct a reintroduction at a future time.

Document the species status and distribution.

- Conduct surveys and obtain ***population*** information.
- Map or obtain maps of the known populations to determine the current and historical distribution as it relates to ecoregions, habitat, geology, and soil type.
- Assess habitat-specific population information (Knight 2012). In each population, count or estimate the percentage of reproductive, juvenile, seedling stages, and if possible, measure growth and reproduction.

Questions to Ask

When Justifying a Reintroduction

A reintroduction may be justified if:

____ Species is extinct in the wild OR;

____ The distribution of the species is known and there are few, small, and declining populations; AND

____ Alternative management options have been considered and conducted, yet have been judged to be inadequate for long-term conservation of the species; AND

____ Threats have been identified; AND

____ Threats from habitat destruction, invasive species, land conversion and/or climate change are imminent and are uncontrollable. Species has high risk of extinction if only managed in situ.

If the species meets any one of the following criteria, then do NOT proceed with reintroduction. Consider ex situ conservation practices (Guerrant et al. 2004). If the unmet criterion is resolved in future, then re-evaluate.

____ Reintroduction will undermine the imperative to protect existing sites.

____ Previous tests indicate that it has not been possible to propagate plants or germinate seeds.

____ High-quality, diverse source material is not available.

____ Existing threats have not been minimized or managed.

____ The reintroduced species may potentially negatively impact species in the recipient site via competition, hybridization or contamination.

____ There is evidence that the reintroduced taxon would harm other threatened and endangered species or conflict with their management.

____ The reintroduction is not supported legally, administratively, or socially.

____ Suitable habitat is not available, nor understood.

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

- Note ***abiotic*** and ***biotic*** conditions in occupied patches. Whenever possible, quantify these factors (for example, near adults and seedlings record the canopy cover, associated species, plant density, soil moisture, light, and other factors).

Ascertain the threats and, when possible, take action to remove, control, or manage them.

- Note specific abiotic and biotic factors that may be causing the population decline. Realize that threats may be direct or indirect (Dalrymple et al. 2012).

- If you are currently monitoring the population, note conditions present so that you will be able to pinpoint changes in future years.

Engage land managers in discussion about best options for the species conservation.

- Attempt or consider all alternative management options before considering reintroduction.

- Discuss which options are feasible to implement.

- Ensure the population will have long-term protection and management (that is, invasive species removal, controlled burns, etc.).

Do not proceed with a reintroduction if you cannot justify it. Use other conservation options for the species.

Do no harm to a recipient community or to existing wild populations.

- Consider whether your reintroduction will do any harm to a recipient community or to existing wild populations. If so, consider alternative conservation strategies.

- Determine whether potential collateral impacts of the species in the recipient site are negligible. There is little threat of ***hybridization***, invasion, or contamination.

- The reintroduction will not undermine the imperative to protect existing populations and their habitats.

Determine that the reintroduction is feasible legally, logistically, and socially.

- Because laws governing rare species protection vary by location and jurisdiction, it is essential to discuss and verify that the reintroduction plan is supported by the law, legal authorities, the recipient site landowner, and the public.

- Ideally, the reintroduction will have been identified as an important step for preserving the species in a legal document, such as the species' recovery plan or a conservation action plan.

C Preparing the Reintroduction

Summary

- Careful planning of biological, ecological, political, and financial support for the reintroduction will help ensure success.
- Designing a reintroduction entails linking genetic source to the recipient site characteristics.
- Conducting reintroductions as experiments will result in a lesson learned and can help build plant reintroduction science.

Although it is impossible to say definitively, we believe that many failed ***reintroductions*** could have succeeded if appropriate preparation had been undertaken. Being prepared for a reintroduction requires a good strategy coupled with large investments of time and resources. This requires commodities that are often in short supply in our rapidly changing world—patience and persistence. It may not be possible to know all factors we describe below, but the more that is known the higher the likelihood of success, and practitioners should at least be aware of the gaps in their knowledge.

Reviewing your reintroduction plan by addressing the following questions will allow you to assess your degree of preparedness. This comprehensive list is designed to help practitioners identify gaps in their knowledge. Once knowledge gaps are identified, there is an opportunity to weigh whether there is adequate information to proceed. The risk of proceeding without the knowledge can be assessed along with the risk of taking no action and losing the species. We recommend that r*eintroductions be conducted as experiments precisely designed to fill in these knowledge gaps*. In this way, each reintroduction can not only help future actions for the target species but may in turn help others doing plant reintroductions around the world.

Previous CPC publications have addressed detailed preparations for reintroductions with regard to demography, genetics, and horticultural practice (Falk and Holsinger 1991; Falk et al. 1996; Guerrant 1996). Specific guidance for ***ex situ*** collection and management is essential preparation for reintroductions (Guerrant et al. 2004). Our aim here is to provide guidance for establishing sustainable ***populations*** in the wild where they may have opportunities for adaptation, evolution and interactions within a natural ecosystem. Although it is necessary to describe the steps of the plan sequentially, often several steps are conducted simultaneously. (See the "Questions to Ask When Planning a Reintroduction.")

Questions to Ask

When Planning a Reintroduction

____ Is the taxon already living at the recipient site, was it historically present there, or is this a completely new location?

____ Have you considered legal issues, logistics, and land management? (McDonald 1996)

____ Is the biology and ecology of the species understood? (Menges 2008; Maschinski, Albrecht et al. 2012)

____ Are genetic studies needed? (Neale 2012)

____ Have germination protocol and propagation methods been determined? (Guerrant 1996; Guerrant et al. 2004; Haskins and Pence 2012)

____ Has a suitable recipient site been identified and are land managers supportive? (Fiedler and Laven 1996; Maschinski, Albrecht et al. 2012)

____ Are pollinators known and present?

____ Are plants susceptible to herbivory? Will they be protected?

____ Have threats been reduced or eliminated?

____ How many plants or seeds are available and how many are needed? (Guerrant 1996; Albrecht and Maschinski 2012; Knight 2012)

____ What is the experimental design? (Falk et al. 1996)

____ How will success be measured? (Pavlik 1996; Monks et al. 2012)

____ What kind of aftercare for plant and site management will be needed and how frequently should it be performed?

____ What is the involvement of the land manager/owner?

____ What is the monitoring design and plan for reporting results?

____ In what ways will you involve the public in your project? (Maschinski, Wright et al., 2012)

____ Suitable habitat is not available, nor understood.

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

Making the Plan

Design the reintroduction as an experiment and seek peer review.

- Identify the project leader and key collaborators, who will be responsible for planning, supporting, implementing, site management, monitoring and reporting findings of the project.

- Identify areas of expertise needed to execute the reintroduction. If not represented in the collaborative group, then seek outside experts to join the team. For example, enlist the help of a scientist with experience in experimental design and statistical analysis to ensure that you have adequate replication to answer your research question. Or you may need to ask an experienced horticulturist to help you grow sufficient numbers of plants.

- Plan the reintroduction based upon the best scientific information available. Rely on peers to review your reintroduction plan and provide feedback and alternative points of view. Finding peers to review your reintroduction plan may be easy or difficult depending upon where you reside. Rely on the global community to assist you (see the "Potential Reviewers for Reintroduction Plans" box).

Potential Reviewers for Reintroduction Plans

In some regions, there are panels of plant conservation experts who review reintroduction plans as a part of ongoing legislative process. For example, the North Carolina Plant Conservation Program (http://www.ncagr.gov/plantindustry/plant/plantconserve/index.htm) requests and evaluates reintroduction plans as part of the process of granting legal permission to proceed with a plant reintroduction in the state of North Carolina, US.

Experts operating in different areas of the world are also available. The Center for Plant Conservation provides a resource to learn about reintroductions that have been done and is a source for potential peer reviewers (info@saveplants.org).

The Re-introduction Specialist Group IUCN (http://www.iucnsscrsg.org/) has a *Re-introduction Practitioner's Directory 1998* intended to facilitate communication between individuals and institutions undertaking animal and plant reintroductions.

The Global Restoration Network (http://www.globalrestorationnetwork.org/) provides a web-based information hub linking research, projects, and practitioners.

Questions to Ask

When Designing Reintroduction Experiments

____ What additional knowledge is needed about the species biology or other factors? How can the reintroductions be planned as experiments to address these unknowns?

____ What is the experimental design? How much replication is needed for adequate statistical power? How will the study be analyzed?

____ Have you considered testing aspects of ecological theory, such as ***founder*** events, small population dynamics, establishment-phase ***competition***, dispersal and disturbance ecology, ***succession***, ***metapopulation dynamics***, ***patch dynamics*** on ***population persistence***, resilience and stability over time?

____ Using the reintroduced population as a cohort, will you examine natural variation in survival, mortality, and recruitment and tie these to environmental factors?

____ Will the reintroduction test key habitat gradients of light, moisture, elevation, or temperature?

____ Will the underlying environmental drivers of population growth be measured? (Knight 2012)

____ Will genetic factors be part of the experimental design?

____ What traits will be monitored and how will they be analyzed?

____ Will the reintroduction further our knowledge of key principles related to rare species' ability to cope with climate change?

____ Are you testing factors within a single site or across multiple sites?

____ Has a monitoring plan been developed? How long will monitoring be conducted? Have you considered an ***adaptive monitoring plan***? What will the duration of the experiment be?

____ Have you developed a clear unambiguous datasheet to track reintroduced plant growth, reproduction and survival? If the monitoring persists for decades, will your successors be able to interpret the data you have collected?

____ Will the data be housed within your institution or elsewhere so that your successors will able to use it?

____ How will the plants be mapped and marked/numbered?

____ If plants are susceptible to herbivory, will their response be included in the design or should the plants be protected?

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

- Train and adequately manage all personnel and volunteers that are involved.

- Consider addressing theoretical questions in the reintroduction experiment/project to advance the field of reintroduction biology. (See the "Questions to Consider When Designing Reintroduction Experiments" box.)

- Define goals of reintroduction related to the recovery of the species. Set objectives.

- Develop methods, decide the plant and population attributes that will be measured, determine monitoring protocol, frequency and duration, and reference the analysis.

The Law, the Land, and Funding

Obtain legal permission to conduct the reintroduction.

- Familiarize yourself with the laws and regulations associated with a reintroduction. Note that these may differ for ***augmentations***, reintroductions, and/or introductions.

- In some locations you may be required to obtain one or many permits before conducting a reintroduction (for example, from the landowner/ land manager, local, regional or national authorities).

- Often a carefully written plan for the reintroduction is required for the permit.

- Note expiration date of all permits involved and requirements for periodic reports or updates to permitting agency.

- If reintroduction is done as a ***mitigation***, it is critical that all preliminary planning steps be taken within legal parameters. (See Falk et al. 1996 for extensive discussion regarding mitigation.)

Involve landowners and land managers.

- Ensure that landowners and land managers are involved and supportive of the project and can account for possible changes in the future.

- Discuss the long-term support and management of the proposed recipient habitat with land managers. Lack of management can doom a restored population to fail.

- Develop a written agreement outlining who will be responsible for what action and any special protocols that need to be followed by parties working on the site.

- Set a schedule to meet with the recovery team periodically to assess the species' condition and the status of the restored population.

- If future changes require intervention, determine a process for evaluating impacts on the restored population. For some agencies, it may be necessary to develop a protocol or decision tree to trigger management action.

- Develop a mechanism for handling any conflicts that may arise (for example, management for one species is detrimental to another species, etc.).

Secure adequate funding to support the project.

◗ Ideally, funding should be garnered for implementation and for several years, if not decades, following the installation. At the very least, parties proposing a species' reintroduction should be committed to seek long-term funding support for the project. This requires that you have detailed the cost of implementing, monitoring, and management of the restored population. Committed partners, who are willing to provide in-kind services and/or volunteer citizens, who are willing to monitor the restored population will help make this aspect feasible.

◗ Determining the outcome of a reintroduction takes time. Expect to devote >10 years to monitoring to determine whether a population is sustainable (Monks et al. 2012). There are few recorded reintroductions that have created sustainable populations in which multiple generations have been completed within 25 years (Dalrymple et al. 2012). Key life-cycle events such as next generation seedling recruitment and reproductive maturation can take years to decades in long-lived species. Thus, a few decades may be required before fates of reintroduction can be determined.

Understanding Species' Biology

Knowing the biology and ecology of your ***taxon*** will benefit the reintroduction plan and experimental design. We advise gathering information from the literature on your target taxon and closely related ***congeners***. If possible, conduct experiments if there are gaps in your knowledge. (See the North Carolina Reintroduction Documentation Form, http://ncbg.unc.edu/uploads/files/Reintroductionguidelines.pdf).

Know the species' biology and ecology.

◗ Knowing the ***mating system*** will determine whether source material should come from a single population or from mixed populations and the spatial pattern of ***outplanting***. For example, due to remnant populations lacking compatible ***alleles*** for successful reproduction, reintroductions done with Florida ziziphus required carefully selecting compatible individuals from more than one location to achieve reproductive success (Weekley et al. 1999; Weekley et al. 2002). In contrast, *Schiedea obovata*, which is capable of ***selfing*** or outcrossing, requires keeping all outplantings separate (Kawelo et al. 2012). Highly ***inbreeding*** taxa are more likely to form ***ecotypes*** than ***outcrossing*** species.

◗ If a species is ***dioecious***, the spatial design of plantings should place male and female plants in close proximity (for example, *Zanthoxylum coriaceum* in Maschinski et al. 2010).

◗ Species or conditions that may require special techniques for growing and implementing a reintroduction include: ***edaphic endemics***, species with ***specialist pollinators***, and species requiring symbionts for ***germination*** and growth.

- ***Edaphic endemics***: In *Astragalus bibullatus* an edaphic specialist of limestone cedar glades, translocations are only successful when conducted on a specific

type of limestone even though multiple types of limestone occur in the historic range of the species. The species can only be propagated in very well-drained soil and must be watered from below to prevent disease (Albrecht, Missouri Botanical Garden, personal observation).

- ***Specialist pollinators***: Using pollinator baiting techniques at potential reintroduction sites can ensure pollinators are present before the outplanting occurs (Reiter et al. 2016). Lack of pollinators limits orchid distributions, thus knowing pollinators are present before conducting a reintroduction is advised (Phillips et al. 2014).
- ***Mutualists***: Providing inoculated soils containing mycorrhizal fungi may help establish the outplanted population (Haskins and Keel 2012). Because some taxa require ***symbionts*** to germinate or grow (Ogura-Tsujita and Yukawa 2008; Janes 2009; Haskins and Pence 2012) knowing whether there are ***obligate mutualists*** will influence reintroduction success. Attempting to germinate or grow such species without their obligate mutualists will fail. Providing inoculated soils containing mycorrhizal fungi may help establish the outplanted population (Haskins and Keel 2012).

Site Selection

Choosing a recipient site should be done with great care and intention. Several conditions influence a species' ability to colonize a new site including functional ecosystem processes, appropriate associated species, and ongoing management to remove threats and maintain ecosystem health. In general, seek a recipient site with great similarity to the place where the rare species is thriving. Knowing the site history may help explain existing conditions. Although it is impossible to know with certainty what a site will become in the future, as much as possible practitioners should try to imagine the future conditions the reintroduced population will face. Ongoing management and threat abatement are essential for maintaining conditions conducive to population sustainability.

In addition, it is important to think about any recipient site in the context of the species' whole distribution. Because corridors may facilitate migration and dispersal between patches, especially with the onset of climate change (Noss 2001), a reintroduced population can serve an important function of connecting existing populations either by forming a stepping-stone between patches or by expanding the size of existing patches. Connecting 15 or more patches will improve chances for the entire metapopulation capacity (see Hanski and Ovaskainen 2000). (See the "Questions to Ask about the Recipient Site" box.)

Choose a suitable recipient site.

◗ Determine the cause of declines in ***wild populations***. Ensure that threats can be ameliorated in the recipient reintroduction site (Dalrymple et al. 2012; Knight 2012).

Questions to Ask

About the Recipient Site

____ Have you researched the history of the recipient site?

____ Have you incorporated species-specific factors related to optimal population growth to assess suitable recipient sites for your taxon?

____ Have you identified species-specific environmental and community factors in occupied versus unoccupied patches?

____ Have you ranked several potential suitable recipient sites to determine the best place for the reintroduction to occur?

____ Is there still suitable habitat left within the species' range? (See Falk et al. 1996 for discussion of range.)

____ Are recipient sites of sufficient quality and with sufficient long-term protection to ensure the long-term security of the reintroduced population?

____ Are threats absent or adequately managed at the site?

____ What were the previous threats that may have caused the species to become extirpated from site?

____ What is the potential for future threats?

____ Is current and future land use of the recipient site and surrounding sites compatible with sustainability of the reintroduced population?

____ Are potentially hybridizing ***congeners*** present at recipient site? Which ones? Are they present at nearby sites? Are they present within the target species' range?

____ Is the recipient site within the species' climate envelope now? Are there models suggesting the location will be safely within the climate envelope in the future?

____ What site preparation is required before the plants can be installed (for example, canopy thinning, invasive removal, etc.)? Will habitat manipulation continue after plants are installed?

____ Does the species require habitat conditions that no longer exist on site (for example, fire, periodic inundation, etc.)? Can those conditions be mimicked?

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

- Evaluate potential reintroduction sites using a recipient site assessment or quantitative assessment (Maschinski, Falk et al. 2012). Base your evaluation on the natural habitat where a population has positive (or at least stable) growth rate (Dalrymple et al. 2012; Knight 2012).

- To choose among several potential sites, rank reintroduction sites incorporating logistics or ease of implementation, quality of habitat, and management influencing the species' ability to persist at a site (Maschinski, Falk et al. 2012; see Figure 4.1).

- To account for uncertainty, incorporate heterogeneity into the reintroduction plan. Use multiple sites and multiple ***microsites*** (even outside of our expectations) to test heterogeneity of conditions needed for optimal growth for all life stages of a species (Dalrymple et al. 2012; Maschinski, Falk et al. 2012; Maschinski, Albrecht et al. 2012).

- Because the fine-scale requirements for individual plant growth and optimal population growth are often unknown, using microsite as an experimental factor is good practice. Measure abiotic conditions (for example, soil, precipitation, temperature) and biotic conditions (for example, predators, mutualists, invasive species) at the reintroduction site that are associated with plant performance and population growth (Knight 2012; Maschinski, Falk et al. 2012). Ensure that there are adequate areas for population expansion (that is, microsites within the recipient site and adjacent suitable habitat outside of the recipient site).

- Realize that if environments conducive to positive population growth are rare or non-existent, additional reintroduction activities, beyond simply reintroducing ***propagules***, will be necessary (Knight 2012; Maschinski, Falk et al. 2012).

- Note that using existing populations and their habitat conditions as reference points for reintroductions will not always be appropriate if the species does not have positive growth rate at these locations (Possley et al. 2009; Dalrymple et al. 2012; Knight 2012; Maschinski, Falk et al. 2012; Maschinski, Wright et al., 2012).

- It will be essential to use an experimental context to determine key factors necessary for positive population growth.

- Reference points may not be available within core habitat under climate change conditions (Dalrymple et al. 2012). Similarly, geographic distribution may not be a good reference for fundamental ***niche space***. For this reason, known historic range may not necessarily be the only guide to assess optimal habitats for successful reintroduction (Maschinski, Falk et al. 2012; Maschinski, Wright et al., 2012).

FIGURE 4.1

Recipient site assessment based upon ranking criteria related to logistics and habitat quality. The assessment can be used to score a single or to prioritize among multiple sites. Scores ≥ 27 ≤ 54 are acceptable reintroduction sites. When choosing among multiple sites, the best site will have the lowest total score and no single criterion scoring 3. (Adapted from Maschinski, Falk et al. 2012.)

Date:	Observers
Site:	Description:

Criteria for prioritizing potential restoration site	3	2	1	Score
Category 1: Logistics, implementation, management				
A) Status of relationship with land owner and management	none	some	good	
B) Commitment level of agency to protect introduced population	none	some	good	
C) Willingness of agency to manage habitat for target species	none	some	good	
D) Site preparation, threats removed	no	partially		
E) Amount of public access/susceptibility to human disturbance	high	medium	low	
F) Accessibility for planting logistics and future monitoring	poor	fair	good	
G) Water source present		no	yes	
Category 1 Total				
Category 2 Quality habitat characteristics				
A) Percentage of associated species common with extant sites	0-40%	41-71%	71-100%	
B) Quantity and diversity of aggressive invasive plant species	high	medium	low	
C) Current and future impact of invasives	high	medium	low	
D) Size of potential reintroduction area	small	medium	large	
E) Quality of adjacent habitat	poor	fair	good	
F) Quantity of good-quality habitat adjacent to reintroduction site	none	some	abundant	
G) Soil texture similar to extant sites	no	partial	yes	
H) Soil nutrients similar to extant sites	no	partial	yes	
I) Canopy cover optimal for target species	no	partial	yes	
J) Hydrology similar to extant sites	no	partial	yes	
K) Topography similar to extant sites	no	partial	yes	
L) Target species presence at site	never	historic	current	
M) Special requirements of target species present	no	partial	yes	

Criteria for prioritizing potential restoration site	3	2	1	Score
N) Mutualists present	no	partial	yes	
O) Herbivores present	no	partial	yes	
P) Ecosystem processes functional	no	partial	yes	
Q) Number of potential translocation areas within site	1	2	3	
R) Proximity to existing wild populations	>10km	5–10km	<5km	
S) Natural disturbance regime	excessively high or low	moderate	normal	
*				
Category 2 Total				
All Criteria Total				

* Additional habitat feature of interest for this species (e.g., seasonal flooding, salinity level, nurse plant present, etc.)

NOTES: For evaluating a single site: Add total scores; 27 is a perfect score. Scores > 27 < 54 are acceptable reintroduction sites. Any criterion with score of 3 should be improved before moving forward.

For choosing among multiple sites: The best site has the lowest total score and no single criterion scoring 3.

Questions to Ask

About Habitat or Landscape Level Considerations

____ Does the recipient site contribute to natural patterns of heterogeneity in the species' distribution?

____ Have you considered habitat connectivity? Is healthy suitable habitat nearby that will allow for the restored population to expand in area and number of individuals? Is adjacent property suitable habitat? Is adjacent property protected?

____ Are there ***metapopulation*** possibilities? Have you accounted for between site factors as well as within site factors? Is the site located in close proximity to extant populations or other reintroduced populations?

____ What are the distances between the proposed reintroduction and nearby wild populations?

____ What benefits or detriments do the nearby sites give the restored population?

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

Create a sustainable population.

- Improve the probability of creating a sustainable population by considering the metapopulation context of your reintroduction site.

- Choose recipient sites that have connectivity and increase the probability of dispersal (Maschinski, Falk et al. 2012).

- Consider landscape level phenomena. (See the "Questions to Ask About Habitat or Landscape Level Considerations" box.)

Genetics Considerations

Ideally, the genetic composition of the source material needs to be a balance between representing the local gene pool and creating a new broadly genetically diverse population. Reasons to consider genetic studies in a reintroduction plan include helping to make decisions about appropriate location(s) for collecting source material, confirming whether hybridization may be a potential problem, confirming the species taxonomy, or determining whether to use mixed or single population source material (Falk and Holsinger 1991; Falk et al. 1996; Neale 2012). For example, you may wish to pursue genetic studies if you suspect there are hybridization problems, if the species looks different in different locations, if one or more populations of

the species has distinct ecology from the majority of populations, or if it is difficult to distinguish this species from a congener. You may also wish to conduct genetic studies if you know or suspect that your species has variable ploidy levels across populations (Kramer et al. 2018).

Conducting a molecular genetics study can help elucidate the ***mating system***; the degree of natural inbreeding; the level of genetic divergence among collection sites or subpopulations; area of seed and pollen dispersal; the degree of genetic relationship or co-ancestry between adult plants in natural conditions; and the neighborhood within which adults are genetically related (Crossa and Venkovsky 2011). (See Part 3, "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection.")

Often, it will be necessary to work with local geneticists at botanic gardens, universities and/or government facilities to do the genetic studies. Although costs for genetic analysis are becoming more reasonable with technological advances, be aware that adequate funding will be required for proper genetic work. Complementary to genetic studies are ***hand-pollination*** studies, ***common garden experiments***, or ***reciprocal transplant*** studies. The latter will allow researchers to understand the performance of the species for a particular source in a new setting. Each has advantages and disadvantages.

When are genetic studies needed?

- Ascertain whether genetic studies are needed before conducting the reintroduction and, if possible, conduct studies to measure ***genetic structure*** of the focal species (Neale 2012).

- A genetic assessment of wild populations is advised before conducting a reintroduction if the species meets any of the following criteria in the box "When Are Genetic Studies Needed?"

- Once genetic data is available, review compatible management options (Ottewell et al. 2016).

- In the absence of genetic data, it is valuable to utilize information on species life-history traits, such as habit and breeding system, to inform reintroduction decisions (Neale 2012).

Use a genetically diverse founding population.

- Use a large genetically diverse founding population to improve chances of establishing a self-sustaining population (Guerrant 1996).

- To compensate for propagule losses due to mortality during reintroduction, start with an estimate of desired numbers of individuals surviving to reproduction in a new founding population. Then, account for expected losses during establishment. Some of these calculated losses can be mitigated by maintaining backup ***clonal*** material.

Questions to Ask

When are Genetic Studies Needed?

Assessing the genetic diversity of wild populations can reveal insights about the biology of the species, however genetic studies can be expensive and may not always be necessary. They can include either molecular work (***genotyping, sequencing, genome*** or ***ploidy*** analysis) or common garden studies. These types of studies are advisable before collecting a rare species or before conducting a reintroduction if the wild populations have any of the following characteristics:

Within-population issues

____ Population has fewer than 50 individuals flowering and setting fruit.

____ The species is clonal.

____ Little or no viable seed is being set.

____ There are potential taxonomic concerns (taxonomic ambiguity, potential hybrids, or variation in ***ploidy***).

Issues across the species' range

____ The species is declining and little is known about the biology or life history of the species.

____ The species has highly fragmented and isolated populations.

____ The species looks different in different locations.

____ One or more populations of the species has distinct ecology from the majority of populations.

(Maschinski, Albrecht et al. 2012)

◗ When growing the material for purposes of a reintroduction or other reintroductions, keep in mind the reproductive biology of the species. (See Part 3, "Genetic Guidelines for Acquiring, Maintaining, and Using a Conservation Collection.") For example, obtaining 10 female plants of a dioecious species may require planting twice as many seeds as the expected germinant count if the sex ratio is 50:50.

Use founders with evenly represented family lines.

◗ Collect and maintain seeds from each maternal line separately. In this way, it is possible to know and intentionally control even representation of the different founders.

◗ Minimize "unconscious" or ***artificial selection*** during seed increases or augmentation of natural populations. Note that variation in germination and growth of maternal lines should be expected. Resist the temptation to over-represent the winners—

Questions to Ask

About Wild Populations

____ What is the ***genetic structure*** of the wild populations?

____ What is the dispersal capability of the species?

____ If hybridization is a concern, what are the ploidy levels of the wild populations (McKay et al. 2005)?

____ Does the species suffer symptoms of inbreeding depression?

____ Is there evidence of outbreeding depression?

____ Based upon special ecology, unique morphology (that is, ecotypes) or spatial disconnection from other populations, do you suspect that a population has local adaptation?

____ Based upon the presence of a congener in the wild population and/or variable morphology, do you suspect that the species is hybridizing with a congener?

(McKay et al. 2005; Neale 2012)

those abundantly available, vigorously growing maternal lines that may skew the diversity of the population—but rather consciously maintain even family line representation (Guerrant et al. 2004; McKay et al. 2005).

Choose founders from a single source or mixed populations.

- Sometimes it may be appropriate to use a single-source population, while other times it may be appropriate to mix populations for the founders.

- The decision of whether to mix source populations or keep them separate should consider several factors: condition and context of the wild population(s), ***mating system***, dispersal mode, ***ploidy*** level, and ***genetic structure***. (See box "Questions to Ask Related to Wild Populations," Figure 3.2, "Summary of Collecting Recommendations for Numbers of Populations to Sample," and Figure 3.3, "Summary of Collecting Recommendations for Numbers of Individuals to Sample within a Population.")

- Traditionally, it is recommended to use founders from only a single wild population that is ecologically similar to the recipient site in order to preserve locally adapted genes. For example, if the species is an ***obligate outcrosser*** and is locally adapted to a site at very fine scale, then mixing populations may cause ***outbreeding depression*** (Neale 2012). This is especially true if there are known genetic differences between existing populations or if populations have more than 100 individuals, have distinct ecology, and have been separated for more than 20 generations (Frankham et al. 2011).

- Mixing source material may be necessary if there is no appropriate ecological recipient site that matches extant population site, if the available source material is limited, or if there is evidence of low genetic diversity or ***inbreeding depression*** in the source population (Dalrymple et al. 2012; Neale 2012). We recommend mixing source material if the taxon has extant populations of less than 100 individuals with no chromosomal differences, no distinct ecological differences, and if populations have been separated less than 500 years (Frankham et al. 2011).

- If mixing sources, keep track of each individual source through collection, production, and reintroduction to allow for rapid response should any issues arise.

Consider genetic rescue.

- When the wild or reintroduced population has low genetic diversity and signs of inbreeding depression, consider genetic rescue (Frankham 2015).

- Infusing new genetic stock into a wild or reintroduced population (***genetic rescue***) may be necessary to overcome detrimental effects of ***inbreeding*** (Frankham 2015). Introducing new individuals or genes (from pollen) could increase genetic diversity and fitness of a small, inbred population (DeMauro 1993; White et al. 2018).

- Aim to release equal numbers of individuals from each source population early in the reintroduction to promote balanced ***admixture*** in the descendant population (Havens et al. 2004; White et al. 2018).

- For species critically imperiled by threats that are genetically linked, genetic rescue may also comprise insertion of advantageous genes as is being done in crop development (Rinaldo and Ayliffe 2015).

Source Material and Horticulture

The source material used for any reintroduction may determine its fate. To give the new population the best chance to persist against future stochastic or catastrophic events, it is important to use plants that are adapted to site conditions, have adequate genetic diversity and good health (Falk et al. 1996; Guerrant 1996; USFWS 2000; Guerrant et al. 2004; Neale 2012). Review and account for genetic concerns of source material from collection through propagation in the nursery to ***outplanting*** in field. This requires that you simultaneously evaluate and match recipient site characteristics (see "Choose a suitable recipient site" and "Create a sustainable population") to genetic stock available for the reintroduction.

Review the US Fish and Wildlife Service Policies.

- Review the USFWS Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act (USFWS 2000). (See "USFWS Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act" pdf.)

Plan the source material.

- Review and plan the source material that will be appropriate to introduce to a particular site (Basey et al. 2015).

- Identify the potential source material(s) available for reintroduction. Note collection site ecological conditions, community structure, and proximity to the proposed recipient site (Maschinski, Falk et al. 2012).

- Collect or retrieve from a seed bank the source material whose location has similar climatic and environmental conditions to the recipient site(s). This is particularly important if the species has distinctly different appearance (or ecotypes) within wild population sites. Detailed information recorded on accession forms at time of the collection is essential for this evaluation.

- The extent of gene flow between populations varies by species. Some may have isolated, locally adapted patches within a small area, whereas others may have great gene flow over great distances. Therefore, there isn't a simple relationship between distance and genetic relatedness (Richards et al. 2016).

- Use genetically heterogeneous founders to improve the ability to cope with varying conditions (Falk et al. 1996; Guerrant et al. 2004; Neale 2012). Theoretically, high levels of genetic diversity will equip the new population with ***adaptive*** potential needed to withstand ***stochastic*** and ***deterministic events*** including climate change, and can defend against potential genetic pitfalls of small populations such as founders effect and inbreeding depression. (See "Using a single-source population versus mixing populations.")

- Genetic rescues may use targeted genotypes to restore fitness at a recipient site rather than focus specifically on maximizing genetic diversity in the founder population. (See "Consider genetic rescue.")

Questions to Ask

Regarding the Genetics of Source Material

____ From which wild population(s) should the material be collected for use in the reintroduction?

____ What is the basis for collecting source material from a particular location?

____ How will the source material be sampled?

____ What is the genetic composition of the material reintroduced?

____ Should material come from an ex situ source, only one wild source population, or mixed population sources?

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

Use ex situ source material.

- CPC recommends using ***ex situ*** source material before collecting new material from the wild (Guerrant et al. 2004).

- Using ex situ propagules will minimize adverse impacts on wild populations (Guerrant et al. 2004). Over several years it may be beneficial to add fresh stock to increase diversity and age structure (Guerrant et al. 2004) and improve the chances for successful establishment of the reintroduced population (Duquesnel et al. 2017).

- Compelling reasons not to use ex situ propagules include: a) the collection site is ecologically very different from the recipient site, b) there is a more appropriate source population that can withstand collection, or c) the ex situ propagules you have available are not genetically diverse.

- Bulking up ex situ collections through vegetative reproduction or seed bulking is often very feasible. When producing propagules for reintroduction, be aware of potential selection and genetic bottlenecks that may occur (Basey et al. 2015).

- If ex situ material is not available, collect no more than 10% of seed produced in any year from wild populations to avoid harm to the wild populations with >50 plants. Collect from all individuals within the population if there are < 50 plants. Capturing broad genetic diversity may require collecting in different years and across the range of the fruiting season. (See Guerrant et al. (2004) for specific guidance regarding ex situ collection and management and Part 1B, "Collecting Seeds from Wild Rare Plant Populations.")

Choose the best propagule type.

- Choose the best propagule type and size of founders based upon the species' life history, recipient site characteristics, and logistics.

- It is possible to use seeds or whole plants for any reintroduction, however there are advantages and disadvantages of each (Table 4.1).

- Seeds may be an easily collected, bulked in the nursery setting, and provide a rich source of genetic diversity for use in reintroductions. If seeds are orthodox, they are relatively inexpensive, easily and compactly stored prior to use. When seeds germinate at the recipient site, they are a bioindicator that germination is possible there. However, typically only a small percentage of seeds (1–10%) germinate in wild conditions and a small percentage of reintroductions have established with seeds (Albrecht and Maschinski 2012, Dalrymple et al. 2012, Guerrant 2012). Therefore, founder population size for seeds will require thousands to tens of thousands of seeds. Further, the time required to mature to reproductive stage from a seed varies with species' life history. For most species, the most vulnerable life stages to mortality factors are the seed and seedling stages (Grubb 1977). The longer the seed or seedling stage remains in the wild, the more mortality should be expected.

TABLE 4.1. Advantages and Disadvantages of Using Seeds or Whole Plants for a Reintroduction

Qualities	Seeds		Whole Plants	
	Advantages	Disadvantages	Advantages	Disadvantages
Acquisition and Quantities Required	Easily collected and easily sown	1000s to 10,000s required; ease of installation and care is misunderstood. "They'll take care of themselves" is not necessarily true.	In comparison to seeds, fewer individuals can comprise the founder population and they will have overcome the perils that seedlings face in the wild.	In comparison to seeds, fewer individuals can comprise the founder population, hence there may be less genetic diversity represented.
Propagation	Easily bulked to produce next generation in nursery	Nursery produced seeds may be adapted to nursery rather than wild conditions.	Plants can be propagated from seeds or cuttings.	Propagation requires adequate space.
Genetic Diversity	Rich source of diversity	Survival of seeds and seedlings in wild may be quite low.	Survival and maturation is good in nursery and in wild. Controlled propagation can provided more control over genetic structure of founder population.	Generally, because of space constraints, the total diversity represented in a whole plant collection will be less than that of seeds.
Cost	Relatively inexpensive	Many seeds and/ or seedlings will not survive long in the wild, therefore there is a resource cost.	High survival is expected in the greenhouse. One hundred seeds may yield 95 plants if germinated in a greenhouse, while only a single seedling may emerge in the field.	Time to achieve appropriate size for planting in wild has labor and resources cost.
Maturation Time	Annuals and short-lived species can grow to reproductive maturity in the first year or two.	Maturation time from seed to adult may be lengthy for long-lived species. The longer the seed or seedling stage remains in the wild, the greater chance of mortality.	Growing plants in a nursery setting can accelerate growth to maturity.	Plants with large root systems may be challenging to transplant in the field.
Ex Situ Space Requirements	Easily and compactly stored prior to use, if the species has orthodox seeds	If the species has desiccation or freezing intolerant seeds, storage requirements are specialized or the seeds must be used immediately.		Whole plants require much more space for production than seeds.
***Bioindicators* of Appropriate Recipient Site**	When they germinate at the recipient site, they are a bioindicator that germination can happen at the recipient site.	Typically, only a small percentage of seeds (1%–10%) germinate in wild conditions and a smaller percentage establish.	Large plants tend to have good survival at the recipient site.	The location where a transplant is placed may not be optimal for next generation seed to germinate.

◗ To improve the likelihood of success of a seed reintroduction, use thousands, use dormancy-breaking treatments if appropriate, protect seeds and seedlings from herbivory, and irrigate for months as is the practice for perennial whole plants (Bainbridge 2007, Maschinski et al. 2017).

◗ For annual and short-lived species, seeds are often the best choice. Transplanting annual plants as seedlings or adults to the field is fraught with perils, as plants would not survive well or would require extreme care and watering on a daily basis if natural rainfall did not occur daily.

◗ For species with intermediate lifespans (usually herbaceous perennials), whole plants have been shown to be most successful. Grow plants as large as is feasible to manage for transport to the reintroduction site and planting. Using physically large founders increases the likelihood of establishing a persistent population (Guerrant et al. 2004; Albrecht and Maschinski 2012). An exception to this is if habitats, such as rock outcrops, do not allow digging or transplanting whole plants. If this is the case, then seeds would be the best choice.

◗ If the species is long-lived, reintroduce plants of varying size and life-stage to account for variable success of stages in different microsites (Albrecht and Maschinski 2012). Using different-stage plants will result in a more diverse population structure in the present and future and will increase the probability of finding the optimal conditions for the whole population to grow. For example, use juveniles and reproductive plants in the reintroduction. Sometimes, the two will have different microsite requirements (Wendelberger and Maschinski 2016). Generally, the largest plants one can manage to transplant will have greatest survival, as was the case with *Amorpha herbacea* var. *crenulat*a (Wendelberger et al. 2008).

◗ To improve the likelihood of success of a whole plant reintroduction, use large numbers, protect new transplants from herbivory, and provide irrigation aftercare for months.

◗ For many trees, foresters have found the best survival and most cost effective size for transplanting thousands of trees is a long-rooted seedling (in a container that forces deep root growth). The best timing for planting is when trees are dormant for temperate species (North Carolina Division of Forest Resources 2009), while for tropical species planting in the rainy season is advised. Tree roots are best established from the seedling or small container size, as they tend to get root-bound and suffer from circular root patterns in containers. Palm trees are an exception. Large *Pseudophoenix sargentii* juveniles in 3–10 gallon containers reintroduced to the Florida Keys had higher survival than seedlings (Maschinski and Duquesnel 2007).

Confirm that successful propagation is possible.

◗ Confirm that the species can be successfully propagated and that adequate numbers of high quality, healthy, genetically diverse source material is available.

◗ A critical step to accomplish prior to reintroduction is mastering the art of propagating large quantities of the species, acclimatizing them, and growing them ex situ.

A declining species that has not been propagated such that large numbers exist in ex situ nursery stock is simply not a good candidate for reintroduction.

- Acknowledge that you are not ready to proceed if you have not mastered this step.

Allow enough time to generate the source material.

- Allow enough time to generate adequate numbers of source material prior to initiating the reintroduction. Depending on the species, this may take several months to several years.

Keep detailed documentation on all source material used.

- Keep detailed documentation on all source material used to restore populations. This documentation should be linked to permanent plant labels/ID tags attached to the reintroduced plants. Store these data in multiple locations.

Don't use all material for the reintroduction.

- Keep some material in reserve.

- Genetically diverse source material should be safely backed up in an ex situ location so that regardless of whether reintroduction succeeds or fails there is still ***germplasm*** conserved.

Use good horticultural practice.

- Acclimate plants to novel conditions (Haskins and Pence 2012). Transitions from culture medium to soil and from greenhouse to outdoors will require a period of adjustment to reduce the chance of shock.

- Take phytosanitary precautions to insure that diseases will not be inadvertently transmitted.

- Use native soils from the wild site (if possible) during nursery production. Native soils may require augmentation with sterile perlite or vermiculite to achieve consistency necessary to be container-grown. The benefit of native soils is that they potentially contain beneficial microbes; however, pathogens may also be transferred with native soil. Follow good nursery hygiene practices accordingly. We advise separating plants with native soil from the rest of the nursery in quarantine.

- Weed pots containing plants destined for the reintroduction to reduce the chance of introducing weeds to reintroduction site.

- If using propagules that were derived from tissue culture, acclimatization will be important. We recommend gradually decreasing humidity, while subjecting cultures to ventilation or air exchanges before transfer to soil. Alternatively, methods could include increasing ambient CO_2, decreasing sugar levels in the cultures, or treating with growth regulators to increase stress tolerance. (Haskins and Pence 2012)

Questions to Ask

Related to Planning for Population Growth

____ What founder population size will be used?

____ What size and stage structure of plants will be used?

____ How will the founding population be spatially configured to favor demographic persistence and stability?

____ What is known about population growth, recruitment, and survivorship in wild habitats and what environmental or community factors are correlated with population growth rates?

____ How will population growth, recruitment, and survivorship be monitored in the reintroduced population? And by whom?

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

◗ Grow in controlled nursery conditions to maximize plant health and grow to appropriate size prior to moving to the reintroduction site.

Planning for Population Growth

Use as many founding individuals as is feasible.

◗ Use as many founding individuals as is feasible (50+ individual plants or thousands of seeds) to bolster population growth (Guerrant 1996; Albrecht and Maschinski 2012). Increasing the numbers of reproductive adults early in population establishment increases the chances of next generation recruitment (Albrecht et al. 2018).

◗ Develop a demographic model for the species to determine the optimum founder size (Knight 2012).

◗ When working with perennial herbs and sites in highly competitive environments like grasslands, founder population sizes will need to be larger than 50. Introduce enough individuals (seeds or juveniles) to be able to break through demographic and environmental stochasticity of low populations to achieve a viable population (Knight 2012). Planting higher numbers of individuals increases the probability that the population will persist and perhaps spread (Reichard et al. 2012). This may occur because of higher numbers of total seeds produced or perhaps because, even with some mortality after planting, sufficient numbers of individuals remain to reproduce.

◗ Multiple outplantings over many years may be required to build up a population structure and size that sustains population growth over the long-term.

- Use seed bulking at the nursery to generate enough seed for a reintroduction. This provides an opportunity to document F_1 characteristics, such as variation in timing of germination, which can be compared to the wild population.

Create experimental conditions to improve germination.

- Seek or create conditions experimentally with the intention of improving germination and the establishment and survival of next generation seedlings (Albrecht and Maschinski 2012).

- Although used in large-scale restoration projects, to date there have been few published or reported reintroductions using seeds that have incorporated experimental designs with techniques to improve success of field seed germination and establishment, such as ***microcatchments*** (for example, Bainbridge 2007). Similarly, there are few reports of manipulating site conditions for the next generation seedlings. As this is a critical part of establishing a sustainable population, more attention should be placed on this step in the reintroduction process.

D Implementing the Reintroduction

Summary

- Good logistical preparation will make installation day run smoothly.
- Ensure that the plants or seed plots are labeled, mapped, and recorded in such a way that they can be monitored for many years into the future.
- Implementation day is a great day to involve the public. Be sure to demonstrate planting or sowing techniques to safeguard the rare species.

The logistics of the ***reintroduction*** day often entail coordinating many details and people. It can be a time of great celebration as a high point in the steps towards a species' recovery. Particularly when involving volunteers or inexperienced personnel, coordinating logistics well can result in an extremely satisfying event.

Plan how the reintroduction will be implemented.

Plan timing, materials, personnel, and logistics needed to implement the reintroduction. (See the "Questions to Ask Regarding Logistics for Implementation".)

Improve site conditions for the reintroduced species.

- If necessary, remove invasive species or thin canopy to improve site conditions for the reintroduced species.

- Often it will be easiest to prepare the site prior to and on a different day than the reintroduction. If site preparation increases the potential risk of invasion by undesirable species or exotics, the site should be monitored for several months and ***outplantings*** should be delayed until the risk of invasion is considered low.

- Multiple site preparation treatments may be required to ensure ideal conditions for reintroduced plants.

Use a system, such as color coding, to easily distinguish plants in different experimental treatments.

- Select durable, long-lasting tags for labeling plants.

- Particularly if you have a large number of plants and a large number of people helping with the installation of the reintroduction, it is important to be able to distinguish plants from different treatments. For example, if you are testing plants that had ***mycorrhizal inoculum*** versus those that did not, clearly mark plants before moving to the field and clearly mark the location at the site where plants of each group should be planted.

Questions to Ask

Regarding Logistics for Implementation

___ What is the best season to transplant or sow seeds? Keep in mind that best season for rainfall may also be the hottest time of the year and plants may require more attention.

___ Have you invited participation from enough staff, volunteers, community members, agency and landowners, or land managers to execute the reintroduction?

___ Have permits been acquired and are they up-to-date?

___ How will you ensure that plants will be able to be tracked for many years in the future? Are plants tagged and positions recorded with GPS?

___ How will you transport plants to the recipient site? Do you have necessary off-road equipment for transport away from roadways?

___ What is the planting layout design?

___ How are you going to water plants?

___ Have you notified the press or have you arranged for photos to be taken of the event? (Note that there may be circumstances when the exact location of the conservation translocation must not be publicized to prevent unauthorized collection of the taxon; however, good conservation news with general descriptions of the reintroduction can be used to engender public enthusiasm for plant conservation. If you are uncertain, talk to your regulatory agency prior to notifying the press.)

(Vallee et al. 2004)

Select microsites carefully.

- Select ***microsites*** carefully or conduct an experiment to test different microsites.

- Even when there is reasonably good information about the environmental attributes associated with the species occurrence, test plantings can show which ***microhabitat*** conditions are optimal for growth and survival and long-term population growth (Maschinski, Falk et al. 2012).

- Note aspects of the landscape topography, ecosystem dynamics, and patterns that may help determine the locations with greatest likelihood of sustaining a reintroduced population (Maschinski, Falk et al. 2012).

Plant in a spatial pattern that will promote effective pollination, seed production, and recruitment.

- Plant density strongly influences variation in ***outcrossing*** (or ***selfing***) among plants; therefore, plant in a spatial pattern that will encourage the appropriate breeding system of your species (Monks et al. 2012). Planting individuals in small clusters throughout the population, instead of fewer larger clusters, may lead to increased spread in the ***population*** (Reichard et al. 2012). Keep spatial design in mind in any experimental design.

- Understanding a target species' tolerance for competition and disturbance, as well as habitat composition and structure, can help inform spatial and temporal placement of any reintroduction (Maschinski, Falk et al. 2012; Maschinski, Wright et al., 2012). For example, if the target species is not a good competitor, planting into open spaces with few other species present is beneficial.

Ensure that you have enough help to treat the site and/or install plants.

- This is a wonderful opportunity for student and citizen volunteers of all ages.

- Ensure that individuals installing plants are provided with adequate training and supervision.

- Bring snacks and water.

Consider pretreating reintroduced seeds.

- If using seeds, consider pretreating seeds to release them from dormancy and sow into permanently marked plots or ***transects*** (Maschinski et al. 2017).

E After the Installation

Summary

- A reintroduction will have a higher chance of successful establishment if it receives water and weeding after installation.
- Keeping land managers apprised of the performance of the rare species and engaging them in active site management is critical for long-term population persistence.
- Developing and implementing a long-term monitoring plan is needed to document the success of the reintroduction.

After the time-intensive process of preparing for the ***reintroduction*** and installing it, practitioners often breathe a sigh of relief when the plants or seeds are finally in the ground. However, it is important to realize that the work is not over at this step. Survival and ***population*** persistence of the reintroduction depends upon aftercare and no one will be able to learn about the reintroduction unless it is monitored long-term and findings are reported back to the conservation community. The great thing is that aftercare is likely to improve successful establishment and reduce the species' risk of extinction. Monitoring helps document this success, so it is worth it!

Conduct Aftercare of the Restored Population

Water plants and seeds until established.

- Account for the amount of effort and time required to transport water for supplemental watering.

Periodically weed until plants are well-established.

Ongoing site management is important.

- Collaborators committed to long-term site management should review the status of the site periodically to ascertain whether management is needed.

FAQ Should I manipulate my planting site after the reintroduction?

- Control invasive weeds and competing vegetation.
- Control overabundant herbivores. Cage plants, if necessary.
- Restore historical disturbance regimes such as fire.
- Periodically review the site surveys to detect unforeseen issues (for example, trampling, theft, herbivory, pest insects, vandalism, or maintenance personnel abuse of plants.)

Questions to Ask

Regarding Post Planting

___ What aftercare will be needed and how frequently will this require attention?

___ What habitat management and threat abatement is needed? How frequently?

___ Has a monitoring plan been prepared and reviewed?

___ How will success be measured?

___ Are sufficient funds available for aftercare?

___ Do permits cover aftercare activities?

(Vallee et al. 2004)

Design Appropriate Monitoring Plans

A well-designed monitoring plan is an essential component of any reintroduction program. To ensure the long-term persistence of a species in the face of environmental change, a long-term monitoring plan is necessary to evaluate how reintroduced populations respond to infrequent events (for example, drought) and to detect changes in the population that might take years to express (for example, ***inbreeding depression*** in long-lived perennials or replenishing of the soil seed bank). Our goal is not to provide an exhaustive review of how to monitor plant populations, but rather to provide standards for the minimum amount of information needed to evaluate the long-term fate of reintroduced populations. A long-term monitoring strategy will depend upon a number of factors including the trajectory of population growth, the life-history of the focal species, monitoring resources available, and the goals and objectives of the experimental components of the project.

Use the reintroduction to learn more about the species.

- Use the reintroduction as an opportunity to learn more about the species, its habitat requirements, and its ***biotic*** interactions.

- Incorporate the factors of interest into monitoring plan. Note conditions at the time of installation.

- Document how pollinators and other animals interact with translocated species to improve understanding of the community function in the ecosystem.

Develop a monitoring plan.

- Although all monitoring plans must be tailored to individual projects in order to obtain data relevant to the experimental design and objectives, all reintroduction monitoring plans include basic components needed to provide information relevant to species' biology and techniques for managing rare plant populations (Table 4.2).

- A well-designed monitoring plan with clear objectives provides information on the species' biology and techniques for managing rare plant populations. It should be easily understood by your successors, therefore record details as if you are writing for institutional memory.

- If any changes are made to the monitoring plan, then document changes in detail.

Gather demographic data.

- Gather demographic data on the reintroduced population, unless it is not appropriate for the life-history of the target species (Morris and Doak 2002).

- Demographic monitoring of individuals is the method of choice for achieving the central objectives of most rare plant reintroduction projects.

- Specifically, we recommend measuring survival, growth, and reproduction on each plant preferably over multiple generations (Monks et al. 2012).

TABLE 4.2. List of actions essential to monitoring plans for reintroduced plant populations. These are the minimum items to consider when establishing a monitoring plan.

Action	Description
1) Develop clear monitoring objects.	Take into account the life history of the focal species, propagule stage(s) planted, biological and project goals (Pavlik 1996).
2) Define sample units.	Use individuals or transplants for demographic monitoring or plot/transect based methods for monitoring demographic structure. All transplants and plots permanently marked and mapped, preferably with GPS.
3) Determine appropriate monitoring frequency.	Monitoring period should match key phenological phases (e.g., peak fruiting and flowering) and life-history of the focal species.
4) Monitor vital rates.	Follow the fates (survival, growth, fecundity, and recruitment) of transplanted individuals and their progeny or quantitatively track abundance of stage classes (seedling, juvenile, non-reproductive adult, reproductive adult).
5) Evaluate fecundity.	Measure seed production by counting the number of fruits per plant and estimate the number of seeds per fruit through sub-sampling. Compare results to reference or natural populations.
6) Survey new habitat patches for dispersal and spread.	Search for seedlings at each census both near and far from sample units. Add new recruits to demographic studies, subsample if recruitment densities are large. Conduct searches for the focal species in suitable habitat patches within and beyond the initial planting site. Establish new sample units to monitor the growth and development of new patches/ populations.
7) Monitor wild reference populations.	Simultaneously monitor reintroduced and natural populations to gain insight into key factors that underlie restoration success. Natural populations should be monitored across several sites and during the same years to capture variation in vital rates for comparison to reintroduced populations.
8) Monitor threats.	Document evidence of changes in: exotic species distribution and abundance, successional patterns, hydrology, disturbance regimes, land management, herbivores, predators, and disease.
9) Prepare backup plan to relocate lost sample units.	Document all sites and plots with GPS and supplement with precise directions that includes compass directions and measured distance from permanent visible landmarks (Elzinga et al. 1998). Produce GIS layers and maps if possible.
10) Archive monitoring data and provide metadata.	Enter, store, and backup all monitoring data in digital files. A minimum of two copies of raw data sheets should be kept on paper file, preferably in separate locations. One copy should be accessible to take into the field during subsequent monitoring events. Metadata should be assembled during the project and continually updated.

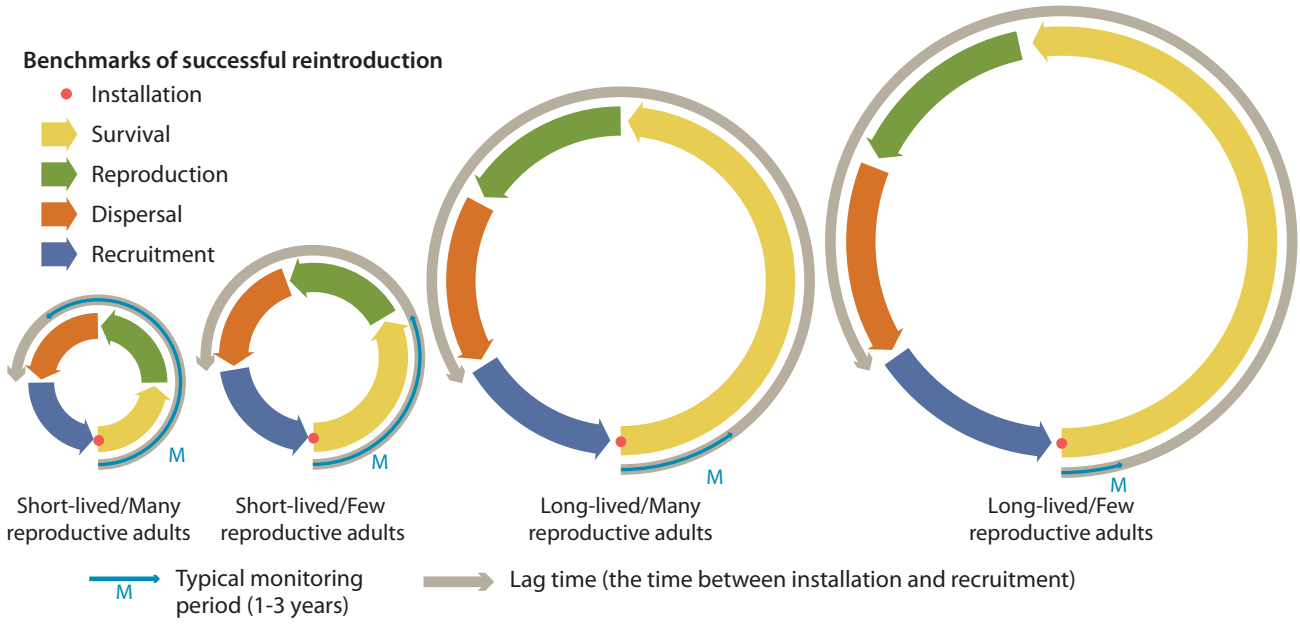


FIGURE 4.2 **Benchmarks of successful reintroduction.** Bars indicate the four benchmarks of a reintroduction: survival, reproduction, recruitment, and dispersal, where dispersal encompasses movement to a new location and establishment. For founders installed as whole plants, the first benchmark is survival, however if founders are seeds, there is an added step. The first benchmark is recruitment, followed by survival, reproductive maturity, next generation recruitment, and dispersal. Species life history and reproductive adult abundance influence duration of time needed to achieve benchmarks. The ability to detect success is constrained by a typical monitoring period of 1-3 years versus the time required to detect recruitment. Turquoise blue arrows denote typical monitoring period, which may be brief and limited by project funding. Grey arrow around circumference of circle indicates lag time to next generation recruitment.

- For demographic modeling and tracking the success of the reintroduction, determine life history stages (typically seedlings, juveniles, non-reproductive adults and reproductive adults) and note when benchmarks are achieved (See Figure 4.2).

FAQ How will I know if the reintroduction is a success?

- Count the number of seedlings, juveniles, non-reproductive adults, and reproductive adults in your reintroduced population.

- If you plan to develop and compare population dynamic models for the reintroduced population and natural populations, then the frequency of monitoring will need to be at a rate that accurately charts movement of an individual from one life stage to another.

FAQ How often/how long should I monitor my reintroduction?

- Define how large an area you will need to search for recruits.

Monitor wild reference populations.

- Whenever possible, monitor wild reference populations to compare to the reintroduced population (Bell et al. 2003; Colas et al. 2008; Menges 2008).

◗ Reference populations will give context for the reintroduced population's ***vital rates*** and aid in identifying the vital rates that are driving population trends (Morris and Doak 2002).

◗ In ***augmentations***, the fate of augmented individuals and naturally occurring ones should be distinguished in demographic or quantitative censuses when possible to determine whether transplants are performing differently than naturally occurring individuals in the population.

◗ If available, multiple ***reference populations*** should be monitored to capture the full range of variation in vital rates possible across different sites and years.

Adopt an appropriate monitoring strategy.

◗ Adopt a monitoring strategy that is appropriate for the life history of your target species and the founding propagule used.

a) For long-lived perennial plants, monitoring plans will need to accommodate changes in population structure over time.

- Specifically note when transplants transition into larger size classes and sexually reproduce.
- Tag new seedlings as they recruit into the population.
- Searches beyond the transplant plots or transects will need to be conducted to document dispersal, seedling recruitment and ***metapopulation dynamics*** adequately.
- Most monitoring of perennial plants will need to be done at least annually to obtain annualized vital rates. More frequent visits may be necessary to quantify disparate parts of the life cycle such as survival, ***fecundity***, and seed ***germination***.
- For long-lived species (for example, trees), monitoring on an annual basis may not be necessary to detect changes in population trends.

b) For annuals and short-lived species, monitoring plans will need to accommodate temporal and spatial fluctuations in population size. (Albrecht and Maschinski 2012; Dalrymple et al. 2012).

- Track counts of reproductive versus non-reproductive plants that emerge in permanently marked plots or transects across years.
- In annual species, dormancy and germination are often driven by climatic cues that vary from year to year, resulting in wide annual fluctuations in distribution and abundance.

c) The method used to monitor seeds will depend upon the sample unit.

- When sample sizes are small, seeds can be tracked individually. In most cases, however, sow seeds directly into plots so that cohorts can be followed.

d) If demographic monitoring of individuals is not possible, monitor stages or size classes that are most important in maintaining population growth.

- If the importance of the vital rates is known for your taxa, you can concentrate on the most important vital rate and note changes across years to understand population trends.
- If populations begin to decline, then monitoring individuals in all stage classes may be needed to understand mechanisms that are driving the decline and determine what management actions are needed to reverse the decline.

f) If demographic monitoring is difficult or impractical, we recommend doing census counts of all or key life-history stages to detect population trends (Menges and Gordon 1996). Examples of species characteristics that may challenge typical monitoring practice include clonal reproduction, seed or plant dormancy or other cryptic life-history stages (for example, tiny seedlings, corms, bulbs).

g) As subsequent generations disperse seed, restricting the census to the original sown plots would fail to capture local dispersal. It will be important to note which microsites are suitable for germination and survival.

- Regular counts of individuals within grids or belt transects that cover broad areas within the habitat may be needed to fully capture changes in the spatial distribution and abundance over the longer-term and to assess population trends effectively (Young et al. 2008).

Monitor for at least 3 years and if possible for 10 plus years.

◗ Long-term monitoring provides information necessary to evaluate how reintroduced populations respond to rare events (for example, drought) that were infrequent or nonexistent during the early phase of population establishment. It can reveal genetic issues that might play out only after multiple generations (for example, inbreeding). (Falk et al. 1996; Dalrymple et al. 2012.)

◗ Ultimately, long-term monitoring is needed to predict the fate of the reintroduced population and determine the mechanisms driving population viability (Albrecht et al. 2011). To develop population viability models and predict population trajectories, a minimum of 3 years of monitoring data are required. To predict long-term trends (10–100 years) and determine whether reintroduced population is potentially self-sustaining under current environmental conditions, extended periods of monitoring are necessary, see Figure 4.2.

◗ Demographic data will be needed to provide population size estimates for reintroduction plans whose objective is to achieve a specific population size or stage structure.

◗ A long-term monitoring strategy will depend upon a number of factors including the trajectory of population growth, the life-history of the focal species, monitoring resources available, and the goals and objectives of the experimental components of the project. (See Elzinga et al. 1998 for more details.)

◗ Enlist the help of public volunteers to accomplish long-term monitoring (Maschinski, Wright et al. 2012). Whenever possible, include land managers in monitoring to foster a close connection with the reintroduced population.

Use redundancy to mark individuals and plots. Assume that some sample units will be lost over time.

◗ Although an essential element in all reintroduction plans, long-term monitoring of reintroduced populations can pose formidable challenges. Over time, natural or anthropogenic disturbances can impede access to sites or complicate relocating sample units. For example, plots and transect boundaries or demographic markers can be lost due to fire, flood, downfalls, burial, vandalism, animal impacts, etc.

◗ Losses can be mitigated with a good insurance plan, which can be used to re-establish or re-locate the boundaries of sample units or tagged individuals when necessary. Whether using plot-based methods or monitoring individuals demographically, there are several ways to ensure the accurate relocation of lost plot markers, transects, and tagged individuals. (See pages 190–191 in Elzinga et al. (1998) for more details.) Submeter GPS points are also helpful.

Determine how success will be measured and have realistic goals.

◗ Expand definition of success. Identify short-, mid- and long-term success that pertain to the target species and its habitat.

◗ Remember to think about project success and biological success (Pavlik 1996).

◗ Comparative mating system studies combined with pollination biology can be carried out over relatively short timeframes (one or two flowering seasons) and can be used to give vital clues to potential recruitment and reproductive success in subsequent generations (Monks et al. 2012).

◗ Use molecular markers to assess key population processes such as mating system variation and genetic variation in reintroduced populations and, where possible, compare to wild populations to predict reintroduction success (Monks et al. 2012).

Monitoring intensity may change over time.

◗ As short-term goals are achieved in a reintroduction program, monitoring intensity may change from experimental to observational.

◗ For example, when reintroducing the perennial forb *Helenium viriginicum* to sinkhole ponds in the Ozarks, Rimer and McCue (2005) initially set out to determine how planting position and maternal lines affected establishment rates of transplants over a 2-year period. Individuals of the species were grown ex situ, transplanted in a replicated experimental design, and then the fates of transplants were followed demographically. After completing the initial goals of the reintroduction, the populations grew rapidly due to vegetative reproduction and successful seedling recruitment, making it impractical to differentiate transplants and new recruits in subsequent censuses. Because the short-term goals of the experimental design were accomplished, the populations grew rapidly, and the species was capable of completing its life cycle in this location, the monitoring protocol switched to count estimates and surveys for new threats rather than full-scale demographic monitoring of individuals.


ENVIRONMENTAL

Questions to Ask

Documentation Needed to Justify and Decide Whether to Conduct a Reintroduction

___ Survey and status updates are complete. Status includes degree of protection, threats, and management options for the extant populations.

___ Specific information on the number of populations has been collated within the last 18 months.

___ Counts or estimates of the number of individuals in each population have been done.

___ The age structure of the populations is known.

___ The relationship of populations in a metapopulation context is compiled.

___ Surveys identifying suitable habitat are complete.

___ Suitable recipient sites have been assessed and ranked.

___ Long-term protection and management plans are documented for suitable recipient sites.

___ Sufficient money is secured to conduct the reintroduction.

(Falk et al. 1996; Vallee et al. 2004; Maschinski, Albrecht et al. 2012)

Likewise, transitioning to observational monitoring may lead to less frequent data collection (for example, annual rather than quarterly) than was needed during the more intense experimental stage.

Analyze data and report results in a timely fashion.

- Report results through publishing or publicizing via social media, newsletters, and websites.

Documentation

Documentation is an essential component of reintroduction, and we encourage practitioners to regard their reintroductions not only as activities done for the preservation of species, but as experiments. To this end, we encourage careful documentation so that the reintroduction is justified, that good decisions can be made about preparedness prior to the reintroduction event, that appropriate monitoring can be implemented, and that the data can be analyzed to determine project success. These steps are important to represent accurately the reintroduction from a legal and scientific perspective. (See Dalrymple et al. 2012). (See Part 5 "Documentation and Data Sharing" and North Carolina Reintroduction Documentation Form http://ncbg.unc.edu/uploads/files/Reintroductionguidelines.pdf.)

FAQ Frequently Asked Questions

Should I manipulate my planting site after the reintroduction? Provide supplemental water until plants are displaying visual signs of establishment (that is, they display new growth, good color, remaining vertical) and appear well-rooted (that is, they display resistance to gentle tugging). The planting site should be manipulated as needed to maintain ideal growing conditions for your plant, including removal, mowing, pruning of surrounding plants. Strive to achieve these conditions by maintaining or restoring historical disturbance regimes via prescribed fire, grazing, etc.

How will I know if the reintroduction is a success? Establishment and survival of reintroduced plants should be viewed as short-term success in and of itself. Long-term success is typically accomplished when populations are self-sustaining (increasing in number, cross-pollination, minimal intervention requirements). Having comparative wild populations that are considered "successful" can help to shape expectations.

How often/how long should I monitor my reintroduction? Consider your reintroductions as a long-term commitment. Plan to monitor them for a minimum of 5-10 years and ensure agreements are in place with land managers to accomplish this goal. Monitoring plans should begin with a basic understanding of your plant's life history (life span, growing season, flowering/fruiting season, seedling emergence, etc.). Local climate and weather patterns and changes thereof should be considered when timing the monitoring. Look for other factors such as herbivory, vandalism, invasive species, etc., that may influence monitoring/aftercare frequency. In general, visiting more frequently after your planting is recommended to foster establishment, but can later be reduced based on the expected dynamics of the population. Initial frequent (monthly) monitoring should help you learn about population dynamics if these are not already understood and can help you make decisions on the frequency of future visits. For example, observed plants with highly variable flowering times and seedling recruitment may require more frequent monitoring to understand true population dynamics, while species with strict annual flowering seasons may only require one visit. Slow growing, long-lived species -once established- often can be monitored annually or less.

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Glossary Terms

abiotic—Non-living factors that influence plant growth, function and survival (for example, soil, water, amount of sunlight, nutrients, etc.).

adaptive—capable of genetic change by natural selection.

adaptive monitoring plan—Long-term plan for measuring attributes such as survival, growth, and reproduction of a reintroduced population that can evolve or change when answers to initial questions (or hypotheses) are met and new questions arise across time.

admixture—Interbreeding between two or more previously isolated populations, sometimes resulting in introducing foreign or unadapted genes.

allele—DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to- many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity.

artificial selection—The process of modifying organisms by selection in breeding controlled by humans (for example, choosing a plant with numerous fruits and removing low fruit-producing plants in a breeding program will artificially select for fruit production).

augmentations— (also known as enhancement, enrichment, replenishment, or restocking) Adding seeds or individual plants to a population that were propagated from that same population, with the aim of increasing population size or genetic diversity and thereby improving viability; re-creating a recently extirpated population with individuals propagated from that population. Often an ex situ facility is the intermediary between the original collection from a population to propagation in a nursery setting before the propagules are placed back into the wild population.

bioindicator—A species or ecological community that is so closely associated with particular environmental conditions that its presence is indicative of these conditions in a particular environment. For example, the presence of seedlings indicates proper conditions are present for germination

biotic—Living factors that influence plant growth, function and survival (for example, predators, fungi, pollinators).

clonal—Type of asexual reproduction in plants that produces new individuals with the same genetic makeup as the mother plant (unless unusual mutations occur). Examples include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

common garden—An experimental technique wherein plants from more than one location are grown together in a single (hence common) garden. This method allows researchers to determine if differences observed in the geographically separated populations have a genetic basis.

competition—An interaction between living organisms or the same or different species for a common resources, territory, or mate that occurs in a limited supply.

congeners (congenerics) —Members of the same genus.

conservation translocation—A definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of indigenous range including conservation introductions, comprising assisted colonization and ecological replacement.

deterministic event—A predictable or known event (for example, cold temperatures in winter north of the equator are predictable).

dioecy—Having male and female flowers on different individuals.

ecotypes—A distinct form or race of a plant occupying a particular habitat.

edaphic endemics—Plants that grow only on a specific soil type.

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

F1 generation—First generation.

F2 generation—Second generation.

fecundity—The number of seeds or asexual propagules produced by an individual plant or population.

founder(s)—The individual(s) that starts a new population.

genetic rescue—Increasing genetic diversity from infusing new genes into a population. If population is inbred, this practice may increase chances that some plants will survive.

genetic structure—A measure of the differences across and within populations of a species. Populations of a species may have similar or different genetic composition. Measures of this pattern have conservation implications for providing source material for a conservation translocation.

genome—A complete set of genes or genetic material present in a cell or organism.

genotype—The genetic constitution of an individual (genotyping is determining the genetic constitution of an individual).

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

germplasm—Living seeds or tissues from which plants can be grown.

hand-pollination (hand pollinated) —A process where pollen is manually and deliberately transferred to the receptive portion of a flower (the stigma). This technique is often used to control the parentage and to maximize pollen transfer in hopes of achieving fertilization and good seed set.

hybridization—Cross species fertilization. In the context of conservation, it is often undesirable to have hybridization occur in a cultivated or wild setting, as the more common parent will likely swamp the genepool of the rare species.

in situ—In wild habitat.

iinbreeding—Mating between closely related individuals. Over many generations, genetic disorders may arise.

inbreeding depression—Reduced fitness of progeny resulting from breeding of related individuals.

mating system—The way that plants produce seeds. Some plants can produce seeds in multiple ways, while others are restricted to a single mating system. The types of mating system include outcrossing or cross-pollination (a flower receives pollen from another plant of the same species), autogamy or self-fertilization (a flower receives pollen from the same plant) and apomixis (asexual reproduction without fertilization that is only possible with evolution of a modified flower). Mixed mating systems, in which plants use two or even all three mating systems, are not uncommon.

metapopulation—A group of spatially separated populations of the same species that interact (for example, the interaction can be pollen or seeds moving between plant populations).

metapopulation dynamics—A change across time within a group of separated but interacting populations that influence the overall persistence of a species.

microcatchments—Small, localized wells or depressions around a plant that serve to gather and hold water. These are recommended for plants or seeds installed in dry habitats.

microhabitat(s) —Very localized abiotic (soil, light, and moisture) and biotic (associated plants, insects, and other animals). The nature of a microhabitat may greatly influence seedling and adult plant growth and survival. See also microsite(s).

microsite(s) —Very localized abiotic (soil, light, and moisture) and biotic (associated plants, insects, and other animals). The nature of a microhabitat may greatly influence seedling and adult plant growth and survival. See also microhabitat(s).

mitigation—In the context of CPC protocols, mitigation is a legal term for an action that is taken to offset the adverse impacts of development on U.S. listed species. For example, a parcel land where a species occurs may be preserved as mitigation for developing a portion of the species' habitat.

mutualists—two organisms that exist in a relationship in which both benefit.

mycorrhizal inoculum—A fungi that forms a symbiotic relationship with a plant's roots and increases water and nutrient absorption.

niche space—The ecological space occupied by a species (Hutchinson definition); an ecological role (Grinnel definition); a species' response to and effect on environment (Elton definition).

obligate mutualists—Organisms that are required to rely on one another for survival.

obligate outcrosser—Pollen from a different plant (not self) is required for successful seed set.

outbreeding—A condition where flowers of one plant receives pollen from another plant of the same species.

outbreeding depression—Low fitness of progeny resulting from mating between two genetically distant (and usually physically distant) plants.

outcrossing—The form of plant reproduction that requires pollen from another plant of the same species to form seeds.

outplanting—Transplanting from a botanical garden nursery (ex situ setting) to a wild setting for purposes of reducing the extinction risk of a species and allowing persistence in a natural setting.

patch dynamics—Spatial and temporal changes within and among patches of vegetated or bare spaces that make up a landscape.

ploidy—The number of sets of chromosomes in a cell.

population—A group of potentially interbreeding individuals that share a common ancestry or gene pool.

population persistence—A measure of effective conservation is to have a population grow and reproduce sustainably over time in a wild setting.

propagule— A general term to describe any plant material that can function to propagate a new plant, including seeds, stems, corms, tubers, or spores.

reciprocal transplant—An experimental method which involves introducing plants from each of two or more environments into the other(s). The method can be used to test whether differences in appearance, for example, between populations have a genetic versus environmental basis.

reference populations—The population used as a basis for comparison. In the context of reintroduction, the growth and survival of plants in the wild population can be compared to the reintroduced population as a measure of success.

reintroduction(s)—intentional movement of species into habitat it previously occupied.

selfing—Pollen from one flower fertilizes the same flower and successfully sets seed.

sequencing—Refers to genetic procedure to determine the composition and order of genes of an individual as in genotyping.

specialist pollinators—Organism adapted in structure and behavior to gather and transfer pollen to flowers of one or a few related species of plant.

stochastic event—Unpredictable or chance event.

succession— the process of change of an ecological community in the composition and structure of species over time. The time scale can be decades (for example, after a wildfire), or even millions of years after a mass extinction.

symbiont—An organism living in close association with another.

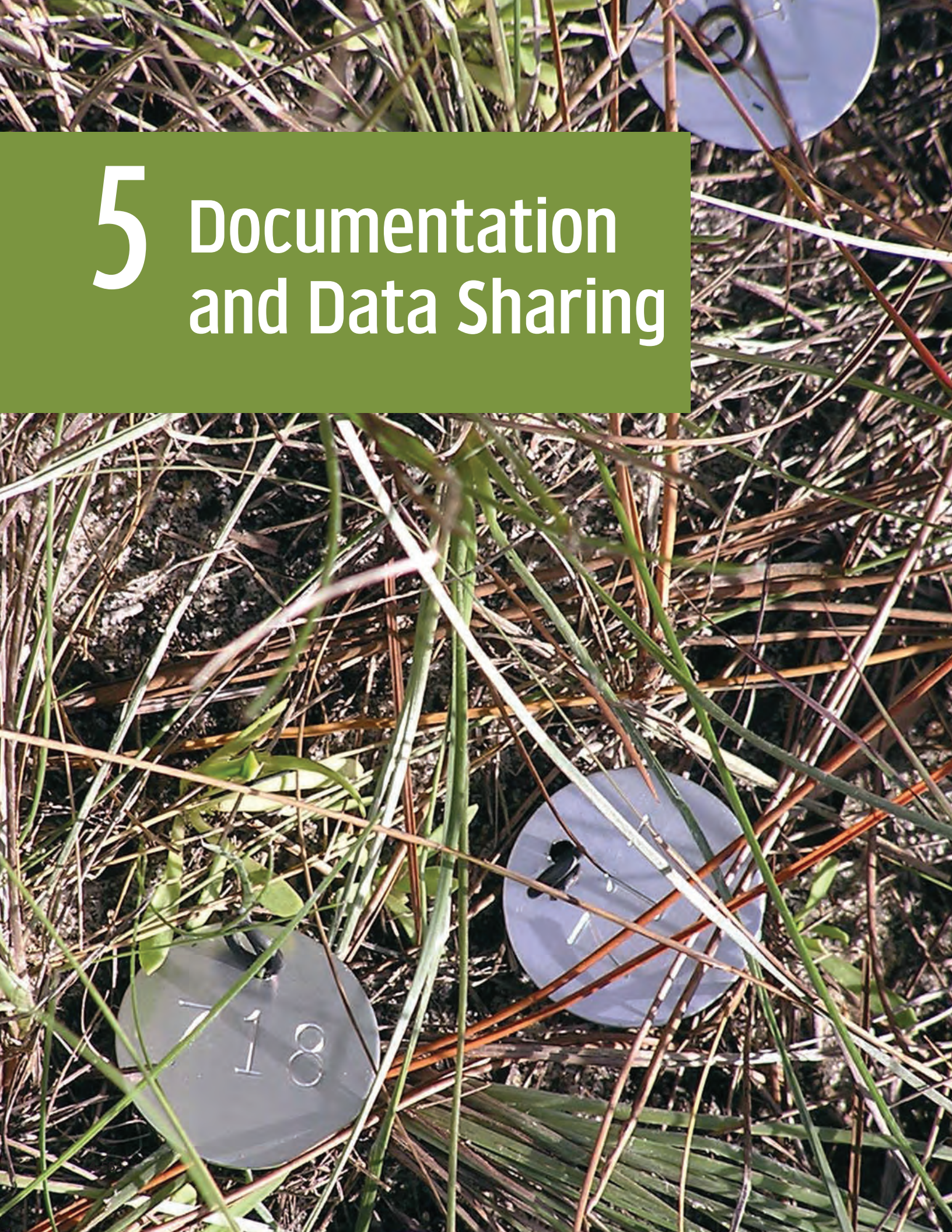
taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

transects—A line of known length used as a way to monitor vegetation.

vital rates—Measure of change that influence population growth (for example, birth rate, survival of an age class from one year to the next, death rate).

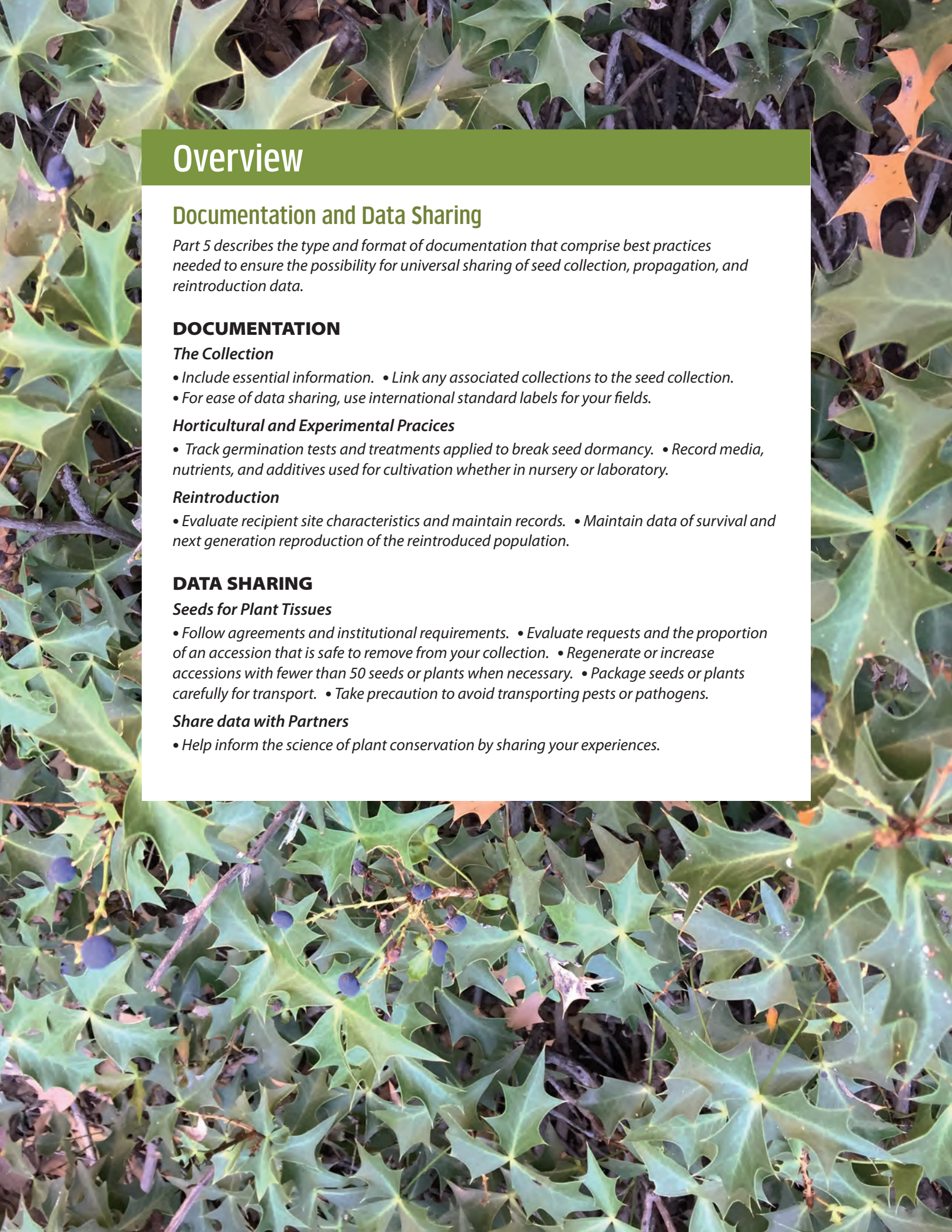
wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.

5 Documentation and Data Sharing



Documentation and Data Sharing

Center for Plant Conservation Best Practices



Overview

Documentation and Data Sharing

Part 5 describes the type and format of documentation that comprise best practices needed to ensure the possibility for universal sharing of seed collection, propagation, and reintroduction data.

DOCUMENTATION

The Collection

• Include essential information. • Link any associated collections to the seed collection. • For ease of data sharing, use international standard labels for your fields.

Horticultural and Experimental Pracices

• Track germination tests and treatments applied to break seed dormancy. • Record media, nutrients, and additives used for cultivation whether in nursery or laboratory.

Reintroduction

• Evaluate recipient site characteristics and maintain records. • Maintain data of survival and next generation reproduction of the reintroduced population.

DATA SHARING

Seeds for Plant Tissues

• Follow agreements and institutional requirements. • Evaluate requests and the proportion of an accession that is safe to remove from your collection. • Regenerate or increase accessions with fewer than 50 seeds or plants when necessary. • Package seeds or plants carefully for transport. • Take precaution to avoid transporting pests or pathogens.

Share data with Partners

• Help inform the science of plant conservation by sharing your experiences.

A Introduction

Documentation is essential to represent the scientific and legal accuracy of our ***conservation collections***. Several steps in the process ensure the optimal use of the conservation collection (see Overview). The rapidly improving information technology and bioinformatics fields are increasing the speed and convenience of data input and data sharing for both research and conservation actions. However, key to the efficacy of any database is timely input and ongoing updates from practitioners. All the great tools in the world will never surpass the knowledge of the individuals, who know rare plant species in their ***wild populations***, in ***ex situ*** gardens, or in a reintroduction or ***conservation translocation***. The Center for Plant Conservation encourages practitioners to use available tools to place valuable insights from an individual mind into a database where it may have uses far beyond the individual experience or time. The power of collectively shared and carefully documented data is enormous. With accurate data, we can help guide conservation actions to save more plants.

B Documentation

Center for Plant Conservation Best Practices

SUMMARY

- Conservation collections require appropriate documentation to retain their highest ***conservation value***.
- When documentation is kept according to international standards, it can be easily shared with other institutions.
- Conservation translocations may require years or decades to establish; therefore, records kept at the institution will help ensure that the efforts and success of the endeavor can be assessed and contribute to reintroduction science.

Conservation collections must have documentation to have highest value to support species survival in the wild. Increasingly important is our ability to input information rapidly into a database so that the information will be available for internal and external use. It is important for collectors and researchers to document collection information at time of collection, during care at our facilities, and after it returns to the wild. When done well, these data form the basis of the best plant conservation practice that can be accessible to partners throughout the world.

Document the collection appropriately.

- Essential accession information includes: institution name, accession number, collector, collection date, species name, family, locality information, ***georeferenced*** latitude and longitude, site ownership, permit documentation, and population information (the total number of individuals in the population, number of reproductive individuals, and number of individuals sampled for seeds that were harvested). (See CPC Field Collection Form.)

  - Providing habitat information may provide clues to ***germination*** or ***tissue culture*** requirements of the species. Recommended fields include light and moisture conditions, soil type, slope orientation, and associated species. Provide photos of habitat and plant in its habitat.
  - Be sure to document any associated collections (for example, leaf litter, soil, mycorrhizal fungi) and maintain the link through processing of samples.
  - Gather and report additional accession data according to their institutional protocols. Complying with International Transfer Format for Botanic Garden Plant Records (https://www.biodiversitylibrary.org/bibliography/45427#/summary) and/or ***Darwin Core*** standards (http://rs.tdwg.org/dwc/) will allow easy transfer of information to partners.

- Complete one field form per accession. Multiple accession numbers and field forms only need to be created for collections made from populations, which are separated by at least 1 kilometer.

- Transmit accession data to CPC and if storing seeds at NLGRP, transmit accession data to ARS-USDA National Laboratory for Genetic Resources Preservation (NLGRP) via online form provided to Participating Institutions through the CPC PI portal, which can be accessed at www.saveplants.org/login.

Document the conservation collection treatment at your facility.

- Track germination tests conducted, propagation details, soil media used for propagation, and any horticultural steps taken to care for the species.

- Record growth to maturity details. Note timing of germination, flowering, and seed set.

Document experimental protocols carefully.

- Whenever steps of protocol are compared to controls, report survival of controls and treated groups. Remember that steps that are NOT successful will help future practitioners.

- Note the age of the material used for tissue culture or ***cryopreservation***.

- Take photos of your shoot tips ***in vitro*** culture.

- Note average shoot tip size and photograph this.

- Note condition of tips and any appearance differences across treatments (hairy, shape, leaves present or not, etc.). Note survival of phenotypes.

- Track the type of medium used for pre-culture, stock and recovery culture; note any additives used (ABA, antibiotic, etc.); ***cryoprotectant*** used; cold hardening treatment; cooling rate and ***vitrification*** method used; and any modifications in standard procedures.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Documentation for Orthodox Seeds

4.7.1 Passport data of 100 percent of the accessions should be documented using FAO/Bioversity multi-crop passport descriptors.

4.7.2 All data and information generated in the genebank relating to all aspects of conservation and use of the material should be recorded in a suitably designed database.

Standards for Documentation for Field Genebanks

5.8.1 Passport data for all accessions should be documented using the FAO/Bioversity multi-crop passport descriptors. In addition, accession information should also include inventory, map and plot location, regeneration, characterization, evaluation, orders, distribution data and user feedback.

5.8.2 Field management processes and cultural practices should be recorded and documented.

5.8.3 Data from 5.8.1. and 5.8.2 should be stored and changes updated in an appropriate database system and international data standards adopted.

Standards for documentation in vitro and cryopreservation

6.6.1 Passport data for all accessions should be documented using the FAO/Bioversity multi-crop passport descriptors. In addition, accession information should also include inventory, orders, distribution and data user feedback.

6.6.2 Management data and information generated in the genebank should be recorded in a suitable database, and characterization and evaluation data (C/E data) should be included when recorded.

6.6.3 Data from 6.6.1. and 6.6.2 should be stored and changes updated in an appropriate database system and international data standards adopted.

MSB Partnership Collections *(Millennium Seed Bank Partnership 2015)*

Data management

5.1 A data management system, using recognised seed bank data standards, is in use and capable of export in standard format.

Document details of the recipient site and reintroduction.

- The recipient site characteristics, location, and predicted climate envelope may influence reintroduction success. Thus, careful documentation is important. (See Figure 4.2, Maschinski, Falk et al. 2012.)

- Specific details of the reintroduction are a necessary contribution to the science and are essential for long-term monitoring. (See Maschinski, Albrecht et al. 2012, North Carolina Reintroduction Documentation Form, http://ncbg.unc.edu/uploads/files/Reintroductionguidelines.pdf).

Share publications with CPC Network partners.

Distributing Samples and Information

Center for Plant Conservation Best Practices

Summary

- Conservation collections ideally serve the conservation of the species in the wild. Distributions made for this purpose are encouraged.
- Permits, the collector's institution collection policy, and the storage agreement with banking facilities will govern the distribution details for seeds, tissues, or whole plants in the future.
- Distribute in a manner that maintains collection health.

Samples are primarily collected for repatriation to the wild or for research that facilitates the species ***reintroduction*** to the wild. Participating Institutions may wish to have accessions distributed for these purposes and should not feel obligated to distribute for any other purpose.

Follow permit requirements for distribution.

- It is best to clarify details regarding seed or in vitro culture distribution with land owners at the time permission is requested. For example, some landowners may request that seeds or in vitro cultured plants be available for future restoration projects.

FAQ

How do I decide how many seeds to return to land managers who granted permission for me to collect seeds from the public property they manage?

- Understand any restrictions that may be imposed regarding distribution of seeds or in vitro cultured plants.

Follow your institution requirements for distribution.

- If your institution does not have a material transfer policy and agreement, work with administration to create one. Note that CPC Participating institutions are included under the *Material Transfer Research Agreement* with the National Laboratory for Genetic Resources Preservation. A CPC Participating Institution may obtain a separate ***Material Transfer Agreement*** for ***black-box storage***.

- Indicate the final destination of surviving plants (if for research), use and ownership of any F_1 seed propagated, and information sharing relevant to the conservation of the ***taxon***, etc.

Understand distribution requirements of your agreements.

- Seeds and relevant accession information may be distributed in compliance with agreements, national laws, permits, and policies of the seed bank and the participating institution (for example, CPC PIs MTRA form, which can be accessed at www.saveplants.org/login).

- In vitro cultures may be distributed according to agreements held with the laboratory.

Distribute seeds or in vitro cultures sparingly.

- Evaluate and distribute the minimum number of seeds or in vitro cultured plants based on the users' research needs, the biology of the species, and the quantity of seeds or plants available.

- At the time a request is made, assess the quantity of seeds available at the seed bank or in vitro cultures in laboratory and compare to the quantity needed for the researcher's research question. Some research needs may require as few as five viable ***propagules***, while others require large quantities. Population genetic research, propagation trials, and research on species with unknown ***germination*** requirements may require large numbers of seed or plant tissues.

FAQ How can I determine a bona fide request for distribution of my seeds?

- Accessions with fewer than 50 seeds or in vitro cultured plants may require regeneration/ increase by the Participating Institution or a partner before they can serve a reintroduction or research purpose. Mutual agreement between the user and the Participating Institution will determine the nature and financial details of the regeneration.

Package items for distribution carefully.

- Package seeds or plant tissue for distribution considering weather and arrival conditions and including all necessary data.

- Place packaged seeds into a plastic Ziploc bag to keep them dry, clearly marking each envelope with the full taxon name, accession number, and quantity of seeds. Place seed envelopes in a padded box or Styrofoam container for shipment. Because seeds are fragile, protect them from excessive movement, crushing, and temperature extremes. Do not freeze prior to shipping.

- If transporting plant stem cuttings, wrap stems in moist paper towel or sphagnum moss to transport to laboratory.

- In vitro cultured plants can be transported in growing tubes.

- Include a printed, dated form describing the full contents of the shipment with the package. Where appropriate, also send shipment and accessions data to the recipient electronically. Germplasm sent to the National Laboratory for Genetic Resources Preservation (NLGRP) research should be accompanied by the submission of the electronic accession form available in the CPC PI portal (http://saveplants.org/login/pi-portal/).

- Prior to sending seeds or plants, communicate with recipient to align timing of package arrival with the availability of personnel to receive the shipment. Take care not to ship for a weekend arrival or postal holiday. Ship seeds overnight. Notify the receiver and send the tracking number.

- Obtain all relevant customs paperwork and necessary permits prior to shipment.

- Make arrangements as necessary as to which party will bear shipping costs.

International Standards

Reference for CPC Guidelines

FAO Genebank Standards for Plant Genetic Diversity *(FAO 2014)*

Standards for Distribution and Exchange

4.8.1 Seeds should be distributed in compliance with national laws and relevant international treaties and conventions.

4.8.2 Seed samples should be provided with all relevant documents required by recipient country.

4.8.3 The time span between receipt of a request for seeds and the dispatch of the seeds should be kept to a minimum.

4.8.4 For most species, a sample of a minimum of 30–50 viable seeds should be supplied for accessions with sufficient seeds in stock. For accessions with too little seed at the time of request and in the absence of a suitable alternative accession, samples should be supplied after regeneration/ multiplication, based on a renewed request. For some species and some research uses, smaller numbers of seeds should be an acceptable distribution sample size.

MSB Partnership Collections *(Millennium Seed Bank Partnership 2015)*

Distribution

6.1 Collections are available for use [under an appropriate Material Supply Agreement], at least in country where banked.

6.2 A distribution policy, with appropriate risk management for pests, diseases and potentially invasive species, is in place and applied.

Consider appropriate risk management precautions.

- Prior to distribution to the wild, consider appropriate risk management precautions for pests, diseases, and potentially invasive species.

- Review the CPC's protocol for reintroductions and work through the checklist provided (See Part 4. "Rare Plant Reintroduction and Other Conservation Translocations.")

Share information with partners.

Germination protocols, viability of seeds in storage over varying lengths of time, and seed characteristics are important details to share with conservation partners. Because there is still much to learn about storing seeds of rare species, we encourage you to publish your findings.

FAQ

Frequently Asked Questions

How do I decide how many seeds to return to land managers who granted permission for me to collect seeds from the public property they manage? Abide by any agreements that were made prior to collection (when permission was initially given).

If the land manager desires to augment a declining wild population, CPC recommends beginning with a discussion to review the status of the species and the condition of the habitat before planning an ***augmentation***. Agency staff (federal and/or state) may need to be consulted as well.

It may be necessary to increase the number of seeds via regeneration in a greenhouse before attempting to sow into the wild. See Part 1E, "Curating Small Samples: Increasing the Number of Seeds for Storage and Restoration."

How can I determine a bona fide request for distribution of my seeds? Ask for a copy of a research proposal or reintroduction plan and CV from the requestor. CPC recommends distributing seeds only to personnel associated with research institutions or organizations with known high-quality horticulture and where identities may be verified. Referencing a vetted recovery plan also adds value to a proposal or plan.

Equipment List

- GPS
- Foil or glassine envelopes
- Padded envelope, box for shipping

References

Food and Agriculture Organization of the United Nations (FAO). 2014. Genebank standards for plant genetic resources for food and agriculture. Rome, Italy. http://www.fao.org/3/a-i3704e.pdf.

Maschinski, J., M. A. Albrecht, L. Monks, and K. E. Haskins. 2012. Center for Plant Conservation best reintroduction practice guidelines. *In* J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington DC.

Maschinski, J., D. A. Falk, S. J. Wright, J. Possley, J. Roncal, and K. S. Wendelberger. 2012. Optimal locations for plant reintroductions in a changing world. *In* J. Maschinski and K. E. Haskins,editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington DC.

Millennium Seed Bank Partnership (MSB). 2015. Seed conservation standards for "MSB Partnership Collections." Royal Botanic Gardens, Kew, UK).

Glossary Terms

augmentations— (also known as enhancement, enrichment, replenishment, or restocking) Adding seeds or individual plants to a population that were propagated from that same population, with the aim of increasing population size or genetic diversity and thereby improving viability; re-creating a recently extirpated population with individuals propagated from that population. Often an ex situ facility is the intermediary between the original collection from a population to propagation in a nursery setting before the propagules are placed back into the wild population.

black-box storage—The seeds in the seed bank are stored under "black box" arrangements, meaning that overseers of the seed bank will never open or test any of the seed packages.

conservation collection—An ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues or whole plants of at least 50 unrelated mother plants, and breadth, meaning it consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.

conservation translocation—A definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of indigenous range including conservation introductions, comprising assisted colonization and ecological replacement.

conservation value—The degree to which an action might contribute to reducing a species' extinction risk.

cryopreservation—Storing at liquid nitrogen temperatures, usually at –170°C to –180°C (vapor above liquid nitrogen) or at –196°C (in liquid nitrogen) to maintain viability.

cryoprotectant— A substance that prevents tissues from freezing, or prevents damage to cells during freezing.

Darwin Core—A body of standard terms intended to facilitate the sharing of information about biological diversity by providing reference definitions, examples, and commentaries (http://rs.tdwg.org/dwc/).

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

F1 generation—First generation.

georeferenced—Points taken with GPS that can be used to locate a plant again. Herbarium specimens and seed collections when georeferenced can contribute greatly to our understanding of how plant populations may change in the future.

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

in vitro—Micropropagation; plant tissues grown on nutritional medium in a sterile humid glass or plastic container.

Material Transfer Agreement—The agreement that CPC has with the Resources Preservation Unit - Seeds of the National Laboratory for Genetic Resources Preservation, Fort Collins, Colorado, to store national collection rare plant seed accessions in long-term black-box storage.

propagule— A general term to describe any plant material that can function to propagate a new plant, including seeds, stems, corms, tubers, or spores.

reintroduction(s)—intentional movement of species into habitat it previously occupied.

taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

tissue culture—Growing cells in an artificial medium. In plant conservation, the cells may be whole seeds, spores, or meristems and the medium is usually some derivation of agar.

vitrification—To make liquids solid without forming crystals, a "glassy state." Forming ice crystals inside plant cells can be deadly. When a solid state can be achieved without ice, vitrification is the term used to describe ice free cryopreservation.

wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.

Glossary Terms

abiotic—Non-living factors that influence plant growth, function and survival (for example, soil, water, amount of sunlight, nutrients, etc.).

accession—A collection occurring within one plant population at one location that may be collected over several consecutive days. In botanical garden databases, an accession is given a unique number that can be tracked through time.

acclimatize—Harden off a plant so that it can survive under low humidity conditions.

adaptive—capable of genetic change by natural selection.

adaptive monitoring plan—Long-term plan for measuring attributes such as survival, growth, and reproduction of a reintroduced population that can evolve or change when answers to initial questions (or hypotheses) are met and new questions arise across time.

admixture—Interbreeding between two or more previously isolated populations, sometimes resulting in introducing foreign or unadapted genes.

adventitious shoot—A shoot that arises from a point that is not the shoot tip (for example a bud at a leaf axil).

allele—DNA found on one location on a chromosome that corresponds to a trait. Depending upon the plant and the number of paired chromosomes it has, one-to- many alleles may be responsible for traits related to appearance, chemistry, or growth. In genetic tests, the number of unique alleles is one measure of genetic diversity.

ambient conditions—The relative humidity and temperature of the room. When processing seeds for long-term storage, it is a good idea to check the room temperature and humidity. Seeds will have best chance for long-term survival if processed at temperatures below 25°C. Humidity levels can be taken below ambient levels when using a desiccator.

apical meristem—Growing tissues at the tip of a shoot.

artificial selection—The process of modifying organisms by selection in breeding controlled by humans (for example, choosing a plant with numerous fruits and removing low fruit-producing plants in a breeding program will artificially select for fruit production).

asexual—In plants a form of reproduction that does not involve pollen or flowers and therefore new individuals formed by this method have the same genetic makeup (unless unusual mutations occur). Types of asexual reproduction in plants include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

augmentations— (also known as enhancement, enrichment, replenishment, or restocking) Adding seeds or individual plants to a population that were propagated from that same population, with the aim of increasing population size or genetic diversity and thereby improving viability; re-creating a recently extirpated population with individuals propagated from that population. Often an ex situ facility is the intermediary between the original collection from a population to propagation in a nursery setting before the propagules are placed back into the wild population.

auxins—A plant hormone that causes the elongation of cells in shoots and is involved in regulating plant growth.

axillary bud—A bud that grows from the axil of a leaf or node and has the potential to form stems and branches with leaves or reproductive shoots with flowers.

axillary shoot—The stem that grows from an axillary bud at the axil or base of a leaf.

backup facility—A second seed bank or nursery where a representative portion of an accession can be stored.

biodiversity, or biological diversity—The variety of life. This recent concept includes different levels of biological organization. It considers the diversity of species of plants and animals that live in one place, their genetic variability, the ecosystems that these species form part of, and the landscapes or regions where the ecosystems are located. It also includes the ecological and evolutionary processes that occur at the level of genes, species, ecosystems, and landscapes.

bioindicator—A species or ecological community that is so closely associated with particular environmental conditions that its presence is indicative of these conditions in a particular environment. For example, the presence of seedlings indicates proper conditions are present for germination

bioinformatics—An interdisciplinary field of science that combines biology, computer science, mathematics, and statistics to analyze and interpret biological data.

biome—Distinct communities of animals and plants that have formed in response to a shared physical climate and occur across continents (for example, tundra, temperate forest).

biotic—Living factors that influence plant growth, function and survival (for example, predators, fungi, pollinators).

black-box storage—The seeds in the seed bank are stored under "black box" arrangements, meaning that overseers of the seed bank will never open or test any of the seed packages.

breeding system—The method by which a plant can successfully produce seeds. Plants have three basic breeding systems: outbreeding or outcrossing, where pollen from a different individual is needed to fertilize the egg of the maternal plant to produce seeds successfully; selfing or self-fertilization, where pollen from the same individual can fertilize the egg and produce seeds; or apomixis, where seeds can be set without fusion of gametes.

clonal—Type of asexual reproduction in plants that produces new individuals with the same genetic makeup as the mother plant (unless unusual mutations occur). Examples include producing corms or bulbs (as in lilies) or producing roots along a stem that gets buried (as in willows).

common garden—An experimental technique wherein plants from more than one location are grown together in a single (hence common) garden. This method allows researchers to determine if differences observed in the geographically separated populations have a genetic basis.

competition—An interaction between living organisms or the same or different species for a common resources, territory, or mate that occurs in a limited supply.

congeners (congenerics) —Members of the same genus.

conservation collection—An ex situ (offsite) collection of seeds, plant tissues, or whole plants that supports species' survival and reduces the extinction risk of globally and/or regionally rare species. A conservation collection has accurate records of provenance, maternal lines differentiated, and diverse genetic representation of a species' wild populations. To be most useful for species survival in the wild, a conservation collection should have depth, meaning that it contains seeds, tissues or whole plants of at least 50 unrelated mother plants, and breadth, meaning it

consists of accessions from multiple populations across the range of the species. Conservation collections should have tests of initial germination and viability, cultivation protocols developed, and periodic testing of long-term viability. A conservation collection differs from a horticultural collection, which may have few genetically unique individuals, or is solely comprised of unusual appearing forms.

conservation introduction—Defined by IUCN as the intentional movement and release of an organism outside its indigenous range.

conservation status—A formal designation of endangered or threatened status at a state, federal, or international level.

conservation translocation—A definition coined by IUCN to describe intentional movements of organisms within the species' indigenous range (reinforcement or augmentation of existing population and reintroduction into an area once but not currently occupied by the species) and movements outside of indigenous range including conservation introductions, comprising assisted colonization and ecological replacement.

conservation value—The degree to which an action might contribute to reducing a species' extinction risk.

conspecifics—Individuals of the same species.

controlled rate cooling—Precise and steadily paced reduced temperatures imposed on plant tissues prior to cryopreservation in liquid nitrogen. The intention of the process is to avoid injuries to cells and increase survival after exposure to liquid nitrogen.

conventional storage—Storage at freezer temperatures (–18°C ± 3°C or 0°F ± 5°F).

cross-pollination—The transfer of pollen from the anther of a flower of one plant to the stigma of the flower of another plant of the same species.

cryogenic storage—Storage below –130°C in specialized containers holding liquid nitrogen.

cryopreservation—Storing at liquid nitrogen temperatures, usually at –170°C to –180°C (vapor above liquid nitrogen) or at –196°C (in liquid nitrogen) to maintain viability.

cryoprotectant— A substance that prevents tissues from freezing, or prevents damage to cells during freezing.

cultivars—A plant variety that has been produced in cultivation by selective breeding (for example, a "Champagne" mango).

cultivated setting—A place for growing plants with altered amounts of water, changed light conditions, and reduced exposure to predators than the natural conditions experienced by the wild population.

curation package—A separate envelope containing one to a few seeds of each maternal line in an accession that can be used to test germination and longevity of viability after storage over time.

cytotypes—A characteristic of a cell. Organisms of the same species with different cytotypes have different numbers of chromosomes.

Darwin Core—A body of standard terms intended to facilitate the sharing of information about biological diversity by providing reference definitions, examples, and commentaries (http://rs.tdwg.org/dwc/).

desiccation—The process of removing moisture for the purpose of preservation.

desiccators—Sealable enclosures containing desiccants used to equilibrate the moisture of seeds prior to long-term storage. Desiccators create and maintain dry environments to ensure stable moisture content.

deterministic event—A predictable or known event (for example, cold temperatures in winter north of the equator are predictable).

differential scanning calorimetry (DSC) —A technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. This is relevant to seed storage in that lipid composition within a seed can be measured using this technique without harming the seed.

dioecy—Having male and female flowers on different individuals.

DNA banking—Long-term storage of an individual's genetic material. In plants, leaves are common tissue that can be stored.

dormant—a state of slowed activity, in seeds, alive, but not actively growing

drying agent—Any substance that can remove moisture from seeds. Examples include salts and silica gel.

drying temperatures—The temperature at which seeds should be held while drying to the target moisture content. Safe drying temperatures to maximize seed longevity are below 25°C.

ecological—Pertaining to the ecology of an organism, the interactions of living organisms with each other and with their environment.

ecoregion—A geographically defined area with distinctive ecology that may change with changing global conditions.

ecotypes—A distinct form or race of a plant occupying a particular habitat.

edaphic endemics—Plants that grow only on a specific soil type.

effective population size (Ne) —The number of individuals that contribute genes to succeeding generations. This number is typically less than the number of individuals in the whole population, because not all individuals may be reproductive.

embryo—the part of a seed, consisting of one or more cotyledons and precursor tissues for the leaves, stems, and roots.

ex situ—Offsite, away from the wild population, usually referring to collection held in nursery or botanic garden.

exceptional species—Non-orthodox species that cannot be conserved long-term using conventional seed banking methods. This includes species with few or no seeds available for banking, species with seeds that are intolerant of desiccation and freezing, or seeds that can tolerate drying, but not freezing, or species that may only tolerate storage at –20°C for less than 10 years.

explant—Tissue transferred to or from a tissue culture medium.

extirpation—Local extinction, where a species ceases to exist in a chosen geographic area, while it still exists elsewhere.

F1 generation—First generation.

F2 generation—Second generation.

fecundity—The number of seeds or asexual propagules produced by an individual plant or population.

field genebank—Plants grown in the ground for the purpose of conservating genes. In botanical gardens, display collections of trees, shrubs, herbs can be considered field genebanks.

flow cytometry—A laser or impedence-based technology employed to count or sort cells. This can be used to analyze the ploidy levels or numbers of chromosome sets of individual plants in a population.

foil envelopes—Protective packaging that maintains stable moisture levels within the package and is recommended for long-term seed storage.

founder effect—The genetic composition of the individuals that create a new population.

founder(s)—The individual(s) that starts a new population.

gene—A specific DNA sequence that is transferred from a parent to offspring that may determine some characteristic of the offspring.

gene flow—, or migration—Any movement of individuals, and/or the genes from one population to another. It can be described as limited, meaning that the individuals living near one another are closely related (for example, monkshood), or extensive, meaning that it is possible to find traces of genes in an individual that lives very far away (for example, wind-pollinated plants).

genetic diversity—Variation in the DNA sequence between distinct individuals of a given species (or population). The degree of genetic diversity can be compared between individuals or populations. Capturing the breadth of existing genetic diversity is the goal of a conservation collection.

genetic drift—Variation in the relative frequency of different genotypes in a small population, owing to the chance disappearance of particular genes as individuals die or do not reproduce.

genetic erosion—Loss of genetic diversity. For an endangered species with a limited gene pool loss of individuals equates to loss of genes and alleles represented for the species.

genetic rescue—Increasing genetic diversity from infusing new genes into a population. If population is inbred, this practice may increase chances that some plants will survive.

genetic structure—A measure of the differences across and within populations of a species. Populations of a species may have similar or different genetic composition. Measures of this pattern have conservation implications for providing source material for a conservation translocation.

genome—A complete set of genes or genetic material present in a cell or organism.

genomic studies—evaluations of the specific arrangement of DNA on the chromosome.

genotype—The genetic constitution of an individual (genotyping is determining the genetic constitution of an individual).

georeferenced—Points taken with GPS that can be used to locate a plant again. Herbarium specimens and seed collections when georeferenced can contribute greatly to our understanding of how plant populations may change in the future.

germination—When a radical emerges from a seed. Percent germination is the percentage of seeds in a test sample that germinate in a given time. (Seeds germinated by day x/Total number of seeds tested) X 100 = % germination).

germplasm—Living seeds or tissues from which plants can be grown.

hand-pollination (hand pollinated) —A process where pollen is manually and deliberately transferred to the receptive portion of a flower (the stigma). This technique is often used to control the parentage and to maximize pollen transfer in hopes of achieving fertilization and good seed set.

herbarium vouchers—A specimen of a pressed plant that is deposited and catalogued for future reference.

hybridization—Cross species fertilization. In the context of conservation, it is often undesirable to have hybridization occur in a cultivated or wild setting, as the more common parent will likely swamp the genepool of the rare species.

immature seeds—Seeds with underdeveloped embryos. To determine whether a seed is fully developed may require cutting it open and looking under a microscope.

immigrate—To move into an area. In a context of conservation collection health, it is sometimes necessary to allow genes or seeds from a wild population to move into an ex situ population.

in situ—In wild habitat.

in vitro—Micropropagation; plant tissues grown on nutritional medium in a sterile humid glass or plastic container.

inbreeding—Mating between closely related individuals. Over many generations, genetic disorders may arise.

inbreeding depression—Reduced fitness of progeny resulting from breeding of related individuals.

intact embryos—A complete, undamaged embryo.

inter situ—In between ex situ (offsite and fending for itself) and in situ (in wild habitat), the inter situ conservation collection has diverse genetic representation and supplemental care. The term was first used to refer to the restoration of declining species in areas that are outside their current range but within historical ranges, inferred from paleoecological studies, but is now used to describe a semi-wild setting for ensuring species' survival.

intermediate (seed) —Share functional characteristics with recalcitrant seeds and should be stored in liquid nitrogen. When dried to 50%–75% RH, they have a longer shelf-life than storage at 15%– –35% RH levels recommended for orthodox seeds. Seeds age faster when stored at conventional freezer temperatures compared to refrigerated temperatures. Faster aging might be detected within days, months or years, making it difficult to identify which species' seeds are intermediate. Longevity of seeds increases with drying and cooling (as with orthodox seeds), but seeds still age rapidly during conventional storage and will die within about 5 years.

internal seed moisture—Water content of a seed. This can be measured using a process that will destroy the seed: weighing a seed, drying it until there is no change in its mass, and calculating water content mathematically. Obtaining individual seed moisture content is recommended. For very small seeds, this requires a very precise balance.

loci—The location of a particular gene on a chromosome.

long-term storage—At least 5 years. Note that the length of time a species' seed can stay alive in dry, cold storage will vary across species.

long-term storage packages—Foil package containing CPC seed collections with maternal lines separated for a single accession (collection of seeds from a single wild population on a single date) that is intended to stay in storage for at least 5 years.

longevity—How long a seed remains viable.

maternal lines—The offspring (seeds or plants) from a single mother plant are distinguished with unique identifying number, stored in a separate package, and labeled if grown in a nursery. Knowing the number of maternal lines in a conservation collection is an estimate of the genetic diversity represented.

maternal plants—Individual plants producing seeds. Keeping track of seeds from each maternal plant allows for estimates of genetic diversity in a collection and allows for maintaining even representation of maternal lines while growing the accession in the garden's nursery or reintroducing to the wild.

Material Transfer Agreement—The agreement that CPC has with the Resources Preservation Unit - Seeds of the National Laboratory for Genetic Resources Preservation, Fort Collins, Colorado, to store national collection rare plant seed accessions in long-term black-box storage.

Material Transfer Research Agreement—The agreement that CPC has with the Plant Germplasm Preservation Research Unit of the National Laboratory for Genetic Resources Preservation, Fort Collins, Colorado, to conduct research on and store national collection rare plant seed accessions long-term.

mating system—The way that plants produce seeds. Some plants can produce seeds in multiple ways, while others are restricted to a single mating system. The types of mating system include outcrossing or cross-pollination (a flower receives pollen from another plant of the same species), autogamy or self-fertilization (a flower receives pollen from the same plant) and apomixis (asexual reproduction without fertilization that is only possible with evolution of a modified flower). Mixed mating systems, in which plants use two or even all three mating systems, are not uncommon.

meristem—Growing tip of plant.

metapopulation—A group of spatially separated populations of the same species that interact (for example, the interaction can be pollen or seeds moving between plant populations).

metapopulation dynamics—A change across time within a group of separated but interacting populations that influence the overall persistence of a species.

microcatchments—Small, localized wells or depressions around a plant that serve to gather and hold water. These are recommended for plants or seeds installed in dry habitats.

microhabitat(s) —Very localized abiotic (soil, light, and moisture) and biotic (associated plants, insects, and other animals). The nature of a microhabitat may greatly influence seedling and adult plant growth and survival. See also microsite(s).

micropropagation—In vitro; plant tissues grown on nutritional medium in a sterile humid glass or plastic container.

microsatellite markers—Genetic markers consisting of a series of short repeating base pairs of DNA that are variable within populations and can be used to identify individuals or species, or evaluate structure and gene flow between populations.

microsite(s) —Very localized abiotic (soil, light, and moisture) and biotic (associated plants, insects, and other animals). The nature of a microhabitat may greatly influence seedling and adult plant growth and survival. See also microhabitat(s).

mitigation—In the context of CPC protocols, mitigation is a legal term for an action that is taken to offset the adverse impacts of development on U.S. listed species. For example, a parcel land where a species occurs may be preserved as mitigation for developing a portion of the species' habitat.

moisture content—The percentage of water in seed. %Moisture content = ((Weight of fresh sample – Weight of dry sample)/ weight of fresh sample) x 100. The target moisture for storing seeds equals no more than 25% RH (lower risk of failure) and no less than 10% RH (higher risk of failure) at the intended storage temperature.

molecular (studies) —Genetic studies.

monoecy—Having flowers with only one sex (male or female) or flowers of both sexes carried on a single plant.

morphological—Pertaining to the form or structure.

mutualists—two organisms that exist in a relationship in which both benefit.

mycorrhizal inoculum—A fungi that forms a symbiotic relationship with a plant's roots and increases water and nutrient absorption.

natural selection—The process whereby organisms better adapted to their environment tend to survive and produce more offspring than those that are not well adapted.

neighborhood size—The local area within which most matings occur.

niche space—The ecological space occupied by a species (Hutchinson definition); an ecological role (Grinnel definition); a species' response to and effect on environment (Elton definition).

nodes—Intersection along a stem holding one or more leaves, as well as buds which can grow into branches which in turn may produce leaves, cones, or flowers.

non-orthodox species—See exceptional species.

obligate mutualists—Organisms that are required to rely on one another for survival.

obligate outcrosser—Pollen from a different plant (not self) is required for successful seed set.

open pollination (open pollinated)—Allowing flowers to be pollinated naturally by wind, insects, or birds. In studies, open pollination is used as a comparison to hand pollinated trials to determine the highest seed set that could be expected in a flower.

orthodox seed—Seeds that survive with very little water in their cells and also survive prolonged storage at –20°C. Orthodox seeds survive drying at 15% relative humidity which translates to water contents less than 0.08 g H_2O/g total mass (<8%).

outbreeding—A condition where flowers of one plant receives pollen from another plant of the same species.

outbreeding depression—Low fitness of progeny resulting from mating between two genetically distant (and usually physically distant) plants.

outcrossing—The form of plant reproduction that requires pollen from another plant of the same species to form seeds.

outplanting—Transplanting from a botanical garden nursery (ex situ setting) to a wild setting for purposes of reducing the extinction risk of a species and allowing persistence in a natural setting.

patch dynamics—Spatial and temporal changes within and among patches of vegetated or bare spaces that make up a landscape.

perennial—A plant that lives for many years.

phenological—Pertaining to phenology. With changing climate, plants growing in certain locations have changes in the phenology.

phenology—The timing of key life history events in a plant's life, such as flowering or fruiting.

phenotype—The measurable appearance of a trait.

plantlet—Young or small plant.

plasticization— The process of changing structure to make less rigid and crystallized to a form that is pliable and flexible.

ploidy—The number of sets of chromosomes in a cell.

ploidy analysis—Examination of the number of chromosomes in an individual plant or across plants in a population and would be performed if differences in ploidy are expected.

ploidy level—A description of the number of chromosomes in an individual, population or species. For example, diploid organisms have pairs of chromosomes. In plants, multiple sets of chromosomes are possible, even within a single population, especially as a result of hybridization.

pollination mechanism—The method in which pollen is transferred from anthers to the stigma of same or different flowers. Wind, insects, or birds are the most common pollinating mechanisms.

polyploidy variation—Differences in the number of sets of chromosomes present in a species or population.

population—A group of potentially interbreeding individuals that share a common ancestry or gene pool.

population dynamics—The study of changes in size and age structures of populations over time.

population persistence—A measure of effective conservation is to have a population grow and reproduce sustainably over time in a wild setting.

population reproductive output—Total seeds produced in a population within one growing season.

population size—The number of individual plants of all ages in the population.

primary facility—The seed bank or botanical garden where the accession is stored. A duplicate of the same accession is stored at a backup facility.

propagule— A general term to describe any plant material that can function to propagate a new plant, including seeds, stems, corms, tubers, or spores.

provenance—The place of origin.

randomly chosen—A formal process for selecting an unbiased sample.

recalcitrant seed—Seed incapable of conventional storage due to desiccation intolerance and/or freezing intolerance.

reciprocal transplant—An experimental method which involves introducing plants from each of two or more environments into the other(s). The method can be used to test whether differences in appearance, for example, between populations have a genetic versus environmental basis.

recovery staff—Personnel of U.S. Fish and Wildlife Service working toward ending extinction of federally listed species.

recovery team—Experts from U.S. Fish and Wildlife Service, academia, and conservation organizations who voluntarily review a species conservation status and make recommendations for its recovery.

reference populations—The population used as a basis for comparison. In the context of reintroduction, the growth and survival of plants in the wild population can be compared to the reintroduced population as a measure of success.

reintroduction(s)—intentional movement of species into habitat it previously occupied.

reintroduction science—Growing body of experimental evidence related to enhancing a rare species survival by human action to increase the number of individuals or populations in a natural setting.

representative genetic diversity—Best captured by making seed collections across the spatial extent of the population, from plants that are not physically close to one another, and from plants of all sizes and levels of seed output.

rescue collections—When an entire population is threatened, it may need to be removed from its location and brought to an ex situ or offsite facility for care.

safety duplication—A half of a collection that may be stored in a second location as a precaution against losing the accession due to natural catastrophe.

sample—A portion of the population that is collected at one time. CPC recommends collecting a sample of no more than 10% of the seeds produced within a population in a growing season.

sampling—Strategy to use to collect the representative genetic diversity in a population.

seed water content—Water in seeds that is measured from the fresh and dry mass of the seed, fresh mass being at ambient conditions and dry mass being measured after placing the seed in a drying oven at 95 to 100°C. Water content is calculated by the ratio of the fresh minus dry mass to either the fresh (i.e., total mass) or dry mass. Different labs may prefer expressing water content on a total or dry mass basis, and it's a minor difference when water content is less than 0.15 g/g (15%). Putting seeds at 95-100°C is lethal and so measuring water content is a destructive test.

self-compatibility—A condition where a flower of an individual can receive pollen from itself and set good seeds.

self-fertilizing—Pollen from one plant fertilizes a flower of the same plant such that good, viable seed results. This technique is often used experimentally to determine whether this is possible for a species.

self-incompatibility—A condition in flowering plants that prevents self-fertilization and thus encourages seeds to be set when pollen from a different unrelated individual fertilizes the eggs of the mother plant. One of the mechanisms that causes this is a special allele (SI) that prevents pollen from germination on the receptive surface of the flower called the stigma.

selfing—Pollen from one flower fertilizes the same flower and successfully sets seed.

sensitive animal species—Those with protected legal status by state or federal agency.

Sentinel Plant Network—A collaboration between the National Plant Diagnostic Network and the American Public Gardens Association to improve the ability to detect and respond quickly to serious plant pests and diseases.

sequencing—Refers to genetic procedure to determine the composition and order of genes of an individual as in genotyping.

silica gel—A granular, vitreous, porous form of silicon dioxide made synthetically from sodium silicate that can be used to dry seeds.

specialist pollinators—Organism adapted in structure and behavior to gather and transfer pollen to flowers of one or a few related species of plant.

species conservation status— the designated level of endangerment of a species as determined by the U.S. Fish and Wildlife Service, NatureServe, IUCN or state governmental agency.

stochastic event—Unpredictable or chance event.

succession— the process of change of an ecological community in the composition and structure of species over time. The time scale can be decades (for example, after a wildfire), or even millions of years after a mass extinction.

symbiont—An organism living in close association with another.

target storage RH—The relative humidity sought to maximize seed longevity while seeds are held in storage at a particular temperature.

taxon—A taxonomic group of any rank, such as a species, family, or class. Sometimes this term is used rather than species, because it will encompass varieties and subspecies.

taxonomic ambiguity—Unknown or undetermined genus and species designation of an organism.

Thumb Rules or Hundred Rule—Developed by J. F. Harrington (1916–2002) at UC Davis in the 1950s to guide storage conditions for maintaining seed viability, state: "Seed lifespans double for every 1% decrease in water content or 10°F decrease in temperature." According to Harrington's "Hundred Rule," seed viability can be maintained [for 5 to 10 years] if the sum of the relative humidity and temperature (in °F) is less than 100.

tissue culture—Growing cells in an artificial medium. In plant conservation, the cells may be whole seeds, spores, or meristems and the medium is usually some derivation of agar.

transects—A line of known length used as a way to monitor vegetation.

USFWS—U.S. Fish and Wildlife Service.

viability—Ability to live and survive successfully.

viability testing—Systematically checking and counting the number of live seeds in a sample.

vital rates—Measure of change that influence population growth (for example, birth rate, survival of an age class from one year to the next, death rate).

vitrification—To make liquids solid without forming crystals, a "glassy state." Forming ice crystals inside plant cells can be deadly. When a solid state can be achieved without ice, vitrification is the term used to describe ice free cryopreservation.

voucher specimen—A pressed plant sample deposited in an herbarium for future reference.

water vapor transmission rate—The amount of water lost or gained across a storage container membrane in a given period of time.

Weed Risk Assessments—A science-based evaluation of the potential of a plant species to establish, spread, and cause harm in a region. Several weed risk assessments exist for different regions of the U.S. (for example, Hawaii; https://sites.google.com/site/weedriskassessment/home, California: http://www.cal-ipc.org/solutions/research/riskassessment/, and Florida: http://edis.ifas.ufl.edu/ag376).

wild population—The plant population that exists in a natural setting. Note that the wild or natural setting may not be pristine.

wild species—a species that is usually found growing in the wild without human intervention (i.e., in situ). We might also say they are from 'natural populations.' Usually we distinguish wild and domesticated species, the latter has been changed by humans usually for the purposes of cultivation. Arboreta and botanical gardens often grow or store individuals collected in the wild. We would call this "ex situ."

Supplementary Materials

CPC Best Practices Supplementary Materials and Forms

1 Example Monitoring Form

Monitoring Form 2018: San Diego Zoo Native Plant Seed Bank

Basic Information			
Occurrence Accession Code:		**Date of Monitoring:**	/ /
Observers Present:	☐ Stacy Anderson ☐ Joe Davitt ☐ Tobin Weatherson ☐ _____, _____, _____, _____		
Population Characteristics ***(update every visit)***			
Current Population Size:		☐ Estimate ☐ Exact	**Population Notes:**
% Population Vegetative	☐ 0% ☐ 1-24% ☐ 25-49% ☐ 50-74% ☐ 75-100%		
% Population Flowering	☐ 0% ☐ 1-24% ☐ 25-49% ☐ 50-74% ☐ 75-100%		
% Population Fruiting	☐ 0% ☐ 1-24% ☐ 25-49% ☐ 50-74% ☐ 75-100%		
% Population Senescent	☐ 0% ☐ 1-24% ☐ 25-49% ☐ 50-74% ☐ 75-100%		
Threats ***(update once per growing season)***			
Site/Pop. Quality	☐ Excellent ☐ Good ☐ Fair ☐ Poor		
Visible Disturbances			
Threats Present:	☐ Invasive Grasses ☐ Invasive Forbs ☐ Trails/Recreation ☐ Herbivory ☐ Construction ☐ Erosion ☐ Climate (Drought, Heat, Flood) ☐ _____		
Invasive Plant Cover:	☐ 0% ☐ 1-24% ☐ 25-49% ☐ 50-74% ☐ 75-100%		
Notes about Threats		**Predominant Invasive Species**	

Associated Species ***(update once per growing season)***

Acmispon glaber	*Bromus diandrus*	*Deinandra fasciculata*	*Galium angustifolium*	*Rhamnus crocea*
Adenostoma fasciculatum	*Bromus hordeaceus*	*Dichelostemma capitatum*	*Hedypnois cretica*	*Rhus integrifolia*
Apiastrum angustifolium	*Bromus madritensis*	*Eriogonum fasciculatum*	*Hesperoyucca whipllei*	*Salvia apiana*
Artemisia californica	*Bromus rubens*	*Eriophyllum confertiflorum*	*Heteromeles arbutifolia*	*Salvia mellifera*
Avena barbata	*Calochortus splendens*	*Erodium botrys*	*Hirschfeldia incana*	*Selaginella cineracens*
Avena spp.	*Calystegia macrostegia*	*Erodium cicutarium*	*Hypochaeris glabra*	*Sisyrinchium bellum*
Baccharis sarathroides	*Centaurea melitensis*	*Erodium spp.*	*Isocoma menziesii*	*Sonchus asper*
Bloomeria clevelandii	*Chlorogalum parviflorum*	*Festuca myuros*	*Logfia gallica*	*Sonchus oleraceus*
Brachypodium distachyon	*Convolvulus simulans*	*Festuca perennis*	*Lysimachia arvensis*	*Stipa pulchra*
Brassica nigra	*Crassula connata*	*Plantago erecta*	*Malosma laurina*	*Xylococcus bicolor*

_____, _____, _____

Tasks Completed on This Date			
Occurrence Established:	☐ Yes ☐ No	Population Polygon:	☐ Yes ☐ No
Herbarium Voucher:	☐ Yes ☐ No	Soil Sample:	☐ Yes ☐ No
Seed Collections:	☐ Yes ☐ No	Genetic Sample	☐ Yes ☐ No

2 CPC Participating Institution Agreements

for Seed Banking and Information Sharing at the National Laboratory for Genetic Resources Preservation (NLGRP)

One of the benefits CPC provides to its Participating Institutions is an opportunity to store seeds within the network or with our partners at USDA-ARS National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado, or the Millennium Seed Bank.

Note that the NLGRP has two different agreements covering CPC seed storage. A summary of each agreement is given below.

The Material Transfer Agreement (MTA) each CPC PI individually signed with NLGRP (so-called "black-box") places the responsibility on the CPC PI to distribute seeds to any bona fide research request. Seeds collected on National Park Service lands cannot be stored using the MTA with NLGRP, because the NPS distribution policy is inconsistent with the USDA legal requirement for distribution. For distributions outside the US, the primary distribution site is responsible for obtaining phytosanitary certificates and any other necessary permits (for example, CITES, USFWS). Questions regarding this agreement should be addressed to Stephanie Greene, USDA-ARS, NLGRP, Stephanie.greene@ars.usda.gov

The Material Transfer Research Agreement (MTRA) between CPC and NLGRP covers Participating Institution seed collections that contribute to research on seed storage behavior and longevity studies. Note that the curation package will be used to gather information about seed characteristics, germination, and storage requirements (see Part 1C, "CPC Best Practices for Splitting Samples for Safety Duplication Storage and Testing.") This agreement has no distribution requirement. Seeds collected on National Park Service lands can be stored for research purposes under this agreement. The CPC highly recommends institutions store their seeds with the research side of NLGRP, so that the lab can perform research and collection monitoring. (See Material Transfer Research Agreement NLGRP_CPC) Questions regarding this agreement should be addressed to Christina Walters, USDA-ARS, NLGRP, Christina.Walters@ars.usda.gov

NLGRP, CPC seed bank affiliates, and PIs agree to share information gleaned from seed banking with the network upon request.

For easy data transfer, use the online form (http://saveplants.org/login/pi-portal/).

Germination protocols, viability of seeds in storage over varying lengths of time, and seed characteristics are important details to share with conservation partners. Because there is still much to learn about storing seeds of rare species, we encourage you to publish your findings.

3 CPC Field Collection Form

CENTER FOR PLANT CONSERVATION

General Information

1. Completed by:_____ Date:_____
2. Taxon name (scientific): _____
3. Institution: _____
4. Your institution's accession number:_____
5. Herbarium specimen number:_____
6. Collected by: _____ Date of collection: _____

Collection Location (Fill out as many as possible; items 7 and 8 are essential. If you do not have information for item 8, fill out item 12 at a minimum.)

7. State:_____ County:_____
8. Latitude: _____ Longitude: _____
9. Township:_____ Range:_____ Section:_____
10. Elevation:_____
11. USGS Topo Map:_____
12. Land owner name and address:_____

13. Descriptive location _____
_____

Habitat Information

14. Light (circle one)	Open	¼ shade	½ shade	¾ shade	full
15. Slope (circle one)	0–5	6–10	11–40	41–60	>60
16. Exposure	North	South	East	West	
17. Litter	0 cm	<1 cm	1–3 cm	3–8 cm	>8cm

18. Plant community_____
19. Associated species_____
_____

Soil Characteristics

20. Parent Rock
21. Soil test results

P	K	Ca	Mg	CEC	Organic matter	Soil Ph	Soil type

CENTER FOR PLANT CONSERVATION

Population Information

21. Number of plants 1–10 11–30 31–100 101–500 >500

22. Area covered length (m)
width (m)

23. Avg. no. of plants/sq m_____
24. Max. no. of plants/sq m_____

26. No. or % reproductive_____
27. No. or % nonreproductives_____
28. No. or % seedlings_____
29. % reproductives In bud In flower In fruit

30. Threats to this population _____

4 Maternal Line Count

Species Collected

Maternal Line	Eriastrum harwoodii		Acc # 24094		Lot # 5414	Dissection Test Results: 5 of 5 seeds filled with normal, viable emrbryos			
	Total		Active		Base		Backup		100 weight
Number	Quantity	Weight (g)	Quantity	Weight (g)	Quantity	Weight (g)	Quantity	Weight (g	Weight (g)
1	107	**0.048**	21	0.010	44	0.020	42	0.018	
2	227	**0.084**	43	0.016	95	0.035	89	0.033	0.037
3	209	**0.067**	41	0.013	88	0.028	81	0.026	0.032
4	372	**0.160**	74	0.032	149	0.064	149	0.064	0.043
5	88	**0.039**	18	0.008	36	0.016	34	0.015	
6	123	**0.046**	25	0.010	49	0.018	49	0.018	
7	63	**0.026**	14	0.006	24	0.011	25	0.009	
8	156	**0.064**	29	0.012	66	0.027	61	0.025	0.041
9	248	**0.082**	48	0.016	100	0.033	100	0.033	0.033
10	176	**0.060**	35	0.012	71	0.024	71	0.024	0.034
11	115	**0.046**	23	0.010	46	0.018	46	0.018	
12	163	**0.065**	33	0.013	65	0.026	65	0.026	0.040
13	135	**0.046**	26	0.009	56	0.019	53	0.018	0.034
14	173	**0.071**	34	0.014	71	0.029	68	0.028	0.041
15	50	**0.016**	10	0.003	20	0.006	20	0.007	
16	264	**0.087**	52	0.017	106	0.035	106	0.035	0.033
17	86	**0.036**	18	0.007	34	0.015	34	0.014	
18	95	**0.037**	19	0.007	38	0.015	38	0.015	
19	108	**0.038**	22	0.008	43	0.015	43	0.015	
20	89	**0.038**	18	0.008	36	0.015	35	0.015	
21	240	**0.095**	48	0.019	96	0.038	96	0.038	0.039
22	74	**0.012**	14	0.001	30	0.005	30	0.006	0.031
23	136	**0.041**	27	0.008	54	0.017	55	0.016	0.033
24	180	**0.059**	36	0.009	72	0.025	72	0.025	0.040
25	360	**0.150**	72	0.031	144	0.060	144	0.059	0.038
26	116	**0.044**	22	0.009	47	0.017	47	0.018	
27	92	**0.032**	18	0.006	37	0.013	37	0.013	
28	68	**0.024**	14	0.006	27	0.009	27	0.009	
29	136	**0.047**	27	0.009	54	0.019	55	0.019	
30	125	**0.051**	25	0.010	50	0.020	50	0.021	
31	109	**0.035**	21	0.007	44	0.014	44	0.014	
32	135	**0.051**	27	0.009	54	0.022	54	0.020	
33	85	**0.027**	17	0.006	34	0.010	34	0.011	
34	58	**0.017**	12	0.004	23	0.006	23	0.007	
35	50	**0.023**	10	0.005	20	0.009	20	0.009	
36	124	**0.047**	25	0.008	49	0.019	50	0.020	
37	118	**0.043**	23	0.008	47	0.018	48	0.017	
38	48	**0.017**	9	0.003	20	0.007	19	0.007	
39	162	**0.058**	32	0.011	65	0.024	65	0.023	
40	64	**0.022**	14	0.003	25	0.009	25	0.010	
41	150	**0.049**	30	0.010	60	0.020	60	0.019	
42	83	**0.032**	17	0.006	33	0.013	33	0.013	
43	91	**0.035**	18	0.007	36	0.014	37	0.014	
44	99	**0.032**	20	0.007	39	0.013	40	0.012	
45	80	**0.023**	16	0.005	32	0.009	32	0.009	
46	107	**0.034**	21	0.006	43	0.014	43	0.014	
47	95	**0.042**	19	0.007	38	0.017	38	0.018	
48	57	**0.018**	11	0.003	23	0.008	23	0.007	
49	442	**0.171**	89	0.033	176	0.069	177	0.069	

50	143	**0.049**	29	0.009	57	0.019	57	0.021	0.037
51	75	**0.021**	15	0.004	30	0.008	30	0.009	
52	72	**0.027**	15	0.006	30	0.011	27	0.010	
53	60	**0.021**	12	0.003	24	0.009	24	0.009	
54	168	**0.052**	32	0.010	68	0.021	68	0.021	0.031
55	100	**0.034**	24	0.008	47	0.016	29	0.010	0.034
56	88	**0.035**	18	0.007	36	0.014	34	0.014	
57	98	**0.042**	21	0.009	44	0.019	33	0.014	0.043
58	66	**0.005**	13	0.001	26	0.002	27	0.002	
59	366	**0.139**	76	0.029	153	0.058	137	0.052	0.038
60	272	**0.106**	200	0.078	38	0.015	33	0.013	0.039
61	28	**0.005**	6	0.001	11	0.002	11	0.002	
62	246	**0.086**	49	0.017	97	0.034	100	0.035	0.035
63	283	**0.051**	56	0.010	117	0.021	111	0.020	0.018
64	81	**0.023**	27	0.005	31	0.010	23	0.008	
65	75	**0.028**	15	0.005	30	0.012	30	0.011	
66	60	**0.024**	12	0.006	24	0.008	24	0.010	
67	142	**0.051**	31	0.011	61	0.022	50	0.018	0.036
68	103	**0.035**	21	0.007	41	0.014	41	0.014	0.034
69	153	**0.055**	31	0.011	61	0.022	61	0.022	0.036
70	126	**0.049**	23	0.009	49	0.019	54	0.021	0.039
71	68	**0.022**	14	0.003	27	0.009	27	0.010	
72	176	**0.060**	35	0.012	71	0.024	71	0.024	0.034
73	108	**0.039**	25	0.009	50	0.018	33	0.012	0.036
74	60	**0.022**	12	0.004	24	0.009	24	0.009	
75	28	**0.003**	6	0.001	11	0.001	11	0.001	
76	521	**0.219**	107	0.045	212	0.089	202	0.085	0.042
77	70	**0.026**	14	0.005	28	0.010	28	0.011	
78	43	**0.003**	9	0.001	17	0.001	17	0.001	
79	252	**0.068**	48	0.013	96	0.026	107	0.029	0.027
80	112	**0.048**	21	0.009	44	0.019	47	0.020	0.043
81	95	**0.036**	19	0.007	38	0.014	38	0.015	
tlt	**11069**	**3.971**	**2372**	**0.842**	**4400**	**1.583**	**4296**	**1.546**	**0.036**

Column Descriptors:

Accession: Number will be assigned by RSBG; An accession is defined as a collection of one plant population at one location and time period.

The collection may take place over several days. Multiple maternal lines (30-50 individuals) should be targeted for each accession.

Lot numbers: Number will be assigned by RSBG

Total: Total number of seeds in the collection of one species at one site

Active: 20% of the total number of seeds should be designated for the 'active' lot. These seeds are used for testing and distribution

Base: 40% of the total collected seeds should be designated for the 'base' lot.

Backup: 40% of the total collected seeds should be designated for the 'backup' lot.

100 weight: Weight of 100 seeds. For large maternal lines, this can be used to extrapolate total seed quanity by wieght.

Use formula =(weight * 100)/(100 weight)

5 Guidelines for Tissue Collection and Storage Related to Genetic Studies

The collection and preservation of plant tissues in DNA banks can serve as a long-term repository for the genetic material of a species, facilitating a range of genetic studies (phylogenetic, population genetic, and conservation genetic research) into the future. To maintain the integrity of a DNA collection, proper long-term storage is required to prevent degradation by nucleases, oxidation, hydrolysis, and ionizing radiation1,2. The quality of plant tissues or DNA extracts may be best maintained through cryopreservation, where materials are stored in the vapor phase of liquid nitrogen (below –180°C), a temperature at which molecular motion significantly slows. Because liquid nitrogen is cold in and of itself, mechanical failures and electrical outages are less of an issue with liquid nitrogen vats than ultracold freezers3. However, cryopreservation may not be financially or logistically feasible for many institutions.

As an alternative to cryopreservation, institutions may opt to maintain tissue collections in a dried or frozen (–20°C or –80°C) state. Silica gel is a commonly used desiccant that can be used to quickly and easily dry plant material for the purpose of extracting DNA6. If silica gel is used, plant tissues should be dried down as quickly as possible to mitigate DNA degradation. While desiccated tissues can be maintained at room temperature with silica in air-tight containers, at least for short periods5, Hodkinson et al. (2007) recommend maintaining dried plant materials in a –20°C freezer, a practice followed by the Missouri Botanic Garden7. The Smithsonian Institution, on the other hand, stores dried tissues at –80°C. Studies assessing the long-term effects of storing desiccated tissues at various temperatures are lacking, although "the colder the better" is generally the recommendation for fresh tissues and DNA extracts. DNA quality is further maintained by avoiding long-term storage of tissues in newspapers or other acidic materials, in humid conditions, and in contact with light, all of which may degrade DNA5. Storing tissues in air-tight containers is particularly important, as storage in containers with poor seals can reduce the quality of DNA5.

Maintaining fresh plant material in freezers is another viable method for the long-term preservation of tissues. Frost-free freezers that cycle through temperatures should be avoided, as temperature fluctuations can lead to DNA damage2 or protein deterioration8. Many plant tissues should be stable in long-term storage at -70°C to -80°C for the extraction of DNA, RNA, and proteins, while degradation may occur at -20°C9. However, Neubig et al. (2014) found that plant material frozen for 24 years maintained high quality regardless of whether tissues were stored at -20°C or -80°C in a pulverized or intact state5. Storage at -80°C may be the prudent choice to increase molecular integrity of the samples. Because -80°C freezers are prone to mechanical or electrical failures3, appropriate alarm systems and back-up storage space should be in place.

If time, space, and resources allow, lab facilities may choose to store DNA extracts in addition to tissue specimens5, as DNA samples are more stable than tissues9. DNA

extracts are often stored in TE (Tris EDTA)2,9 buffer which prevents contamination by bacteria and degradation via nucleases10. To reduce the number of freeze-thaw cycles and thus a deterioration of DNA quality, frequently accessed samples can be stored in aliquots at -20°C, while archival extracts can be preserved at -80°C1,2,11. In a study investigating optimal storage of orchid leaf samples originally dried with silica, DNA extracts of the tissue stored at -20°C for 7 – 12 years had slightly higher quality than DNA newly extracted from the tissue that was stored in desiccated form in the dark without silica for the same period of time5. DNA extracts may be suitable for PCR for 4 – 7 years when stored at -18°C and for over 4 years when stored at -80°C1. After evaluating various storage buffers and temperatures, Smith and Morin (2005) found that the highest DNA yields were achieved with storage at -80°C or dried at room temperature with trehalose12. The highest DNA quality, on the other hand, was achieved with trehalose either dried or at -80°C, while quality deteriorated with storage at 20°C and 4°C. As is the case with plant tissues, storage at liquid nitrogen temperatures is optimal for preserving the highest quality extracts2. While, DNA extracts may better preserve the integrity of DNA, it is still worthwhile to maintain tissues in cold storage which will allow a wider variety of downstream molecular studies. Most DNA banks store tissues (or cells) and only extract DNA as requested1.

RNA-Seq is a burgeoning field in genomics that allows one to examine the gene-coding portions of genomes and to assess differential expression of genes among treatment groups of interest. Thus, labs may elect to store tissues for RNA in addition to DNA extractions. Tissues are typically flash frozen in liquid nitrogen and either stored at -80°C or at liquid nitrogen temperatures.

There are a multitude of nucleic acid storage methods, many of which are beyond the scope of this document. The long-term preservation of plant tissue samples via desiccation by silica gel and storing flash frozen tissue at -80°C are two methods that are commonly employed. The following recommendations for preserving plant material are based largely on guidelines from the Smithsonian Institution13 and the Global Genome Initiative14, which provide an excellent workflow for the collection and storage of plant tissues intended for DNA or RNA extractions. These guidelines include preserving silica-dried and flash-frozen plant tissues in long-term storage at -80°C. I summarize and expand on these guidelines below and refer the reader to these sources for further reference.

Tissue Desiccation and Storage

Working with Silica Gel Desiccant

While there are many potential reagents for desiccating plant tissues 4, silica gel is a commonly used desiccant that can easily and rapidly dry down plant tissues for the purpose of DNA extractions4,6. Silica gel can act as a skin, eye, and respiratory irritant, and thus appropriate personal protective equipment (gloves, facemask, and goggles) should be worn when working with silica (consult the safety data sheets for your particular product). Silica gel is available in various mesh sizes, where finer

grades (28 – 200 mesh size) can more quickly dry down tissues due to the larger surface area to volume ratios. However, larger grades (2 – 4 mm bead size) should be sufficient for drying, and are less expensive and less likely to be inhaled than smaller mesh13.

Indicating silica gel changes color when saturated and can thus be used to signal when a change of silica gel is required. A ratio of 1:10 indicating:non-indicating (white) silica gel is suggested13. Three types of indicating silica gel are available (colors change from orange to clear/white, orange to green, or blue to pink when saturated). The orange to clear/white indicating beads are recommended, as they are the least toxic13. Once saturated, the silica gel can be dried for reuse by heating in a plant dryer. Follow the manufacturer's instructions for re-drying the beads.

Collecting, Desiccating, and Storing Plant Tissue

What to collect. Young, actively growing leaf tissue is best for DNA extractions9, although extremely young tissue that is fragile should be avoided13. Young tissue is preferable to older tissue because it has a more cells per volume13, is less likely to be colonized by fungal endophytes13, and is less likely to have secondary compounds that could interfere with DNA extractions and inhibit downstream PCR reactions. It is also possible to extract DNA from young shoots, roots, seeds, pollen, or gametophytes9.

How much tissue to collect. From 15 spatially distant individuals (see section on Sample Size), collect 3 – 5 leaves per plant7 (or 10 – 25 cm2 of leaf material from species with large leaves13), provided it is not detrimental to the plant. Because leaf thickness can vary by species, another useful guideline may be to collect at least 100 mg fresh tissue, if not harmful to the plant. DNA extractions generally require 50 mg to less than 100 mg wet tissue per sample (or less than 20 mg dry tissue)15, and it is ideal to have extra material with which to work should optimization of the extraction protocol be required. Thus, 100 mg wet tissue may provide enough material for two extractions. This amount can be adjusted depending on the starting tissue amount recommended by the DNA extraction protocol in use.

How to collect tissues. To avoid DNA degradation, it is advisable to dry down tissues as quickly as possible, preferably with 12 – 48 hours13, although within 12 hours is most ideal6. This can be achieved by storing samples with silica, in a ratio of at least 10:1 of silica gel to total leaf tissue5,6, although more silica may be required for species with thick leaves or high water content. Drying should occur in a cool location at ambient temperature13. Leaf tissue can be placed in separate coin envelopes (2 ¼ x 3 ½ inches) by sample13, properly labeled with sample name, date, species, collection location, and collector. White coin envelopes are recommended, which tend to be less acidic (and thus less degradative to DNA) than brown envelopes13. Envelopes are then placed in a sealable Ziploc plastic bag with the appropriate ratio of silica gel desiccant for the amount of tissue stored. Multiple envelopes can be stored in the same Ziploc bag, preferably grouped by collection location, and archival paperclips can be used to secure envelopes if needed. Alternatively, samples can be collected

into small, sealable polyethylene bags (2 ¼ x 3 ½ inches) for each sample, with an individual aliquot of silica13. Although tissues may dry more quickly, the drawback is that silica is time-consuming to remove when samples are ready for permanent storage and silica cannot be re-used (to prevent contamination)13. See Funk et al. (2017) for a list of products and suppliers.

To determine whether samples have sufficiently dried, samples can be checked after the desired drying time has passed (e.g. 12 hours). Samples are sufficiently dried if they break cleanly when bent6. At this point, most silica except a small amount can be removed6, as excessive storage of tissues with silica gel can lead to DNA degradation9. To optimize the drying process, multiple practices can be followed, as suggested by Funk et al. (2017). It is advisable to move the beads around periodically to maximize their contact with leaf tissues. When placing leaves in envelopes, spread leaves throughout the envelope, as stacking can prevent drying of the interior leaves. Cut particularly thick or waxy leaves into smaller pieces to maximize surface area for drying5. For very large thick mid-ribs, remove with scissors to dry easily and avoid tearing the leaf, which could release enzymes that degrade DNA7. Clean scissors with 70% ethanol between samples to prevent cross-contamination.

Long-term storage. Once tissues are properly dried, most silica can be removed and the samples can be placed under long-term storage conditions. Store samples at -80°C13, if available, or if ultra-cold freezers are not available store at -20°C7,10 or room temperature4 in airtight opaque containers. Coin envelopes or Ziplocs can be stored in boxes with small amounts of silica to absorb any excess moisture9,13. The Smithsonian Institution recommends Lock & Lock boxes (HPL 836) and including a relative humidity indicator card (Sorbent Systems) in each box to monitor humidity, which should not exceed 30%13.

Flash Freezing and Storing Tissue Samples

Flash freezing tissue samples in liquid nitrogen and preserving them in -80°C freezers is another method of preserving high quality DNA or RNA. Tissues can be collected in 8 mL cryogenic tubes (externally threaded with o-rings)13, labeled appropriately with sample and collection information. Cryovials are place in liquid nitrogen-filled storage Dewars at the collection location and are transported to the lab where they are stored in cryoboxes in -80°C freezers. If freezing samples for RNA preservation, apply extra cleanliness procedures, including wearing laboratory gloves and properly disinfecting all tools between samples to minimize nuclease exposure. Because RNA degrades quickly and gene expression changes rapidly, samples should be placed into the liquid nitrogen as quickly as possible after collection. Be sure to wear appropriate PPE (goggles, cryo gloves, long pants, close-toed shoes, lab coat) when working with liquid nitrogen. As an alternative to working with liquid nitrogen, samples can also be preserved in RNA later.

SAMPLE SIZE

DNA. Collecting multiple samples per population opens the possibility of future population genetic analyses (e.g., assessing genetic diversity, gene flow, population structure, population assignment, cryptic species, signals of selection, etc). Traditionally, researchers have suggested collecting as many samples per population as possible. However, some recent studies suggest that with the larger number of single nucleotide polymorphisms (SNPs) that can be assayed through next generation sequencing, it is possible to sample fewer individuals per population16–18. Through simulations of empirical data, Nazareno et al. (2017) found that 6 – 8 samples were sufficient to obtain accurate diversity estimates for a self-incompatible, outcrossing Amazonian plant species when 1000 SNPs were considered17. Similarly, Willing et al. (2012) found that 4 – 6 samples and greater than 1000 SNPs could sufficiently estimate genetic differentiation for a simulated dataset, but suggested that greater that 10 samples are required for outlier analyses16. Nevertheless, a recent simulation comparing trade-offs in sample size versus sequencing depth encouraged sampling as many individuals as possible at the expense of lower sequence coverage, in order to estimate genetic diversity accurately and calculate population differentiation statistics19.

For institutions interested in population genetic studies, we suggest beginning with a sampling target of 15 individuals per population, provided that there is a sufficient number of individuals from which to collect and that such collections would not harm the population. However, the ideal size sample will depend on the specific goals of the study (e.g. the type of genetic estimates desired) and the life history of the species of interest (demographic history, mating system, pollen and seed dispersal syndrome, etc)17. Individuals collected should be spatially distant and thus more likely to be representative of the population's genetic composition.

RNA. Collecting samples for RNA is both effort- and time-intensive. Labs may opt to collect one sample per population or species for RNA preservation to begin, and collect additional samples per population as specific studies require. Having at least one sample per species would allow the creation of a reference transcriptome, which could be useful for identifying genes and variants and may facilitate future studies.

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