The unique role of seed banking and cryobiotechnologies in plant conservation

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Societal Impact Statement
Eroding plant diversity has serious implications for the well-being of humanity and our planet. Conserving plants ex situ requires technologies that are rapidly advancing and readily accessible. The alarming loss of plant habitats has spawned global investment in technologies that focus on either conventional freezer storage, which exploit seed adaptations to survive drying, or on cryogenic platforms, that ensure long-term survival of germplasm that is not amenable to conventional methods. Increasing evidence that germplasm survives for decades provide proof of concept, but also warns of the limited utility of stored germplasm that is not returned to the Earth.

Summary
A future sustainable world requires concerted efforts to conserve plant biodiversity. Using an integrated approach, botanic gardens, arboreta, universities, governmental agencies, and non-governmental organizations are addressing that challenge. Here, we summarize some of the technological advances, in an ever-growing toolbox, that increase the scope of taxa that are conserved ex situ as well as the lifespans of diverse plant tissues that can be used as germplasm. Seed banking continues to be a powerful and efficient tool. Seeds that tolerate extreme drying and low temperature will likely survive at least 100 years using conventional conditions of a common freezer. The extreme tolerance of seeds among diverse taxa has led to the global growth of seed banks to over 1,750 currently, and the conservation of over 50,000 species. Not all plants produce seeds or seeds that survive freezer conditions. Predictive models provide insight into the extent of taxa needing alternative strategies and an initial list of such species is available. These “exceptional” species require cryobiotechnologies (cryogenic storage in liquid nitrogen and in vitro technologies), which provide effective, long-term ex situ conservation for a wide variety of tissues beyond seeds. The application of cryobiotechnologies increases the potential for conserving all plant biodiversity. Restoration of plant biodiversity into the future will require institutional collaborations among living collections, seed banks, and cryobanks to ensure technology transfer, information gathering and sharing, and capacity building in centers of biodiversity.
1 | INTRODUCTION

Ex situ conservation creates reserves of plant diversity through human involvement, cooperation, and emerging technologies. Botanical gardens or field genebanks grow plants in their living collections to provide materials for observation, research, and education (Westwood, Cavender, Meyer, Smith, 2020). A complementary strategy stores germplasm (i.e., propagules that can regenerate a plant) in a state of “suspended animation,” possibly for decades or centuries. Seed banks are a classic example of quiescent collections, initiated mostly to serve agriculture by providing genetic resources for crop breeding (Vavilov, 1987). The technologies and successes of early seed banks encouraged broader application for conservation (Chapman, Miles, & Trivedi, 2019; Falk & Holsinger, 1993; O’Donnell & Sharrock, 2017). Now, nearly 1,750 seed banks exist globally and house about 6 million accessions (i.e., samples having unique identifier information especially taxon, date, and location; Byrne et al., 2018; Hay & Probert, 2013; O’Donnell & Sharrock, 2018). An estimated 50,000–60,000 taxa are now represented in today’s germplasm banks; about 15,000–20,000 taxa in agricultural genebanks (e.g., the USDA National Plant Germplasm System houses nearly 14,000 species (https://npgsweb.ars-grin.gov/gringlobal/query/summary.aspx) in its base collection and many more species in research collections) and about 45,000–55,000 taxa in conservation-based genebanks (O’Donnell & Sharrock, 2018). Species collections that support agriculture often have hundreds to thousands of accessions representing cultivars, genetic stocks, and wild relatives of crops. In contrast, there are usually fewer accessions per species in conservation collections, partly because collecting from the wild is more expensive, and also because accessions from wild populations are harder to manage in genebanks, and use afterwards, due to their heterogeneity, small sample size, unknown growth requirements, and additional needs for non-seed conservation methods (Pence, 2011; Walters, 2015a; Walters, Richards, & Volk, 2018).

2 | WHY ARE SEEDS THE PREFERRED GERmplasm TO BANK?

Seeds are highly adapted to survive harsh conditions and establish when conditions improve. Survival of complete drying is a near-ubiquitous adaptation, possibly derived from the earliest land-inhabiting plants (Gaff & Oliver, 2013). Most seeds acquire desiccation tolerance as they accumulate food reserves within embryo cells (Vertucci & Farrant, 1995; Wang, Liu, Song, & Møller, 2015). Packing the cytoplasm with dry matter maintains cell structures when water is displaced (Walters, 2015b).

Drying seeds stop metabolizing and become essentially quiescent, or cryptobiotic, at ambient relative humidity (Walters, Hill, & Wheeler, 2005). Cryptobiosis in animals, such as tardigrades and brine shrimp, has captured considerable attention because the organism is alive, but doesn’t show it by respiring, responding to environmental stimuli, or growing (Clegg, 2001; Wright, 2001). In plants, the tendency is widespread during sexual reproductive phases and rarer in vegetative cells (Costa et al., 2016; Dinakar & Bartels, 2013). Quiescent collections can store tremendous diversity relatively inexpensively, often using small spaces and standard procedures and achieving long shelf-life (Hay & Probert, 2013; Li & Pritchard, 2009; Walters et al., 2018).

But, shelf-life is not indefinite. Quiescent organisms eventually lose the ability to recover and, in the case of seeds, cannot germinate even when required conditions are met (Fleming, Hill, & Walters, 2019; Rajjou & Debeaujon, 2008; Walters, 2015a, 2015b; Walters, Hill, et al., 2005). In other words, the utility of the sample becomes limited by its achieved lifespan during storage. That survival duration (i.e., longevity) depends on storage conditions, namely, the relative humidity (RH) and temperature (Walters, 2015a; Royal Botanic Gardens Kew, 2019). Longevity of the orthodox seed storage category can be predicted by models for temperatures between 60°C and −20°C and RH between 80% and 30%, and approximately doubles for each 6°C–10°C drop in temperature (Walters, Wheeler, & Stanwood, 2004; Fleming et al., 2019; Royal Botanic Gardens Kew, 2019). Longevity of orthodox seeds during freezer storage remains mostly conjectural; the earliest experiments were started just 50–60 years ago, providing insufficient time to detect change.

Lacking empirical proof that freezer storage was effective, seed banks established before 1970, used refrigerated storage at 5°C–10°C. In the 1980s, a few seed banks pioneered the use of freezer storage at −18°C to −20°C based on the possibility that seed lifespans would increase 4- to 16-fold, a possibility that has been confirmed for a few samples (i.e., Fleming et al., 2019; Walters et al., 2004). Freezer storage is now the international standard for “conventional” seed banking (CPC, 2019; FAO, 2014; MSBP, 2015; Figure 1a,b). We can project that properly dried orthodox seeds that survive for 25–50 years under refrigerated conditions will survive for 100–200 years in the freezer (Walters, Wheeler, & Grotenhuis, 2005). The long survival period of diverse germplasm that is protected from vulnerabilities inherent to living collections and is readily available when needed, at an affordable cost, makes this ex situ conservation strategy implementable at large and small institutions (Li & Pritchard, 2009).

Seeds are preferred germplasm because they are a complete organism, rather than a gamete, and there are many species that naturally survive drying and so naturally enter into a quiescent state. However, pollen of many plant species also tolerates desiccation (Franchi et al., 2011) and so can be a candidate for “conventional” freezer storage. As the male gametophyte, pollen is analogous to...
sperm, which is the most commonly banked germplasm for animals (Mazur, Leibo, & Seidel Jr., 2008). Indeed, pollen is increasingly valued as a germplasm form for ex situ conservation, especially when trying to restore diversity to a population of trees or when trying to cross individuals that flower asynchronously (Towill & Walters, 2000). However, orthodox pollen is not stored using conventional methods, even though longevity exhibits similar temperature and moisture dependencies as seeds (Buitink, Leprince, Hemminga, & Hoekstra, 2000; Walters, Hill, et al., 2005). This is because orthodox pollen is inherently short lived, with many species surviving just weeks to months in the refrigerator at optimum RH (Dafni & Firmage, 2000). A 4- to 16-fold increase in longevity, achieved by placing these grains in the freezer, would only provide viable germplasm for a few years at best. This illustration of the limited utility of freezer storage of pollen underscores the time frame for survival expected from seed banking using conventional methods: seeds are expected to survive several decades. In fact, germplasm in quiescent collections should outlive counterparts stored as living and growing specimens.

### 3 | WHEN DRY GERMLASM DOESN’T STORE WELL

There is increasing recognition that some seeds and other germplasm forms can be intrinsically short-lived, much as is observed for pollen (previous paragraph). Examples include seeds of *Salix* (willow), *Ulmus* (elm), some orchids, and some species native to Hawaii (Ballesteros & Pence, 2017; Chau et al., 2019; Popova et al., 2013; Whigham, O’Neill, Rasmussen, Caldwell, & McCormick, 2006), as well as spores from ferns and mosses that have relatively short lifespans (Ballesteros, Hill, & Walters, 2017; Walters, Hill, et al., 2005). The trait may be associated with mesic woodland and alpine habitats as opposed to seeds from species originating from warm, dry areas (Merritt et al., 2014; Mondoni, Probert, Rossi, Vegini, & Hay, 2010; Probert, Daws, & Hay, 2009; Walters, Wheeler, et al., 2005). Storage at −10°C to −20°C also leads to immediate damage or faster than expected aging in some dry plant germplasm (Ellis, Hong, & Roberts, 1991). The syndrome is associated with crystallization of storage lipids, called triacylglycerols (TAG), that are high in saturated (e.g., palmitic or steric acid) or monounsaturated (e.g., oleic acid) fatty acids (Ballesteros, Hill, Lynch, Pritchard, & Walters, 2018; Crane, Kovach, Gardner, & Walters, 2006; Hamilton, Ashmore, & Pritchard, 2009). Thermal behavior of TAG may explain low shelf-life in the freezer of many temperate nut species (e.g., *Juglans*, *Carya*, *Castanea*, and *Corylus*) and tropical species (e.g., *Cuphea*, *Elaiis*, and *Hevea*), as well as seeds from numerous Hawaiian species (Chau et al., 2019). We recently showed that seeds from safflower (*Carthamus tinctorius*) showed low temperature sensitivity after 20-30 years of storage (Fleming et al., 2019). TAG with high levels of saturated or monounsaturated fatty acids tends to be viscous and crystallizes near 0°C to −10°C.
Reasons for poor longevity during freezer storage remain conjectural, but surely are related to the composition and organization of cellular constituents within the embryo, deposited during development and affected by interacting factors including maternal health, maturity, and post-harvest handling, as well as the storage environment, making seed longevity truly a "complex trait" (https://en.wikipedia.org/wiki/Complex_traits, visited December 2, 2019).

Analogous questions about stability are studied in foods, pharmaceuticals, and materials which seek to reliably predict expiration dates or onset of poor functionality (Yoshioka & Aso, 2007).

In these highly controlled synthetic systems, the formulation and the method of processing are critical in achieving a desired product that lasts for the expected time. Molecules in solids (also called glassy matrices) are immobilized by crowding, and irregular spacing allows movement over short distances. This minute motion is the root cause of degradation (Bhattacharya & Suryanarayanan, 2009), with glasses characterized as “fragile” being extremely vulnerable to slight warming or added water (Yoshioka & Aso, 2007). Longevity of seeds, and dried cytoplasm in general, is likely influenced by similar principles (Figure 2). Hence, pollen and short-lived seeds, such as Salix sp., may indicate glass fragility. Low temperature sensitivity, likely from TAG crystallization, reflects a composite material having components with different thermal properties; destabilizing gaps in the cytoplasmic matrix form as TAG crystallizes and the volume of oil bodies shrink.

Poor-storing dry seeds and other germplasm can be stored cryogenically (Ballesteros et al., 2018; Ballesteros & Pence, 2017; Dulloo et al., 2009; Engelmann, 2011; Hamilton et al., 2009; Pence et al., 2020; Towill & Walters, 2000; Figure 1e). The extremely low-temperature range of liquid nitrogen (LN; −176°C to −196°C) slows molecular motion much more than does −20°C and extends germplasm survival to a few decades (Ballesteros & Pence, 2017; Pence et al., 2020; Figure 1f). Moreover, TAG crystallization can be inhibited by moderate cooling rates (20°C–40°C/min) and the low temperatures afforded by liquid nitrogen do not allow recrystallization (Ballesteros et al., 2018). Storage at −80°C also appears to be effective, although there is considerably less empirical or theoretical support for this storage platform. Use of LN provides an alternative

**FIGURE 2**  Schematic phase diagram illustrating safe (orange) and lethal (yellow) environmental conditions for germplasm storage based on interactions between moisture and temperature. Metabolism occurs in hydrated cytoplasm (green region) and quiescence is induced by solidification, when germplasm is dried and/or cooled. But tolerance of germplasm to these environmental stresses varies considerably, and response to environmental treatments must be considered to effect successful preservation. Orthodox physiology (represented by the bean icon) describes germplasm that survives longer when dried or cooled, treatments that encourage glass formation (solid blue curve). Once solidified, ice formation is not a risk and aging kinetics follow classic temperature dependencies. The papaya icon reflects germplasm with cytoplasm that doesn’t form a stable solid at freezer temperatures, probably for many reasons, one being crystallization of lipids. This germplasm survives exposure to LN temperatures without added treatments; care to cool and warm moderately rapidly (within 1–4 min) has aided recovery (unpublished). The acorn icon represents germplasm, like recalcitrant seeds, that survive considerable drying but not enough to solidify cytoplasm. To safely cool to a solid (orange region), this germplasm must traverse the lethal ice zone (yellow region) within about ½ s, which is facilitated by reducing the size of the specimen to just the excised embryonic axis. Alternative treatments are needed for highly hydrated cells that are also extremely sensitive to water stress. Application of cryoprotectant solutions and rapid cooling to limit exposure time within the lethal ice zone is effective strategies (diagram adapted from CPC, 2019) [Colour figure can be viewed at wileyonlinelibrary.com]
to mechanical freezers that are subject to warming during electrical outages.

4 | SEED RECALCITRANCE

About 10% of flowering plants produce seeds that do not survive sufficient drying to solidify cytoplasm as orthodox seeds do (Wyse & Dickie, 2017). In the genebanking context, these seeds are termed recalcitrant. Despite the name, recalcitrant seeds (or seed parts) can be cryopreserved. The same principles of solidifying cytoplasm are used for them, but cells can only survive partial dehydration and so must be subjected to extremely low temperatures to achieve and maintain the solid state (Walters, 2015b; Walters, Berjak, Pammenter, Kennedy, & Raven, 2013). Incidence of recalcitrance is higher among tropical species, such as Persea (avocado), Cocos (coconut), Cacao (cocoa), Syzygium/Eugenia, and Artocarpus (breadfruit). However, seed recalcitrance is not limited to the tropics, and is a major impediment to seed banking iconic and endangered oak (Quercus sp.), silver maple (Acer saccharinum), buckeye (Aesculus sp.), and wetland species such as wild rice (Zizania sp.) and Howelia aquatilis. Concerns over seed recalcitrance stymied seed banking activities in Hawaii, until it was shown that about 3% of the endemic species produced recalcitrant seeds (Chau et al., 2019).

The strategy to preserve recalcitrant seeds relies on solidifying cytoplasm by a combination of drying and rapid cooling. Lethal ice forms when embryos are dried to their tolerance limits (Wesley-Smith, Walters, Pammenter, & Berjak, 2015), and so freezer storage is unsuitable. But, slowed motion at LN temperatures prevents crystallization (Walters et al., 2013). Cooling partially hydrated tissues at 100°C and 500°C/sec can prevent ice crystal growth (Wesley-Smith et al., 2015; Figure 2). However, cooling of whole recalcitrant seeds, which tend to be large, within half a second is physically impossible and the ability to surgically remove the embryonic axis and recover it in vitro presented breakthrough technology (Walters et al., 2013). This dependency of in vitro and cryotechnologies spawned the evolution of a new scientific discipline called cryobiotechnologies (Pence et al., 2020; Pritchard, 2018). Cryopreserving axes of recalcitrant seeds, especially of species from tropical origins, remain challenging as the imposed stresses appear to induce aberrant metabolism akin to programmed cell death (Wesley-Smith et al., 2015). Advanced protections and recovery media are key to ameliorating degradative metabolism and bolstering organogenesis in surviving cells.

The term “recalcitrant” can cause confusion in seed banking and cryobiotechnology contexts. Recalcitrant seed is sometimes used to describe fastidious germination requirements involving separate and repetitive periods of warm and cool moisture to stimulate growth of a rudimentary embryo that eventually becomes sensitive to dessication as it germinates. We suspect anecdotal references to seed recalcitrance in Asimina/Annona, Impatiens, Helonias, Hydrastis, and Torreya are based on the complex physiology. In the biotechnology sense, recalcitrance describes species that are difficult or impossible, thus far, to initiate into tissue culture (Benson, 2000).

5 | EXCEPTIONAL SPECIES AND PROSPECTS FOR GERMLASM BANKING

Storing seeds in the freezer is not a suitable ex situ conservation strategy for many species, and we call these species "exceptional" (Pence, 2013). As discussed above, freezer storage may not be effective for maintaining viability of seeds that are relatively short lived when dry or that are sensitive to extreme drying and/or freezer temperatures. Moreover, obtaining seeds may not be possible for populations in reproductive failure or for plants, like ferns and mosses, that don’t produce seeds. Preservation of some plants, such as algae in lichens or some orchid species, may require technologies that also preserve obligate symbiotic relationships with the microbiome. Through predictive models and data analyses, it has been estimated that roughly half of the world’s plant species will not be bankable in conventional seed banks and about 60,000 may be of conservation concern (Colville & Pritchard, 2019; Pence, 2013; Pence et al., 2020; Wyse, Dickie, & Willis, 2018). While exceptional species are not yet all identified, a working list is now available online for global input (http://cincinnatizoo.org/global-list-of-threatened-exceptional-plants/). These will require alternative ex situ conservation methods. Cryopreservation presents an effective alternative for the conservation of these species, and cryobiotechnologies may be essential to obtain source materials, recover thawed samples, or assess longevity potential.

Species experiencing reproductive failure in the wild, in which plants do not produce pollen or seeds, require advanced cryobiotechnologies, as do agricultural varieties that must be propagated clonally (Engelmann, 2011; Pence, 2013; Pence et al., 2020). Mating systems, pollinators, gene flow mechanisms, fire suppression, and fragmented populations contribute to the dearth of seeds available for seed banking in seed bearing plants. Seeds and pollen may also be unavailable if plants are remote and inaccessible during flowering or at seed maturity. In cases of isolated or fragmented populations, such as in Hawaii, there may be just a few individual plants extant in the wild; representative sampling requires that germplasm from each plant is obtained.

When seeds are not available, the best option is to obtain cuttings of existing plants and propagate them, usually using microculture (i.e., in vitro) techniques. Most commonly, in vitro shoot cultures are initiated and propagated, and these provide tiny (1–2 mm) shoot tips, which are dissected from the shoot and cryopreserved (Figure 1d). In addition, somatic embryos and tips from root cultures can also be utilized for storage in liquid nitrogen (Carneros, Hernández, Toribio, Díaz-Sala, & Celestino, 2017; Reed, 2008; Simão et al., 2018). Healthy explants and the right medium for growth and proliferation are required for this approach, and species vary in the ease with which such cultures are initiated and grown, either because of endophytes that can contaminate the cultures, the production of inhibiting phenolic compounds in some species that are released upon wounding, or the need of some species for specialized growth medium and conditions (Benson, 2000; Niedz & Bausher, 2002). These biotechnologies can also be used to establish...
in vitro, living collections that are not at risk from biotic and abiotic stresses outdoors; however, maintenance of these collections are labor intensive and many groups seek to cryopreserve their in vitro collections to gain the advantage of quiescence (Pence, 2011, 2013). Despite the challenges, a wide range of species have been cryopreserved as in vitro tissues (Reed, 2008), and reports of long-term (1–2 decades) survival through LN storage are now being reported (Caswell & Kartha, 2009; Pence et al., 2017, 2020). These in vitro methods require a major investment of time and resources before cryopreservation procedures can be implemented, but they offer a viable option when other methods are not workable (Dulloo et al., 2009; Li & Pritchard, 2009; Pence, 2011).

Successful cryopreservation of explants follows the same principle as seeds and pollen: stabilize cellular structure and limit chemical change by solidifying the cytoplasm. Cytoplasmic solidification through air drying causes lethal cell shrinkage and so partial dehydration is accomplished chemically by adding osmotica and cryoprotectant molecules that remove and replace water without massive cellular deformation. In some procedures, partial air drying also helps to remove water that would otherwise form ice. Protecting molecules also delay freezing of the remaining water molecules so that cells can be cooled at achievable rates of a few hundred °C/sec (Volk & Walters, 2006). Vitrifying agents, such as glycerol, ethylene glycol, and dimethyl sulfoxide, have the capacity to induce glassy matrices within cytoplasm without inducing major structural changes to the cell (Volk & Walters, 2006). These compounds, along with sucrose, are major constituents in PVS2 (Plant Vitrification Solution 2), one of the most effective and broadly applicable cryoprotectant solutions used for plant cryopreservation (Sakai, Hirai, & Niino, 2008).

Approaches in the cryopreservation toolbox are varied and flexible. Deciding on a method will depend primarily on the type of tissue available. Can seeds be cryopreserved whole, or is embryo excision or dormant bud storage necessary? If pollen is available, can it be stored? What in vitro methods are needed? Choosing the most effective method requiring the least input of time will allow efficient use of resources and help ensure the conservation of more species (Pence et al., 2020; Smith & Pence, 2017). While the cryogenic genebank is a more recent addition to the conservation toolbox, recognition of the need for increased global capacity is growing, and the Exceptional Plant Conservation Network was established to focus on species that cannot be conserved in conventional seed banks (https://cincinzatizoo.org/epcn).

6 | THE “EXIT PLAN”—CASE STUDIES OF THE BENEFITS OF GENE_BANKED PLANT MATERIAL

Seed banks (and more generally genebanks) for plants are a recognized strategy to conserve genetic diversity, providing insights about Earth’s biodiversity, humans’ need for biodiversity, and the consequences for our planet if we do not protect biodiversity. The concepts presented in this review demonstrate that it is possible to achieve a quiescent state (i.e., preserve) in diverse plant germplasm; however, germplasm will not survive indefinitely. To ensure a return on investment of genebanking, the material must eventually be removed from the genebank and used. Those responsible for genebanking may not be the same as those using the materials; successful genebanking requires partnerships among different groups that have different missions. There are a number of works that have been published over the years to describe these partnerships as well as best practices to bank genetic resources that are “fit for purpose” (Center for Plant Conservation, 2019; Commander et al., 2018; Maschinski & Haskins, 2012; Offord & Meagher, 2009; Pritchard & Probert, 2003; Walters et al., 2018). In this section, we give a few examples of how the authors interact with various partners to successfully use banked germplasm.

In agriculture, the Food and Agriculture Organization of the United Nations (FAO) recognized the importance of ensuring the availability of genetic resources to sustain agriculture and landscapes (FAO, 2019). Case studies of agricultural uses of plant biodiversity, and the role of seed banks in providing materials, grow daily as the need increases to protect crops from emerging diseases, altered landscapes, and changing weather patterns (Byrne et al., 2018; Greene et al., 2019; Khoury, Greene, Krishnan, Miller, & Moreau, 2019). For example, the USDA National Plant Germplasm System yearly receives hundreds of thousands of requests from around the world for information and germplasm and yearly distributes about 250,000 accessions for diverse uses.

Moreover, greater awareness of the rich diversity of plants in North America, and the role of these plants in sustaining resilient ecosystems unique to the United States, has led to national, multiorganizational partnerships, such as Seeds of Success (SOS), a national native seed collection program, led by the Bureau of Land Management. SOS is charged with collecting wildland native seeds for research, development, germplasm conservation, and ecosystem restoration (Haidet & Olwell, 2015). FAO highlighted SOS as a prime example of interagency and private groups’ cooperative efforts to ensure restoration of biodiversity and greater resilience of ecosystems following natural disasters (FAO, 2019, p 30). The SOS seed collection of US native species contributes to the National Seed Strategy, which aims to ensure the availability of genetically appropriate seed to restore viable and productive plant communities and sustainable ecosystems (Plant Conservation Alliance, 2015) and now contains over 25,000 accessions (i.e., samples; Oldfield, 2019; Plant Conservation Alliance, 2015).

Seed banks, such as Project Baseline housed at National Laboratory for Genetic Resources Preservation (NLGRP), can capture and maintain populations for long periods and, thus, provide “time capsules” to evaluate temporal changes in an approach referred to as “resurrection ecology” (Etterson, Franks, et al., 2016; Etterson, Schneider, Soper Gorden, & Weber, 2016; Franks et al., 2008; McGraw, Vavrek, & Bennington, 1991). The Project Baseline collection (baselineseedbank.org) includes numerous populations per species, making it possible to examine interactions among selection
stresses, population history, and genetic composition to influence the rate and extent of evolutionary response across species’ ranges. Over the next 50 years, this unique seed collection will provide material to conduct resurrection experiments that will illuminate how anthropogenic and natural disturbance, including climate change, is driving evolution in wild plant species across both time and space.

The Center for Plant Conservation (CPC), established in 1984, convenes a unique network of botanical gardens and arboreta with additional government and private partners to effect conservation of the US’s most rare or imperiled plant species. As of 2019, over 1,600 of the 4,400 rare plant taxa in the US are curated in at a CPC participating institution. Conservation success stories from these collections abound (Maschinski & Haskins, 2012; Figure 1c): for one, the successful pollination of American chestnut trees from pollen preserved for 20 years, a feat that could restore genetic diversity to new chestnut lines resistant to chestnut blight which killed most trees in the early 1900s (D. Ballesteros and V. C. Pence, unpubl. data). Successful reintroduction of the federally endangered Cumberland sandwort (Minuartia cumberlandensis) required a combination of two conservation genebanking methods, preserved seeds, and in vitro propagation, to establish a new population in the Daniel Boone National Forest (Pence, Blair, Clark, & Taylor, 2011). CPC collections form the basis for a body of research on seed collecting, seed banking, and plant reintroduction that has been synthesized into a set of standards for the plant conservation community in the CPC Best Conservation Practices to Support Species Survival in the Wild (Center for Plant Conservation, 2019). Notably, these conservation methods will be at the heart of an historic, large-scale genebanking project funded by the State of California to collect seed from over 700 currently unbanked rare plant taxa for storage at botanical gardens in the California Plant Rescue collaborative.

7 | CONCLUSION

The rapidly developing technologies that allow plant germplasm banking under conventional or cryogenic platforms broaden the possibilities and promise that humans can safeguard the Earth’s trove of plant diversity. But saving these invaluable resources in freezers and cryovats isn’t enough. Stored germplasm that is forgotten or neglected will eventually die and will not serve a conservation goal. Therefore, an integrated approach that combines preservation technologies with botanical expertise and restoration ecology, as well as organizations from public and private sectors, is essential to protect plants and ensure they keep a home in the world that relies on plants for its own existence.

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AUTHOR CONTRIBUTION

CW outlined and wrote the first draft. VP reviewed, edited, and provided original text in several sections.

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