



# Ex situ collections and their potential for the restoration of extinct plants

Thomas Abeli <sup>1\*</sup>, Sarah Dalrymple,<sup>2</sup> Sandrine Godefroid,<sup>3,4,5</sup> Andrea Mondoni,<sup>6</sup> Jonas V. Müller,<sup>7</sup> Graziano Rossi,<sup>6</sup> and Simone Orsenigo<sup>6</sup>

<sup>1</sup>Department of Science, University of Roma Tre, Viale Guglielmo Marconi 446, Roma 00146, Italy

<sup>2</sup>School of Natural Sciences and Psychology, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, U.K.

<sup>3</sup>Research Department, Botanic Garden Meise, Nieuwelaan 38, Meise 1860, Belgium

<sup>4</sup>Service général de l'Enseignement supérieur et de la Recherche scientifique, Fédération Wallonie-Bruxelles, rue A. Lavallée 1, Brussels, 1080, Belgium

<sup>5</sup>Laboratory of Plant Ecology and Biogeochemistry, Université libre de Bruxelles, CP 244, Boulevard du Triomphe, Brussels 1050, Belgium

<sup>6</sup>Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100, Pavia, Italy

<sup>7</sup>Millennium Seed Bank, Conservation Science, Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, RH17 6TN, West Sussex, U.K.

**Abstract:** The alarming current and predicted species extinction rates have galvanized conservationists in their efforts to avoid future biodiversity losses, but for species extinct in the wild, few options exist. We posed the questions, can these species be restored, and, if so, what role can ex situ plant collections (i.e., botanic gardens, germplasm banks, herbaria) play in the recovery of plant genetic diversity? We reviewed the relevant literature to assess the feasibility of recovering lost plant genetic diversity with using ex situ material and the probability of survival of subsequent translocations. Thirteen attempts to recover species extinct in the wild were found, most of which used material preserved in botanic gardens (12) and seed banks (2). One case of a locally extirpated population was recovered from herbarium material. Eight (60%) of these cases were successful or partially successful translocations of the focal species or population; the other 5 failed or it was too early to determine the outcome. Limiting factors of the use of ex situ source material for the restoration of plant genetic diversity in the wild include the scarcity of source material, low viability and reduced longevity of the material, low genetic variation, lack of evolution (especially for material stored in germplasm banks and herbaria), and socioeconomic factors. However, modern collecting practices present opportunities for plant conservation, such as improved collecting protocols and improved cultivation and storage conditions. Our findings suggest that all types of ex situ collections may contribute effectively to plant species conservation if their use is informed by a thorough understanding of the aforementioned problems. We conclude that the recovery of plant species currently classified as extinct in the wild is not 100% successful, and the possibility of successful reintroduction should not be used to justify insufficient in situ conservation.

**Keywords:** botanical gardens, conservation translocations, de-extinction, herbaria, introduction, reintroduction, resurrection biology, seed banks

Colecciones Ex Situ y su Potencial para la Restauración de Plantas Extintas

**Resumen:** Las alarmantes tasas de extinción actuales y pronosticadas han incitado a los conservacionistas a esforzarse para evitar las futuras pérdidas de biodiversidad, pero para las especies que ya se encuentran extintas en vida silvestre existen pocas opciones. Nos preguntamos si estas especies pueden ser restauradas, y de ser así, qué papel pueden desempeñar las colecciones ex situ de plantas (es decir, jardines botánicos, bancos de germoplasma,

\*email [thomas.abeli@uniroma3.it](mailto:thomas.abeli@uniroma3.it)

**Article impact statement:** Ex situ collections avoid loss of plant diversity, but recovering lost genetic diversity from ex situ material only is highly challenging.

Paper submitted August 2, 2018; revised manuscript accepted July 12, 2019.

herbarios) en la recuperación de la diversidad genética de las plantas. Revisamos la literatura relevante para evaluar la factibilidad de la recuperación de la diversidad genética perdida y la probabilidad de supervivencia subsecuente de las reubicaciones. Encontramos 13 intentos por recuperar especies extintas en vida silvestre, la mayoría de los cuales usó material preservado en jardines botánicos (12) y en bancos de semillas (2). También hubo un caso de una población eliminada localmente que fue recuperada con material de un herbario. Ocho (60%) de estos casos fueron reubicaciones exitosas o parcialmente exitosas de la especie o población focal; los otros cinco fallaron o era demasiado pronto para poder determinar el resultado. Los factores que limitan el uso de material proveniente de colecciones ex situ para la restauración de la diversidad genética de las plantas en vida silvestre incluyen la escasez de material original, la baja viabilidad y la longevidad reducida del material, la baja variación genética, la falta de evolución (especialmente para el material almacenado en herbarios y bancos de germoplasma) y los factores socioeconómicos. A pesar de esto, las prácticas modernas de colección representan una oportunidad para la conservación de las plantas, como los protocolos mejorados de recolección y las condiciones acrecentadas de cultivo y almacenamiento. Nuestros hallazgos sugieren que todos los tipos de colecciones ex situ pueden contribuir efectivamente a la conservación de especies de plantas si su uso está respaldado por un entendimiento a fondo de los problemas antes mencionados. Concluimos que la recuperación de especies de plantas que actualmente están clasificadas como extintas en vida silvestre no es 100% exitosa y que la posibilidad de una reintroducción exitosa no debería utilizarse para justificar una conservación in situ insuficiente.

**Palabras Clave:** bancos de semillas, biología de la resurrección, des-extinción, herbarios, introducción, jardines botánicos, reintroducción, reubicaciones de conservación

## Introduction

Techniques to halt the loss of biodiversity include intentionally moving organisms for conservation purposes (conservation translocation) (IUCN 2013) (Table 1). Translocations such as reintroduction and reinforcement are based on the assumption that the focal species can be restored to an in situ habitat. More interventionist translocations, such as ecological replacement, and de-extinction, or, more accurately, the introduction of proxies of extinct species, have raised concerns that organisms being moved and released into near-natural ecosystems will not be able to fill exactly the ecological niche of the extirpated species (Seddon et al. 2014; Seddon 2017). Distinguishing between de-extinction, ecological replacements, and reintroduction is important to the communication and evaluation of conservation, but distinguishing among interventions can sometimes obscure commonalities that are useful to improving future practice. We reviewed the body of work that evaluates ex situ contributions to plant conservation and examined the many factors relevant to well-established interventions, such as reintroduction. We also considered the implications ex situ collections may have for the debate around de-extinction. We searched the published and gray literature on reintroduction of species formerly declared extinct in the wild for which ex situ material was used as source material for reintroduction. Data sources were the IUCN Red List ([www.iucnredlist.org](http://www.iucnredlist.org)), the Botanic Garden Conservation International Plant Search Database ([https://tools.bgci.org/plant\\_search.php](https://tools.bgci.org/plant_search.php)), Web of Science, SCOPUS, and cross-references among articles.

Our main aim was to assess the feasibility of recovering lost plant genetic diversity from ex situ plant material by evaluating the role of ex situ collections in cases where

a final translocation of a species extinct in the wild was achieved.

In addition to cases from the peer-review literature, we identified unpublished examples of species formerly declared extinct in the wild at the global level and reintroduced through a survey distributed to staff and affiliates of the European Native Seed Conservation Network (ENSCONET), the IUCN Conservation Translocation Specialist Group, and our network of 174 conservation biologists in 38 countries. The survey did not involve any personal data, so an ethical protocol was not needed. The response rate was very low (19 responses), but provided 4 unpublished cases (Table 2).

De-extinction is possible through technological advances in many fields of biology, but the concept has developed within the zoological sciences as a tool to reverse animal extinctions. It is the creation of a proxy of an extinct species (IUCN 2016), whereby the term *proxy* acknowledges that the resurrected individuals are materially different from the focal species of the attempted de-extinction. Animal de-extinction techniques include back breeding, cloning via somatic cell nuclear transfer, and genetic engineering (Shapiro 2017). However, because of the demanding technological requirements for animal de-extinction, the most advanced examples of recovery of ancient genotypes lost from the wild actually involve plants. In contrast to animal de-extinction, the recovery of plant genetic diversity lost from the wild can be achieved relatively easily by propagating seeds and spores and culturing plant tissue.

The analysis of de-extinction is relevant because many of the criticisms leveled at it can also be aimed at restoration from ex situ collections, an action which is the only resort for many species. We examined the known opportunities and constraints in this field to

**Table 1. Definitions of the terminology used in the review of reintroduction of species declared extinct in the wild for which ex situ material was used as source material for reintroduction.**

Term	Meaning	Source
Conservation translocation	intentional movement and release of a living organism with the primary objective of conservation; includes population reinforcement, reintroduction, and conservation introduction	IUCN 2013
De-extinction	used in a limited sense to apply to any attempt to create some proxy of an extinct species, not an exact replica of any extinct species	IUCN 2016
Recovery	used to indicate the process of reestablishing species or populations, extinct in the wild; does not include release of material in a recipient site	this article

explore de-extinction and to devise recommendations for furthering the de-extinction debate.

The recent growth of a date palm (*Phoenix dactylifera*) from seeds found in a Roman archeological site in Israel and dating back to the first century A.D. suggests that genotypes lost long ago can be successfully recovered (Sallon et al. 2008). *Phoenix dactylifera* exceeds previous records of length of seed viability held by *Canna compacta* Rosc. (550 years old) (Lerman & Cigliano 1971) and *Nelumbo nucifera* Gaernt. (1288 years old) (Shen-Miller et al. 1995). These species are overshadowed by *Silene stenophylla*, which was recovered from ovary plant tissues preserved in the Siberian permafrost for 30,000 years (Yashina et al. 2012). The longevity of these plant materials makes a compelling case for the possibility of the recovery of extinct species.

Although these examples highlight the potential for genetic recovery where the species in question is still extant, it raises the issue that the reinstatement of their genetic material may introduce strains that are substantively different from currently extant populations. They therefore serve to illustrate the point that the use of long-preserved genetic material, such as seeds in historical ex situ collections, may be akin to introducing a proxy of the existing species in line with definitions of *de-extinction*.

O'Donnell and Sharrock (2017) state there are about 500 plant species currently preserved ex situ that are either extinct in the wild or have been locally extirpated. Therefore, an analysis of opportunities and constraints resulting from the availability of propagules in ex situ plant collections is essential to evaluate their real potential in recovering lost genetic diversity and for translocation in general.

## Restoration of Genetic Diversity from Living Ex Situ Collections

### Botanic Gardens Sensu Lato

Collections of living specimens include those in public botanic gardens, private gardens, community gardens, arboreta, nurseries, and zoos (hereafter botanic garden)

(Bird et al. 2017). Botanic gardens are very effective in increasing plant stocks through ex situ propagation, and although a large proportion of plants in botanic gardens are common ornamentals, the cultivation of rare and threatened species for conservation purposes (including conservation translocations [Heywood 2017]) (examples in Table 2) has become increasingly important (Mounce et al. 2017).

Despite the growing role of ex situ living plants in conservation, the use of material propagated in botanic gardens presents significant constraints that may jeopardize the success of future interventions. First, genetic variation of ex situ populations may decline after several generations of cultivation due to high inbreeding rates, genetic drift, or a small number of founders originally collected in the wild, especially for very rare species (Maunder et al. 2001a; Wang et al. 2016; Wilson et al. 2017) (Table 3). The most recent collection and management strategies aim to minimize some of these problems by adding specimens to living collections to achieve genetic diversity comparable with that of wild populations (Cibrian-Jaramillo et al. 2013; Christe et al. 2014; Griffith et al. 2015) (Table 3). However, there may be a trade-off between improved genetic diversity and increased costs of maintenance of additional plants. Advanced tools developed for managing the genetic variation in captive animal populations are increasingly applied to ex situ plant collections (e.g., PMx software) (Lacy et al. 2011), and strategic material exchange between botanic gardens worldwide ensures backup collections and a large number of individual plants that can be used for propagation. Swapping material for cross-fertilization effectively maintains genetic diversity ex situ (e.g., Cibrian-Jaramillo et al. 2013), unlike the exchange of clones or inbred individuals that may result in genetically similar stocks (Theaker & Briggs 1993).

The second major constraint in the use of cultivated material affects even the most carefully managed collections: cultivation and horticultural care are known to affect both the evolution of ex situ plant populations and the individual's ability to tolerate stress and the consequences of translocation are poorly understood (Ensslin

**Table 2. Outcome and information on reintroductions of species extinct in the wild sourced from ex situ material.\***

<i>Species</i>	<i>Status</i>	<i>Source collection</i>	<i>Success</i>	<i>Generation length and growth form</i>	<i>Reference</i>
<i>Bromus interruptus</i> (Hack.) Druce	extinct in the wild, U.K.	private garden	successful	annual, herb	Marren 2005
<i>Cyanea superba</i> Cham.	extinct in the wild, Hawaii, U.S.A.	botanic garden	too early to judge	~10 years, tree	H. Kawelo, personal communication
<i>Cylindrocline lorencei</i> A.J.Scott	extinct in the wild, Mauritius	botanic garden	unsuccessful	unknown, tree	S. Buord, personal communication
<i>Diploaxis siettiana</i> Maire	extinct in the wild, Spain	seed bank	successful	annual or biennial, herb	Pérez Latorre et al. 2011
<i>Erica turgida</i> Salisb.	extinct in the wild, South Africa	botanic garden	unsuccessful	unknown, shrub	C. Cowell, personal communication
<i>Erica verticillata</i> P.J. Bergius	extinct in the wild, South Africa	botanic garden; private garden; seed bank	successful	<10 years, shrub	Hitchcock & Rebelo 2017
<i>Eriocaulon beleocharioides</i> Satake	extinct in the wild, Japan	botanic garden	partially successful	annual, herb	Tanaka et al. 2015; N. Tanaka, personal communication
<i>Hibiscadelphus giffardianus</i> Rock	extinct in the wild, Hawaii, US	botanic garden	partially successful	not found, tree	Belfield et al. 2011
<i>Lachanodes arborea</i> (Roxb.) B.Nord.	extinct in the wild, St. Helena, (U.K. Overseas Territories)	local plant nursery	partially successful	unknown, tree	T. Heller, personal communication
<i>Lysimachia minoricensis</i> J.J. Rodr.	extinct in the wild, Minorca, Spain	botanic garden	partially successful	biennial, herb	Galicia Herbada & Fraga Arquimbau 2011
<i>Normania triphylla</i> (Lowe) Lowe	extinct in the wild, Madera, Portugal	botanic garden	unsuccessful	unknown	Delmail et al. 2012
<i>Sophora toromiro</i> Skottsb.	extinct in the wild, Easter Island, Chile	botanic garden	unsuccessful	not found, shrub	World Conservation Monitoring Centre 1998; Maunder et al. 2000
<i>Trochetiopsis erythroxyton</i> (Forst.) Marais	extinct in the wild, St. Helena, (U.K. Overseas Territories)	nursery	partially successful	<20 years, tree	Lambdon & Ellick 2016; T. Heller, personal communication

\*Definitions: successful, reintroduced population is self-sustaining, resulting in a new generation; partially successful, released plants alive but not reproducing; unsuccessful, reintroduced populations disappeared without a new generation. Indications of success or failure derived from the literature or from personal communications with the authors of the reintroductions. Affiliations of colleagues who provided personal communications are in Acknowledgments.

et al. 2015). These cultivation techniques become selective forces affecting genotypes and life-history traits in garden populations of different species. Effects are large on annual and short-lived plants, whereas long-lived perennials, such as trees, are less affected or unaffected (Ensslin et al. 2011; Lauterbach et al. 2012). Selective

forces can be positive, but are likely to be maladaptive when plants are subsequently released into natural habitat (Ensslin et al. 2011). Recent cultivation guidelines aim to reduce the effects of ex situ cultivation problems (Basey et al. 2015). Nursery conditions can reduce plant viability and vigor after several generations, especially

**Table 3. Strengths and weaknesses of and opportunities for ex situ collections as source material for recovery or translocation of lost plant genetic diversity.**

<i>Strength or weakness</i>	<i>Botanic garden</i>	<i>Seed bank</i>	<i>Herbarium</i>
<b>Strength</b>			
propagation facilities	x	x	
collecting strategies ensure genetic wild diversity is represented ex situ	x	x	
cultivation strategies avoid adaptation to ex situ conditions	x		
low cost of maintenance		x	x
safe long-term storage		x	
historic collections (old material preserved)			x
large number of specimens		x	x
<b>Weakness</b>			
reduced genetic variation in old accessions	x	x	x
founder effect	x	x	
unintentional selection (e.g., large seeds, fast germination, dormancy loss, large plants)	x	x	
adaptation to garden conditions	x		
hybridization	x		
genetic drift through recurrent propagation	x		
material susceptible to pests and diseases	x		x
freezing of evolution		x	x
historical importance that limits usability			x
accidental propagule survival			x
<b>Opportunities</b>			
application of advanced technologies (e.g., molecular engineering and synthetic biology)	x	x	x
maternal effects	x		
preconditioning	x		

in short-lived plants, and although ex situ stocks can be revitalized with new propagules from wild or ex situ sources (Navarro et al. 2016), this is of course impossible with species that are already extinct in the wild. Manipulations of growing conditions have proved effective in improving ex situ plant quality through plasticity, use of preconditioning, and induction of transgenerational maternal effects (Brancaleoni et al. 2018).

There have now been many successful translocations of endangered plant species in which plants have been collected from the wild, multiplied in ex situ conditions, and reintroduced to the wild (e.g., Ramsay 1998). Despite this, the potential of botanic gardens to contribute to translocation is not fully realized (Cibrian-Jaramillo et al. 2013). Constraints associated with using living specimens from botanic gardens (Table 3) limit the possibility to reintroduce species (or subspecific taxa) extinct in the wild from botanic garden material only, especially for material collected before modern protocols and collection-management strategies and tools were adopted (Cavender et al. 2015). It is therefore not surprising that of 13 species globally extinct in the wild or locally extirpated that have been reintroduced from botanic gardens 3 (23%) were successfully reintroduced, whereas 31% of introductions failed and 38% were partially successful (Table 2).

### Germplasm Banks

Germplasm banks for plants are mainly represented by seed and spore banks specifically developed to store plant material for conservation and research purposes over the long term (Schoen & Brown 2001). There are over 1750 germplasm banks in the world. Most of them are agricultural gene banks (storing crop diversity) and several are wild plant gene banks. Germplasm banks store about 4.6 million accessions (Hay & Probert 2013).

One of the targets of the Global Strategy for Plant Conservation 2011–2020 is to make 20% of the germplasm bank collections available for in situ conservation translocation actions (<http://www.plants2020.net/gspc-targets/>). Therefore, it is important to evaluate the potential of germplasm bank accessions to contribute to the recovery of lost genetic diversity. Germplasm banks can contribute to plant recovery directly through their collections and propagation facilities and through the conservation expertise of the researchers who curate the collections. This expertise can help drive necessary research on longevity in storage, dormancy breaking, and germination requirements of rare wild species to improve effective seed use (Merritt & Dixon 2011). Stored seed stocks were used as source material for several reintroductions of threatened species (e.g., Cochrane et al. 2007). However, we found only 2 cases of species



extinct in the wild recovered using seed bank material (*Diplotaxis siettiana* and *Erica verticillata*) (Table 2), both of which were successful.

Similar to natural systems, high seedling mortality can negatively affect a conservation translocation by rapidly depleting the plant stocks. To overcome this, several reintroduction guidelines suggest growing plants in a controlled environment before their release as adult or subadult plants (Godefroid et al. 2011b; Maschinski & Albrecht 2017). In this case, propagation and ongoing care should be undertaken in such a way as to minimize detrimental impacts of cultivation discussed in the previous section.

The key target of germplasm banks is to keep seeds and spores alive by preserving their inherent longevity. About 75–80% of seed-bearing species produce orthodox seeds that can survive a drying process under standard conditions (i.e., drying at 15% eRH, 15 °C) and prolonged storage at –20 °C (Walters et al. 2013). Under these conditions, seed germinability could take decades, perhaps centuries, to decline (Walters et al. 2005). Nevertheless, even under optimal storage conditions, loss of seed viability due to seed aging over time is inevitable (Bewley et al. 2013), and this in turn affects seedling emergence and survival. Seed longevity varies between species and different populations of the same species, depending partly on climate. Seeds from plants living in hot, dry sites generally last longer than those from cool, wet climates (Probert et al. 2009; Mondoni et al. 2011). Other important correlates of seed longevity include embryo size and maturity (Probert et al. 2009) and seed dispersal syndrome (Merritt et al. 2014). Additionally, seed longevity has shown transgenerational changes associated with environmentally induced parental effects (Kochanek et al. 2010).

One of the significant advantages of seed banks is the ability to store many species with orthodox seeds in a limited space, reducing collection maintenance costs. Seed banks can flexibly accommodate seeds when they are produced in quantity (in response to unpredictable masting events for example), and multiyear accessions reduce pressures on small wild populations (Cochrane et al. 2007). A problem with seed stocks of rare species is the quantity that may be available for translocation, which is usually very low (Cochrane et al. 2007). Moreover, up to 10% of all angiosperms produce recalcitrant seeds (i.e., seeds that are not desiccation tolerant and that therefore cannot be stored using standard seed banking protocols [Berjak & Pammenter 2008]), and this percentage increases to 36% for critically endangered plant species (Wyse et al. 2018). Ex situ conservation of recalcitrant seeds is sometimes possible with cryogenic technologies, whereby seeds are rapidly cooled at ultralow temperatures, often in liquid nitrogen (–196 °C) (Walters et al. 2008). Cryopreservation may be used for tissues other than seeds (e.g., meristems). However, cryogenic stor-

age is costly and requires specialized infrastructure and highly trained personnel. In addition, recalcitrant seeds require rigorous preparations before being exposed to cryogenic storage (e.g. surgical dissection of embryonic axis) (Engelmann 2011). Consequently, the conservation of species with recalcitrant seeds for large-scale translocation is technically possible, but may not be feasible from a practical or financial standpoint.

## Restoration of Genetic Diversity from Herbarium Specimens

As sources of genetic material for translocation, herbaria compare poorly with living collections, especially germplasm banks, where high-quality storage conditions are applied to promote seed and spore longevity. Nevertheless, the sheer number of preserved specimens, more than 387 million specimens in about 3000 herbaria (Thiers 2018) means their potential to provide genetic material should be considered (Bowles et al. 1993). Indeed, if one does not consider species in seed banks that do not occur in the wild extinct, sensu IUCN (Dalrymple & Abeli 2019), then herbaria represent the sole possibility to resurrect true extinct species.

So far, there have been few attempts to use herbarium specimens in translocation and most research has only explored their potential as a propagule source. Several authors have obtained viable spores and seeds from herbarium vouchers up to 237 years old, which indicates that spores and seeds may remain viable in an herbarium for a long time (e.g., Molnár et al. 2015). In ferns chlorophyllous spores decrease their viability more rapidly than spores that are not green. Studies on angiosperms suggest *Fabaceae* has some of the most long-lived seeds preserved in herbaria, followed by *Poaceae* and *Apiaceae* (Molnár et al. 2015). However, storage conditions seem more important than taxonomic or ecological characteristics in determining seed viability of herbarium specimens (Godefroid et al. 2011b).

Only one attempt has progressed toward the establishment of viable plant populations from propagules gained from herbarium specimens (*Crepis foetida* subsp. *foetida*) (Sears 2011) (Table 2).

Some critical problems limit the use of herbarium spores or seeds (Table 3). Herbarium-sourced translocation material is generally scarce in terms of the number of specimens for rare species and number of spores or seeds preserved within each specimen (Godefroid et al. 2011b). Moreover, spores and seeds typically show low viability, and in old specimens DNA is often degraded (Leino & Edqvist 2010). Godefroid et al. (2011a) explored the feasibility of propagating 26 extinct taxa from the Belgian flora from old herbarium vouchers that had been stored for 23 to 158 years. Of the 2,672 seeds tested, only 8 seeds from a single species germinated, and these did not produce viable seedlings.

Several studies reported germination as a percentage of the sown seeds that germinated, without considering that an unknown proportion of those seeds were already dead at the beginning of germination tests. This prevents the accurate assessment of seed viability. Germination is often reported at radicle emergence (tip of the root tissue penetrates the seed coat). However, Godefroid et al. (2011b) observed radicle emergence in seeds of *Bupleurum tenuissimum* that were >100 years old without any further development of shoots; this further complicates the accurate reporting of germination from herbarium-sourced propagules.

Low seed germination percentages of seeds from herbarium specimens may also be due to a deep secondary-dormancy status induced by unfavorable storage conditions (Merritt et al. 2014). Seed dormancy works as an ecological mechanism that allows seeds to germinate only when conditions are suitable for supporting seedling growth (Finch-Savage & Leubner-Metzger 2006), but it is a constraint when seeds have to be used for plant regeneration (Ladouceur et al. 2017). Proper dormancy-breaking techniques should be applied to induce germination, such as cold or warm stratification and dry after ripening in the case of physiological dormant seeds (Baskin & Baskin 2014). However, when working with rare species, the required information is often not available, and experimentation is therefore necessary (Godefroid et al. 2016), which may rapidly deplete the already limited stock of seeds available from herbarium specimens.

Low viability of herbarium propagules may also be due to pest-control treatments, including the application of chemicals and heat treatments (Godefroid et al. 2011b, 2017). Modern curators avoid the use of chemicals where possible by using sealed containers and periodic freezing (RBGE 2017).

The final, but very important, caveat associated with using herbarium specimens is that delivering conservation benefit may undermine the primary use of herbaria collections; sampling spores or seeds from herbaria may destroy or irreparably damage the specimens, which significantly limits the use of voucher specimens of historical importance for taxonomic descriptions (Graves & Braun 1992; Shiga 2013). Determination of whether that risk is worth taking should be undertaken at species level and account for the availability of specimens for both systematics and conservation.

### General Obstacles to The Recovery of Plant Genetic Diversity Extinct in the Wild

Volis and Blechner (2010) clearly identified the main roles of ex situ collections in conservation: creating a backup of genetic material should in situ conservation actions fail; preserving a significant portion of the genetic diver-

sity of a species; and propagating species for restoration. Botanic gardens and seed banks can be very effective in achieving these goals, whereas the potential of herbaria still needs further investigation.

Botanic gardens play a valuable role in propagating plants for translocation from natural populations (e.g., Baker et al. 2014; Makowski et al. 2016). However, this approach is not possible when a species or other taxon is extinct in the wild, and there are additional constraints that make the recovery and release of genetic diversity lost from the wild difficult (Tables 1 and 3). We summarize these constraints to stimulate a scientific debate on possible solutions.

Unintentional selection after several generations in ex situ cultivation of short-lived species make propagules unsuitable for their reintroduction. The tendency to have low quantities of seed or plant stocks preserved ex situ is often reflected in reduced genetic diversity of ex situ collections (Sarasan et al. 2016) and recovery attempts based on only a very small number of founders (e.g., *Normania triphylla* one individual) (Delmail et al. 2012). From an evolutionary point of view, material stored in germplasm banks and herbaria is only representative of the time at which it was collected, whereas environmental factors impose evolutionary changes in extant plant populations (Lowe et al. 2000). When material from old ex situ collections is propagated, it may no longer be adapted to current abiotic conditions and biotic interactions (e.g., with symbionts, pests, and pollinators [Schoen & Brown 2001]). This is particularly relevant for species that have long been missing from their natural habitat. After the rearrangement of the ecosystem following their extinction, they may no longer find a suitable niche. For example, Yashina et al. (2012) found significant differences in the flower morphology of ancient and extant *Silene stenophylla* plants that may reflect different reproductive strategies.

Despite the fact that most lost genetic variation and extinct species are preserved as herbarium specimens, the value of this material is doubtful because of the unsuitable conditions for the long-term viability of seeds and spores and pest-control treatments.

Spontaneous hybridization between morphologically similar congeners can also occur in ex situ collections because of overlapping flowering period and spatial proximity (Maunder et al. 2004). Such a risk of hybridization may considerably restrict the conservation value of botanic garden collections (Volis 2017). Interspecific hybridization of some ex situ collections intended for reintroduction have already been raised previously in the case of the extinct species *Sinojackia xylocarpa* (Zhang et al. 2009) and *Sophora toromiro* (Püschel et al. 2014).

Of course, there are other obstacles to the recovery of species extinct from the wild that are not directly connected to the type of source material (see Sandler

**Table 4. Summary of obstacles to the recovery of plants extinct in the wild and possible solutions.**

<i>Obstacle</i>	<i>Possible solution</i>
Reluctance from land management authorities, government agencies, local stakeholders	outreach and educational programs
Low success rate of translocation	further research and training of specialized professional figures with multidisciplinary competences
Reason for extinction unknown	further studies encouraged in the lab or in the field to identify specific threats
Low genetic diversity	if possible, accessions of different origin should be included to maximize original genetic diversity; genetic studies of ex situ collections could help in assessing conservation value of material
Challenging propagation	further research needed on development of efficient propagation protocols
Habitat degraded or no longer existing	habitat must first be restored to a state that ensures the viability of target species

2013): lack of habitat and support from the public. Before reintroducing a species extinct in the wild, one must first ensure that its habitat still exists or that it has been appropriately restored. In some cases, this is difficult, if not impossible, because a description of the original native habitat may be lacking (e.g., *Tulipa sprengeri*) (Maunder et al. 2001b). The case of *Bromus bromoideus* illustrates the complexity of recovery programs from a social perspective. Endemic to southern Belgium and northern France, *B. bromoideus* has been extinct in the wild since 1935. Today it exists in 6 ex situ collections, but no translocations are currently planned. The species is associated with a crop that is nowadays rarely used in Western Europe (*Triticum spelta*) and a survey among farmers shows that most of them were not in favour of its translocation (Godefroid et al. 2010).

## Lessons for Practitioners

In recent years, botanic gardens and seed banks have made significant progress towards the conservation of endangered species. In Europe a recent assessment showed that 63% of European threatened species are already conserved ex situ in seed banks (Rivière et al. 2018). However, it appears that relatively few of these are used for translocation actions in the wild. According to the European seed bank database ENSCOBASE (as of 20/06/2018), of the 67620 seed accessions of native plants stored in European seed banks, only 64 accessions (0.09%) were used in translocation programmes (<http://enscobase.maich.gr/>).

To promote the use of ex situ accessions, facilities such as botanic gardens and germplasm banks could modify collection strategies to ensure that the harvested material can be used for translocations in the wild (Walck & Dixon 2009). For high-priority species, seed, spore, and plant collections should follow the most recent protocols to optimize the genetic diversity captured with reference

to the global genetic population structure of the target species (Hoban & Schlarbaum 2014). Ideally, material collections should focus not only on amassing numbers of species when many of these species will be poorly represented, but also on improving the quality of the collections. Collecting should therefore include infraspecific taxonomic levels (e.g., subspecies, ecotypes), different ploidy levels (different chromosome numbers within a species), several populations from across a species' distribution (Akeroyd & Wyse Jackson 1995; Griffith et al. 2015), and both sexes in dioecious species and should represent the interannual variability of seed or spore performance with multiyear collections (Table 4). Alternative techniques, such as cryopreservation and tissue cultures, should be improved to allow recalcitrant-seeded species to be maintained ex situ (Wyse et al. 2018).

Our review highlights the role of herbaria in supporting species conservation, particularly in the recovery of species extinct in the wild. However, survival of plant propagules in herbaria is often accidental. Moreover, herbaria would have difficulties conforming to these new collecting policies; collecting many individuals for an herbarium would seriously jeopardize the chances of survival of endangered species. However, herbaria might play a more valuable conservation role by ensuring the availability of flowering and fruiting plants among their vouchers from which pollen and seeds may be collected and adopting pest treatments with no or limited effects on spore, seed, and pollen viability. Such recommendations are of importance considering the recent debate on synthetic biology applied to conservation (including DNA synthesis [Piaggio et al. 2017]) because advancements in molecular engineering will make herbaria possible sources of genetic material. Herbaria may also provide important information to support restoration programmes, such as dates of occurrence, distribution, and habitat of a focal species.

The final phases of genetic restoration is translocation of propagules or plants to the wild and subsequent



management to promote establishment and regeneration. However, the relatively low success rate of translocation has prompted recommendations for improved practice in many aspects of the intervention (Godefroid et al. 2011a; Dalrymple et al. 2012). Our review suggests how ex situ facilities may be better employed, but we also recommend the integration of conservation and agronomy techniques (e.g., genome editing, marker assisted breeding) that are as yet unused or even unknown in the field of wild plant conservation.

Despite recent advancements in technology, the recovery of plants that are extinct in the wild, and their subsequent translocation, is still a little-used conservation approach due to the logistical and ecological complexity in undertaking such interventions. We have detailed the growing role of ex situ plant collections and deliberately articulated the limitations of the various modes of storage and the implications there are for genetic restoration. These observations lead us to conclude that the recovery of some threatened species may rely on ex situ plant conservation in the future, but successful intervention will not depend on this alone. With plant material secured ex situ, more time is available for engagement with stakeholders, habitat rehabilitation, or the development of suitable propagation techniques. However, the infrastructure, policy, and practice of threatened species management must continue to prioritize in situ species protection with ex situ interventions taking a supporting role, and under no circumstance should the existence of botanic gardens, seed banks, and herbaria be used as a justification against effective in situ species protection through other means.

## Acknowledgments

We are grateful to S. Buord, D. A. Burney, C. Cowell, R. Hai, T. Heller, H. K. Kawelo, S. McDaniel, N. Tanaka, and M. Hyvärinen for the valuable information on the species reported in Table 2 and other species. We thank all our colleagues who replied to the survey, in particular D. Bickerton, M. Maunder, P. Soorae, and P. Smith. We also thank the 3 anonymous reviewers for their useful suggestions. The Grant of Excellence Department, MIUR-Italy (ARTICOLO 1, COMMI 314 – 337 LEGGE 232/2016), is gratefully acknowledged for the support of T.A.

## Literature Cited

- Akeroyd J, Wyse Jackson P. 1995. A handbook for botanic gardens on the reintroduction of plants to the wild. BGCI, Richmond.
- Baker K, et al. 2014. Rescue, ecology and conservation of a rediscovered island endemic fern *Anogramma ascensionis*: ex situ methodologies and a road map for species reintroduction and habitat restoration. *Botanical Journal of the Linnean Society* **174**:461–477.
- Basey AC, Fant JB, Kramer AT. 2015. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. *Native Plants Journal* **16**:37–53.
- Baskin CC, Baskin JM. 2014. Seeds – ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, California.
- Belfield T, Tunison T, Chase J, McDaniel S. 2011. Rare plant stabilization projects at Hawai'i Volcanoes National Park, 1998–2008. Technical report 174. Pacific cooperative studies Unit, University of Hawaii, Mānoa. Available from <http://hdl.handle.net/10125/33199> (accessed March 2018).
- Berjak P, Pammenter NW. 2008. From *Avicennia* to *Zizania*: seed recalcitrance in perspective. *Annals of Botany* **101**:213–228.
- Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H. 2013. Seeds: physiology of development, germination and dormancy. 3rd edition. Springer-Verlag, New York.
- Bird SA, Esseen PJ, Hewitt R. 2017. Reintroductions of native plant species to the United Kingdom. *International Zoo Yearbook* **51**: 32–49.
- Bowles ML, Betz RF, DeMauro MM. 1993. Propagation of rare plants from historic seed collections: implications for species restoration and herbarium management. *Restoration Ecology* **1**:101–106.
- Brancaleoni, L, Gerdol, R, Abeli, T, Corli, A, Rossi, A, & Orsenigo, S. 2018. Nursery pre-treatments positively affects reintroduced plant performance via plant pre-conditioning, but not maternal effects. *Aquatic Conservation: Marine and Freshwater Ecosystems* **28**:641–650.
- Cavender N, Westwood M, Bechtoldt C, Donnelly G, Oldfield M, Rae M, McNamara W. 2015. Strengthening the conservation value of ex situ tree collections. *Oryx* **49**:416–424.
- Christe C, Kozłowski G, Frey D, Fazan L, Bétrisey S, Pirintsos S, Gratzfeld J, Naciri J. 2014. Do living ex situ collections capture the genetic variation of wild populations? A molecular analysis of two relict tree species, *Zelkova abelica* and *Zelkova carpinifolia*. *Biodiversity and Conservation* **23**:2945–2959.
- Cibrian-Jaramillo A, Hird A, Oleas N, Ma H, Meerow AW, Francisco-Ortega J, Griffith MP. 2013. What is the conservation value of a plant in a botanic garden? Using indicators to improve management of ex situ collections. *Botanical Reviews* **79**:559–577.
- Cochrane JA, Crawford AD, Monks LT. 2007. The significance of ex situ seed conservation to reintroduction of threatened plants. *Australian Journal of Botany* **55**:356–361.
- Dalrymple S, Abeli T. 2019. Ex situ seed banks and the IUCN Red List. *Nature Plants* **5**:122–123.
- Dalrymple SE, Banks E, Stewart GB, Pullin AS. 2012. A meta-analysis of threatened plant reintroductions from across the globe. Pages 31–52 in Maschinski J, Haskins KE, editors. *Plant reintroduction in a changing climate - promises and perils*. Island Press, Washington, D.C.
- Delmail D, Labrousse P, Lesouëf J-Y, Le Hir F, Autret J-L, Botineau M. 2012. In vitro establishment and multiplication of the *Normania triphylla* (Lowe) Lowe. *Brazilian Archives of Biology and Technology* **55**:543–547.
- Engelmann F. 2011. Cryopreservation of embryos: an overview. *Methods in Molecular Biology* **710**:155–184.
- Ensslin A, Sandner TM, Matthies D. 2011. Consequences of ex situ cultivation of plants: Genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanical gardens. *Biological Conservation* **144**:272–278.
- Ensslin A, Tschöpe O, Burkart M, Joshi J. 2015. Fitness decline and adaptation to novel environments in ex situ plant collections: current knowledge and future perspectives. *Biological Conservation* **192**:394–401.
- Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**:501–523.
- Galicía Herbada D, Fraga Arquimbau P. 2011. *Lysimachia minoricensis*. The IUCN red list of threatened species. International Union for

- Conservation of Nature, Gland, Switzerland. Available from <http://dxdoiorg/102305/IUCNUK2011-2RLTST61670A12535686en> (accessed March 2018).
- Godefroid S, et al. 2010. Conservation of *Bromus bromoideus*: feasibility study of the reintroduction of a plant extinct in the wild. Page 123 in Abstract book 7th SER Conference of the Society for Ecological Restoration (SER). SER, Washington, D.C.
- Godefroid S, Piazza C, Rossi G, Buord S, Stevens AD, Agurauja R, Cowell C, Vanderborght T. 2011a. How successful are plant species reintroductions? *Biological Conservation* **144**:672–682.
- Godefroid S, Le Pajolec S, Van Rossum F. 2016. Pre-translocation considerations in rare plant reintroductions: implications for designing protocols. *Plant Ecology* **217**:169–182.
- Godefroid S, Van de Vyver A, Stoffelen P, Robbrecht E, Vanderborght T. 2011b. Testing the viability of seeds from old herbarium specimens for conservation purposes. *Taxon* **60**:565–569.
- Godefroid S, Van de Vyver A, Stoffelen P, Vanderborght T. 2017. Effectiveness of dry heat as a seed sterilisation technique: implications for ex situ conservation. *Plant Biosystems* **151**:1054–1061.
- Graves GR, Braun MJ. 1992. Museums: Storehouses of DNA? *Science* **255**:1335–1336.
- Griffith MP, Calonje M, Meerow AW, Tut F, Kramer AT, Hird A, Magellan TM, Husby CE. 2015. Can a botanic garden cycad collection capture the genetic diversity in a wild population? *International Journal of Plant Sciences* **176**:1–10.
- Hay FR, Probert RJ. 2013. Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* **1** (cot030) <https://doi.org/101093/conphys/cot030>.
- Heywood VH. 2017. The future of plant conservation and the role of botanic gardens. *Plant Diversity* **39**:309–313.
- Hitchcock A, Rebelo AG. 2017. The restoration of *Erica verticillata* - a case study in species and habitat restoration and implications for the Cape Flora. *Sibbaldia* **15**:39–63.
- Hoban S, Schlarbaum S. 2014. Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. *Biological Conservation* **177**:90–99.
- IUCN (International Union for Conservation of Nature) SSC (Species Survival Commission). 2013. IUCN guidelines for reintroductions and other conservation translocations. IUCN SSC, Gland, Switzerland.
- IUCN (International Union for Conservation of Nature) SSC (Species Survival Commission). 2016. IUCN SSC guiding principles on creating proxies of extinct species for conservation benefit. Version 10. IUCN SCC, Gland, Switzerland.
- Kochanek J, Buckley YM, Probert RJ, Adkins SW, Steadman KJ. 2010. Prezygotic parental environment modulates seed longevity. *Australian Ecology* **35**:837–848.
- Lacy RC, Ballou JD, Pollack JP. 2011. PMx: software package for demographic and genetic analysis and management of pedigreed populations. *Methods in Ecology and Evolution* **3**:433–437.
- Ladouceur E, Jimenez-Alfaro B, Marin M, De Vitis M, Abbandonato H, Iannetta PPM, Bonomi C, Pritchard HW. 2017. Native seed supply and the restoration species pool. *Conservation Letters* **11**: e12381.
- Lambdon PW, Ellick S. 2016. *Trochetiopsis erythroxyton*. The IUCN red list of threatened species. International Union for Conservation of Nature, Gland, Switzerland. Available from <http://dxdoiorg/102305/IUCNUK2016-1RLTST30560A67371983en> (accessed March 2018).
- Lauterbach D, Burkart M, Gemeinholzer B. 2012. Rapid genetic differentiation between ex situ and their in situ source populations: an example of the endangered *Silene otites* (Caryophyllaceae). *Botanical Journal of the Linnean Society* **168**:64–75.
- Leino MW, Edqvist J. 2010. Germination of 151-year old *Acacia* spp. seeds. *Genetic Resources and Crop Evolution* **57**:741–746.
- Lerman JC, Cigliano EM. 1971. New Carbon-14 evidence for six hundred years old canna compacta seed. *Nature* **232**:568–570.
- Lowe AJ, Gillies ACM, Wilson J, Dawson IK. 2000. Conservation genetics of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis. *Molecular Ecology* **9**:831–841.
- Makowski D, Tomiczak K, Rybczyński JJ, Mikuła A. 2016. Integration of tissue culture and cryopreservation methods for propagation and conservation of the fern *Osmunda regalis* L. *Acta Physiologiae Plantarum* **38**:19.
- Marren P. 2005. Not extinct, only interrupted. *The Plant Talk* **42**:16–17.
- Maschinski J, Albrecht MA. 2017. Center for Plant Conservation's best practice guidelines for the reintroduction of rare plants. *Plant Diversity* **39**:390–395.
- Maunder M, Cowan RS, Stranc P, Fay MF. 2001a. The genetic status and conservation management of two cultivated bulb species extinct in the wild: *Tecophilaea cyanocrocus* Chile and *Tulipa sprengeri* Turkey. *Conservation Genetics* **2**:193–201.
- Maunder M, Culham A, Alden B, Zizka G, Orlic C, Lobin W, Bordeu A, Ramirez JM, Glissmann-Gough S. 2000. Conservation of the toromiro tree: case study in the management of a plant extinct in the wild. *Conservation Biology* **14**:1341–1350.
- Maunder M, Higgins S, Culham A. 2001b. The effectiveness of botanic garden collections in supporting plant conservation: a European case study. *Biodiversity and Conservation* **10**:383–401.
- Maunder M, Hughes C, Hawkins JA, Culham A. 2004. Hybridization in ex situ plant collections: conservation concerns liabilities and opportunities. Pages 325–364 in Guerrant EO, Havens K, Maunder M, editors. 2004. Ex situ plant conservation: supporting species survival in the wild. Island Press, Washington, D.C.
- Merritt DJ, Dixon KW. 2011. Restoration seed banks—a matter of scale. *Science* **332**:424–425.
- Merritt DJ, et al. 2014. A continental-scale study of seed lifespan in experimental storage examining seed, plant, and environmental traits associated with longevity. *Biodiversity & Conservation* **23**:1081–1104.
- Molnár VA, Sonkoly J, Lovas-Kiss Á, Fekete R, Takács A, Somlyay L, Toeroek P. 2015. Seed of the threatened annual legume, *Astragalus contortuplicatus*, can survive over 130 years of dry storage. *Preslia* **87**:319–328.
- Mondoni A, Probert R, Rossi G, Vegini E, Hay FR. 2011. Seeds of alpine plants are short-lived: implications for long-term conservation. *Annals of Botany* **107**:171–179.
- Mounce R, Smith P, Brockington S. 2017. Ex situ conservation of plant diversity in the world's botanical gardens. *Nature Plants* **3**:795–802.
- Navarro A, Perez Rovira P, Oltra JE, Ferrando I, Ferrer-Gallego PP, Escriba MC, Albert FG, Laguna E. 2016. Creating new populations to conserve the endangered *Silene cambessedesii* in the Iberian Peninsula. Pages 262–266 in Soorae PS, editor. Global re-introduction perspectives: additional case-studies from around the globe. International Union for Conservation of Nature Species Survival Commission Re-introduction Specialist Group, Abu Dhabi, United Arab Emirates.
- O'Donnell K, Sharrock S. 2017. The contribution of botanic gardens to ex situ conservation through seed banking. *Plant Diversity* **39**:373–378.
- Pérez Latorre AV, Cabezudo B, Mota Poveda J, Peñas J, Navas P. 2011. *Diplotaxis siettiana* The IUCN red list of threatened species. International Union for Conservation of Nature, Gland, Switzerland. Available from <http://dxdoiorg/102305/IUCNUK2011-1RLTST61631A12529074en> (accessed March 2018).
- Piaggio AJ, et al. 2017. Is it time for synthetic biodiversity conservation? *Trends in Ecology & Evolution* **32**:97–107.
- Probert RJ, Daws MI, Hay FR. 2009. Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* **104**:57–69.
- Püschel TA, Jaime Espejo J, Sanzana M-J, Benítez HA. 2014. Analysing the floral elements of the lost tree of Easter Island: a morphometric comparison between the remaining ex-situ lines of the

- endemic extinct species *sophora toromiro*. PLOS ONE 9 (e115548) <https://doi.org/10.1371/journal.pone.0115548>.
- Ramsay MM. 1998. Re-establishment of the lady's slipper orchid (*Cypripedium calceolus* L.) in Britain. Botanical Journal of the Linnean Society 126:173–181.
- Rivière S, Breman E, Kiehn M, Carta A, Müller JV. 2018. How to meet the 2020 GSPC target 8 in Europe: priority-setting for seed banking of native threatened plants. Biodiversity & Conservation 27:1873–1890.
- Royal Botanic Garden Edinburgh (RBGE). 2017. RBGE guide to collecting herbarium specimens in the field. RBGE, Edinburgh.
- Sallon S, Solowey E, Cohen Y, Korchinsky R, Egli M, Woodhatch I, Simchoni O, Kislev M. 2008. Germination, genetics, and growth of an ancient date seed. Science 320:1464.
- Sandler R. 2013. The ethics of reviving long extinct species. Conservation Biology 28:354–360.
- Sarasan V, Buord S, Pellice J, Sanchez M, Cowan RS, Lesouëf J-Y. 2016. Approaches to develop a road map for the long-term conservation of an island endemic genus *Cylindrocline*. Acta Physiologia Plantarum 38:1–10.
- Schoen DJ, Brown ADH. 2001. The conservation of wild plant species in seed banks. BioScience 51:960–966.
- Sears J. 2011. Re-introduction of stinking hawk's-beard into South-East England, UK. Pages 234–238 in Soorae PS, editor. Global re-introduction perspectives: additional case-studies from around the globe. International Union for Conservation of Nature Species Survival Commission, Re-introduction Specialist Group, Abu Dhabi, United Arab Emirates.
- Seddon P. 2017. The ecology of de-extinction. Functional Ecology 31:992–995.
- Seddon P, Moehrenschrager A, Ewen J. 2014. Reintroducing resurrected species: selecting de-extinction candidates. Trends in Ecology & Evolution 29:140–147.
- Shapiro B. 2017. Pathways to de-extinction: How close can we get to resurrection of an extinct species? Functional Ecology 31:996–1002.
- Shen-Miller J, Mudgett MB, Schopf JW, Clarke S, Berger R. 1995. Exceptional seed longevity and robust growth: ancient sacred lotus from China. American Journal of Botany 82:1367–1380.
- Shiga T. 2013. A relationship among curator, collector, and user surrounding natural history specimens: toward a better specimen preservation, collection and usage. Japanese Journal of Ecology 63:375–383.
- Tanaka N, Ono H, Nagata S. 2015. Floral visitors of *Eriocaulon beleocharioides* (Eriocaulaceae), an extinct aquatic species in the wild. Bulletin of the National Museum of Natural Sciences, Series B 41:179–182.
- Theaker AJ, Briggs D. 1993. Genecological studies of groundsel *Senecio vulgaris* L. IV Rate of development in plants from different habitat types. New Phytologist 123:185–194.
- Thiers B. 2018. Index herbariorum. New York Botanical Garden, New York. Available from <http://sweetgum.nybg.org/science/ih/> (accessed March 2018).
- Volis S. 2017. Conservation utility of botanic garden living collections: setting a strategy and appropriate methodology. Plant Diversity 39:365–372.
- Volis S, Blechner M. 2010. Quasi in situ: a bridge between ex situ and in situ conservation of plants. Biodiversity & Conservation 19:2441–2454.
- Walck J, Dixon K. 2009. Time to future-proof plants in storage. Nature 462:721.
- Walters C, Berjak P, Pammenter N, Kennedy K, Raven P. 2013. Preservation of recalcitrant seeds. Science 339:915–916.
- Walters C, Wheeler LM, Grotenhuis JM. 2005. Longevity of seeds stored in a genebank: species characteristics. Seed Science Research 15:1–20.
- Walters C, Wesley-Smith J, Crane J, Hill LM, Chmielarz P, Pammenter NW, Berjak P. 2008. Cryopreservation of recalcitrant i.e. desiccation-sensitive seeds. Pages 465–484 in Reed BM, editor. Plant cryopreservation: a practical guide. Springer, New York.
- Wang B, Ma Y, Chen G, Li C, Dao Z, Sun W. 2016. Rescuing *Magnolia sinica* (Magnoliaceae), a critically endangered species endemic to Yunnan, China. Oryx 50:446–449.
- Wilson WD, Hutchinson JT, Ostrand KG. 2017. Genetic diversity assessment of in situ and ex situ Texas wild rice *Zizania texana* populations, an endangered plant. Aquatic Botany 136:212–219.
- World Conservation Monitoring Centre. 1998. *Sophora toromiro*. The IUCN red list of threatened species. Available from <https://www.iucnredlist.org/species/30392/9535225#conservation-actions> (accessed October 2018).
- Wyse SV, Dickie JB, Willis KJ. 2018. Seed banking not an option for many threatened plants. Nature Plants 4:848–850.
- Yashina S, Gubin S, Maksimovich S, Yashina A, Gakhova E, Gilichinsky D. 2012. Regeneration of whole fertile plants from 30,000-year-old fruit tissue buried in Siberian permafrost. Proceedings of the National Academy Science 109:4008–4013.
- Zhang JJ, Ye Q-G, Yao X-H, Huang H-W. 2009. Spontaneous interspecific hybridization and patterns of pollen dispersal in ex situ populations of a tree species (*Sinojackia xylocarpa*) that is extinct in the wild. Conservation Biology 24:246–255.