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A pragmatic and prudent consensus on the resurrection of extinct plant species using herbarium specimens

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Abstract

Herbaria may represent remarkable sources of viable diaspores for recovering lost genetic variation and extinct plant species, but the application of rewilding extinct species using these collections has not been explored in detail. De-extinction in plants may be achieved by germinating viable diaspores or culturing tissues preserved in herbarium specimens. Germination of old diaspores (fruits, seeds, spores) preserved in sub-optimal uncontrolled storage conditions demonstrates that the recovery of extinct plants from herbarium specimens may be possible. Plant de-extinction via herbarium specimens relies on the availability of samples containing viable diaspores, and on the inclination of curators and the conservation community to use such material for this purpose. We developed an internet-based survey to assess (i) whether the scientific community would consent to the use of herbarium specimens of extinct species to attempt de-extinction, and (ii) the limitations of removing diaspores from specimens. Despite the risk of potential damages to valuable specimens from historical collections when harvesting diaspores, a consensus for using specimens of extinct plant species emerged. Most respondents would permit the collection of a low number of diaspores, preferably from duplicate specimens and only if the integrity of the specimen is preserved. These considerations would be more restrictive for type specimens and those of historical value. These results help to formalise a decision framework for the grant and use of material from natural history collections and a pragmatic approach to attempt to resurrect extinct species from herbarium specimens.

Keywords
biodiversity loss; herbaria; natural history collections; old diaspores; seed longevity; species resurrection.

INTRODUCTION

De-extinction is defined by the IUCN SSC (2016) as the creation of a proxy or functionally equivalent of an extinct species and is considered a potential conservation tool for restoring biological diversity and lost ecological functions (Shapiro, 2017).
Besides the abovementioned benefits, de-extinction poses several concerns like decreased support for preventing extinctions, uncertainty about invasiveness, unexpected resurrection of ancient pathogens, hybridisation, ethical and socio-economic impacts, and re-extinction (IUCN SSC, 2016). In animals, de-extinction may be achieved e.g., via cloning, back-breeding and/or genetic engineering (Shapiro, 2017), which make its application difficult and poses several ethical concerns (Slater&Clatterbuck, 2018). Recovery of extinct plant species, however, is comparatively easier to perform than animal species. It is (theoretically) possible to recover an extinct plant by germinating old diaspores (i.e., fruits, seeds, spores) and plant tissue culturing (Abeli & al., 2020). In this paper, we, therefore, use the term “de-extinction” to indicate the process of recovering true extinct species (not proxies), without including the release of resurrected material in the wild, which falls under the domain of translocation ecology (Abeli & al., 2020; Dalrymple & al., 2020).
There are several examples of old diaspores, mainly seeds, germinating after hundreds or thousands of years. Impressive examples are represented by Lupinus arcticus S.Watson and Silene stenophylla Ledeb. from the Pleistocene (Porsild & al., 1967; Yashina & al., 2012), 2000-year-old seeds of Phoenix dactylifera L. (Sallon & al., 2008), and several other seeds germinated after more than 100 years of sub-optimal uncontrolled storage conditions (e.g., Daws & al., 2007; Molnár & al., 2015). Although none of the ancient seeds germinated so far belonged to an extinct species (Molnár & al., 2015), the germination of old seeds demonstrates that recovering extinct plants may be possible.
Herbaria, with about 390 million specimens preserved worldwide (Thiers, 2020), represent remarkable sources of viable diaspores that can be used for recovering lost genetic variation (Bowles & al., 1993; Godefroid & al., 2011; Magrini, 2011; Nakahama & al., 2015) and facilitate the de-extinction of plant species lost to anthropogenic threats (Albani Rocchetti & al., 2021), in compliance with international and local regulation (i.e., the Convention on Biological Diversity [UNEP, 1992] and the Nagoya Protocol [SEB, 2011]), which application differs at the national level (Sherman & Henry, 2020).
To realise the potential of herbaria for plant de-extinction, it is necessary: (i) to assess the actual presence and quality (i.e., bearing viable diaspores) of specimens of extinct plants in herbaria; and (ii) to verify whether, and with what stipulations, herbarium curators and the scientific community (conservation biologists in primis) would agree to the use of preserved specimens of extinct species to attempt de-extinction. Regarding the first topic, a preliminary search in digitised herbaria (accessible at GBIF, JACQ, Intermountain Biota) showed that there are at least 217 specimens of 65 plant species (out of 345 extinct species) bearing seeds (G. Albani Rocchetti, in prep.). This suggests that the potential of herbaria for de-extinction is not only theoretical and that further research on the opportunity to use diaspores from these specimens is necessary.
Therefore, our study aims to address the latter topic through an internet-based opinion survey of herbarium curators and experts. More specifically, the study aims to assess the general viewpoint on plant de-extinction using herbarium material in relation to the position and expertise of respondents, and opinion on collecting material from different kinds of herbarium specimens (e.g., nomenclatural types, duplicates, specimens of historical value, etc.). This study also aims to

propose a novel decision tree for granting access to diaspores from natural history collections. Implications of this study are crucial to evaluate whether the de-extinction of plant species from archived herbarium specimens can proceed further or should be abandoned.

MATERIALS AND METHODS

Our survey was developed in collaboration with curators of several Italian herbaria. The 12-question survey was anonymous and constructed in Google Forms (suppl. Appendix S1). Questions 1 to 9 represented the core of our survey and aimed at investigating the respondents' opinion on the importance of herbaria in different research fields and specifically on the collection of diaspores to attempt de-extinction. Questions 10 to 12 aimed at making an anonymous profile of the respondents (i.e., age, professional position, and field of expertise). Since questions 10 to 12 could have more than one answer, the total responses exceeded 100%. Answers were analysed by pooling responses from all respondents. Additionally, to highlight different points of view among conservation biologists and herbarium curators, answers to questions 2 to 8 were also analysed separately for respondents that declared to be conservation biologists (only), herbarium curators (only), and those who declared to be both.
The poll completion required about 10 minutes (see suppl. Appendix S1 for the complete survey). The survey was sent to: (i) the entire mailing list of INDEX Herbariorum (http://sweetgum.nybg.org/science/ih/herbarium-list/); (ii) the mailing lists of the following network of seed banks, herbaria, and botanic gardens: CORIMBO (Coordinamento della Rete Italiana dei Musei Botanici), ENSCONET (The European Native Seed Conservation Network), GENMEDA (The Network of Mediterranean Plant Conservation Centres), RIBES (Rete Italiana Banche del Germoplasma), and SBI (Società Botanica Italiana); (iii) a list of personal contacts of the authors, who were asked to complete the survey and also to forward to additional contacts for completion. For this reason, the exact number of people contacted is unknown, which makes it difficult to calculate the percentage of survey responders. An approximate response rate of 24% was calculated based on the number of responses received (462) and the number of e-mail requests sent directly by the authors (1,928). The survey was made available for two months, from 16 January to 16 March 2020. Most of the questions were quiz-like, except for questions 2, 3, 9, 11, and 12, which allowed short, open answers (suppl. Appendix S1).

RESULTS

Profile of the respondents. — Nearly 60% of respondents were curators of collections (i.e., herbaria, museums, botanic gardens, and/or seed banks), 47% were researchers or professors. Moreover, 18%declared to be both curator and researcher/professor. Most respondents declared to be botanists (60%), followed by taxonomists (31%), ecologists (26%) and conservation biologists (24%) (Fig. 1). Other Categories were less represented (Fig. 1).
Opinions concerning the use of herbaria for de-extinction.— Answers to the first question indicated that herbarium specimens are considered most important for phytogeography (90% of respondents assigned "important" to "very important" values), followed by history & ethnobotany (86.4%), global change biology (82%), genetics (81%), plant conservation (80.3%), ecology (77.3%), and de-extinction (55.2%) (Fig. 2A). Moreover, 80% of respondents would agree to permit diaspore collection from specimens of extinct species for de-extinction purposes, although with some restrictions (Fig. 3 Q2). Forty-five per cent of respondents would agree to diaspore collection only from duplicates (i.e., part of a single gathering of a single species or infraspecific taxon, e.g., isotypes) and other original material (i.e., syntypes, paratypes), 8% would allow diaspore collection but only: (i) if the number of specimens/diaspores available is considered adequate through a careful evaluation of the specimen, (ii) if the extinction dynamics suggest that de-

extinction is justified, or (iii) if there is a chance of germinating the diaspores. In contrast, only 5% of respondents would not agree to collect any diaspores (Fig. 3 Q2).

The percentage of respondents supporting diaspore collection decreases to 66% when the sampling involves type specimens (Fig. 3 Q3). In particular, 22%of respondents would not allow collection from holotypes but would allow collection from other type specimens providing sufficient material is available (Fig. 3 Q3).

Further insight on the general opinion of diaspore removal is given by answers to questions 4 and 5: 68% of respondents would be favourable to remove only 1 or 2 diaspores from a specimen bearing few diaspores and only 3% of respondents would permit the removal of all diaspores. Nearly 25% of respondents would not permit the removal of any diaspores (Fig. 3 Q4). In contrast (question 5), in the case of specimens bearing spare or detached diaspores, most respondents would permit the removal of between 10% and 75% of spare diaspores (Fig. 3 Q5). When more specimens of an extinct species are available, most respondents would permit the collection of 10%–25%of available diaspores (Fig. 3Q6). In contrast, when a single specimen of an extinct species is available, 18% of respondents would not permit the collection of any diaspore, and most respondents (56%)would permit the collection of less than 10% of available diaspores (Fig. 3 Q7).

Regarding the historical importance of herbarium specimens (Fig. 2B), most respondents would not agree to permit collection from older specimens (17% for 19th century and 26% for 18th century), 29% of respondents would not remove diaspores from specimens collected by eminent scientists and explorers, while only 6% would not permit the collection of diaspores from 20th century specimens.

Comparison between responses from conservation biologists and herbarium curators. — Answers to questions 2 to 8 provided by curators and conservation biologists are reported in Table 1. These data suggest a more cautious approach advocated by curators versus conservation biologists. Further differentiation is seen by curators versus conservation biologists when type material is involved (Table 1 Q3). Most curators would permit the collection of less than 25% of available diaspores, whilst most conservation biologists would permit up to 50% of diaspores (Table 1 Q5, Q6). Regarding the use of historical collections, similar restrictions to other specimens were proposed.

DISCUSSION

Recovery of extinct plants from herbarium specimens relies on the availability of specimens containing viable diaspores and on the inclination of curators and the conservation community to use such material for the purpose. Here, we investigated the latter aspect to clarify potential impediments to the initial use of source plant material preserved in herbaria for de-extinction. A key point to emerge in our assessment is the risk of damaging valuable specimens when collecting diaspores, without any certainty of success in extinct species recovery.

An emerging interest and the general consensus. — Although respondents were inclined to consider using herbarium specimens for de-extinction less important than more traditional uses, it is nevertheless interesting to note that more than 50% of the respondents considered this application important or very important. Indeed, herbaria have recently gained relevance for the conservation of plants, with applications ranging from climate change effects to exploitation analyses (Meineke & al., 2018; Albani Rocchetti & al., 2021). Similarly, specimen digitisation is creating a wealth of new and expanded uses of these precious resources (Hedrick & al., 2020). It could become a part of sampling protocols to conserve images of the specimen before and after propagule sampling, damage, or modification, as suggested by two respondents. Thus, our survey highlights the interest of scientists, curators, and other conservation experts to use herbarium specimens to resurrect extinct plant species.

Such interest is not only theoretical, but it concretises in a prudent consensus in the use of diaspores contained in herbarium specimens to attempt germination and recovery of extinct plant species.

Answers to our survey revealed some caution in using herbarium material for de-extinction purposes, especially when old and valuable specimens are involved. From the answers to questions 3 to 7, most respondents posed some restrictions on removing diaspores. Most respondents would agree to allow the collection of a low number of diaspores, preferably from duplicate specimens (especially if nomenclatural types are involved), and only if the integrity of the specimen is preserved. There is a consensus also in not allowing the use of type specimens and specimens of historical value, especially holotypes. In particular, a sampling from the latter type of category would be admitted only by seven respondents, who however would allow sampling from other type categories. Thirty per cent of respondents would not permit removal from any specimen dating from the 19th century or before.

Considerations on plant species characteristics, specimen preservation, and diaspore viability. — An important aspect emerging from our survey is the concern that diaspores in older specimens may not be viable and, in any case, germination potential may be low or null (e.g., Porteous & al., 2019). Reduced seed viability due to age is inevitable (Bewley & al., 2013). For orthodox seeds (i.e., desiccation-tolerant seeds), the longevity in storage may vary considerably across species (Walters & al., 2005), decreasing predictably with increasing temperature and moisture content (Ellis & Roberts, 1980). Furthermore, there are important correlates of seed longevity with the climate of species origin (Probert & al., 2009; Mondoni & al., 2011), embryo size (Probert & al., 2009), and seed dispersal syndrome (Merritt & al., 2014). On the other hand, desiccation-sensitive (recalcitrant) seeds do not survive drying; therefore, they are intrinsically short lived (Walters & al., 2013). Consequently, based on these pieces of evidence, we hypothesise that plants from naturally dry habitats, with large embryos and little endosperm may provide more chances for de-extinction success. Moreover, specimens collected before the 1970s have often been treated with DNA-damaging substances for pest control (e.g., mercury chloride, etc.). Therefore, while diaspores from herbarium specimens may still be able to germinate, they may not fully mature into seedlings. For instance, Godefroid & al. (2011) recorded radicle germination, but no shoot emergence, in old seeds of Bupleurum tenuissimum L., a species extinct in Belgium. Nevertheless, it has been demonstrated that germination of old seeds can occur after decades of nonoptimal storage conditions (Molnár & al., 2015 and references cited therein). Some respondents suggested that the collections of extinct species'diaspores should be allowed only if a germination protocol already exists, at least among closely related species. This important consideration should stimulate more in-depth research on plant species with phylogenetic proximity to extinct species targeted for de-extinction.

From our comparison between the responses of herbarium curators versus conservation biologists, substantial agreement in the use of herbarium specimens for de-extinction emerged. Conservation biologists are more prone to permit the collection of diaspores from specimens of extinct species than curators; curators tend to be more cautious in the percentages of diaspores that can be removed, especially when type specimens are involved.

Decision framework for the use of diaspores from natural history collections.—To date, international and national protocols for regulating disruptive sampling from natural history collections (e.g., herbaria, fruit collections) do not exist, although some local attempts have been done (e.g., Teece & al., 2002; Herbarium Sample Collection Protocol of the Florida Institute of Technology, https://research.fit.edu/applied-biogeography/protocols/; DNA sampling protocol of the National Herbarium Nederland, https://www.wur.nl/).We, therefore, propose a novel decision tree (Fig. 4) based on the results obtained from our survey, with the aim of providing the first

guideline for evaluating the grant and use of diaspores from natural history collections, especially herbaria. This framework can be applied both in the case of sampling for general research purposes and specific conservation actions. For the latter case, prior evaluation of the availability of other propagules sources (i.e., living plants in the wild, in botanic gardens, propagating tissue culture, and diaspores stored in germplasm banks) shall be performed to assess the current state of conservation of the target species or population. The use of alternative sources should have priority over natural history collections sampling, due to the disruptive nature of this action, the rarity and importance of many collections, and the higher probability of success when using seed bank and/or botanic garden material.

Biological requirements shall also be evaluated, as diaspores' viability depends on its storage behaviour and longevity: species that produce orthodox, long-lived diaspores should have priority over species with recalcitrant/intermediate, and short lived diaspores, as storage conditions of herbaria are not ideal for germplasm conservation, which instead requires lower humidity and temperature conditions (10%–20% humidity at _20°C for long-term storage; FAO, 2013). Finally, specific features and availability of the specimens shall be considered on a single-case basis: as suggested by our respondents, diaspore collection from duplicate material, recent (after 20th century) and bearing multiple diaspores is less problematic than collecting from type and original material, ancient specimens and specimens collected by eminent scientists. Moreover, we propose that in the first case up to 25% of the available diaspores could be collected, while in the latter case the diaspores sampling should not exceed 10% of the available material.

Final considerations. — In conclusion, a pragmatic and prudent consensus in using herbarium diaspores for de-extinction suggests that research on the recovery of extinct plants should proceed. The conditions and limitations of the use of herbarium material for plant de-extinction that emerge from our study represent an approach that minimises the risk to specimens and the loss of valuable material without precluding research on de-extinction.

In summary:

- In the absence of guidelines for selecting candidates for plant de-extinction (in contrast to animal species; Seddon & al., 2014), specimens for diaspore collection should be evaluated case by case, with the limitation of damages to valuable specimens as a priority.
- The development of techniques that facilitate the identification of viable diaspores before they are detached from aspecimen would greatly improve the applicability of plant de-extinction. In this regard, non-destructive methods for accurate seed quality assessment already exist (e.g., spectroscopy, hyperspectral imaging, thermal imaging, electronic nose, and soft X-ray imaging, see Rahman & Cho, 2016). However, most of these techniques are calibrated for only one or a few (usually crop or other economically important) species and are unlikely to be useful across the wide diversity of physical and chemical features of the propagules of extinct species.
- The ecological reasons behind de-extinction are even more important than their technical feasibility. A few respondents pointed out that de-extinction should not be attempted if the original habitat of a species has been totally altered or destroyed. We highlight, however, that de-extinction may not necessarily imply translocation or that resurrected individuals constitute an ecologically functional species. The recovery of an extinct plant species may have important implications for biodiversity conservation, even if reintroduction is not attempted (Dalrymple&Abeli, 2019). A recovered species may contribute to improving techniques and technologies to reverse biodiversity loss, to understand ecological and evolutionary processes, and to educate, even if only cultivated in a botanic garden.

- If de-extinction is attempted to reintroduce a species, one of the main issues includes the minimum number of propagules necessary for achieving a true recovery/restoration of a species, as the number of available or usable propagules in herbarium specimens is limited. Although it is difficult to indicate a minimum number of propagules that should be resurrected, some successful reintroductions started from one or few individuals. For instance, Symonanthus bancroftii (F.Muell.) Haegi was successfully reintroduced from a single male and a female plant (He & al., 2007); Cyanea superba Cham. was reintroduced from seeds collected from the last wild plant, which died in 2002 (Adamski & al., 2020). Finally, as most of the currently extinct species disappeared due to habitat loss, they might only be translocated if their original habitats are restored. However, the release of species lost long ago may impact the recipient habitats in an unpredictable way; these risks must be carefully evaluated (Genovesi & Simberloff, 2020).

AUTHOR CONTRIBUTIONS

GAR, TA and GC conceived the idea and wrote the manuscript, GAR analysed the data. All the other authors contributed to the development and distribution of the survey and contributed to data interpretation and to the text.—GAR, https://orcid.org/0000-0001-7361-9179; CD, https://orcid.org/0000-0001-8747-1101; GC, https://orcid.org/ 0000-0002-0551-7100; GB, https://orcid.org/0000-0002-1714-3978; GFa, https://orcid.org/0000-0003-1817-7966; GFe, https://orcid.org/00 00-0003-4762-5043; BF, https://orcid.org/0000-0001-6451-4025; GG, https://orcid.org/0000-0002-2501-456X; DG, https://orcid.org/0000-0003-4677-3730; GGdG, https://orcid.org/0000-0003-4719-3711; MI,https://orcid.org/0000-0002-2771-2935; SM, https://orcid.org/0000-0002-7966-7574; AMa, https://orcid.org/0000-0002-5124-7631; AMo, https://orcid.org/0000-0002-4605-6304; SO, https://orcid.org/0000-0003-0348-9115; LP, https://orcid.org/0000-0001-9008-273X; TA, https://orcid.org/0000-0003-3096-2035

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Table 1. Answers to questions 1 to 8.

	Q1. In your opinion, in addition to systematics, taxonomy and nomenclature, how important are herbarium specimens in the following areas? (from 1 = not important, to 5 = very important)						
	De-extinction	Ecology	Genetics	Global change biology	History and ethnobotany	Phytogeography	Plant conservation
1	6%	2%	3%	2%	2%	2%	3%
2	16%	5%	3%	5%	3%	1%	4%
3	23%	16%	13%	11%	8%	7%	12%
4	19%	30%	29%	23%	21%	15%	25%
5	36%	48%	52%	59%	66%	75%	55%

	Q2. Considering that it is not possible to know *a priori* the viability of diaspores preserved in a herbarium specimen, would you agree to collect diaspores from specimens of an extinct species with the aim to try de-extinction?			
	Yes	Yes, but only from its duplicates, if any	No	It depends
HC	43%	41%	3%	13%
CB	61%	32%	5%	2%
CC	46%	43%	5%	7%

	Q3. Considering that diaspore removal from herbarium specimens implies a partial damage to the sample, would you consider acceptable diaspore removal from type specimens and other original materials specimens?		
	Yes	No	It depends
HC	38%	34%	28%
CB	71%	11%	18%
CC	33%	36%	31%

	Q4. In the case of a herbarium specimen holding very few diaspores (i.e., 1–5), which number of diaspore removal would you consider acceptable?			
	None	1–2	3–4	5
HC	24%	69%	5%	2%
CB	11%	78%	11%	0%
CC	17%	71%	6%	5%

	Q5. In the case a herbarium specimen bears spare/detached diaspores (i.e., removal does not imply a damage to the specimen), which percentage of diaspore removal would you consider acceptable?					
	None	<10%	10%–25%	25%–50%	50%–75%	75%–100%
HC	1%	24%	38%	21%	12%	6%
CB	4%	16%	22%	31%	11%	16%
CC	0%	29%	27%	17%	21%	6%

	Q6. Considering that diaspore removal from herbarium specimens implies a partial damage to the sample, in the case several herbarium specimens containing diaspores of an extinct plant species are available, which percentage of diaspore removal would you consider acceptable?					
	None	<10%	10%–25%	25%–50%	50%–75%	75%–100%
HC	3%	32%	36%	18%	9%	3%
CB	4%	18%	27%	29%	13%	9%
CC	3%	30%	33%	17%	8%	8%

	Q7. Considering that diaspore removal from herbarium specimens implies a partial damage to the sample, in the case a single herbarium specimen containing diaspores of an extinct plant species is available, which percentage of diaspore removal would you consider acceptable?					
	None	<10%	10%–25%	25%–50%	50%–75%	75%–100%
HC	15%	48%	25%	7%	4%	2%

(Continues)

Table 1. Continued.

	Q7. Considering that diaspore removal from herbarium specimens implies a partial damage to the sample, in the case a single herbarium specimen containing diaspores of an extinct plant species is available, which percentage of diaspore removal would you consider acceptable?					
	None	<10%	10%–25%	25%–50%	50%–75%	75%–100%
CB	13%	27%	27%	20%	11%	2%
CC	22%	33%	21%	11%	6%	6%

	Q8. In the case of the following different types of historical herbarium specimens, would you consider acceptable a partial damage of the specimens for diaspore removal?			
		Yes	Yes, but only from its duplicates, if any	No
Before 1800	HC	25%	44%	31%
	CB	29%	56%	16%
	CC	24%	49%	27%
Between 1800 and early 1900	HC	31%	47%	22%
	CB	40%	51%	9%
	CC	29%	56%	16%
After 1900	HC	48%	46%	6%
	CB	60%	51%	2%
	CC	44%	49%	6%
Collection of eminent people	HC	23%	45%	32%
	CB	27%	51%	22%
	CC	22%	51%	27%

Answers to question 1 provided by all respondents, to questions 2–8 provided by herbarium curators (HC), conservation biologists (CB) and respondents that declared to be both (CC). Number of answers: HC = 199, CB = 45, CC = 63.

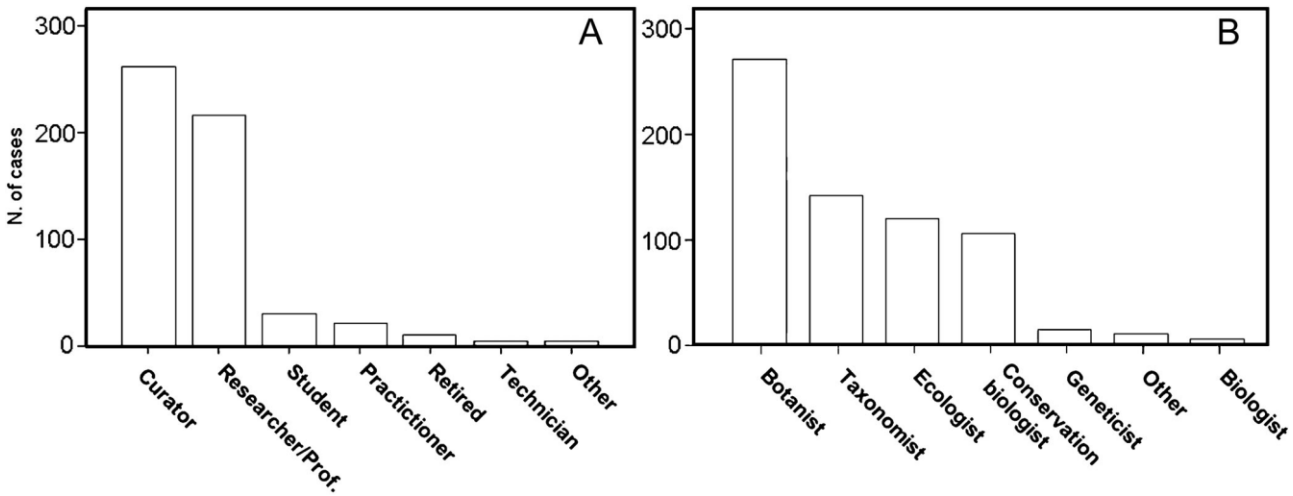


Fig. 1. Professional profile of respondents. A, Current role; B, Field of expertise.

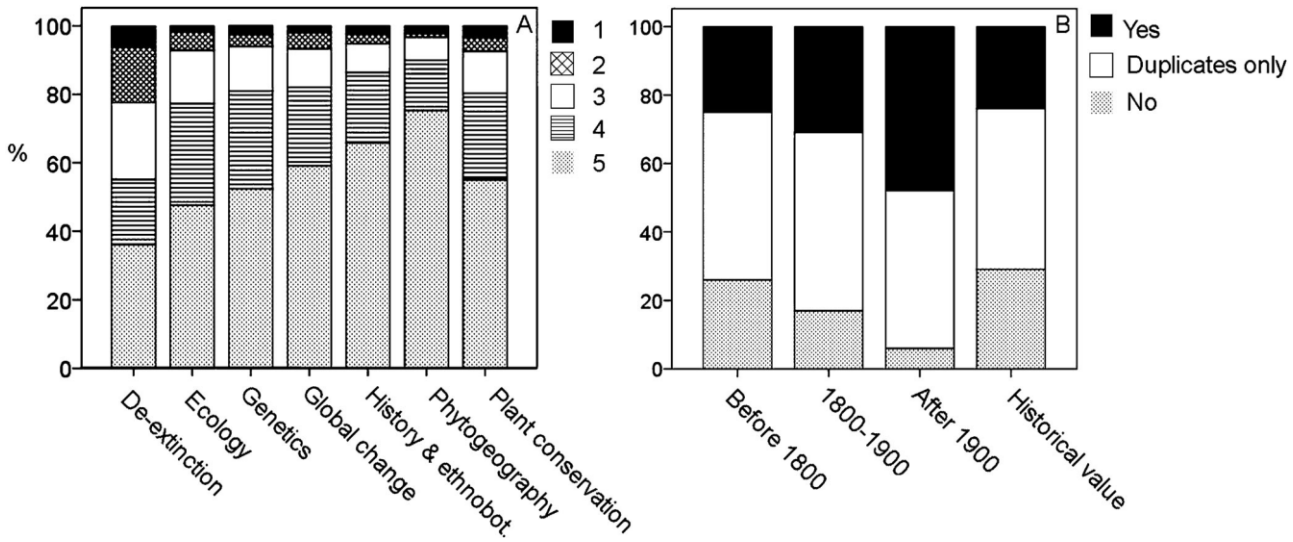


Fig. 2. Answers to questions 1 and 8. A, Answers to question 1 on the importance of herbaria for seven main research fields. 1 = not important at all, 5 = very important; B, Answers to question 8 on the agreement to collect diaspores from herbarium specimens of historical importance.

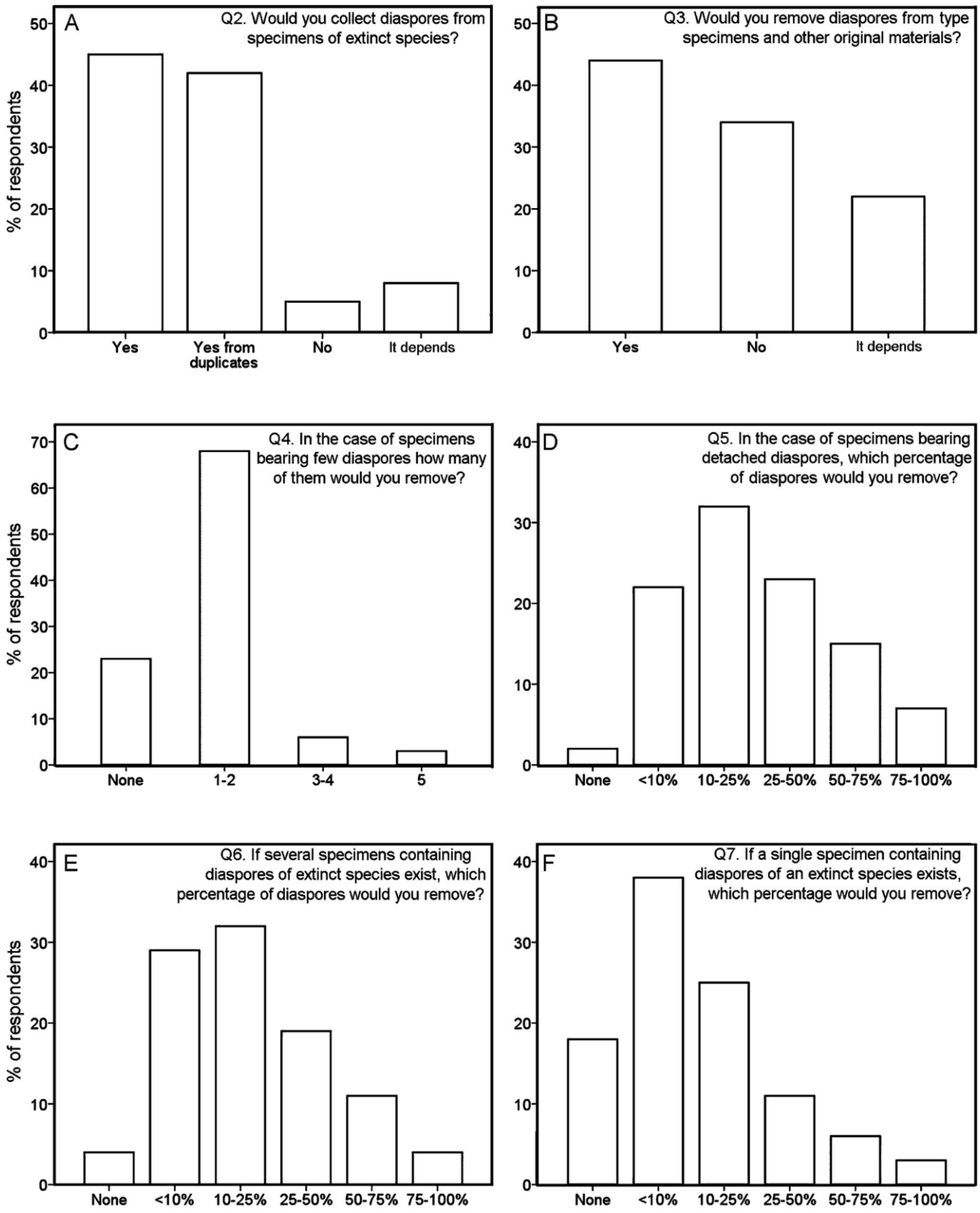


Fig. 3. Respondents' answers to questions 2 to 7. Note that questions reported in the graph were contracted to fit the graph size. The original questions are available in suppl. Appendix S1.

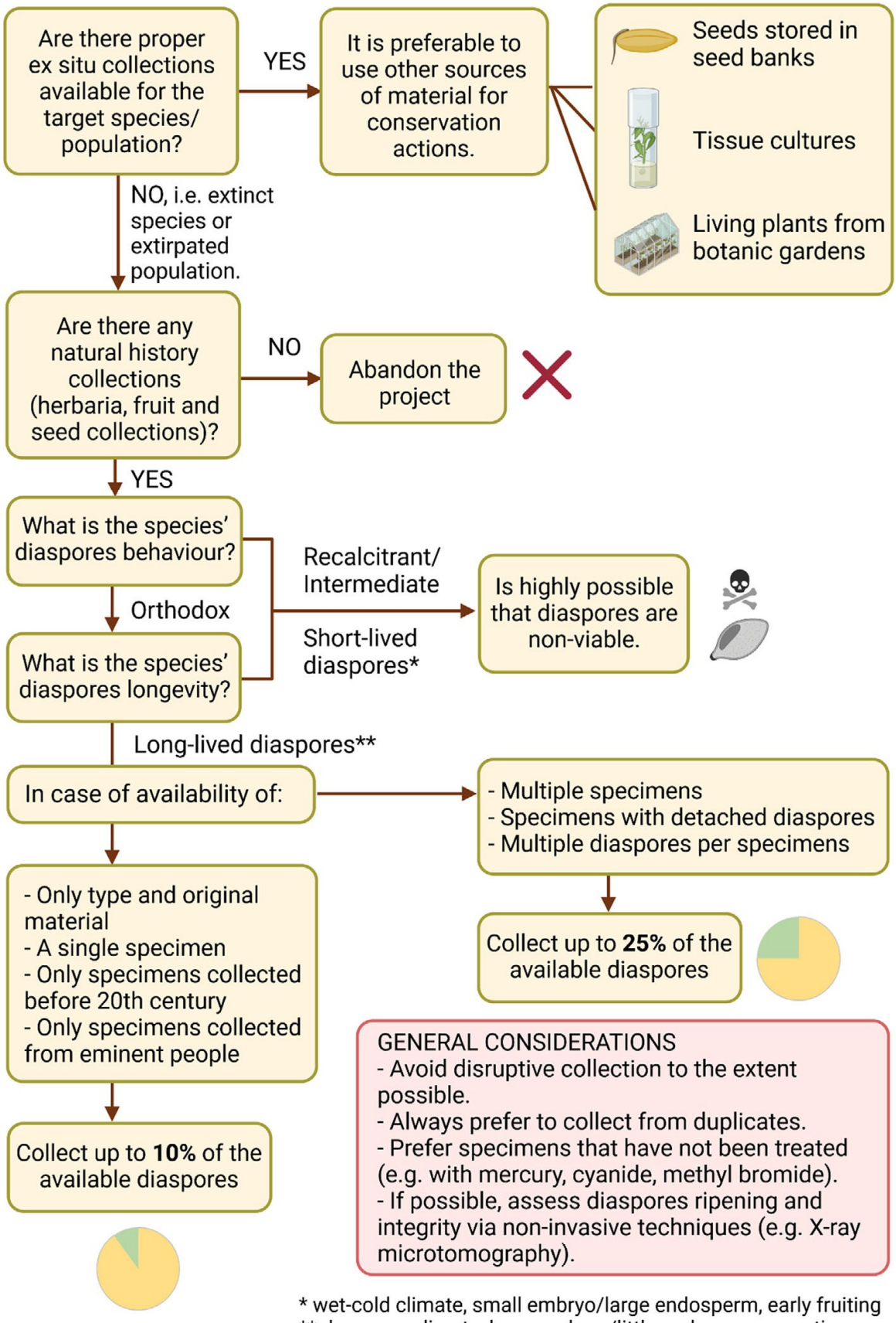


Fig. 4. Decision tree. Framework for evaluating the grant and use of diaspores from natural history collections, especially herbaria.