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SPECIAL ISSUE: STANDARDS FOR NATIVE SEEDS IN ECOLOGICAL RESTORATION

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Cover: Top left: Seed collection of Buriti (Mauritia flexuosa by indigenous collectors of the Xavante ethnicity for the Xingu Seed Network in the Southeastern Amazon, Brazil (photo credit: Rogério Assis); **Bottom left:** Germination on agar on Petri dish of Eucalyptus todtiana seeds, species native to Western Australia (photo credit: Kingsley Dixon); **Right:** Propagation of collected seed material at the Agricultural Research and Education Centre, Raumberg-Gumpenstein, Austria (photo credit: Bernhard Krautzer).

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THANK YOU NOTE

We gratefully acknowledge that this Special Issue was conceived and developed by the Board and membership of the International Network for Seed Based Restoration (INSR), a section of the Society for Ecological Restoration. INSR also acknowledges inputs into the documents' structure and content from many individuals and organisations with special mention of the guidance provided by ISTA through Mr Gil Waibel. The publication of this Special Issue was made possible through support of the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (ICI150100041).

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SHORT COMMUNICATION

Foreword: International Standards for Native Seeds in Ecological Restoration

Adam T. Cross[1,2], Simone Pedrini[1], Kingsley W. Dixon[1]

Restoration practitioners must increasingly incorporate seed procurement models and seed use planning early in project development, despite insufficient guidance about what are reasonable expectations for the sourcing and use of native seeds. This special issue presents a series of articles examining each key step in the native seed supply chain, and provides a framework for the "standards" that need to be applied to native seed batches if the native seed supply chain is to achieve the levels of reliability and transparency required. These Standards provide seed buyers, end users, and funding bodies with a level of confidence and reliability in the sourcing of quality native seeds, and a pathway toward global best practice in native seed use.

Key words: ecological restoration, seed biology, seed collection, seed dormancy, seed storage, rehabilitation

Global initiatives in ecological restoration and forest landscape restoration (as defined in the International Standards for the Practice of Ecological Restoration; Gann et al. 2019) are increasing in both number and scale. Native seeds are the foundation of many ecological restoration projects (Nevill et al. 2018), and as the scale of restoration projects continue to increase, so too the demand for large quantities of native seeds is expected to grow. While the specific seed requirements of individual projects and initiatives will vary depending upon geographic location and land use context, the efficient and effective use of native seeds is a cornerstone of ecological restoration (Kirmer et al. 2012; Erickson et al. 2017). However, the success of restoration projects continues to be constrained by seed-related factors including limited seed availability, highly variable and often poor seed quality, inappropriate seed storage conditions, and low rates of seedling establishment in the field (e.g. Turner et al. 2006; James et al. 2013).

It is clear that the sustainable collection or procurement of native seeds in the required volumes and diversity for ecological restoration projects represents a significant constraint for restoration practitioners around the world (Merritt & Dixon 2011; Menz et al. 2013; Nevill et al. 2018). Additionally, the high rates of seed wastage associated with suboptimal native seed use are a major economic constraint, as the cost of native seeds can be considerable (Merritt & Dixon 2011; Nevill et al. 2018; Masarei et al. 2019). It is becoming evident that successful restoration requires practitioners incorporate seed procurement models and seed use planning into restoration projects at the earliest stages to ensure that seed demands can be matched by seed supply. Such planning may be extremely complex, accounting for many factors including seasonal variability in local climate and plant phenology, and may need to be undertaken on a case-by-case basis as the required seed volumes and species diversity of seed mixes will be dependent upon the scale and requirements of each project site.

To meet the demand for native seeds there is a push to develop native seed supply chains that are reliable, sustainable, and transparent. We need standardized expectations and terminology and consistent methodologies to ensure that different restoration projects can source adequate quantities of native seeds. Such seeds need to reflect appropriate origin and diversity with native seed batches processed, stored, and treated (dormancy release, seed enhancement technologies) to make every seed count, and to ensure that seeds are delivered to the right location at the most appropriate time. These factors are often unclear or poorly defined: What constitutes "native" for a given ecological restoration project? What is the right source of seeds for the requirements of a particular site? How can native seeds be collected and produced in a sustainable manner? What are the most reliable methods for testing the quality of native seed batches and how should "quality" be defined for native seeds? Which seed enhancement options are available, and what is the most appropriate or effective for the needs of a particular project? When is the most appropriate time to sow native seeds?

Seed use in the agricultural and forestry sectors is governed by regulatory seed standards. These standards offer internationally recognized seed testing practices (e.g. AOSA 2019; ISTA 2019),

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and provide buyers with confidence of the quality (purity, viability, germinability, and genotype) of purchased seeds. However, comparable standards guiding the collection, production, quality testing, storage, and sale of native seeds are lacking in most countries. This leaves little by way of guidance for seed users about what are reasonable expectations for the quality of native seed batches. This extends to the supply of information by seed suppliers about the type and alleviation of seed dormancy, methods to promote seed germination, and seed enhancements to optimize seed sowing and seedling establishment.

There is a clear need for unambiguous guidance around the supply and use of native seeds destined for global restoration programs (Fig. 1). This special issue addresses that need, by presenting a series of overview articles on topics relevant to industry, restoration practitioners, and regulators. The overview articles examine each key step in the native seed supply chain: (1) seed sourcing and procurement models (Erickson & Halford 2020); (2) the fundamentals of native seed collection from natural populations and the establishment of seed production systems (Pedrini et al. 2020); (3) established practices and protocols for cleaning, processing, and assessing the quality of native seeds (Frischie et al. 2020); (4) methodologies for short- and long-term seed storage, and for determining the longevity and quality of stored seed collections (De Vitis et al. 2020); (5) an overview of seed dormancy classification, with examples of how dormancy alleviation techniques can be applied at scale for restoration projects (Kildisheva et al. 2020); (6) how seed enhancement technologies can improve the efficiency of native seed use (Pedrini et al. 2020); and (7) strategies, considerations, and current technologies in delivering seeds to site at field scales (Shaw et al. 2020). The information presented in each of the overview articles then builds the framework of the final synthesis article that details the "standards" that need to be applied to native seed batches if the native seed supply chain is to achieve a level of reliability and transparency (see "Principles and Standards for Native Seed in Ecological Restoration"; Pedrini & Dixon 2020). This synthesis document provides seed users with practical tools to develop and structure seed supply systems, and aims to provide restoration practitioners with standard operating procedures for testing and reporting the quality of native seed batches. This synthesis document is a companion to and draws upon the International Standards for the Practice of Ecological Restoration (Gann et al. 2019), a foundational document that guides ecological restoration practice globally.

The underpinning principle for the International Standards for Native Seeds in Ecological Restoration (the Standards) is to provide buyers, end users and funding bodies with a level of confidence and reliability in the sourcing of quality native seeds similar to that enjoyed for crop and forestry species. Both suppliers and consumers of native seeds need assurance of the expectations surrounding seed use. By providing a common nomenclature and testing protocols and guidance in the

Figure 1. Schematic diagram of the interaction between restorative activities and key steps in the native seed supply chain. Seed needs and seed procurement strategies should be identified and assessed in the early phases of restoration planning. The native seed supply chain is then activated, with seeds sourced either from sustainable collection from natural populations or from seed production systems. Sourced seeds should then ideally undergo seed processing and quality testing, and be stored under appropriate conditions to maintain viability if required. Seed dormancy may need to be alleviated prior to the delivery of seeds to site, and appropriate seed enhancement techniques may improve seed delivery and the success of seedling establishment. Seeding should follow site preparation activities, and be conducted in the appropriate season. Monitoring activities should be undertaken following seeding, to facilitate adaptive management if required and provide evidence of ecosystem trajectory at the site. Graphic by S. Pedrini.

deployment of native seeds, producers will be able to efficiently tailor their production methods to meet the specific requirements of end users. Similarly, restoration practitioners require certainty about the origin, quality, and value of seed batches they are purchasing which is now possible with these native seed standards. While the Standards are not intended to be mandatory, they aim to guide industry, regulatory authorities, and governments to adopt standards in native seed use.

This first edition of the Standards is intended to be a living document that will be updated and improved over time in consultation with native seed scientists, restoration practitioners, and native seed suppliers. The International Network for Seed-based Restoration (INSR 2020), a thematic section of the Society for Ecological Restoration, would be the ideal platform for future discussion, implementation, and sharing of these updates with the global native seed community.

If we as a society are to achieve the lofty aspirations of ecological recovery anticipated in the coming decades (Cross et al. 2019; Aronson et al. 2020), while avoiding the environmental harm likely to result from unethical sourcing of native seeds (Nevill et al. 2018), we must develop seed use efficiencies, reduce seed procurement costs, and improve the environmental outcomes of seed-based restoration. These Standards provide a pathway forward for the global native seed industry to adopt, adapt, and transition practices to align with global best practice in native seed.

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STRATEGIC ISSUES ARTICLE

Seed planning, sourcing, and procurement

Vicky J. Erickson[1,2], Anne Halford[3]

Ensuring the availability of adequate seed supplies of species and sources appropriate for restoration projects and programs necessitates extensive science-based planning. The selection of target species requires a review of disturbance conditions and reference areas, development of a reference model, and consideration of specific objectives, timeframes, available resources, and budgets as well as the performance of prospective species in past restoration efforts. Identification of seed sources adapted to site conditions is critical to provide for short-term establishment and long-term sustainability. Seed zones and plant movement guidelines provide tools for sourcing plant materials with reduced risk of maladaptation. A seed zone framework also facilitates seed use planning and contributes to stability and predictability of the commercial market, thereby reducing costs and improving the availability of adapted seed supplies. Calculating the amount of seed required for each species is based on seed quality (viability, purity), seed weight, expected seedling establishment, and desired composition of the seeding. If adequate collections from wildland stands are not feasible, then seed increase in seed fields or use of nursery stock may be warranted. Adherence to seed collection and seed production protocols for conserving genetic diversity is critical to protect genetic resources and buffer new seedings and plantings against environmental stressors. Maintenance of genetic diversity becomes even more critical considering current or expected climate change impacts. Collaboration and partnerships can benefit seed selection and procurement programs through sharing of information, coordination in project planning, and increasing the availability of native seed.

Key words: climate change, direct seeding, genetic diversity, native seed needs assessment, native seed procurement, seed zones, workhorse species

Implications for Practice

- Early planning for seed needs based on site evaluation and examination of reference areas enables procurement of adequate quantities of seed of adapted species and seed sources.
- Seed zone maps and related tools, where available, can aid in selecting seed sources and lower the risk of maladaptation.
- Maintaining genetic diversity from seed collection through field increase and planting is crucial for reducing the risk of project failure.
- Seed source selection and management practices to maintain diversity and adaptive capacity are critical for effective response to climate change.
- Coordination of short- and long-term seed procurement needs improves availability of necessary seed sources.

Introduction

Early planning for future seed needs is essential for ensuring that sufficient quantities of the appropriate species and provenances will be available for restoration projects and programs when and where it is needed. Depending on the plant species, source requirements, quantities desired, and method of procurement, it can take 3 years or more (Fig. 1, from Armstrong et al. 2017) to acquire the target amount of plant material. A missed window of seed harvesting can result in delays of several years due to seed crop periodicity, and unpredictable weather and other factors. It becomes all the more important to determine seed needs for planned projects (e.g. roadside revegetation, pollinator and other wildlife habitat enhancement, invasive weed management), and for emergency restoration needs when there is a high likelihood of unplanned disturbances such as wildfires or flooding. This article focuses on considerations for determining seed requirements for individual projects as well as multi-year, larger-scale needs for a specific planning area (e.g. seed zones

Author contributions: VJE wrote the seed need planning and seed sourcing sections; AH co-wrote the seed procurement section and contributed to the abstract and conclusions.

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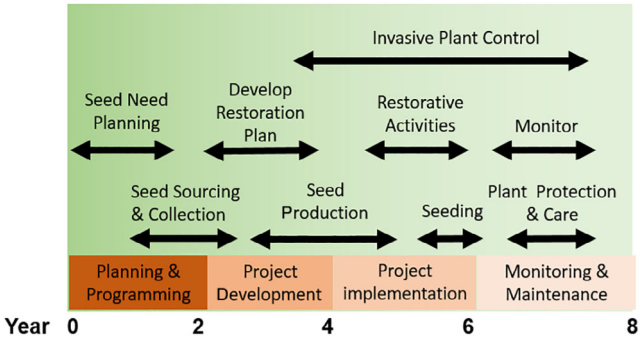

Figure 1 Schematic timeline for planning, implementing and conducting restoration project activities (from Armstrong et al. 2017). Timelines are approximate (e.g. monitoring may be required for longer periods of time to comply with permit requirements or to better understand restoration success).

or other biogeographic area). It also describes methods for sourcing seeds for current and future climates to ensure use of adapted, genetically diverse material, as well as options for procuring needed quantities of seeds of the desired species and sources.

Seed Need Planning

The selection of target species for a particular planting project or seed planning area can depend on management needs and timeframes, as well as strategies or initiatives that emphasize specific objectives such as pollinator habitat enhancement, erosion control, or protection of at-risk species. Development of a reference model and selection of reference sites may provide useful guidance for establishing restoration goals and evaluating progress toward meeting those goals (see Gann et al. 2019). Reference models may include historical records of plant communities or species assemblages at contemporary reference sites. In situations where it would be extremely challenging to return ecosystems to historical conditions and ecological trajectories, reference models may be adapted to guide restoration to new target conditions and to accommodate ongoing transformations caused by climate change and other disturbances (Hiers et al. 2012; Armstrong et al. 2017; Gann et al. 2019).

Key environmental factors to consider when designing seed mixes include local temperature and moisture regimes, soil conditions, species abundance, and where applicable, successional status. Planting a diversity of species and life forms (e.g. annual and perennial grass and forb species, shrubs, trees, nitrogen fixers, wetland species, species with overlapping and sequential bloom periods) appropriate to current or anticipated future environmental conditions is generally desirable (Whisenant 1999; Dion et al. 2017) and may improve treatment effectiveness (e.g. increased abundance and diversity of pollinator visitations), resilience to disturbances such as climate change, and resistance to invasive plant encroachment (Norland et al. 2015). Data and resources for evaluating which native species will be most successful in achieving management objectives for a particular project or seed banking program include:

- Comprehensive plant surveys of project sites, and nearby reference areaswith similar environmental conditions or that approximate anticipated future climates or post-disturbance trajectory of highly disturbed or altered sites (e.g. increased sunlight and temperatures, reduced water availability, invasive plant competition, altered soil conditions, etc.) (Gann et al. 2019).
- Local botanical experts.
- Nursery managers and seed producers.
- Plant propagation manuals and online resources.
- Herbarium and historical records.
- Literature, online tools, and applications describing local flora and plant communities.
- GIS analytical tools and databases that identify suitable species for local areas (e.g. Ecoregional Revegetation Application, http://www.nativerevegetation.org/era/) or that map species distributions for current and projected future climates (e.g. Species Habitat Tool, https://specieshabitattool.org/spht/).

Other important factors to consider when selecting restoration species, especially when large quantities of plant materials are required, are the extent of wildland stands, the cost and ease of wildland seed collection, plant performance and seed production capabilities in nursery and agronomic environments, and availability of appropriate sources in the commercial market (Atkinson et al. 2018). Commonly used restoration species are often referred to as "workhorse" species (Erickson 2008). These are species that establish and thrive in a wide range of sites and ecological settings, often with little assistance from irrigation or fertilizer. Developing seed sources for native species with unknown or poorly understood propagation requirements is likely to increase costs and require longer timeframes. Despite these constraints, more specialized species may still receive emphasis if they fulfill a desired ecological function or management objective (e.g. host plants for pollinators), are culturally important, or are needed for projects containing unique microclimates or soils types (e.g. wetlands/riparian areas, serpentine soils).

Quantity of Seed Required for Direct Seeding

The total amount of seed required for a restoration project or seed planning area is dependent on the projected restoration acreage, the desired plant density of each target species, and key physical and biological seed attributes such as germination and seed purity percentages and the number of seeds per kilogram. Additional reserves for contingency seedings may be required if site resource or environmental conditions are expected to adversely affect seedling survival. For many grass and forb species, seed is applied directly on project sites. If appropriate seed is unavailable in the commercial market or if wildland seed collections are inadequate for direct use, seed may first be grown in nurseries or seed-increase fields where plants can be cultured and harvested to produce larger quantities

Table 1 Calculation of pure live seed required for a direct seeding project. Note: Purity and germination can be derived using information contained in Pedrini and Dixon (2020) and The Royal Botanic Gardens, Kew Seed Information Database (SID) (RBG Kew 2019).

A	Number of seeds/kg[a]	17,640,000 seeds/kg	
B	Purity[a]	60%	
C	Germination[a]	85%	
D	A * (B/100) * (C/100)	9,000,000 PLS/kg	Pure live seeds (PLS) per bulk kilogram of seed
E	Field survival	3%	Estimate of the pure live seeds that become seedlings (as low as 3% for harsh sites and up to 25% for excellent sites)
F	Target seedling density	269 seedlings/m^2	Desired number of seedlings per square meter, all species (108–323/m^2 for grasses and forbs)
G	Target composition	10%	Percent of total plants composed of ANMA
H	(F * E) * G =	893 PLS/m^2	PLS of ANMA to sow per m^2
I	(10,000 * H)/D	1 kg/ha	Kilograms of ANMA to sow on a per ha basis
J	Area to seed	10 ha	Total area for seed mix
K	I * J =	10 kg	Total ANMA needed

[a]Available data for the species. Certified seed laboratory results for the seed lot should be used for project calculations when available.

of seed. Plants may be maintained for a period of one to several years depending on the species and projected seed needs. Field-grown seeds can then be planted directly in project sites or stored for future use in warehouses or freezers.

For each restoration project or seed planning area, the amount of seed required can be calculated as shown in Table 1 for western pearly everlasting (*Anaphalis margaritacea*) (Armstrong et al. 2017; NRCS 2019). Depending on the quantities required and the timeframe of the needs, the seeds may be wildland collected or obtained through establishment of seed-increase fields.

Quantity of Seed Needed for Establishing Seed Increase Fields

Seed increase fields may be established if appropriate sources are unavailable in the commercial market or if seed demand is greater than what can feasibly or economically be met within the required timeframe through wildland collections. Table 2 (Armstrong et al. 2017) provides an example of the steps involved and the information required for determining the amount of wild seed to provide a grower in order to produce the desired amount of seed. More precise guidance can be obtained directly from the seed producer because sowing rates, seed yields, and production timeframes can vary greatly depending on grower location, cultural and harvesting practices, and experience with the target species. In nearly all cases, the wildland seed provided to a grower should be tested at a certified seed testing laboratory, if available, to determine important attributes such as seed germination/viability, the number of seeds per kilogram, seed purity, and the amount of nontarget or noxious weed species.

The quantity of wild seed collected in a single year is frequently inadequate for establishing a seed-increase field. In these situations, one option is to store seed and make additional collections across multiple years until a sufficient amount of seed is available for field establishment. Another approach is to sow a small plot of wildland seed, and then harvest the first-generation seed to establish a larger seed increase field. Small collections may also be first sown in a nursery (e.g. in pots circa 16–33 cm^3 in size), and then transplanted into a seed production field at low densities (<3 seedlings per meter). This strategy reduces the overall amount of wild seed needed for field establishment as well as the time to first harvest. The fields may also be more productive than direct sown fields because the plants are evenly spaced and larger.

Quantity of Seed Needed for Nursery Seedling Production

Successful restoration of many tree and shrub species often requires the use of planting stock with established root systems, especially in areas with heavy grazing pressure or on harsh or disturbed sites such as roadsides. In these circumstances, seeds (or vegetative cuttings) are grown for several months or years in nursery beds or greenhouses. A variety of stocktypes are used, from bareroot and container seedlings that can be produced in less than 1 year, to larger transplanted containerized stock that have a longer production cycle but greater survival and growth in stressful environments due to their larger size. Sedges (*Carex* spp.), rushes (*Juncus* spp.), and many other wetland taxa are often collected and propagated using both seed and seedling production strategies.

The amount of seeds needed to produce a target number of "shippable" (acceptable) seedlings in a nursery is determined by seed germination and purity percentages, the number of seeds per kilogram, and the nursery factor. If seed testing results are not available, approximations of germination, purity, and seeds per kilogram can be obtained from published references, seed bank databases, and seed laboratory and extractory managers. The nursery factor for estimating the proportion of viable seeds that will produce "shippable" seedlings is based on nursery experience and culturing practices. For more difficult-to-grow species, nursery factors may be less than 50%. Nursery factors for target species as well as guidance on the quantity of seed needed to meet the seedling order can be obtained from nursery managers.

Table 2 Calculation of pure live seed required for establishment of seed increase fields.

A	Seed production needs	10 kg	From seed needs plan (see also Table 1)
B	Years in production	2 years	Seed production can span several years depending on lead time of project
C	Sowing rates	1.1 kg/ha	Consult with seed producer or reference tables
D	Annual seed yields	56 kg ha yr^{-1}	Consult with seed producer or reference tables
E	A/B/D	0.09 ha	Area seed producer needs to sow
F	E * C =	0.1 kg	Cleaned wild seeds that seed producer needs to sow
G	Cleaned-to-rough-cleaned seed ratio	33%	Estimated
H	100/G * F	0.3 kg	Rough weight of seeds to collect

The amount of seed needed to produce a target number of "shippable" seedlings can be estimated using the following equation (Armstrong et al. 2017):

$$\text{Quantity of seedlings needed} : [(\%\ \text{germ}/100)*(\%\ \text{purity}/100)*(\text{seeds}/\text{kg})*(\text{nursery factor}/100)].$$

Seed Sourcing

Selection of genetically appropriate seed sources is crucial for effective and responsible restoration, both in the short and long term. If plant materials are poorly matched to local site and environmental conditions, projects may fail or be unsustainable over time due to poor regeneration potential, genetic degradation, disrupted plant–pollinator relationships, or loss of resiliency and adaptive capacity in coping with environmental stressors such as invasive plants and climate change (Hufford & Mazer 2003; Broadhurst et al. 2008; Bischoff et al. 2010; Havens et al. 2015; Bucharova 2017). Having knowledge of seed origin and the genetic diversity and background of available plant material is an important first step for ensuring adapted and resilient plant populations.

Although seed of local origin or provenance is generally considered to have the greatest adaptive potential (McKay et al. 2005; Crémieux et al. 2010; Mijnsbrugge et al. 2010), genetic research indicates that geographic distance is generally a poor predictor of adaptive differentiation (Leimu & Fischer 2008; Richardson et al. 2015). This means there is no fixed distance or rule of thumb for determining where plant material may be successfully moved from its site of origin. Instead, "local" is best defined by the climate and environmental similarity of the source material relative to the planting site where it will be transferred (Hufford & Mazer 2003; Savolainen et al. 2004; Johnson et al. 2010).

Maintaining Adaptive Capacity: Seed Zones and Transfer Guidelines

Plant adaptation is influenced by a wide array of climatic and environmental factors such as precipitation, aridity, temperature, aspect, and soil characteristics. The degree of adaptation may vary greatly among species, from genetic generalists that can abide broad movement across environmental gradients to genetic specialists that are more tightly adapted to local conditions and regions (Rehfeldt 1994; Johnson et al. 2010). Common garden experiments and reciprocal transplant studies are empirical approaches for investigating species-specific adaptive strategies and patterns of genetic variation in a given geographic area (e.g. Campbell 1986; Sorensen 1992). In these studies, variation in important adaptive traits involving survival, growth, and fecundity is correlated to climate and environmental variables of the plant sources included in the experiment. The results can then be used to create species-specific seed transfer guidelines and delineate discrete regions of similar environments (seed zones) within which plant materials can be moved with little risk of maladaptation at new planting locations (Fig. 2). Seed zones have a long history in forestry, especially in the United States and Europe, but have only recently been developed for herbaceous species used in restoration (e.g. Erickson et al. 2004; Horning et al. 2008; Johnson et al. 2013; St. Clair et al. 2013; Bower et al. 2014; Bucharova 2017; Durka et al. 2017). New online tools such as SeedZone Mapper (https://www.fs.fed.us/wwetac/threatmap/TRMSeedZoneData.php) have been developed to catalogue

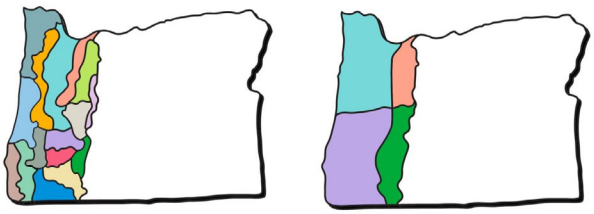

Figure 2 Species-specific seedzones for: (A) Douglas-fir (*Pseudotsuga menziesii*, a genetic specialist) and (B) western red cedar (*Thuja plicata*, a genetic generalist) in western Oregon, U.S.A. The size and configuration of the seed zones for the two species reflect differing patterns of adaptive genetic variation across the landscape, as determined from common garden studies. Seed zones for genetic specialists like Douglas-fir are much smaller, with more restrictive seed movement relative to western red cedar and other generalist species that can tolerate broad movement with little risk of maladaptation.

available seed zone information and allow end-users to view and download GIS data for further use in seed collection and restoration planning.

In addition to enhancing restoration outcomes, seed zones can generate efficiencies and economy of scale in seed and plant production systems, as well as stability and predictability in the commercial market. A seed zone framework greatly facilitates seed use planning and creates opportunities for the sharing and exchange of plant material among land owners and seed banking programs and partners. Collectively these attributes help reduce plant material and overall restoration costs, leading to the increased availability and use of genetically appropriate plant materials in restoration. In spite of the many benefits, seed zones are generally lacking for many herbaceous species required in restoration. In these cases, ecoregional approaches that delineate land areas encompassing similar geology, climate, soils, hydrology and vegetation or other geographic descriptors may be useful proxies for directing seed movement and the collection and sourcing of plant materials. In the United States, generalized provisional seed zones (Bower et al. 2014) have been developed using climate data (winter minimum temperature and aridity) along with ecoregional boundaries to delineate areas that have similar climates but differ ecologically. The provisional zones serve as a useful starting point for ensuring adaptability and protecting genetic resources, especially when used in conjunction with species-specific genetic and ecological information in addition to local knowledge. The ecoregional approach has also been utilized in several European countries, including Austria, Czech Republic, France, Germany, and Switzerland (Fig. 3) (De Vitis & St. Clair 2018).

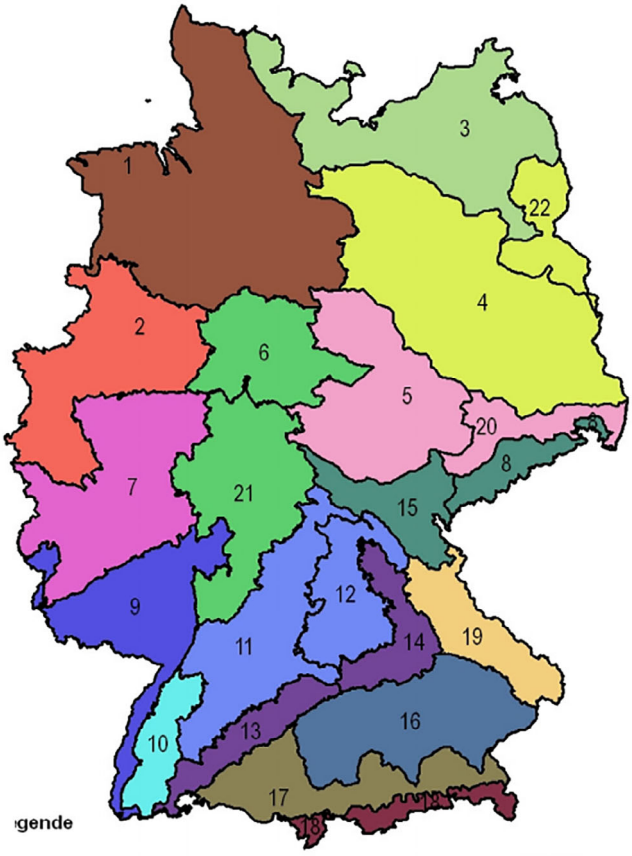

Figure 3 The 22 German regions of seed origin based on climate and local factors (Prasse et al. 2010).

Maintaining Genetic Diversity

An additional important concern in native plant material development and use regards the sampling and maintenance of genetic diversity. All phases of seed and plant production, from wild collection, processing, grow-out, and harvesting, should employ methods that conserve inherent genetic diversity. This will not only protect genetic resources, but also help improve initial restoration success and provide resiliency against environmental pressures and changing conditions in the future (Rogers & Montalvo 2004; Basey et al. 2015). In addition, the restored population must include a sufficient number of unrelated parents to minimize the potential for adverse impacts due to inbreeding. Restoration practitioners should be mindful of genetic diversity needs and concerns whether they are purchasing plant materials or collecting and propagating their own sources. When seed is purchased in the commercial market, the most suitable plant material for a particular project can be assessed through review of government websites and published literature and by consultations with reputable seed producers and brokers. Important factors to consider include seed origin and certification class (if available). In the United States, many of the more recent native species germplasm releases are certified as "Source Identified" to indicate that no selection or genetic modification has occurred in the original wildland parent population or in subsequent generations grown in seed-increase fields or seed production areas and orchards (Young et al. 2003).

Although no single protocol for plant material collection and propagation is guaranteed to safeguard genetic integrity in all situations, following are some general guidelines for consideration when purchasing or collecting/growing seed and seedlings (adapted from Armstrong et al. 2017; see also Rogers & Montalvo 2004; Basey et al. 2015):

Number of unrelated parents. Collecting seed or cuttings from 50 or more unrelated parent plants is often recommended as a general guideline for obtaining a representative sampling of genetic diversity in a population. A similar amount of seed or cuttings should be collected from each plant. If parental contributions are unequal, a larger number of parent plants should be sampled to increase diversity. When collecting cuttings for vegetative propagation of dioecious species, practitioners should strive for a balanced male–female ratio to ensure that both sexes are adequately represented in the collection.

Number of collection sites. Collecting seeds or cuttings from multiple areas within a seed zone will help provide a representative sampling of among-population genetic diversity. Ideally, collection sites would span the full range of environmental and climatic conditions within a seed zone or management area. An approximately equal number of parents should be sampled

within each area. Collecting material from larger populations and avoidance of isolated, fragmented stands where inbreeding or past genetic bottlenecks may reduce genetic diversity are other sampling strategies that can enhance genetic diversity.

Individual parents within a collection site. To reduce the risk of collecting from related individuals (e.g. siblings or clones of the same plant), seed and cuttings should be obtained from plants that are well separated from one another (Vekemans & Hardy 2004; Rhodes et al. 2014). Genetic diversity and representation can also be improved by collecting from plants well dispersed throughout the collection site. In outcrossing species, an important consideration for maintaining genetic diversity is to avoid collecting from isolated plants that may have reduced opportunity for cross-pollination with a wide array of pollen donors. Other recommendations for safeguarding genetic integrity and diversity include collecting plant material throughout the entire flowering period and avoiding inadvertent selection that could result in a disproportionate representation of certain plant types (e.g. earlier flowering, larger sized, or heavier seed producers).

After seed collection, a number of cultural practices and biases in subsequent stages of the plant production cycle can also potentially affect the genetic integrity and diversity of the source material (Schroder & Prasse 2013). Following are some of the more obvious situations to avoid or minimize:

- Bias in selecting seed or plants for crop establishment based on their size or morphology.
- Irrigation, fertilization. or cultural practices that favor certain plant types, causing artificial selection (e.g. trait shifts or reductions in the diversity of the population).
- Harvesting practices that favor certain phenotypes through timing, frequency, or type of harvest method (hand, mechanical).
- Intentional or unintentional removal of viable seed during the seed cleaning process (e.g. seed sizing, grading large seed from small, selection based on seed color).
- Seed storage conditions that cause loss of viable seeds over time (e.g. large fluctuations in temperature or humidity).

Mixing seed crops from different harvest years or re-collecting wild sources on an ongoing basis for establishment of new production fields are other effective strategies for guarding against the degradation of genetic diversity in native plant materials and restored populations.

Seed Sourcing for Changing Climates

For many regions of the world, changing climates will require plant populations to rapidly respond to new environmental conditions and pressures, including habitat alteration and fragmentation, precipitation and temperature extremes, uncharacteristic wildfires, stresses from invasive plant species, and new and intensifying insect and disease infestations. Seeding and planting will become increasingly important tools for mitigating these impacts, and for re-aligning species and populations to keep pace with changing climates and altered disturbance regimes. Although some species and populations may be more vulnerable to climate change (Fig. 4) and the specific effects are highly context dependent (Hufford & Mazer 2003; Broadhurst et al. 2008; St. Clair & Howe 2011), resiliency, diversity, and adaptability will remain overarching strategies for sourcing plant materials for future climates. Methods that enhance diversity, such as the use of diverse species and seed sources and creation of structural diversity within stands and across landscapes, are crucial safeguards for ensuring successful restoration in both the short and long term. Other important objectives are the maintenance of large populations with high connectivity to promote gene flow of adapted genes (via seed and pollen) in the direction of trending climates (e.g. lower to higher latitude or elevation changes). Maintaining and sharing accurate records of plant material sources, combined with well-designed monitoring strategies, will be essential for informing and adjusting restoration practices over time.

Many plant populations are already growing outside their optimal climate as a result of environmental changes that have outpaced the rate of species' response capabilities (adaptational lag, Aitken et al. 2008; Gray & Hamann 2013). In these situations, seed sourcing protocols may be modified to shift emphasis from using only seed from local sources to selecting seed (or a portion of the seed) based on similarities with projected future climate or to climate changes that have already occurred in the recent past. Matching seed sources to climates is made more feasible by the advent of GIS mapping programs that use existing data and climate projections to predict which seed sources will be best adapted to a given planting site, or which planting sites will be most suitable for a given seed source. In North America, the Seedlot Selection Tool (Fig. 5) (https://seedlotselectiontool.org/sst) and the Climate Smart Restoration Tool (climaterestorationtool.org/csrt/) are becoming widely used for tree and shrub/herbaceous species, respectively. A similar application, ResTOOL, is available for plant material selection and restoration of tropical dry forests in Columbia (http://www.restool.org/en/index.php).

"Climate smart" seed sourcing strategies based on near-term climate projections (e.g. 10–20 year planning horizon) will reduce the uncertainty and risk associated with reliance on climate projections for the more distant future. This will also promote the use of plant material that will be optimally adapted to environmental conditions during the highly vulnerable early stages of seed and seedling establishment. For many geographic regions, the direction of plant movement for changing climates will be from the warmer, drier environments of lower latitudes and elevations to higher latitudes and elevations where conditions are cooler and wetter. Flexibility in creating custom seedlots for future climates is greatly facilitated by protocols that collect and bulk seedlots across a narrow range of environments (e.g. temperature or precipitation bands).

Seed Procurement

Once the species, sources, and quantities of seed required for a specific project or long-term program have been determined, procurement strategies and plans must be carefully developed.

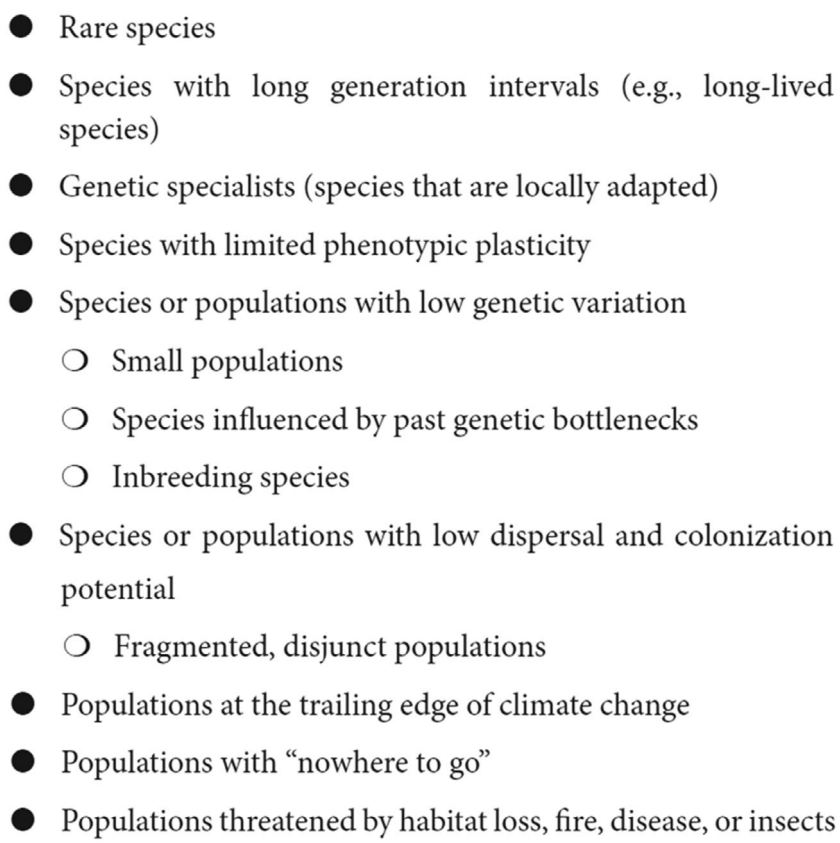

Figure 4 Species and populations most vulnerable to climate change (from St. Clair & Howe 2011).

Several options are available, and decisions require consideration of funding, timelines, and available resources. Some plant materials may be immediately available (off-the-shelf purchases, seed in storage), whereas acquisition of others can require several years depending upon the seed sources selected and time requirements for wildland collection or agricultural seed production. Where available, seed certification and testing standards help to strengthen procurement plans. Common types of procurement tools used for acquiring seed from collectors and seed producers are described in Pedrini et al. (2020).

Improving Seed Availability Through Collaboration and Partnerships

Seed procurement and banking programs are likely to be most successful and cost effective if managers are able to coordinate and prioritize multi-year seed needs in conjunction with other resource disciplines, agencies, and landowners within a seed planning area (seed zone or other biogeographic area). This more integrated and comprehensive approach to seed planning can benefit a wide range of resource needs, protect against overharvesting, and lead to an increased availability of native seed when and where it is most urgently needed for restoring disturbed sites and ecosystems. Other critical factors affecting seed planning and procurement success include supporting infrastructure such as proper storage facilities (warehouse, freezer), seed processing facilities, and nurseries, as well as seed producers who can operate at a scale appropriate to the production needs of the clients. While not "seed need planning" per se, access to supporting infrastructure is essential for successful native plant material programs and remains a serious constraint to planning efforts and seed supplies in many areas. In these cases, partnerships and careful coordination become all the more important in providing for needs through the creation of opportunities to share in planning costs, infrastructure investments, and native seed production.

Conclusions

Successful seedings and seed programs rely on early and thorough planning to identify seed needs. Examination of a comprehensive site evaluation, reference areas in various stages of recovery, and other available resources aid in identifying seed needs for individual projects. Species selected to meet restoration goals should have a history of use in restoration, but some specialist species may be essential and require research or the attention of a skilled propagator. Appropriate sources for each species are selected using available seed zone maps or related tools along with knowledge of the species ecology and potential response to climate change. The species, sources, and quantity of seed required can then be incorporated into the scheduling and budgeting processes early on as 2–3 years may be required

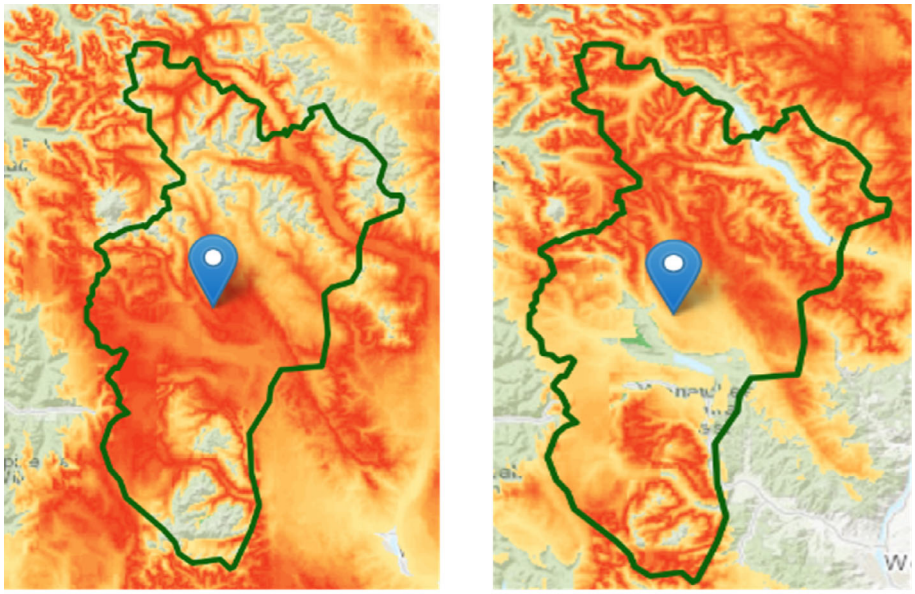

Figure 5 Projections from the Seedlot Selection Tool (https://seedlotselectiontool.org/sst/) illustrating the climate match of a seed source (pin drop) to potential planting sites within the seed zone (green boundary). The areas in the dark red portion of the color gradient reflect the best match of the seed source to: (A) current climate conditions within the seed zone, and (B) the projected climate at mid-century. The climate variable used in the projections is winter minimum temperature. Note that the dark red area of best use for the seed source shifts from 1,100–1,500 m in elevation under current climate conditions to elevations >1,800 m by mid-century.

to obtain some seed sources. Procurement of appropriate plant materials may require wildland collection and in some cases increase in agricultural seed fields or nurseries. Collection and increase should follow established protocols and guidelines to obtain and maintain maximal genetic diversity and fitness to improve the resistance and resilience of restored communities.

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PRACTICAL ARTICLE

Collection and production of native seeds for ecological restoration

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The global push to achieve ecosystem restoration targets has resulted in an increased demand for native seeds that current production systems are not able to fulfill. In many countries, seeds used in ecological restoration are often sourced from natural populations. Though providing seed that is reflective of the genetic diversity of a species, wild harvesting often cannot meet the demands for large-scale restoration and may also result in depletion of native seed resources through over harvesting. To improve seed production and decrease seed costs, seed production systems have been established in several countries to generate native seeds based on agricultural or horticultural production methods or by managing natural populations. However, there is a need to expand these production systems which have a primary focus on herbaceous species to also include slower maturing shrub and tree seed. Here we propose that to reduce the threat of overharvest on the viability of natural populations, seed collection from natural populations should be replaced or supplemented by seed production systems. This overview of seed production systems demonstrates how to maximize production and minimize unintended selection bias so that native seed batches maintain genetic diversity and adaptability to underpin the success of ecological restoration programs.

Key words: ethical seed collecting, managed natural plant populations, native seed farm, seed harvesting

Implications for Practice

- Seed collection from natural populations can be used to provide seeds for ecological restoration; however, collection should be performed sustainably to avoid affecting the reproductive capability of the source population.
- Multiplication of native seeds under cultivated production settings should replace or supplement collection from natural populations, whenever feasible, as it reduces impact on natural populations and allows for higher productivity, improved quality, reliability, and reduced seed cost.
- If cultivated production is not feasible, natural populations can be managed to optimize seed production.
- Seed collection and production should aim to avoid active selection for certain traits and limit unintended selection, to maintain the genetic variability of the seed batch.

Introduction

There are three main approaches for supplying native seeds for restoration projects: (1) seed collection from natural/wild populations, (2) harvest from managed populations, and (3) cultivated seed production systems (such as native seed farms). These three seed supply strategies lie along a continuum where increasing inputs are required. The methods present different advantages and limitations and are not exclusive; these sources may be used in complementary and strategic combinations (Fig. 1).

A key aspect of native seed supply for ecological restoration is to adequately capture the genetic diversity representative of a natural population and ensure that such diversity is maintained throughout the supply chain until seeds are deployed to a restoration site (Broadhurst et al. 2008; Erickson & Halford 2020). In recent decades, conservation seed bank initiatives worldwide have developed guidelines and procedures for collecting native seeds in order to adequately capture genetic variability, while avoiding damage to the source populations' reproductive capability (FloraBank 1999: ENSCONET 2009; USDI Bureau of Land Management 2018). These protocols

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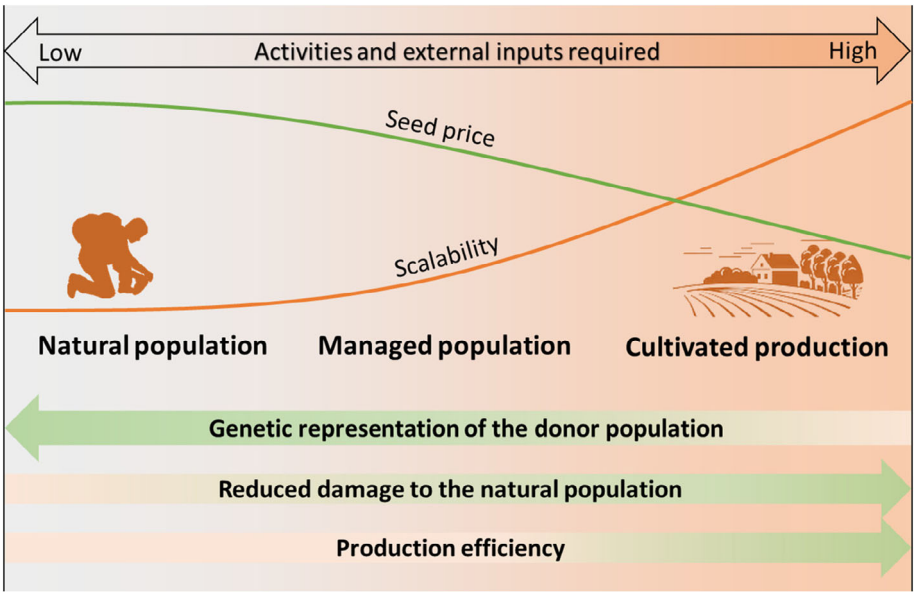

Figure 1. Native seeds supply approaches for ecological restoration. The three approaches commonly used to supply seed for ecological restoration sit on a continuum of increased management activities intensity (e.g. weeding, fencing, plowing, weed mat, sowing). These approaches allow producers to increase seed supply while reducing seed price. Each approach has advantages and disadvantages and might not be always feasible for all species/ecosystems. (Image by Simone Pedrini).

are a useful reference point for planning and collecting seeds for ecological restoration. However, the quantity of seed per collection for conservation purposes is seldom sufficient to meet the demand for native seeds in landscape-scale restoration (Merritt & Dixon 2011) where substantial quantities of seed from large numbers of plants may be harvested (e.g. tens of thousands of plants for mechanical field grass harvest) and so revised collection protocols are required.

However, the rapidly expanding demand for native seed often surpasses the quantity that can be sustainably collected from natural populations (Nevill et al. 2018) where factors such as low or erratic seed set and quality, seed predation, habitat fragmentation, and invasive species all contribute to harvest limitations and increasing seed costs (Broadhurst et al. 2015). Some of these factors can be controlled, or manipulated, by managing natural populations with activities which include weed control, watering, fencing, and fertilization with the aim to improve harvest efficiency and seed yield. This approach can be applied in certain situations or ecosystems (e.g. some tree and shrub species in forest ecosystems, or species-rich grasslands); however, as with seed collection in the wild, such collection volumes requires large areas of relatively intact wild areas to be effective.

The establishment of native seed crops, where seeds are produced in cultivation settings (akin to agricultural or horticultural production), has the potential to meet the rising demand for native seeds (Delpratt & Gibson-Roy 2015; Nevill et al. 2016; Jiménez-Alfaro et al. 2020). Seeds collected from the wild are multiplied using methods and techniques similar to the production of seeds of domesticated cultivars (e.g. cereal crops, fodder species, vegetables, trees for forestry). However, in contrast to traditional farming or horticulture methods, production of native seeds requires special consideration to avoid, as far as practical, selection of specific traits (Pedrini & Dixon 2020), and to maintain the genetic variation found within natural founding populations as the primary means for ensuring evolutionary and adaptive potential (Basey et al. 2015). Collecting seeds from planted, restored habitats is another alternative, but should be undertaken with caution as the genetic diversity of the restored plant population may be unknown or low. In some cases, suboptimal genetic diversity is mitigated if the restored area is adjacent to remnant natural habitat, thus permitting gene flow from external pollination (Pakkad et al. 2007; Jalonen et al. 2018).

Whenever feasible, collection from natural populations should be replaced or supplemented by production in cultivated settings or managed natural populations, as these sources can provide a reliable and cheaper source of native seed, while limiting potential negative impacts to natural populations.

This article is intended to be an overview of current best practices for the collection and production of native seed and, when read in conjunction with other articles in this special issue (particularly genetic considerations in seed sourcing as reviewed by Erickson and Halford (2020), provides the framework for improving our understanding of the seed supply chain.

Seed Collection From Natural Populations

The native seed supply chain commences with the collection of seeds from natural populations.

Collection can be used as a foundation for seed multiplication in cultivated seed production systems, to enrich managed natural populations or for deployment to a restoration site via direct seeding (Broadhurst et al. 2008). When collecting from the wild,

the two overriding concerns are: (1) to avoid negative impacts on the native donor population and (2) to retain appropriate genetic diversity of the donor population.

Pre-Planning

Considered and cautious pre-planning is a key requisite for achieving high-quality, well-documented seed collections. Prior to collecting seed from natural populations, it is important to obtain information on a species biology (e.g. breeding system, estimated flowering and fruiting dates) and likely distribution. Depending on identified project needs/strategies, seed may be collected from one or many natural donor sites, and these need to be identified in advance. Locating appropriate donor sites may require investigating national or local seed herbarium records and botanical or forestry databases, and dialogue with experts (local or otherwise) and other seed collectors. Avoid, or be cognizant of, planted populations where vegetation is of unknown or inappropriate origin. Before conducting surveys or collections in the field, all required consents and permits must be obtained. As these can take some time to process, permit requests should be submitted in a timely manner to avoid the risk of delays in collection. Collectors must be adequately trained in plant/seed identification and risk assessments should be prepared prior to field-based operations.

Site Surveys

Ideally, donor sites should be surveyed in advance of collection to confirm the location and site attributes (e.g. weed loads, access issues, terrain) and to determine whether adequate plant numbers of each target species are present to provide collections of sufficient quantity and genetic diversity (Way & Gold 2014). If possible, the populations should be monitored from flowering onward to ensure that seed collection dates coincide with seed maturity. However, in many cases, the constant surveillance of a population is impractical due to cost and distance, so collectors should seek additional information from local sources to determine optimum collection times. To optimize quality and longevity, seeds of native species should usually be collected at, or close to, the point of natural seed dispersal. Some species disperse over a very short period, so fruit maturity and weather conditions must be monitored carefully to avoid losing potential harvests (for example, wind-dispersed species like *Eriophorum* spp. may disperse seed rapidly on a windy day). Many other species disperse seed over extended periods and so harvests would be ongoing, to capture variation in traits along different maturity dates (ENSCONET 2009).

Pre-Collection Assessments

Particular care should be taken to avoid over harvesting natural populations, especially for small, rare, endangered, or isolated populations (Broadhurst et al. 2017; Nevill et al. 2018). Aim to carry out a pre-collection assessment immediately prior to collecting, to establish collecting limits. As a precautionary measure, aim to collect no more than 20% of the seeds that are mature at the time of collection (Way 2003; Pedrini & Dixon 2020) and for multiple collections from the same population, less than 20% of the total seed produced in any 1 year (ENSCONET 2009). For annual species, whose population survival relies on seeds dispersed in any given year, no more than 5–10% of the entire yearly production should be collected, and collection from the same population in consecutive years should be avoided (Meissen et al. 2015). Use the pre-collection assessment to determine the population size (number of plants, plant density), evaluate seed production per plant or area, and obtain an estimate of total seed production, then set the collection goal to be less than the prescribed percentage, as indicated above.

The pre-collection assessment should also estimate seed quality by cut testing a sample of seeds to calculate the percentage of empty or infested seeds, thus estimating the minimum quantity that needs to be collected to achieve the desired number of viable seeds. When wild seed sources are limited, it may be appropriate to collect and store seed over a number of years (under suitable conditions) until sufficient seed is accumulated to initiate the restoration program (DeVitis et al. 2020).

Collection

Collection may involve single or multiple species. A wide range of collection techniques may be used, depending on the target species' phenology and growth form, population size, amount of seed required, and local terrain. Hand-harvesting methods include plucking, stripping, clipping, shaking, and vacuuming, while mechanical methods include brush, vacuum, and combine harvesting. Seeds of tree species are best collected directly from the tree, if possible, as seeds found on the ground may be of low quality (e.g. old, moldy, or insect infested). Collect the seeds into containers/bags that keep the seeds as cool and aerated as possible (Supplement S1). Safety, communication, and biohazard equipment are also essential—useful checklists are provided by Way and Gold (2014) and FloraBank (1999). Appropriate post-harvest management and storage are critical for maintaining seed viability, see DeVitis et al. (2020) and Frischie et al. (2020) for details.

To allow for the optimal, informed use of seed collections, detailed data should be recorded about the seed lot and source population (Supplement S1). Collection of herbarium voucher specimens before or during seed harvest allows for verification of the material collected. The herbarium specimen should include fruits or flowers and bear the same collection number ID as the seed collection.

Seed Collectors

Collection of native seeds from natural populations can be performed by professional seed collectors (Supplement S1), native seed suppliers/producers, restoration contractors, private landowners, public land managers, researchers, conservation seed bank personnel, nonprofit organizations, and

Figure 2. Nödzö'u Group in Brazil: Xavante women collecting seeds of Buriti (*Mauritia flexuosa*). The Xavante have a seed-collecting tradition and have participated in the Xingu Seed Network since 2013. (Source: Xingu Seed Network, picture by Rogerio Assis).

volunteers. Inexperienced collectors will require appropriate training and leadership by qualified personnel. The involvement of local indigenous people with intimate knowledge of native species can be invaluable (Fig. 2) while, at the same time, providing important employment opportunities and revenue, thus helping to stabilize seed markets and increasing the reliability of native seed supplies for restoration (de Urzedo et al. 2016, 2019, 2020).

Managed Natural Populations for Seed Production

For some species and ecosystems, seed collection from natural populations can be improved by actively controlling environmental or ecological variables. For example, a population may be managed by removing or controlling unwanted species or encouraging (even planting) the spread of desired plants to achieve larger or more concentrated areas of the target species to be harvested. Management activities can also include irrigation, alteration of nutrient characteristics (e.g. fertilization or biomass removal), reduction of herbivore impacts and managing seed predation (e.g. by fencing, bird netting and other means of deterrence), and imposed disturbance measures (e.g. controlled burning or grazing). These approaches suit some native vegetation systems and land managers better than cultivated seed production. For example, highland areas of Scotland are managed on an ongoing basis to maintain the ideal conditions for animal hunting by arresting the succession to woodland with patch burning on a 15–20 year rotation, encouraging growth of young shoots of heather (*Calluna vulgaris*) and other heathland species (Mallik & Gimingham 1983). This creates large stands of uniform vegetation (often dominated by heather) at the ideal age for seed production allowing collection with a brush harvester.

Managing natural populations can also increase opportunities for sequential harvesting and overall seed yields (e.g. by promoting new growth or extending fruiting periods). A managed natural seed population could be considered and treated similarly to an unmanaged population, especially in terms of phenology, ecology, pollinators, herbivores, and demography. Managed populations (in particular herbaceous species) where multiple species coexist can be harvested simultaneously (Scotton et al. 2012) to create a mixed species seed mix. Bulk mechanical collection is a common harvest practice for herbaceous ground layer vegetation (i.e. grasses and forbs growing in close association) where these exist in suitable size and quality and on terrain amenable to harvesting (Shaw & Jensen 2014; Gibson-Roy & Delpratt 2015). Mechanical brush or vacuum harvesting is often used as an efficient and effective method for acquiring large seed quantities (especially for grassland and meadow species).

Cultivated Seed Production

Multiplication of native seeds using cultivated production approaches employing agricultural and/or horticultural practices is now an emerging sector in many parts of the world (DeVitis et al. 2017; Gibson-Roy 2018; White et al. 2018; Hancock et al. 2020). The development of cultivated seed production systems, from small-scale container-bed orchards, to large, field-scale farm operations allows for the multiplication of initially small founding collections through to native seed production plots which greatly enhance native seed supply and prevent or reduce impacts of overharvesting from natural populations (Kiehl et al. 2014; Gibson-Roy & Delpratt 2015). Where restoration markets exist to support the high capital outlay (DeVitis et al. 2017; Gibson-Roy 2018; White et al. 2018) installation of seed production areas on former agricultural land can provide ready benefits in terms of suitable terrain, existing infrastructure, and for many herbaceous species, rapid growth and maturity free from competition (Supplement S2). For example, farm production of native seeds for grassland species is comparable to domesticated perennial forage cultivars (e.g. *Lolium perenne*, *Trifolium pratense*) (Mainz & Wieden 2019). Usually, for production at farming scale for native species, a relatively uniform substrate is prepared prior to seeding, and the growing environment is carefully managed to maximize seed production (Fig. 3). Well-planned and implemented cultural practices, such as pruning, biomass manipulation, weed control, irrigation, pest/disease control, and fertilizer applications, are used to promote flowering, facilitate harvesting, and improve yields. In these settings

Figure 3. Seed farms: (A) Field of Wyeth buckwheat (*Eriogonum heracleoides*) in OR, U.S.A. (B) Mechanical sowing of the annual cornflower (*Cyanus segetum*) in Brechin, Scotland. (C) Rows of European native forbs *Achillea millefolium* and *Silene dioica* in Freising, Germany. (D) Planting of *Allocasurina acutivalvis* seedlings into weed mat in Morawa, Western Australia, in an indigenous-owned and operated native seed farm.

native seeds are typically (but not always) directly sown on cleared, tilled, friable seedbeds by purpose built seeding equipment (e.g. placed in rows or surface broadcast). An alternative production system used at small to field scale is to cover bare soil with weed-mat and plant green stock into cut openings (it is also possible to direct sow into the openings for highly germinable species). This approach is effective for a large range of species and functional types (e.g. perennial species, herbaceous or woody) resulting in a major reduction in weed competition while fallen seed can be easily harvested from the weed mat (i.e. sweeping and/or vacuum).

In spite of some similarities in cultural practices, farm-scale native seed production systems differ significantly from those of conventional crop production systems where selection and breeding programs have modified plant and seed traits to achieve specific characteristics that typically include reduced dormancy, increased synchronicity of ripening or shattering, ease of harvest, processing, and storage. These selections result in uniform plants, often with reduced genetic diversity, and with crop production practices that are tailored to maintain this uniformity. Conversely, native seed production systems for use in ecological restoration typically aim to capture and retain a representative and appropriate range of natural genetic variation displayed in founding populations to allow, as much as is possible, the restored populations to evolve and adapt to prevailing environmental changes (Pedrini & Dixon 2020). With these differences in mind, it is necessary to understand and manage native seed production systems in a way that adopts and modifies relevant agriculture and horticulture systems and equipment to effectively produce native seed of appropriate genetic character and germinability (Supplement S3; Shaw et al. 2012; Shaw & Jensen 2014).

Best Practices to Retain Genetic Diversity

It is important to acknowledge that any seed harvest activity, whether from natural populations (managed or unmanaged) or under cultivated seed systems, may carry the risk of some degree of genetic selection. However, a number of precautions can be taken at each steps of seed procurement to limit, as far as practical, the impact of trait selection.

Collect the Genetic Diversity Representative of a Natural Population

Native seed collection guidelines often recommend that harvest should be made from at least 50 individuals in a population (Brown & Marshall 1995). Recent studies (Hoban & Strand

2015; Hoban et al. 2018) explore the use of simulation models to predict the capture of genetic diversity with different sampling strategies and suggest the adoption of collection guidelines tailored to particular taxa that take into account biological factors such as selfing and seed dispersal strategies.

For taxa with known polyploidy, such as many grasses, it is important (where possible or where the information exists) to determine the ploidy number of the potential source populations and avoid establishing seed production plots with seed from multiple populations with different ploidy levels, because the seed produced by cross fertilization will not produce fertile plants and thus would not persist in restoration plantings (Kramer et al. 2018).

When collecting from natural populations (especially by hand) seeds should be harvested as randomly as possible, avoiding the collection of particular traits, and, if feasible, over several days during the seed dispersal period (The Royal Botanic Gardens Kew 2001; ENSCONET 2009). For large populations of open pollinated species in a uniform landscape, it is often easier to collect in a more systematic way, sampling at regular intervals along a transect or in patterns when using mechanical harvesters to ensure an even contribution of seeds from maternal genotypes.

Guarding Against Genetic and Phenotypic Drift in Cultivated Production

Cultivated native seed production can have the risk of drift in the genetic and phenotypic make-up from the original population due to unintended selection, usually trending towards a narrowing of genetic diversity (Espeland et al. 2017). Native seed producers should be aware of cultural practices that increase the likelihood of active selection for specific traits, for example when growing plants for production (e.g. size, speed of development), at crop harvest (e.g. favoring periods of more synchronous seed set, plant at certain heights, plants with highest yields), or seed processing (e.g. seed shape, size, color) and, as far as practical, take the necessary measures to limit these unintended selection points (Basey et al. 2015). Another practice utilized by seed growers to reduce this risk is by allowing only a defined maximum of generations (e.g. 2–5) before the seed production plot needs to be reseeded with newly collected wild seeds (Gibson-Roy et al. 2010; Association of German Wild Seed and Wild Plant Producers 2017). When tested on five grassland species, this approach proved effective for maintaining the genetic diversity of four species out of five, mostly long-lived and out-breeding perennials, while for *Medicago lupulina*, a short-lived, selfing perennial species, genetic and phenotypic drift was detected after five generations (Nagel et al. 2019). Such studies and results suggest that these

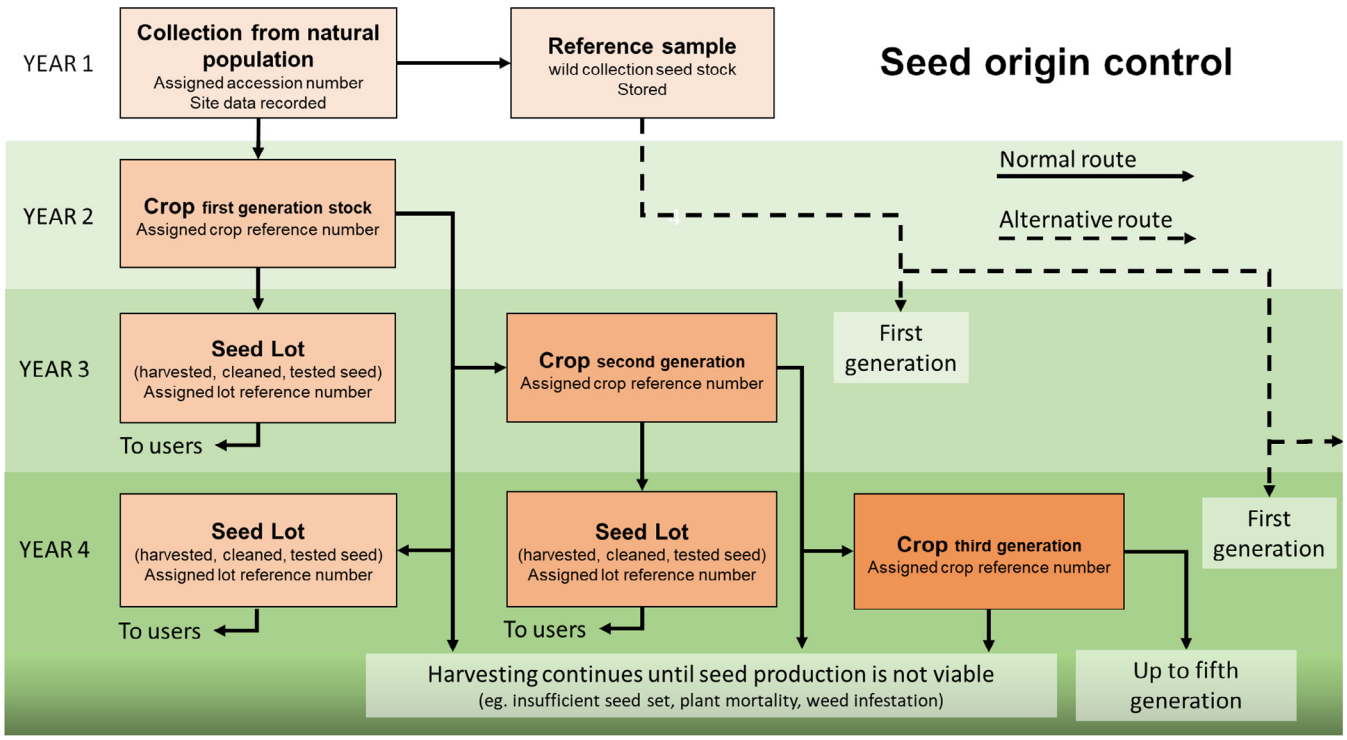

Figure 4. Origin control for seed farm production. All stages are recorded so that seeds can be traced to the original collection. Multiplication of the farm stock should be limited to a maximum five generations from collection. After five generations, a new collection should be made. This graphic is applicable to perennial species that produce seed in the first year. For annual species, harvest of a seed lot is limited to the first year. For perennial and woody species where seed production can occur two or more years after crop establishment, the time scale (on the left) should be extended accordingly. (Original graphic provided courtesy of Scotia Seeds).

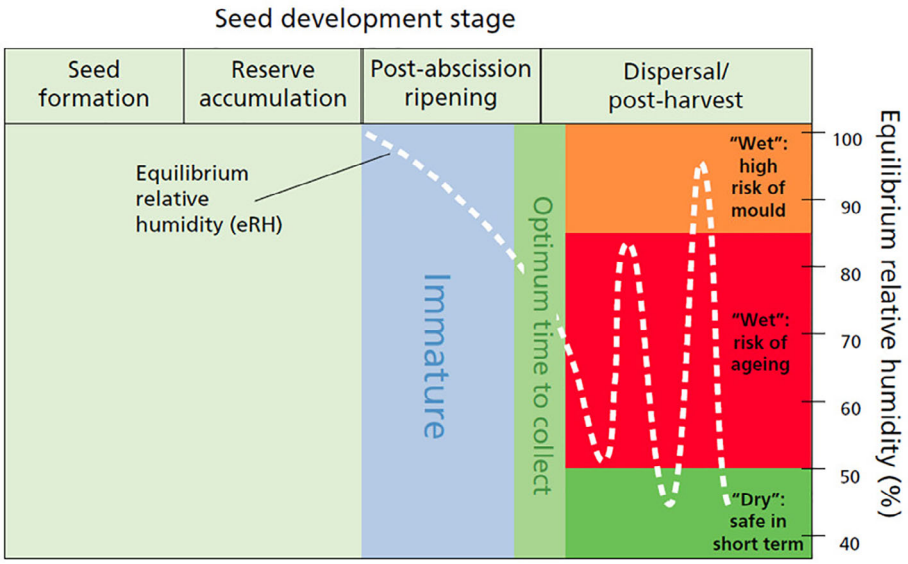

Figure 5. Effect of post-harvest seed moisture status on seed quality. The dashed line shows fluctuations in equilibrium relative humidity with ambient conditions. Copyright 2014 Board of Trustees of the Royal Botanic Gardens, Kew.

Figure 6. Seed collection from natural populations. (A) Seed collecting from *Abies procera*, by U.S. Forest Service climbers in Washington state. Image: M. Way. (B) Collecting tree seed with a pruning pole for RBG Kew's U.K. National Tree Seed Project. (C) Mechanical collection of *Calluna vulgaris* with handheld brush harvester. (D) Vacuum seed harvesting of *Leontodon hispidus*. (Images A,B: Copyright Board of Trustees, RBG Kew. Image C by S. Pedrini. Image D by Marcello De Vitis).

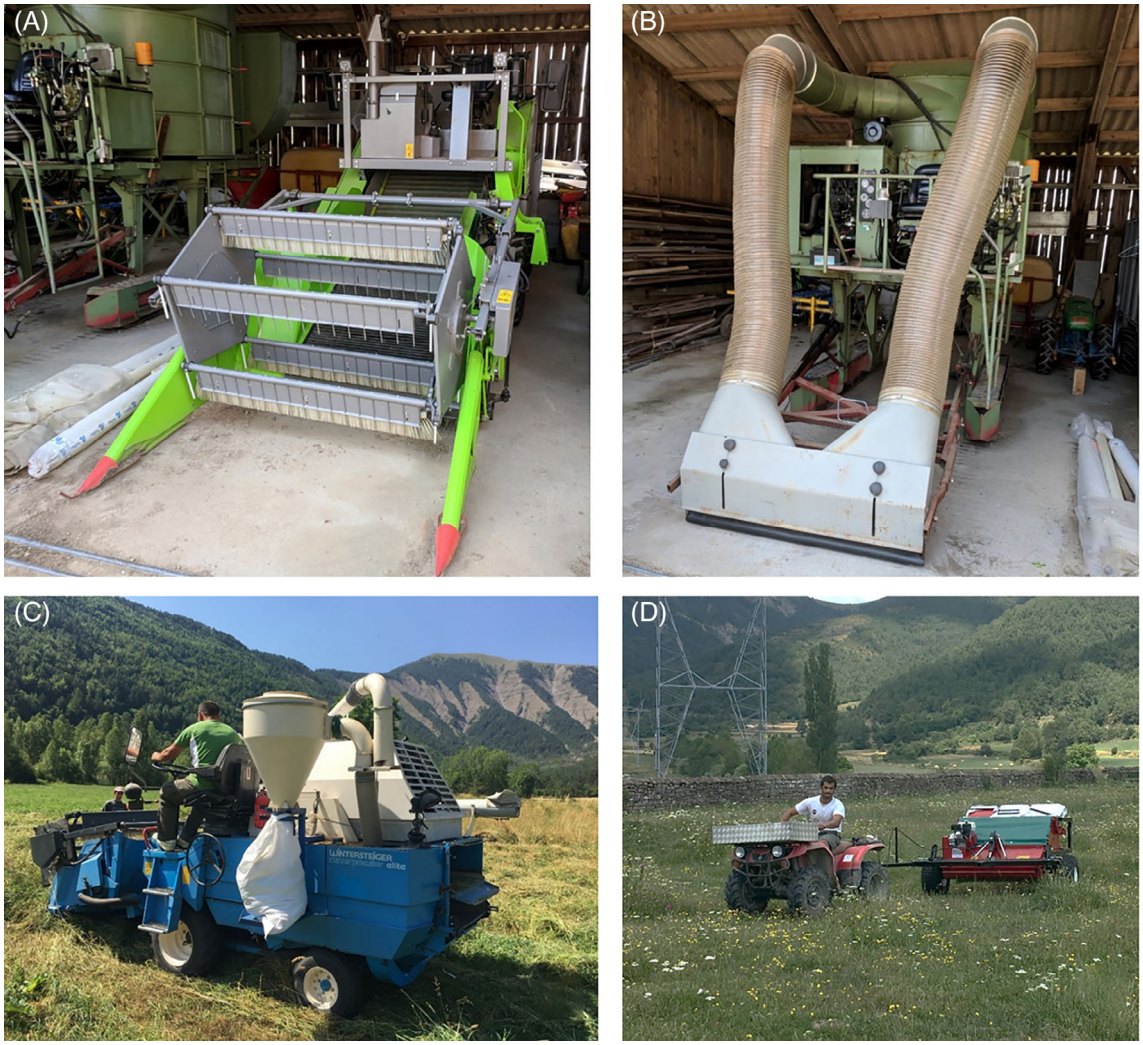

Figure 7. Native seed harvesting machinery. (A) Mini combine harvester. (B) Vacuum harvester from Wildblumenburri in Lenggenwil (Switzerland). (C) Combine harvester and (D) brush harvester used in a hay meadow located in the Pyrenees (Spain). (Pictures (A) and (B) by Simone Pedrini and, (C) and (D) by Candido Gálvez-Ramírez).

approaches are usually effective, but over time and where research and testing capacity are available, the number of generations recommended for cultivated production crops for different taxa (according to life form and reproductive strategies) should ideally be determined on a species by species and region by region basis.

Certification and Traceability

Certification schemes such as the pre-variety germplasm certification program in the United States (Young et al. 2003) or VWW Regiosaaten® in Germany (Mainz & Wieden 2019) are important mechanisms for authenticating the origin of founder seed grown in cultivated seed production settings and that appropriate cultural practices have been adopted in production systems to retain the genetic diversity of those natural founding populations.

Essential to any cultivated native seed production system is a well-structured recording and labelling system that allows for the tracing of a collection from source to field and the path through the production (multiplication) cycle until deployed (Fig. 4). Such systems allow accurate and consistent descriptions of the seed batch through the production cycle. Ideally, they entail recording of essential data to seed growers from source, through cultivation, to harvest yields and seed testing (Guest 2018; Pedrini & Dixon 2020).

Harvesting Techniques and Equipment

Whether seeds are obtained from natural/managed populations or from cultivated seed production systems, similar harvest methodologies and techniques are used; however, the scale of operations, the frequency of harvest, and the volume of seed harvested can be quite different.

Table 1. Comparison of different harvesting methods to be used in managed seed production. *Various hand tools and back-pack or portable vacuum harvesters may be used.

Harvesting Method	*Application*	*Limitations*	*Species (examples)*
Manual*	Small plots to produce foundation seed. Wild populations with limited access. Native stands and planted orchards.	Slow, time consuming. Difficult to harvest large quantities. High cost	All species.
Brush harvester	Species with: Readily dehiscent seed Indeterminate ripening Advantage: multiple harvests possible.	Seed of especially tall or short plants missed.	Grasses. Tall dicots with exposed inflorescences.
Cutting and threshing	Species with: Dehiscence or shattering	Requires significant equipment to manage volume. Cleaning may be difficult, time-consuming, and costly.	Legumes with distinct and rapid dehiscence
Combine harvester	Species with: Short flowering period and indehiscent fruits Easily threshed seeds in favorable seasons Collections of wild populations of some herbaceous species	High fixed and equipment costs. Difficult to use on uneven or steep land. Immature seeds are also collected.	Brassicaceae Asteraceae Plantaginaceae Caryophyllaceae
Vacuum harvester	Species with rapid and fairly uniform dehiscence. Seeds that are: exposed, light, and often possess a coma or pappus. Low-growing or creeping species sown over landscape fabric.	Limited equipment availability. Low collection efficiency.	*Nigella* spp. *Matricaria* spp. *Trigonella* spp. Asteraceae

Ideally, harvest is performed when seeds reach or are near maturity, which is usually the point of natural dispersal. Seeds collected too early may be undeveloped and could lose viability when dried or fail to germinate (Fig. 5). However, seed from certain species can be collected with fruits and stems intact and left in dry storage conditions for seed to mature (Delpratt & Gibson-Roy 2015).

Seed maturity indices include changes in color, fruit dehiscence, and seeds becoming hard and dry. It is highly recommended to clearly separate seed from fruits or coverings and to conduct cut test or pinch test before harvesting, to gauge fill, viability, and optimal timing of harvest as apparently "normal" fruits may be inviable. For many species, clarifying that the endosperm is firm and not soft is a key indication that seed is progressing towards maturity.

Small-Scale Seed Harvesting

Harvesting seed by hand is generally required to collect individual species within multispecies populations, those fruiting earlier or later than other species present in the same population, and at sites with difficult access or rugged terrain. Hand collection is used for seed collection from natural/managed populations and in cultivated production systems where crops are small, or species are of high value (e.g. seed price or species of conservation concern). Simple hand collection techniques include plucking, stripping, raking, clipping, or shaking seed from plants.

If seed dispersal/dehiscence occurs over longer periods seed can be captured using seed traps or bagging. Examples of such are provided by Way and Gold (2014) and Cochrane et al. (2009). Ideally seed is taken directly from the plant rather than from the ground beneath where it may be old, moldy, infested with insects, at or near the point of germination, or from a different or nontarget species altogether. For seed retained at height, it is normally possible to bring seed to ground level using extendable pole pruners, catapults, or throw lines (with appropriate training and safeguards in place climbing may also be necessary) (Fig. 6). In all cases, ensure that proper safety and personal protective equipment is used (Kallow 2014).

Mechanical Seed Harvesting

In most cases, seed harvest from larger scale cultivated production systems is performed with mechanical equipment. Mechanical devices can range from small handheld equipment (e.g. vacuums or brush harvesters) (Fig. 6) through to large agricultural sized machines (Fig. 7). The choice of machine is typically dictated by a range of factors including type and spatial area of the crop, configuration of the crop bed, type of seed, and quantity of seed. A wide variety of agricultural and horticultural harvesters have been adapted for field-scale native seed harvest such as mini-combine harvesters, tractor-mounted brush harvesters, and vacuum harvesters.

Brush harvesters use rotating brushes (of different length, type, density) to displace seed/fruits from plants. Where unripened seed remains on plants further passes may be performed during one season to capture species with different maturation times (Adams et al. 2016) or new seed that is produced at a later stage. Vacuum harvesting—using handheld or vehicle-drawn

equipment—is a technique frequently employed for small, low-growing plants with diffuse, fine seed. This method is also effective for collecting seed directly from the ground (FloraBank 1999) especially where seed has fallen onto laid weed mat (ideally at or soon after dispersal to minimize the risk of seed loss through wind or from predation).

Brush and vacuum harvesters enable seed to be collected at different heights and times without damaging the plant. In contrast, harvesting equipment that cut stems, such as combine harvesters, may gather all seeds, regardless of their maturity state (noting that some species can mature post-harvest). If harvest options are limited to cutting methods, harvesting could be conducted on different parts of the crop at different times. Detailed species knowledge regarding timing of flowering and seed set will help growers to tailor harvest approaches and methods for species and production settings, thus ideally maximizing efficiency, yields, quality, and genetic diversity (Table 1).

Conclusions

Globally, vast quantities of seeds from a great diversity of plant species are critical for meeting large-scale restoration goals. Those species that exhibit the capacity for effective seed production (as managed natural populations or through cultivated seed production systems) and those with a specific need for increase (regardless of ease), such as rare or threatened species, should be candidates for investigation for these purposes so that effective and efficient ecological restoration can be conducted at-scale across the world. However, full ecological restoration based on the eight guiding principles in Gann et al. (2019) may ultimately require increasingly comprehensive suite of species be understood and integrated into seed production systems.

Native seed production is an emerging area across the world and significant research and adaptive management will be required to refine and enhance current methods and outcomes. Many standard agricultural and horticultural techniques have proven effective for use with a relatively narrow suite of wild species, but large information gaps remain for many others (Hancock et al. 2020). Nevertheless, cultivated seed production represents an area of unique potential for the supply of the most fundamental resource for ecological restoration—seed. To this end, managed natural populations and cultivated production systems provide an outstanding opportunity for delivering the quantity and quality of seed required for large-scale global restoration and every effort should be made to encourage and support their development.

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Supporting Information

The following information may be found in the online version of this article:

Supplement S1. Seed collection
Supplement S2. Seed production
Supplement S3. Pollinators, pestand disease management

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PRACTICAL ARTICLE

Ensuring seed quality in ecological restoration: native seed cleaning and testing

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Seeds are a critical and limited resource for restoring biodiversity and ecological function to degraded and fragmented ecosystems. Cleaning and quality testing are two key steps in the native seed supply chain. Optimizing the practices used in these steps can ensure seed quality. Post-collection handling of seeds can have a profound impact on their viability, longevity in storage, and establishment potential. The first section of this article describes seed cleaning, outlines key considerations, and details traditional and novel approaches. Despite the growth of the native seed industry and the need for seed quality standards, existing equipment and standards largely target agricultural, horticultural, and commercial forestry species. Native plant species typically have complex seed traits, making it difficult to directly transfer existing cleaning and quality standards to these species. Furthermore, in ecological restoration projects, where diversity is valued over uniformity crop standards can be unsuitable. We provide an overview and recommendations for seed quality testing (sampling, purity, viability, germinability, vigor), identity reporting, and seed transfer as well as highlight the need to implement internationally recognized standards for certification for native seeds. Novel and improved cleaning and testing methods are needed for native species from a range of ecosystems to meet the challenges and goals of the United Nations Decade on Ecosystem Restoration. The guidelines outlined in this article along with others in the Special Issue of Restoration Ecology "Standards for Native Seeds in Ecological Restoration" can serve as a foundation for this critical work.

Key words: germination, native seed industry, purity, quality standards, seed conditioning, viability

Implications for Practice

- The diversity of morphological and physiological traits among seeds of native species requires a variety of cleaning techniques and equipment to achieve optimal seed lot quality and representative genetic diversity.
- Seed testing provides assurance about the value of a seed lot and is important for calculating seeding rates for ecological restoration.
- The biology and uses of native seeds are distinct from those of agricultural species; cleaning and testing methods for native seeds are needed.
- However, standardized rules for native seed testing are currently lacking for many species, jurisdictions, and ecosystems.
- When third-party seed testing services are unavailable, the seed supplier should provide testing services.

Introduction

Given the unprecedented rates of ecosystem degradation exacerbated by a changing climate, several large-scale efforts such as the United Nations Decade on Ecosystem Restoration, the Trillion Tree Campaign, and FAO REDD+ are working to reverse degradation globally (Food and Agriculture Organization of the United Nations 2020; Plant for the Planet 2020; United Nations 2020). Most ecological restoration relies on the use of direct seeding or planting stock, which requires high input of viable, and genetically appropriate, native plant seeds (Broadhurst et al. 2015, 2016; Gann et al. 2019). Despite the growing demand, there is limited guidance and regulation surrounding the collection, cleaning, and quality testing of native plant seeds (Ryan et al. 2008; Marin et al. 2017), which limits restoration success and increases economic and biological costs. In the longer term, addressing this gap will require investment in developing seed quality standards and certification schemes for native plant seed use in restoration to ensure the ability to meet global restoration targets. While existing standards used in agriculture, horticulture, and commercial forestry can serve as a basis—these methods will need to be modified to address unique

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biological and physiological traits associated with native plant seeds (Pedrini & Dixon 2020). In the short term, assuring that restoration practitioners and planners have adequate information to ensure quality throughout the seed supply chain is critical to the success of restoration outcomes. In this article we describe key elements associated with seed cleaning and quality testing of native plant seeds and offer recommendations.

Post-Collection Seed Cleaning

Following collection or harvest (Pedrini et al. 2020a), seeds and associated materials are typically dried, processed or cleaned, and packaged for immediate use, short-term storage, or long-term conservation banking. Seed handling and storage practices used immediately after harvest and during cleaning can impact seed viability, longevity, and dormancy status (Hay & Probert 2013). Specific recommendations for post-harvest storage are provided by De Vitis et al. (2020).

Seed Cleaning

Seed cleaning (also known as processing, or conditioning) is the removal of inert matter, seeds of undesirable species, and nonviable seeds from a seed lot (or batch) (Houseal 2007; Bonner et al. 2008; Bartow 2015). Appropriate seed cleaning reduces potential vectors for pathogens and pests, reduces seed lot volume, facilitates moisture management (important for viability maintenance), reduces storage costs, increases seed lot purity and quality, simplifies seed handling, improves flowability through mechanized equipment, and allows for the application of seed enhancement treatments such as seed coating (Pfaff et al. 2002; Houseal 2007; ENSCONET 2009; Bartow 2015; Guzzomi et al. 2016; Pedrini et al. 2020b).

Seed cleaning is as much an art as it is a science. This is due to the high diversity of seed traits (e.g. shape, mass, surface texture, covering structures, appendages, dormancy class) and dispersal units (e.g. dehiscent and indehiscent fruits and florets) that present a key challenge to effective and efficient cleaning of native plant seeds (Evans & Dennehy 2005; Erickson et al. 2016a; Saatkamp et al. 2019). While not all seeds must be removed from the dispersal unit or undergo additional cleaning, most do because seed cleaning can optimize storage capacity and duration (De Vitis et al. 2020). The diversity of seed traits means that each species requires different cleaning approaches or equipment—factors that should be carefully considered to maintain genetic diversity and reduce potential damage to seeds.

Seed cleaning typically follows this sequence: (1) extraction or the removal of seeds from the attached structures or fruits and (2) the separation of seeds from inert material by density, shape, and/or surface texture, to improve seed lot purity (or the proportion of filled seed units within a seed lot) (Pfaff et al. 2002; Houseal 2007; Terry & Sutcliffe 2014; Bartow 2015). The equipment used for seed cleaning ranges from simple tools and techniques to high-tech engineered machinery (Fig. 1) (Houseal 2007; Bonner et al. 2008; Terry & Sutcliffe 2014; Bartow 2015). Sometimes, natural cues that promote seed dispersal can inform the development of seed cleaning techniques. For example, for serotinous species (e.g. *Banksia* spp., *Pinus* spp.) exposure to wildfire causes seed release. Mimicking such approaches (e.g. exposure to hot air or hot water) can facilitate seed release in these species (Krugman & Jenkinson 1974; Baskin & Baskin 2014).

Traditional Seed Cleaning Approaches

The first stage in seed cleaning, extracting seeds from attached structures, uses threshing (for dry fruits) or gentle maceration (for wet fruits) (Figs. 1 &2). The most basic and accessible method for cleaning seeds is to break apart the dispersal units and pick seeds out by hand. Simple tools like rubber mats, wood blocks covered with sandpaper or rubber, rolling pins, sieves, fans, or the wind are other low-cost methods. Fruits can be threshed through a variety of physical means or in the case of wet fruits, soaking and rinsing. Mechanized cleaning methods increase the efficiency and uniformity of seed cleaning. There are many thresher designs, typically with a chamber or space where seed units are struck or squeezed to break them apart. Macerators mix water and fleshy fruits together and stir or beat them to dissolve away the pulp.

The second stage in seed cleaning, separating seeds from inert matter or other species of seeds, relies on differences in physical characteristics such as size, density, surface texture, shape, or color and uses a range of basic to highly engineered equipment (Figs. 1 & 2) (Center for Plant Conservation 2020; Pfaff et al. 2002; Houseal 2007; Borders & Lee-Mäder 2014; Terry & Sutcliffe 2014; Bartow 2015).

Alternative and Innovative Seed Cleaning Approaches

Several novel methods with particular promise for species that are otherwise challenging to clean are flash flaming and acid digestion (Stevens et al. 2015; Guzzomi et al. 2016; Ling et al. 2019; Pedrini et al. 2019). Flash flaming uses a modified rotary seed coater to briefly and intermittently expose seeds to an open flame to remove awns or other appendages without damaging seed embryos. Removing the awns and appendages improves seed handling, allows for subsequent seed coating, and increases germination rates in the field (Guzzomi et al. 2016; Ling et al. 2019). However, this process requires careful calibration for each species to avoid damaging seeds or affecting dormancy status (Pedrini et al. 2019). Acid digestion is another approach that has showed improvements in seed handling and germination on Australian native grass species (Stevens et al. 2015; Pedrini et al. 2019). This method needs to be calibrated to the optimal concentration and exposure length for maximum digestion of appendages without compromising viability. Although both flash flaming and acid digestion have shown potential in pilot trials, these methods have yet to be tested at an industrial scale.

Other Cleaning Considerations

All cleaning approaches should be evaluated with respect to potential impacts on seed viability, storage longevity, germination, or establishment (Erickson et al. 2016a). For example, in many seeds with indehiscent fruits, seed extraction may damage the seed, or requires multiple steps such as soaking or heating

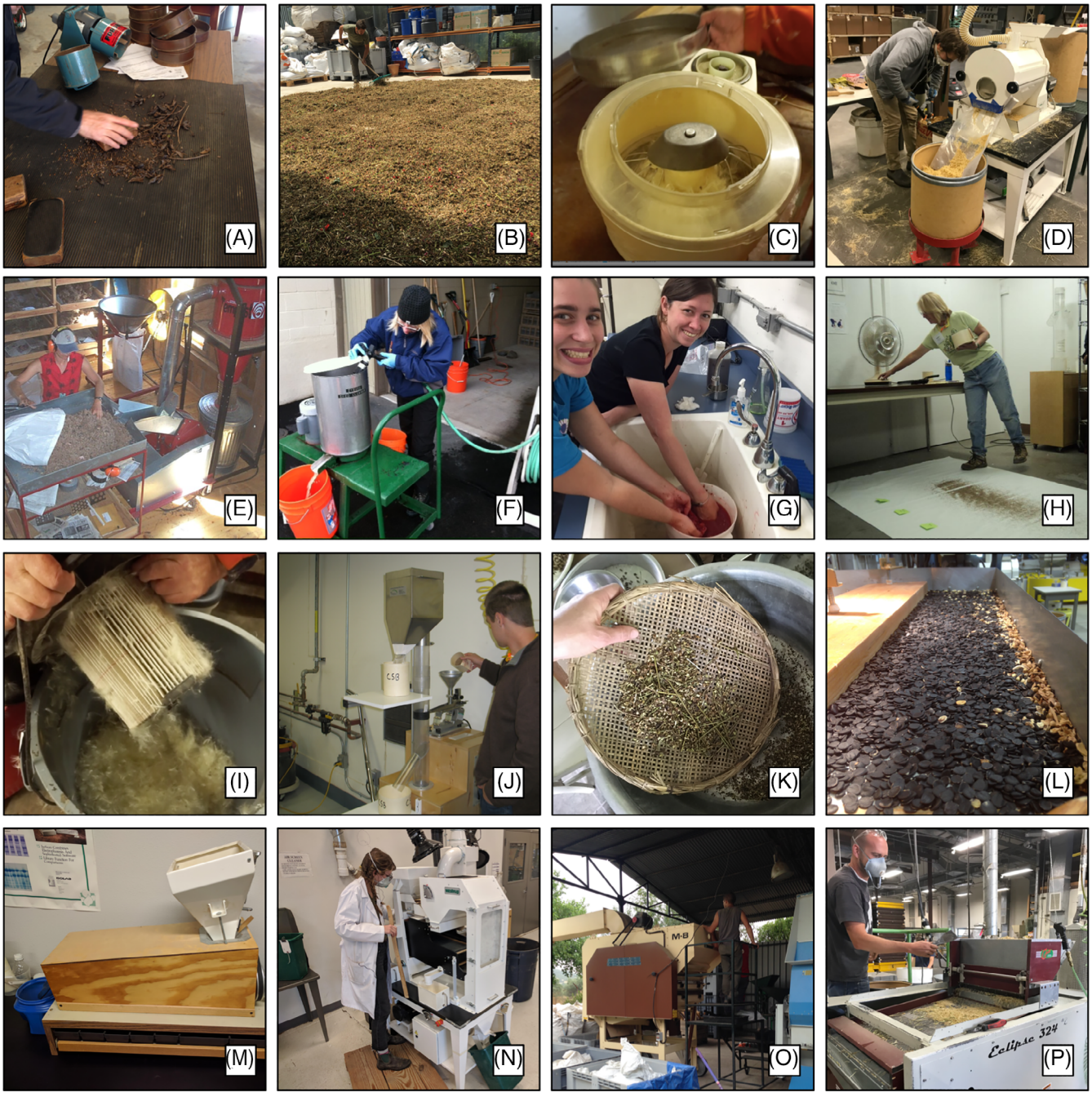

Figure 1. Techniques and equipment frequently used in seed processing. I. Seed extraction: (A) Gentle beating, rolling, or manual separation from attached fruits or florets (S. Frischie/USDA Beltsville Plant Materials Center, U.S.); (B) Exposure to warm or dry temperature to open dehiscent fruits (C. Galvez/Semillas Silvestres, S.L., Spain); (C) Mortar/cement mixers, rock tumblers, kitchen blenders, or food processors with plastic blades (M. Skinner/Skinner Native Seeds, Canada); (D) Brush machine or debearder (USDA Forest Service Bend Seed Extractory, U.S.); (E) Hammer mill (B. Kleiman/Nachusa Grasslands, U.S.); (F) and (G) Macerators or grinders (USDA Forest Service Bend Seed Extractory, U.S.); II. Separation of seed from undesirable material: (H) Fan (USDA Forest Service Bend Seed Extractory, U.S.); (I) Vacuum (M. Skinner/Skinner Native Seeds, Canada); (J) Continuous seed blower (USDA Forest Service Bend Seed Extractory, U.S.); (K) Sieves and screens (S. Frischie); (L) Gravity table (USDA Forest Service Bend Seed Extractory, U.S.); (M) Velvet roller mill (Laura Fischer Walter, Tallgrass Prairie Center, University of Northern Iowa, U.S.); (N) Air screen cleaner or fanning mill (Laura Fischer Walter, Tallgrass Prairie Center, University of Northern Iowa, U.S.); (O) Air screen cleaner or fanning mill (S. Frischie/Semillas Silvestres, S.L., Spain); (P) Air screen cleaner or fanning mill (USDA Forest Service Bend Seed Extractory, U.S.). Other types of equipment, not pictured: thresher, acid digestion, flash flaming, manual separation, indent cylinder, spiral separator, color separator, aspirator, blower.

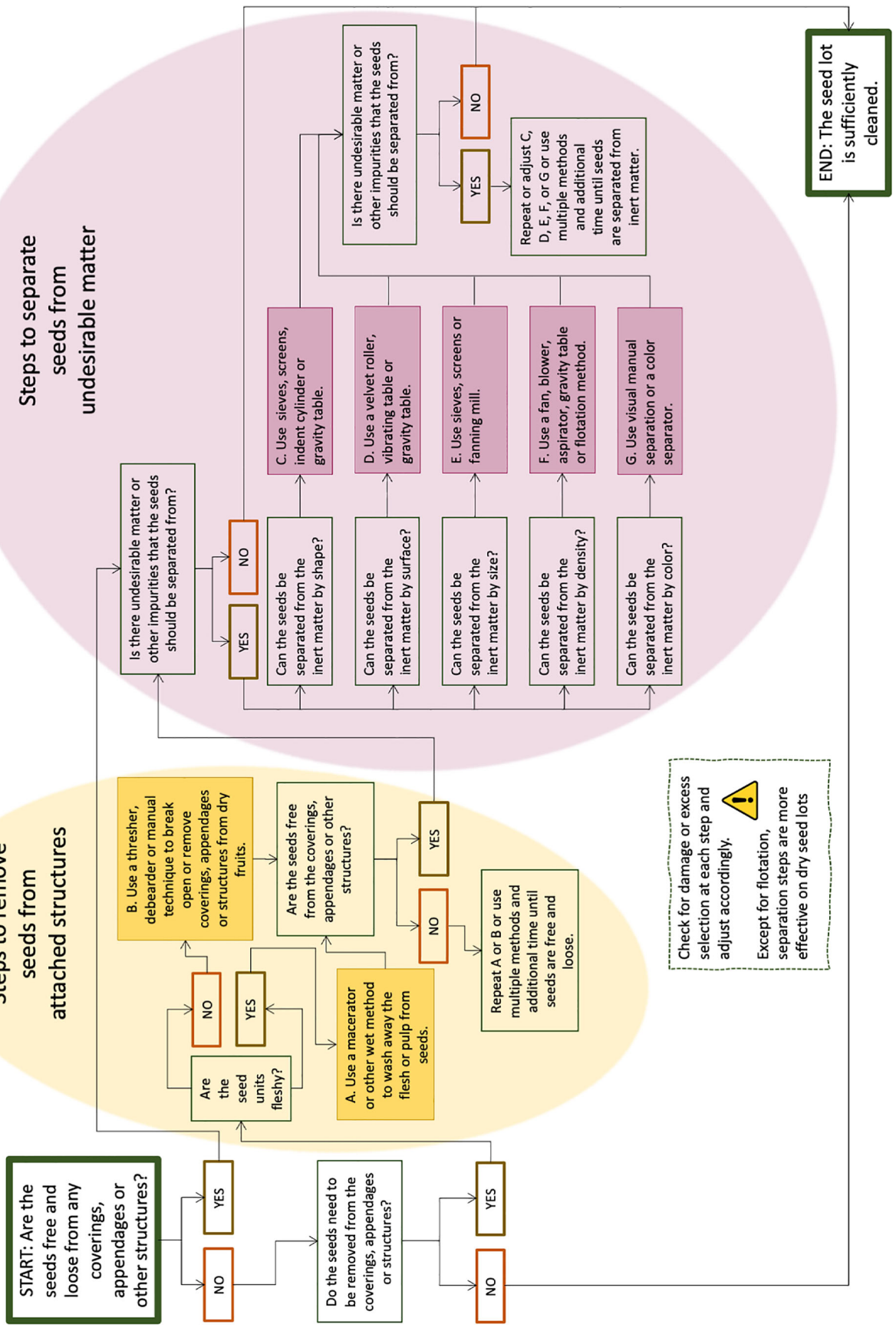

Figure 2. Flowchart showing the steps and processes for cleaning native seed lots.

(for serotinous species) in addition to mechanical extraction (Bonner et al. 2008). In some cases, particularly when storage is not required, whole fruits may be sown as a way of overcoming problems associated with cleaning. When working with a new method, species, or seed population, preliminary trials that compare germination of seeds following cleaning procedures (e.g. appendage or covering structure removal) and control seeds (e.g. no cleaning, or standard cleaning procedures) should be performed (Erickson et al. 2016a).

It is also important to minimize artificial selection during cleaning (Basey et al. 2015; Rogers & McGuire 2015). To avoid overcleaning, one should monitor seed quality carefully, to distinguish between traits that may indicate low seed viability versus genetic variability. Minor differences in seed morphological traits can reflect genetic differences that may be important for plant survival and recruitment, particularly for plant populations in variable and dynamic environments (Basey et al. 2015; Rogers & McGuire 2015). Evaluating a sub-sample for seed quality after each cleaning step (e.g. visual inspection, cut test, x-ray imaging, tetrazolium staining) can help inform how the cleaning approach can be modified to maximize the removal of undesirable matter without sacrificing genetic diversity or seed quality (following section and Fig. 2).

Additional factors, such as the intended use of a seed lot in ecological restoration or conservation (e.g. commercial sale, conservation in a germplasm bank, in-house use, propagation), also contribute to the decision about the degree of seed cleaning needed. Seed lots that are commercially available may be subject to laws for labeling and seed trade within particular jurisdictions and may be held to a higher standard of purity and identity than seeds that are not traded (Agricultural Marketing Service 2011; Mainz & Wieden 2019). Conversely, with small seed lots, seed banks must balance maintaining optimal seed viability with conserving maximum genetic diversity and thus may allow for a slightly less pure seed lot, if inert materials do not jeopardize seed longevity in storage (Terry & Sutcliffe 2014).

The Status of Quality Testing of Native Seeds

The agricultural seed industry has developed detailed and comprehensive guidelines, rules, and protocols for testing seed quality of agricultural, forestry, horticultural, and other commercial species/varieties (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). These widely accepted standards have enabled the creation of an extensive network of accredited seed testing laboratories that can provide independent seed lot quality analysis to verify that minimum seed quality requirement, usually set by regulations. Such a system creates assurances and expectations for customers about how the purchased seeds will germinate and establish.

For the native seed industry, factors such as species diversity (most of which do not have accepted rules for testing under agricultural seed standards), high interspecific variability (Ronnenberg et al. 2007; Hamasha & Hensen 2009), and complex morphological and physiological seed traits (Commander et al. 2009; Baskin & Baskin 2014; Erickson et al. 2016b; Kildisheva et al. 2019) have so far limited the application of seed quality testing under the accepted international frameworks of International Seed Testing Association (ISTA) (Ryan et al. 2008; Pedrini & Dixon 2020). For example, in the United States, attempts have been made to provide seed testing guidelines for native seeds (Native Seed Task Force 2011) and some seed testing laboratories are now able to offer independent seed quality analysis. However, guidelines and official rules are lacking for most taxa and geographies.

In some cases, these challenges have given suppliers and customers the perception that native seed quality cannot be tested effectively, resulting in the sale of seed lots with few or no seed quality measures reported (Ryan et al. 2008). As a result, seed users with limited understanding of native seeds may operate under the assumption that seeds purchased are viable and readily germinable as would be expected for crop species. This approach often results in ill-informed decisions regarding seeding rates and timing (Erickson & Halford 2020) and jeopardizes the success of seed-based restoration projects (Shaw et al. 2020). Given the frequency of unexpected restoration failures, some users have assumed and accepted low seed quality as an intrinsic property of native seeds with no expectation that seed quality measures are required at the point of sale. This has sometimes led to the use of non-native species on difficult-to-restore sites, further contributing to ecosystem degradation. To ensure that critical information for restoration planning is available, seed quality testing is vital for both the native seed supplier and user and should be considered a key component of the native seed supply chain (Hay & Probert 2013).

Native Seed Quality Testing Recommendations

Of the tens of thousands of native species used in restoration, accepted seed testing rules exist for only a few hundred (Association of Official Seed Analysts 2018; International Seed Testing Association 2020).

The seed testing procedures outlined in the following section are derived from the officially recognized seed testing rules and guidelines adopted by ISTA and the Association of Official Seed Analysts (AOSA) (Association of Official Seed Analysts 2018; International Seed Testing Association 2020) and consultation with native seed scientists and suppliers. However, some elements of the standard testing procedure in the ISTA and AOSA documents have been modified or made less restrictive to accommodate the diversity and complexity of native seed traits. Adaptations were also made following the protocols and guidelines developed by the European seed bank consortium (ENSCONET 2009), the Native Seed Quality Task Force in the United States (Native Seed Task Force 2011), and the Australian Florabank initiative (Mortlock & Australian Tree Seed Centre 1999; Mortlock 2000). A detailed overview of how and when various testing approaches should be used is provided by Pedrini and Dixon (2020).

Sampling

Quality testing is performed on a sample of a seed lot. It is important to ensure that the sample used is representative of

the entire seed lot. Samples that are biased or unrepresentative will not provide repeatable or meaningful results. Likewise, any subsequent subsampling should also follow rules for mixing and dividing in an unbiased and representative manner. This step is especially important for species that produce a high quantity of inert plant material (Pedrini & Dixon 2020).

Purity

Purity is usually the first test performed on a seed lot. The aim of this test is to determine the percentage of pure seeds, inert material, and seeds of other species present in the sample. Purity is recorded as a percentage of pure seed units (PSU) by weight in a seed lot (Association of Official Seed Analysts 2018). The concept of PSU is helpful because seeds of many species are enclosed in external structures (e.g. florets, pericarps, and fruits) and, at times, more than one seed can be found within a unit. A well-defined PSU ensures repeatability among labs in seed testing. Both AOSA and ISTA have specific definitions that state what the PSU is for a particular species. If a native species is being tested for which no official PSU has been defined, it is crucial that the analyst performing the test includes a clear definition of what was used as the PSU (specifically what attached structures are included and the appearance and size range of a unit) before proceeding with purity and viability tests. The purity test is performed by visual inspection and/or pressure with tweezers and by separating and weighing the different fractions (manually, or by using sieves and air separator). Unlike ISTA and AOSA guidelines which are based on agricultural standards, Pedrini and Dixon (2020) recommend that native seed units that appear underdeveloped, shriveled, damaged, broken, predated, or infected should not be considered PSU. If a seed batch has a high percentage of inert materials and seeds of other species, the purity may be improved by optimizing the seed cleaning step (Fig. 3).

Viability Testing

The viability test is performed on PSU and provides an estimate of the portion of the seed batch that is viable and potentially able to germinate, known as the viable seed unit (VSU) (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). Viability testing can be accomplished using different criteria (physical or biochemical) and methods or combine multiple methods (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). The selection of the testing approach will depend on the level of information needed and resources available.

A simple, yet effective, means to estimate seed fill (presence of a fully developed embryo)is by performing a cut test. Seed fill is a preliminary estimate of potential viability. In a cut test, seeds are cut with a scalpel blade or other sharp instrument and visually examined, preferably under a dissecting microscope. If the endosperm appears white, turgid, and solid and the embryo is intact the seed could be considered potentially viable. If discoloration or shrinkage is detected and the embryo is damaged or detached the seed is most likely non-viable.

Another testing procedure, x-ray imaging, provides a fast and accurate means to determine seed fill and internal integrity in seed; however, the equipment required for this procedure is expensive. Both the cut test and x-ray require some form of calibration, experience, and care in interpreting the results. Like the

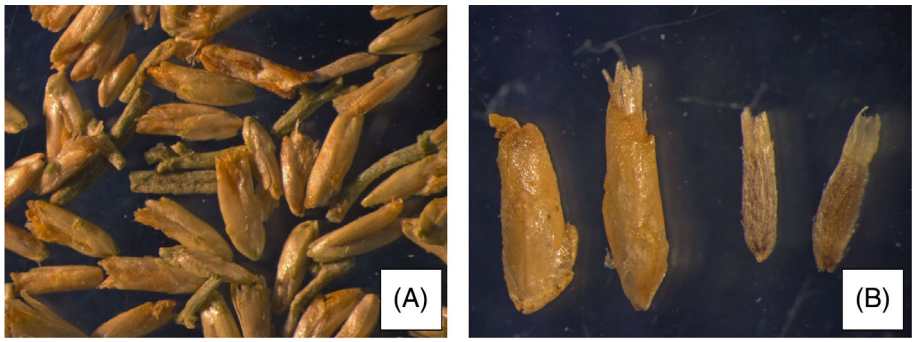

Figure 3. A comparison of different definitions of pure seed unit (PSU). Intact seed heads (A) and individual achenes (B) from a seed sample of *Gutierrizia microcephala* (Asteraceae). One can see the substantial difference in the purity and viability results depending on the pure seed unit definition. In this example, the PLS (pure live seed) based on achenes is three times that of the calculation based on seed heads. This example also illustrates why PLS calculations should be based on purity and viability tests of the same subsample. Never use the results of one purity test with the results from a different viability test to calculate PLS. This can result in drastic miscalculations for a seed lot, especially when the pure seed unit definition has not been standardized, as is the case with many native seeds.

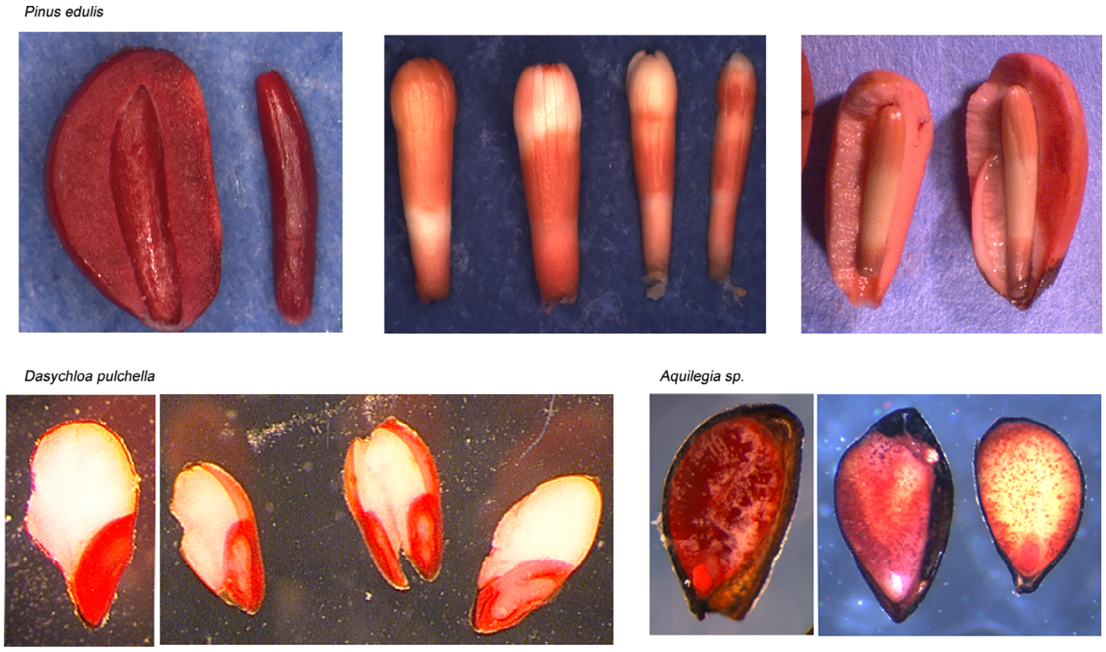

Figure 4. To properly interpret tetrazolium staining patterns, it is important to understand embryo types, anatomy, and whether or not the nutritive tissue is living for each taxon. Sample preparation is also critical to avoid cutting artifacts if possible and to recognize the appearance of them when they occur. Three taxa and the variations in staining patterns are shown. Top: *Pinus edulis* (Pinaceae), nutritive tissue must stain. Left: normal. Center and right: abnormal. Bottom: *Dasychloa pulchella* (Poaceae), nutritive tissue normally does not stain. Left: normal. Right: abnormal. *Aquilegia sp*. (Ranunculaceae), nutritive tissue must stain and the rudimentary embryo at the base must stain. Left: normal, with whitish cutting artifact on the nutritive tissue. Right: both abnormal, one with unstained embryo and one with unstained nutritive tissue and stained embryo.

cut test, the x-ray is not a true viability test, as both viable and non-viable filled seeds can look the same. Assessment of seed fill through a cut test or x-ray imaging is not a replacement for germination or tetrazolium (TZ) tests for viability. A TZ test is performed by imbibing seed to soften seed tissues followed by incision, embryo excision, or the removal of the seed coat to expose the inner tissues of the seed. Seeds are then soaked in a solution of 2,3,5-triphenyl tetrazolium chloride (usually between 1 and 4%) for 2–24 hours. Tetrazolium chloride reacts with the hydrogen ions released by live tissues during respiration and forms an insoluble red compound. This process stains the tissue in a specific pattern that allows for the detection and assessment of overall proportion of vital components of the seed, such as the embryo or endosperm (Fig. 4).

TZ testing has been shown to correlate closely with germination and cut test values of plant species native to Europe and Australia, respectively (Ooi et al. 2004; Marin et al. 2017). This method is particularly useful for species that may be dormant (Ooi et al. 2004); however, the interpretation of the staining pattern requires a sound understanding of species-specific seed morphology and physiology because the staining can occur more slowly and be less pronounced than for crop species (Fig. 4) (Paynter & Dixon 1990). Unfortunately, for most native species, protocols for performing TZ testing and interpretation are not yet widely available (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). However, many available protocols can be adapted following minor preliminary validation (Miller 2010).

Because germination is the ultimate representation of viability in the natural environment, germination tests (see next section) are often interpreted as synonymous with viability. However, because seeds of many native plant species exhibit dormancy (Baskin & Baskin 2014; Kildisheva et al. 2020), interpretation of germination data should be done in tandem with other measures (Pedrini & Dixon 2020). For example, if seeds are non-dormant and germination conditions are well understood, a germination test can be considered equivalent to

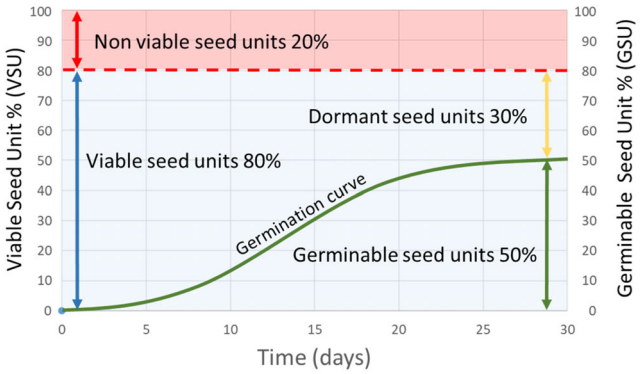

Figure 5. Theoretical representation to show how a germination test combined with a viability test is used to determine the fraction of the seed lot that can be considered viable (blue), germinable (green), dormant (yellow), and non-viable (red).

viability; however, it is recommended that all un-germinated seeds that remain at the end of a test period are subject to a cut or TZ test (to estimate final viability) and are accounted for in the calculation of total germination percentage (Pedrini & Dixon 2020). Conversely, if a large proportion of seeds in a seed lot are dormant, dormancy must be released prior to conducting the germination test. While not always possible, for some species this can be done relatively quickly by applying physical (e.g. scarification) or chemical (e.g. gibberellic acid, potassium nitrate, karrikinolide, or smoke water) treatments, depending on the species-specific dormancy mechanism (Erickson et al. 2016a; Kildisheva et al. 2019).

In addition to traditional methods for viability testing, alternative seed testing techniques to assess seed lot viability are being developed. For example, the electrical conductivity test (EC) test (Marin et al. 2018) and low-tech "pop" test (Tilley et al. 2011) may be used in some cases (Appendix S1).

Germinability

This test is performed on PSU and shows what portion of the seed sample can germinate at a given moment. This portion is known as the germinable seed unit (GSU). The test is performed by placing the seeds in a moist environment, at the optimal temperature and light conditions for inducing germination. Optimal germination conditions and time for germination can vary greatly among species. For species that do not have official testing rules, this information may be available from published sources or online databases (Royal Botanic Gardens Kew 2020). If such information is not available, preliminary germination tests can be used to determine the optimal germination conditions and expected duration of the germination test. Under official seed testing rules, a seed is considered to have germinated when the essential structures (root, cotyledon, epicotyl) of a seedling have emerged and can be evaluated as functional and indicative of the ability to produce a normal plant under favorable conditions (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). This definition of germination is based on the origins of seed testing for agricultural species. However, a different concept of germination may be more suited to native species. For example, researchers studying native seeds and some conservation seed banks consider radicle emergence of 1–2 mm as germination (ENSCONET 2009; Pedrini & Dixon 2020). Currently, radicle emergence is not a valid definition of germination by accredited seed testing labs nor can test results using that definition be labeled for sale (Association of Official Seed Certifying Agencies 2020; Association of Official Seed Analysts 2018; International Seed Testing Association 2020).

Once germinated, the seed can then be removed or can remain in the germination container if further information on seedling development (such as inspection for seedling defects or abnormalities) is required. Germination is recorded over time, to obtain information on germination rate, and at the end of the test, to record final germination. The test is terminated when the percentage of seed germination over time has remained unchanged and no more germination is recorded. Typically, the maximum duration of a commercial germination test is 4–6 weeks (Baskin & Baskin 2014; Kildisheva et al. 2020). The portion of non-germinated seeds at the end of the germination experiment could be either dormant or non-viable. By comparing the results of the viability test (VSU) with the one of the germinability tests, it is possible to obtain an estimate of the dormant seed unit (DSU), which is the percentage of PSU affected by dormancy (Fig. 5) (Pedrini & Dixon 2020). Alternatively, the non-germinated seed can be tested for viability with the methods previously described to derive the portion of non-viable and dormant seed units.

Once values of seed purity (PSU%) and viability (VSU%) have been obtained, they are multiplied to return the pure live seed (PLS%). This value is the main outcome of seed quality testing and should be used for determining the value of seed lots and for calculating seeding rates (Pedrini & Dixon 2020; Vogel 2002).

Vigor Test

Vigor tests are a specialized germination test. While germination tests measure viability under optimum conditions, vigor tests usually measure germination under conditions that induce plant stress. For seed lots with high germination percentage, vigor tests distinguish which lots are likely to maintain quality during storage from those that should be used sooner to avoid deterioration (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). The use of this test is limited by the wide ecotypic variability in dormancy and germination requirements of native seed lots, and the lack of required reference samples. Historically, vigor tests have only been recommended for species that germinate readily. Recently, vigor tests have become useful in evaluating the effectiveness of seed enhancement treatments such as priming and coating (Pedrini et al. 2020b). The types of vigor tests include cold test, accelerated aging, and radicle emergence (Association of Official Seed Analysts 2018; International Seed Testing Association 2020). Both ISTA and AOSA have vigor testing handbooks available describing methods currently in use for crops that could be adapted for native species (Association of Official Seed Analysts 2018; International Seed Testing Association 2020).

Legal Frameworks and the Role of Third-Party Agencies

Accurate seed quality metrics (such as PLS%) help native seed users determine appropriate seeding rates and understand the likelihood of success for each seed lot. Thus, understanding the methods and units represented by the various seed quality tests is important for accurately reporting and interpreting results. Because the legal or regulatory requirements for seed quality reporting vary by species, seed use, and jurisdiction, native seed collectors and producers should familiarize themselves with local and national requirements (Agricultural Marketing Service 2011; Abbandonato et al. 2018; Mainz & Wieden 2019). These laws aim to ensure that consumers have accurate information about the seeds they are purchasing and

that the seed lot is free of noxious weed seeds. Wild ecosystems are especially vulnerable to the spread of weeds, which can occur through large-scale seeding with weed-contaminated lots.

Another component of native seed lot value for restoration use is information on the taxonomic identification, source population (genetic origin), and estimated genetic diversity (Kramer et al. 2019). This should be reported in tandem with seed quality metrics (Abbandonato et al. 2018; Rantala-Sykes & Campbell 2019). For example, a high-quality seed lot (e.g. PLS of 99%) that has not been labeled or correctly identified to species level is of limited value to a restoration practitioner with requirements for matching species to the site. For a detailed description of seed quality reporting metrics and guidelines, see Pedrini and Dixon (2020).

The recent growth of native seed markets world-wide highlights the need to develop internationally recognized accreditation and certification schemes surrounding the collection, cleaning, and quality testing of native plant seeds (Ryan et al. 2008; Marin et al. 2017; Abbandonato et al. 2018).

ISTA and AOSA provide laboratory accreditation and membership to ensure adherence to standardized rules for seed testing among third-party testing laboratories. The Society of Commercial Seed Technologists (SCST) certifies and registers seed analysts.

Certification programs, like those in Germany and Austria and many U.S. states, are designed to identify and track plant material along the supply chain (Association of Official Seed Certifying Agencies 2020; De Vitis et al. 2017; Mainz & Wieden 2019). Implementation and adaptation of such standards at local, national, and trans-national levels would ultimately allow for the development of a reliable native seed supply chain to meet the restoration goals outlined by the United Nations and others (United Nations 2020). A forthcoming online resource, *Testing Wild Seeds*, is under development by ISTA, Royal Botanic Gardens Kew Millennium Seed Bank Partnership, AOSA, and SCST. Explicitly for native seeds, this online resource will include a range of descriptive information about seed morphology, protocols, testing methodologies, glossary, and photos. The anticipated site launch is expected for early 2021.

Conclusions and Recommendations

Appropriate cleaning and accurate quality assessment techniques are critical links in the native seed supply chain—with direct impacts on the success of restoration outcomes. Cleaning and quality considerations for native plant seeds are often more nuanced than crop species, due to the complex seed morphological and physiological traits and the limited knowledge and experience of seed laboratories in working with the diversity of species used in restoration. Meeting global restoration targets will require the development of new approaches and techniques for a diversity of native plant taxa. Furthermore, to ensure consistent quality and to build trust, seed quality and sourcing standards, accreditation, and certification schemes must be developed and implemented as a standard part of the native seed supply chain. These steps will require significant institutional investment in the infrastructure and training programs in the course of this decade (Ryan et al. 2008; Marin et al. 2017; Pedrini & Dixon 2020).

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Supporting Information

The following information may be found in the online version of this article:

Appendix S1. Alternative seed viability testing methods.

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PRACTICAL ARTICLE

Seed storage: maintaining seed viability and vigor for restoration use

Marcello De Vitis[1,2], Fiona R. Hay[3], John B. Dickie[4], Clare Trivedi[5], Jaeyong Choi[6], Rob Fiegener[7]

Effective seed storage after sourcing (harvesting or purchasing) is critical to restoration practitioners and native seed producers, as it is key to maintaining seed viability. Inadequate seed storage can lead to a waste of both natural and economic resources when seeds of poor quality are sown. When working with native species with unknown storage behavior, general assumptions can be made based on studies on related species, and standard practices may be applied with caution; however, an investigation should be conducted to understand if specific storage requirements are needed and for how long seeds can be stored before they lose significant viability. In this paper of the Special Issue *Standards for Native Seeds in Ecological Restoration*, we provide an overview of the key concepts in seed storage and the steps to take for effective storage of native seeds for restoration use.

Key words: ecological restoration, native seed, seed banking, seed longevity, seed moisture content, seed storage behavior

Implications for Practice

- Appropriate seed storage is critical to maintain seed viability and to increase success in restoration activities.
- When working with an unknown species, the seed storage behavior should be determined using a protocol, in order to know how to properly store its seeds.
- After seed collection and before seed storage, using research-based protocols, seed moisture content should be assessed and, according to the seed storage behavior, seeds should be dried to the appropriate level of moisture content and relative humidity and packaged for storage in an appropriate airtight container.
- For orthodox species, seeds can be usually stored at −18°C for more than 5 years.
- For recalcitrant species, seeds should be stored moist at ≥10°C for less than 1 year.

Introduction

Native seeds may not always be intended for use immediately following their collection, and often need to be stored for varying amounts of time before their delivery to the restoration site or use in propagation programs. However, seeds age during storage, resulting in decline in quality and ultimately loss of viability if storage conditions are not appropriate (Harrington 1972).

The deleterious effects of seed aging occur largely due to oxidative processes (Walters et al. 2010), which can lead to deterioration of the proteins (including enzymes; Goel et al. 2003), lipids (and hence cellular membranes: Harman & Mattick 1976), RNA (Fleming et al. 2019), and DNA (El-Maarouf-Bouteau et al. 2011). All of these adversely affect cellular and metabolic integrity of seeds and seedlings (Kranner 2013). Increasing seed age can reduce germination vigor as the seed metabolic system begins to break down, resulting in seeds being slow or even unable to germinate, and poor seedling development and lower establishment for aged seeds that do germinate. Thus, effective seed storage relies on slowing down seeds' normal metabolism as much as possible without incurring damage.

Moisture, temperature, and the proportion of oxygen are key environmental factors that affect seed deterioration and loss of viability. Reducing seed moisture content (MC) to certain thresholds increases longevity in a predictable manner for approximately 90% of species (Roberts 1973). These species are classified as being "orthodox" in their seed storage requirements, and generally retain viability and germinability even

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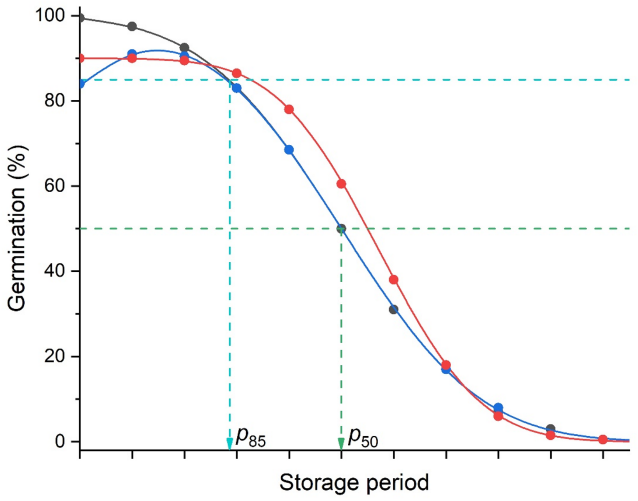

Figure 1. Seed survival curves showing the pattern of viability loss (decline in ability to germinate upon removal from storage) for orthodox seeds during storage at constant moisture content and temperature. The black symbols and line show the "typical" sigmoidal pattern. Also indicated are the time when ability to germinate falls to 85% (p_{85}), the viability standard used by most crop gene banks, and the time when ability to germinate falls to 50% (p_{50}), which is often used as a measure of seed longevity. The blue symbols and line show the survival curve for a seed lot that has some dormant seeds at the start of storage; the dormancy is broken during storage. Lastly, the red symbols and line show the survival curve for a seed lot that shows less than 100% germination at the start of storage but the viability is nonetheless maintained during the first period of storage, before declining.

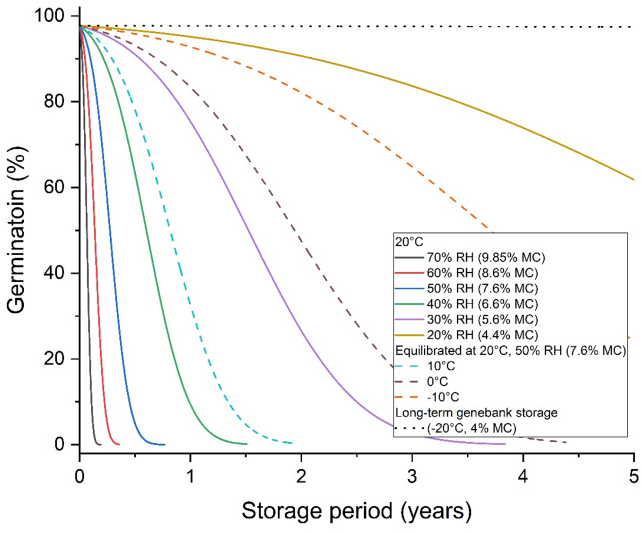

Figure 2. Predicted survival curves for seeds of foxglove (*Digitalis purpurea* L.) stored at 20°C and 20–70% RH. Also shown are the corresponding predicted seed moisture content (MC; % fresh weight) and survival curves for seeds equilibrated at 20°C and 50% RH, then sealed inside an air-tight container before storage at −10°C to +10°C. predictions are based on calculations made using the Seed viability constants menu of the Seed information database (Royal Botanic Gardens Kew 2020).

after storage for long periods under suitably dry, cool conditions (Figs. 1 & 2).

For orthodox seeds, the quantitative effects of both drying and cooling have been modeled in the improved viability equations of Ellis and Roberts (see Pritchard & Dickie 2003). In general, for each 1% decrease in seed moisture (when seed MC ranges between 5 and 14%) and for each 5°C decrease in storage temperature (between 0°C and 50°C) the life of the seed is doubled (Harrington 1972). Basic principles for orthodox seed storage are thus, low seed MC and low temperature. For their short-term storage (<18 months; Hong & Ellis 1996), a temperature between 0°C and 5°C is sufficient to maintain the viability of dry seeds. For longer periods of storage, seeds should be stored at −18°C to −20°C (Hong & Ellis 1996). Seeds should be dried to 3–7% MC (fresh weight basis; see below) and placed in airtight containers (Food and Agriculture Organization (FAO)/International Plant Genetic Resources Institution 1994).

At the other extreme, species that produce seeds that are damaged by and do not survive dehydration are classified as "recalcitrant" (Roberts 1973; Wyse & Dickie 2017). Recalcitrant storage behaviors are more prevalent in woody species from the moist tropics, and the possibilities for successful long-term seed banking of these plants at low temperature (<5°C) may be relatively limited (see Elliot et al. 2013; p 165–166). The viability of recalcitrant seeds can be maintained when the seeds are only allowed to dry slightly, if at all, with oxygen freely available, and at MCs just less than fully imbibed and above the value which results in chilling damage (the value at which the seeds are shed or in equilibrium with 98–99% relative humidity [RH]), at optimum storage temperatures which vary from about 7°C to 17°C among species of tropical origin, and between −3°C and 5°C among those adapted to temperate climates (Hong et al. 1996). The values of the lowest safe MC vary between 23 and 61.5% (fresh weight basis; Hong & Ellis 1996), and the seeds may require rewetting occasionally (Dumroese et al. 2009). Since nondormant recalcitrant seeds need oxygen to maintain their metabolism, the containers should allow gas exchange. For this purpose, 0.075–1.0 mm (3–4 mils) thickness polyethylene bags can be used, as they allow a good water vapor transmission rate for seeds (Bonner & Karrfalt 2008; see Box 1).

Between these two extremes represented by orthodox and recalcitrant seeds there is a continuum of storage behaviors, referred to as "intermediate" species (Ellis et al. 1990; Walters 2015), which exhibits characteristics of both groups and that usually tolerate drying only in part or under specific circumstances. These species may still maintain good levels (i.e. 70%) of seed viability after short- or long-term storage at 5°C (Chau et al. 2019). For restoration applications, short- and medium-term seed storage may be sufficient.

Conducting seed storage behavior experiments can help assigning a species to a particular category based on seed responses to desiccation and storage at different temperatures

Box 1 Facilities and Equipment for Seed Storage

Facilities required for seed storage depend on the amount of seeds to be stored and the expected length of storage. Most long- (10–100 years or more) and medium-term (from 18 months to 5 or 6 years; Hong & Ellis 1996) seed storage will have cold storage facilities with a climate control (temperature and humidity) system, and temporary processing and holding facilities. Short-term seed storage (from 3 to 18 months; Hong & Ellis 1996) needs to consider moisture control, rodents, insects, fungi, and fire (Justice & Bass 1978), which also applies to longer-term seed banks. For storage at temperatures above 0°C, home refrigerators may be sufficient, provided the RH is maintained at the desired level and constantly monitored.

Freezer storage is usually maintained at −18°C to −20°C. Where possible, backup generators and safety alarms in case of power failure are beneficial risk-management tools. Depending on the size and the need of the operation, and on the resources available, solutions for cold storage can range from standard home to walk-in refrigerators and freezers.

Orthodox seeds should be dried and stored in sealed moisture-proof containers that prohibit absorption of moisture from the atmosphere. On the other hand, recalcitrant seeds need good air movement during their short-term storage. Glass and moisture-proof plastics are useful containers but note the breakage risk of glass.

Seeds can be dried using desiccants such as zeolites, silica gel, charcoal, or even rice. For example, silica gel under optimal conditions can absorb up to 33% of its dry weight.

Humidity can be monitored through the use of indicators such as humidity indicator cards, but also silica gel, if it contains indicators that turn color when a certain amount of water has been absorbed (typically when RH of the air is ≥25%). In case silica gel is used, it should not be placed in direct contact with the seeds to avoid their damage.

Setting up a well-equipped seed storage facility can be costly as it requires significant infrastructure investment, but it can also be inappropriate for small-scale restoration projects that do not require long-term seed storage and large amounts of seeds. Seed banking can be very versatile, and even low-budget equipment, if used properly, can reach international standards for seed storage protocols on a small scale. For example, the Blue Drum Kits projects provide low-tech drying equipment for seed collection, processing, and storage (Martens 2018).

when they are at full maturity, but before germination begins (Fig. 3).

Regardless of the seed storage behavior, standard practices must be followed starting from seed collection and during postharvest seed management, prior to storing seeds, in order to ensure that a seed lot of good quality (i.e. high seed viability) reaches the storage facilities. In this article, we provide a compendium of best practices, tools, and standards for the steps between postharvest seed handling and seed storage, for applications in restoration.

Postharvest Seed Management and Short-Term Storage

Seeds should be collected at maturity, at the point of natural dispersal (Hay & Smith 2003), as seeds collected too early

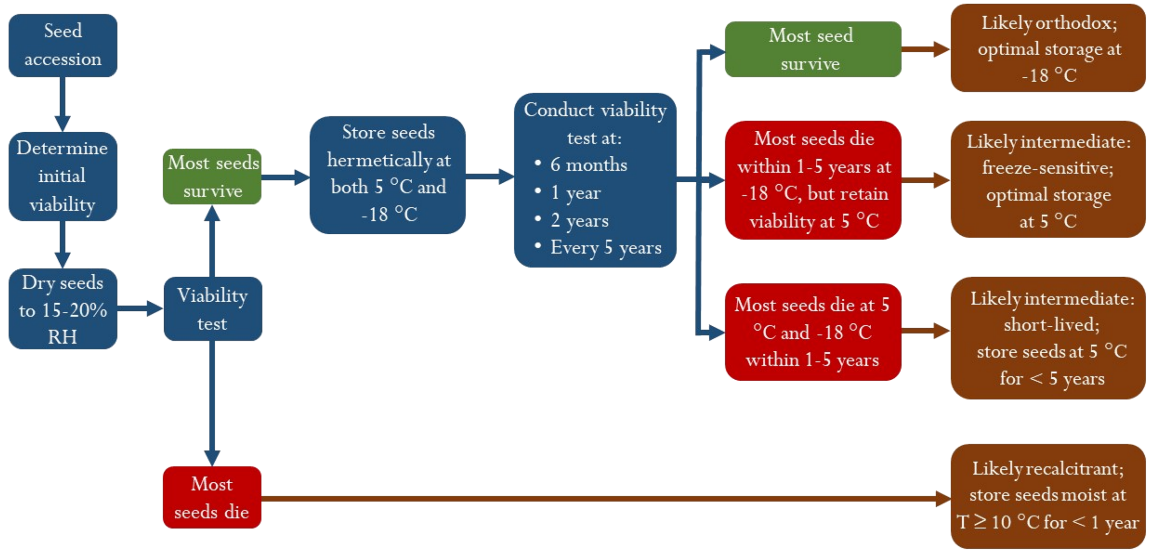

Figure 3. Protocol to determine seed storage behavior of an unknown species (adapted from Chau et al. 2019).

will be undeveloped and will lose viability when dried, or even fail to germinate altogether, whereas seeds collected too late may have reduced viability (see Pedrini et al. 2020).

The key to successful postharvest management and storage of orthodox seed collections is to understand and control the loss and absorption of moisture between seeds and the air surrounding them.

The use of a hygrometer provides a reliable, quick, and nondestructive method to measure equilibrium RH (eRH) of a seed sample, in the field or in the laboratory (Gold 2014). The seed eRH is the RH of air at equilibrium with seeds held in a sealed chamber (Gold & Manger 2014). If the hygrometer is used in the field, it should be kept in the shade, to avoid warming of the sample chamber and sensor, and seeds should be allowed at least 30 minutes in the sample chamber to reach equilibrium. Ideally, measurements should be taken under controlled conditions (e.g. 15% RH and 15°C) and with seeds at the same temperature than the sample chamber (Gold & Manger 2014). It is also possible to buy hygrometers which control temperature, although these are not suitable for field use.

After fruit/seed harvesting, depending on the maturity stage and the moisture status, the material should be handled in the most appropriate way to avoid any viability loss. For orthodox seeds from nonfleshy fruits, if seeds are collected when immature and wet (eRH of 85–100%) and still within the fruits, they should be held intact under shaded ambient conditions for 1–2 weeks for continued ripening, under either dry (daytime RH <50%) or humid (daytime RH >50%) ambient conditions; when seeds are collected at natural dispersal time (mature seeds) and the ambient conditions are dry (daytime RH <50%), if they are damp (eRH >50%), they should be left to dry in a thin layer, but if they are dry (eRH <50%), they can be held in loosely packed mesh or paper bags; in both cases (damp or dry seeds at dry ambient conditions), they should be kept drying in a well-ventilated, shaded location and stored in airtight containers overnight to minimize moisture uptake as the ambient humidity of the air can increase with cooler night-time temperatures; when either damp or dry mature seeds are collected under humid (daytime RH >50%) ambient conditions, they should be transferred to the seed bank as soon as possible or be dried with a desiccant (see Box 1) or placed in an air-conditioned room (Hay & Probert 2011).

If material is dried on carefully selected sizes of wire screen, seeds may fall through and be collected with greater ease.

Seeds that have been dried to equilibrium with ambient conditions of less than 70% RH are usually dry enough to store for short periods with minimal risk of losses due to fungal attack, but viability may drop within a year (Bradford et al. 2016). Seeds that have been dried to and kept at <50% RH will likely maintain viability for several years, while seeds maintained in a controlled dry environment at <25% RH often maintain viability for decades (Adams et al. 2016).

Fleshy fruits should be kept in aerated plastic bags until processing, with the bags opened regularly to avoid mold and fermentation; if flesh needs to be removed, this should be done using a sieve and cool running water; once cleaned and dry, these can be treated as dry seeds (Gold 2014).

Seed MC Calculation

A classic method to measure seed MC, although destructive, is to weigh the seeds both before and after drying them in the oven at 103°C for 17 hours (ISTA 2020). MC is usually calculated as a percentage of the total starting weight of the seed sample (i.e. fresh weight basis), but it may also be calculated as a proportion of the dry weight (dry weight basis). Here we provide both equations.

Fresh basis:

$$MC_{fb} = \frac{W_f - W_d}{W_f} \times 100$$

Dry basis:

$$MC_{db} = \frac{W_f - W_d}{W_d} \times 100$$

with MC_{fb} and MC_{db} being the MC calculated on fresh and dry basis, respectively; and W_f and W_d being the fresh and the dry weight of the same seed sample, respectively. In most seed studies, the basis of MC calculation is not stated and it is assumed to be on a fresh basis, according to the International Seed Testing Association (Bewley & Black 2012). Taking three or more samples as replicates for the MC determination would give a more accurate estimate of the MC of the harvested seed bulk. If the study species produces a large enough seed that could be weighed with accuracy, then the single seed could represent the replicate (and it would be better to sample five or more individual seeds). If the study species produces smaller seeds (<2 mg), and depending on the accuracy and precision of the scale used, it can be useful to first estimate the weight of 100 or 1,000 seeds and then take the measurement with a minimum of three or more replicate seed samples equivalent to 100 or 1,000 seeds, depending on seed availability.

Seed Longevity

Seed longevity is a measure of how long seeds can be stored and remain viable under a given set of conditions. Seed longevity in storage varies greatly among species (e.g. seed composition; Hong et al. 1996) and is also determined by the cumulative effect of environment during seed maturation and harvesting, the time of seed harvest (Hong & Ellis 1996), and the way seeds are handled immediately after harvest (e.g. duration and environment of drying and prestorage environment; Hay & Probert 2011). Several studies describe the relative longevity of seeds in medium- and long-term gene bank storage (Walters et al. 2005; Hay et al. 2013; Ellis et al. 2018). However, these studies relate to crop species with little information available for how long the seeds of native species can be stored without declines in viability and seedling vigor.

Under identical storage conditions, a seed collection of high initial viability would have greater longevity than a collection of the same species with a lower initial viability (e.g. Hay & Probert 1995). As a general rule, maximizing viability and, therefore, optimal potential longevity, is achieved by collecting seeds at or close to the timing of natural seed dispersal (Hay &

Box 2 Seed Storage Experiments

Seed storage experiments (SSEs) involve storing seeds under specific temperature and moisture conditions. They can provide valuable information to predict storability within short time frames. Conditions recommended to perform SSEs to assess relative seed longevity are 45°C and a seed MC that corresponds with 60% RH (Hay et al. 2019). SSEs will normally involve allowing seeds that have already dried to lower MC, to take up moisture, usually at a lower temperature (20°C), to avoid significant viability loss before the SSE starts. SSEs can be done either by placing seeds in aliquots which will be used for viability testing, over water and monitoring their change in weight (if seed MC is known), or by placing seeds over a 60% RH nonsaturated solution of LiCl (Hay et al. 2008) in a sealed container.

Samples are removed after different periods of time (typically up to 60–100 days) to assess viability and determine when viability falls to 50% during the SSE (p_{50}; Fig. 1). The equilibration period required may vary, but 1 week is usual for many orthodox seeds; seed equilibrium RH can be checked using a water activity instrument or seed MC determined using traditional methods (see section "Seed MC Calculation"), if there are sufficient seeds available for destructive testing.

Seeds to be used in the SSE must be transferred to moisture-proof containers or bags once they have equilibrated to the elevated moisture levels. These containers and bags are then exposed to the rapid aging temperature of 45°C. If bulk seeds are stored such that they are exposed to the air, then the aliquots of seeds for the SSE should be similarly stored in an open environment, over a 60% RH nonsaturated solution of LiCl (for valid comparisons across studies, for example, Probert et al. 2009; Merritt et al. 2014) in a sealed container that is placed at 45°C.

The individual aliquots of seeds are removed after 1, 2, 5, 9, 20, 30, 50, 75, 100, and 125 days (Newton et al. 2014) or 2, 10, 15, and 30 days if the seed lot is expected to have poor longevity and few seeds available (Davies et al. 2006) to test the ability of the seeds to germinate. This germination data is then analyzed, usually through probit analysis, to estimate the p_{50} and provide an estimate for relative seed longevity (Figs. 1 & 2). The rankings can then be used to make appropriate decisions regarding use and/or viability monitoring (if appropriate) during storage.

Caution must be used in extrapolating predictions from the models to survival under chilled or sub-zero storage (Pritchard & Dickie 2003; FAO 2013); however, with the presumed shorter storage periods required for most restoration applications, interpretation of results can be less conservative, making SSEs a particularly useful tool in these cases (see http://data.kew.org/sid/viability/index.html).

Smith 2003). For more information and guidance on native seed collection, see Pedrini et al. (2020).

For orthodox seeds, which can be dried without damage to low MC, and over a wide range of environments, the longevity increases with decrease in seed storage MC and temperature in a quantifiable and predictable way (Roberts 1973). Under optimal conditions of low MC and low temperature, viable seeds can show very long life spans (e.g. up to 100 years). The longevity of recalcitrant seeds, on the other hand, is short, and can range from a few weeks to a few months, for species adapted to tropical environments, or longer periods, for example, a few years, for species adapted to temperate environments (Hong et al. 1996 and literature therein).

It is hence reasonable to think that orthodox seeds, collected at the right time of maturity and handled and stored properly, could be used for postdisturbance restoration after many years from collection, while recalcitrant seeds would need to be collected shortly before their use.

Working With Unknown Species

The largest available dataset on seed desiccation sensitivity is the Seed Information Database (SID; Royal Botanic Gardens Kew 2020). Of the 18,174 taxa included in this database, 96% are desiccant tolerant; however, this dataset is strongly biased toward species that can be stored using conventional seed banking practices (Wyse & Dickie 2017). Wyse and Dickie (2017), using two different models (habitat- and taxonomy-based), estimated that approximately 8% of world's seed-plant species produce desiccation-sensitive seeds.

When working with a species for which seed storage behavior is not known, the best way to proceed would be to investigate specific requirements and behavior; however, when this is not possible, it could be helpful to refer to the storage behavior of closely related taxa. Adapting the original guidelines by Hong and Ellis (1996), Chau et al. (2019) developed a protocol to determine freeze-sensitive seed storage behavior (Fig. 3). This protocol involves the following steps: (1) determine initial seed viability of the study species after collection; (2) dry the seeds to 15–20% RH at ambient temperature (20°C); (3) perform a second viability test: if most of the seeds die the species is likely recalcitrant; if most of the seeds are viable then proceed storing them hermetically at both 5°C and −18°C; (4) conduct viability tests after 6 months, 1 year, 2 years, and every 5 years: if most of the seeds die at 5°C and −18°C within 1–5 years, the species is likely intermediate with short-lived seed storage behavior and seeds should be stored at 5°C for less than 5 years; if most of the seeds die within 1–5 years at −18°C but retain viability at 5°C, the species is likely intermediate with freeze-sensitive seed storage behavior and seeds should be stored at 5°C; if most of the seeds survive, the species is likely orthodox with optimal storage at −18°C (Fig. 3). If a species appears to be recalcitrant or short-lived, further testing at different desiccation levels could help identify more specific requirements.

To predict a species' seed storability, performing seed storage experiments is also useful (see Box 2).

Conclusions

The standards for seed storage here described, based on the available knowledge developed through decades of research work, represent the current best practice for native seeds for restoration use. They are meant to support and guide seed laboratory staff and restoration practitioners in the proper management of seed supplies after their harvest. When sourcing seeds for restoration, it is fundamental that every single decision and step regarding the activities prior to storage (i.e. seed harvest and postharvest management) is taken considering best practices to ensure that seeds of the highest possible quality enter the storage facilities. Then, proper protocols are critical to assess seed longevity and maintain high levels of seed viability under storage, and ultimately to supply native seeds of high quality for seed-based restoration projects. Seeds that are handled and stored improperly will have a shorter lifespan and die, and the restoration will fail. As new technologies are developed and knowledge on native seeds is advanced, these standards will likely be refined and improved.

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PRACTICAL ARTICLE

Dormancy and germination: making every seed count in restoration

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From 50 to 90% of wild plant species worldwide produce seeds that are dormant upon maturity, with specific dormancy traits driven by species' occurrence geography, growth form, and genetic factors. While dormancy is a beneficial adaptation for intact natural systems, it can limit plant recruitment in restoration scenarios because seeds may take several seasons to lose dormancy and consequently show low or erratic germination. During this time, seed predation, weed competition, soil erosion, and seed viability loss can lead to plant re-establishment failure. Understanding and considering seed dormancy and germination traits in restoration planning are thus critical to ensuring effective seed management and seed use efficiency. There are five known dormancy classes (physiological, physical, combinational, morphological, and morphophysiological), each requiring specific cues to alleviate dormancy and enable germination. The dormancy status of a seed can be determined through a series of simple steps that account for initial seed quality and assess germination across a range of environmental conditions. In this article, we outline the steps of the dormancy classification process and the various corresponding methodologies for ex situ dormancy alleviation. We also highlight the importance of record-keeping and reporting of seed accession information (e.g. geographic coordinates of the seed collection location, cleaning and quality information, storage conditions, and dormancy testing data) to ensure that these factors are adequately considered in restoration planning.

Key words: dormancy classification, dormancy cycling, seed fill, seed quality, seed testing

Implications for Practice

- Seed dormancy occurs in more than 50% of wild plant species. The lack of understanding and consideration of dormancy and germination traits in restoration planning often contributes to plant establishment failure.
- Seed quality, dormancy, and germination traits can be assessed following a series of standard seed testing steps.
- To improve outcomes, considerations outlined in this article should be a standard component of any seed-based restoration planning.

Introduction

Unlike crop plants that are subject to extensive breeding, the seeds of many wild plant species exhibit some degree of seed dormancy. Seed dormancy regulates germination through various physical and/or physiological means imposed by the seed coat, or within the embryo (Baskin & Baskin 2014). Dormancy can facilitate the persistence of seeds through unfavorable periods ensuring germination occurs when environmental conditions are most likely to lead to seedling establishment. Freshly collected, viable seeds are considered to be dormant if they do not germinate within 4 to 6 weeks under conditions that can be considered ideal (e.g. sufficient moisture and suitable temperatures) to support the germination process (Baskin & Baskin 12004*b*; Baskin & Baskin 12004*c*).

The loss of dormancy is driven by the detection of environmental cues such as temporal changes in moisture and temperature, which seeds can "sense" through a number of mechanisms (Baskin & Baskin 2014). However, for some species with complex germination requirements, even after dormancy has been

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lost, germination will only ensue under specific environmental conditions such as light or dark (indicating the degree of openings or disturbance in vegetation) or in response to chemical cues such as smoke compounds, nitrates, or ethylene (indicating favorable germination conditions). The requirements for dormancy alleviation and germination stimulation vary between seed dormancy classes, and in some cases between different populations of the same species (Ellison 2001; Tieu et al. 12001*b*).

When seed dormancy and germination requirements of species are not adequately considered in restoration planning, they can lead to high levels (more than 90%) of plant establishment failure and seed wastage (James et al. 2011; Merritt & Dixon 2011; Commander et al. 2013; James et al. 2013). To improve restoration success and achieve project goals at a reasonable cost, every seed must have the best opportunity to germinate and establish (Turner et al. 2013).

Worldwide, 50–90% of wild plants produce seeds that are dormant upon maturity, with the specific dormancy traits contingent on factors including environmental conditions, geographic distribution, growth form, and genetics (Baskin & Baskin 2014). Seed dormancy is an evolutionary adaptation that can benefit long-term survival under intact natural conditions (Willis et al. 2014), but in the context of restoration where rapid plant re-establishment is critical to prevent further degradation, dormancy can pose a significant challenge (Turner et al. 2013). Because seeds may take several seasons to lose dormancy, when sown onto a restoration site following disturbance, they become susceptible to seed predators and pathogens, viability loss, and weed competition—which can lead to plant re-establishment failure. This can significantly reduce restoration success, particularly when working with more challenging species and complex plant communities (Broadhurst et al. 2016). Additionally, specialized dormancy and germination requirements can also constrain efforts to increase the scale and diversity of ex situ native seed production, limiting the ability of practitioners to work with multiple species at larger scales (Miller et al. 2017; Ladouceur et al. 2018). Understanding and considering seed dormancy and germination traits in restoration planning can help ensure seeds are managed in a way that promotes germination during periods that are most conducive to plant recruitment. The ability to define the seed dormancy class is the first step in determining the most effective means of dormancy alleviation and should be considered foundational knowledge for all restoration practitioners working with native seeds.

Seed Dormancy Classes

Five main classes of seed dormancy are currently recognized (Table 1), although in some cases these are further divided into sub-levels (Baskin & Baskin 12004*b*; Baskin & Baskin 12004*c*; Gama-Arachchige et al. 2013). Physiological dormancy (PD) is the most common form of seed dormancy worldwide, occurring in gymnosperms and all major angiosperm clades (or groups of species posited to have evolved from a common ancestor) (Baskin & Baskin 12003*b*; Finch-Savage & Leubner-Metzger 2006; Willis et al. 2014). The embryo of seeds with PD is fully developed (Fig. 1, Table 1) but has a low growth potential. Due to this low growth potential, the embryo cannot overcome the mechanical constraints of the surrounding tissues (e.g. endosperm, seed coat, or fruit coat) without receiving cues from the surrounding environment. These cues initiate internal chemical signaling (resulting from changes in the ratio and sensitivity of internal seed hormones), which promotes dormancy loss and germination (Baskin & Baskin 12004*b*). PD is often alleviated by periods of cold or warm stratification or warm dry after-ripening. Three levels of PD are recognized: deep, intermediate, and nondeep (Baskin & Baskin 2014).

The outer surface of the fruit or seed coats of physically dormant (PY) seeds is typically covered by at least one (usually ≤200 μM) layer of palisade (or palisade-like) cells (Fig. 2). These impermeable palisade layers are made up of sclereid cells that have thick lignified secondary walls, which resist water penetration into the seed (Langkamp 1987; Baskin et al. 2000; Gama-Arachchige et al. 2013). PY is released when the water-impermeable layer is degraded or damaged to the point that water uptake (imbibition) can occur. In natural conditions, this degradation often occurs in a specialized area of the seed, called the "water gap" (Baskin & Baskin 2014).

Seeds with combinational dormancy (PY + PD) have both a water-impermeable seed or fruit coat and a physiologically dormant embryo (Baskin et al. 2000; Baskin & Baskin 12004*a*). This dormancy class is relatively uncommon (Baskin & Baskin 12003*b*). Dormancy alleviation of PY + PD is a two-step process. First, it requires the impermeable palisade cell layer to be compromised to allow imbibition of water into the seed. Second, seeds must receive an environmental signal to promote sufficient embryo growth to overcome the mechanical restraint of the surrounding tissues (Baskin & Baskin 2014).

Morphologically dormant (MD) seed embryos are not fully developed at maturity (underdeveloped and small relative to the size of the endosperm) and must grow/mature prior to germination (Baskin & Baskin 12004*c*; Baskin & Baskin 2014). Embryos can be either undifferentiated (no clear structure; Fig. 3) or underdeveloped but differentiated with some rudimentary structures visible (i.e. radicle and cotyledons; Fig. 4). In seeds with MD, germination can be particularly slow even given the optimum germination conditions due to the required period of embryo development/growth prior to radicle emergence (Baskin & Baskin 12004*b*; Baskin & Baskin 12004*c*; Erickson et al. 2016).

Seeds with morphophysiological dormancy (MPD) have underdeveloped (or undifferentiated) embryos that are also physiologically dormant, and require an environmental signal to stimulate embryo growth as a precursor to final development (Baskin & Baskin 12004*b*; da Silva et al. 2007). MPD is a complex dormancy class, further subdivided into nine levels on the basis of the environmental conditions required for embryo growth (Baskin & Baskin 2014). The additional physiological component to dormancy means that radicle emergence requires significantly more time than that of seeds with MD alone (Baskin & Baskin 12004*c*; Scholten et al. 2009; Baskin & Baskin 2014; Erickson et al. 2016; Dalziell et al. 2018).

Table 1. Classes of seed dormancy, adapted from Baskin and Baskin (2014) and Finch-Savage and Leubner-Metzger (2006). A number of genera include species with unusual or unknown dormancy states that defy known approaches of dormancy release. This includes species in *Astroloma*, *Leucopogon*, *Cosmelia*, *Epacris* (Ericaceae) with drupaceous fruits; dryland nut seeded Cyperaceae; many Australian Restionaceae; *Boronia* and *Philotheca* (Rutaceae) (Merritt et al. 2007). The families listed in the table are not meant to be an exhaustive and comprehensive list, but examples of families containing some species with a particular dormancy type.

Seed Dormancy Class	*Seed Characteristics*	*Examples of Plant Families Containing Species With a Known Seed Dormancy Class*
Nondormancy (ND)	Seeds imbibe water and germinate readily (within 4 weeks) over the widest range of environmental conditions possible for the species	Amaranthaceae, Asteraceae, Begoniaceae, Brassicaceae, Bromeliaceae, Dipterocarpaceae, Fagaceae, Lauraceae, Pinaceae, Rubiaceae, Velloziaceae, Xyridaceae
Physiological dormancy (PD)	Seeds imbibe water and possess fully developed embryos with a low growth potential, sometimes in combination with a mechanical constraint from the seed/fruit covering layers	Aceraceae, Amaranthaceae, Asteraceae, Balsaminaceae, Brassicaceae, Byblidaceae, Caryophyllaceae, Commelinaceae, Cucurbitaceae, Cupressaceae, Dioncophyllaceae, Droseraceae, Drosophyllaceae, Ephedraceae, Ericaceae, Euphorbiaceae, Fagaceae, Iridaceae, Lamiaceae, Lauraceae, Lentibulariaceae, Melastomataceae, Myrtaceae, Nymphaceae, Oleaceae, Pinaceae, Plantaginaceae, Poaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Solanaceae, Ulmaceae, Urticaceae, Violaceae, Vitaceae
Physical dormancy (PY)	The seed or fruit coat is impermeable (preventing the uptake of water)	Anacardiaceae, Biebersteiniaceae, Bixaceae, Cannaceae, Cistaceae, Convolvulaceae, Cucurbitaceae, Dipterocarpaceae, Fabaceae, Geraniaceae, Lauraceae, Malvaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae, Sarcolaenaceae, Sphaerosepalaceae, Surianaceae
Combinational dormancy (PY + PD)	The seed or fruit coat is impermeable (preventing the uptake of water) and seed embryos are physiologically dormant	Anacardiaceae, Fabaceae, Geraniaceae, Rhamnaceae, Sapindaceae
Morphological dormancy (MD)	Seeds readily imbibe water; however, embryos are underdeveloped but differentiated and require time to grow before germination	Annonaceae, Apiaceae, Arecaceae, Aristolochiaceae, Campanulaceae, Caprifoliaceae, Cycaceae, Gentianaceae, Iridaceae, Lentibulariaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Sarraceniaceae, Vitaceae
Morphophysiological dormancy (MPD)	Seeds readily imbibe water but have embryos that are underdeveloped and/or undifferentiated and physiologically dormant	Allicaceae, Annonaceae, Apiaceae, Araliaceae, Ericaceae, Gentianiaceae, Ginkgoaceae, Lentibulariaceae, Liliaceae, Magnoliaceae, Papaveraceae, Primulaceae, Ranunculaceae, Taxaceae, Zamiaceae

Figure 1. Internal seed morphology of *Ricinocarpos brevis* (Euphorbiaceae), a critically endangered species producing seeds with physiological seed dormancy and a fully developed linear embryo (Image: A. Fontaine).

Dormancy Cycling

The seeds of many species with PD or MPD can cycle between nondormant and dormant states (Baskin & Baskin 2014; Finch-Savage & Footitt 2017). This process occurs over weeks or months, usually in the soil seed bank. The seeds of many species are capable of cycling seasonally over many years before germination finally occurs. Dormancy cycling generally ensues in response to environmental cues (e.g. changes in light conditions or soil temperature and moisture), as these conditions become either more suitable (moving into the optimal growing season) or less suitable (moving away from the optimal growing season) to support germination (Baskin & Baskin 12004*c*; Duarte & Garcia 2015). Dormancy cycling has also been reported for seeds stored under constant temperature and moisture, suggesting the presence of an "endogenous rhythm" or a "biological clock" within seeds that is somewhat independent of changing environmental conditions (Froud-Williams et al. 1986; Jones

Figure 2. Internal seed morphology of *Adansonia gregorii* (Malvaceae), a species producing seeds with a folded embryo and physical seed dormancy. The water-impervious layer of cells (palisade) is located in the outer testa, which is clearly distinguishable in the insert as a lighter band just under the surface of the seed coat. The palisade layer in this species is just approximately 150 μM in thickness (Image: A. Fontaine).

Figure 3. Internal seed morphology of *Burchardia congesta* (Colchicaceae), a species producing seeds with a small undifferentiated embryo <1 mm in length compared to the rest of the seed which is >2 mm long. This species has MD (Image: A. Fontaine).

et al. 1998; Tieu et al. 12001*a*). Seeds that are partially through a dormancy cycle, either on their way to becoming fully dormant or on their way to becoming completely nondormant, may be conditionally dormant (Baskin & Baskin 12004*b*). These seeds may still be cued to germinate, but only under a much more limited set of conditions (i.e. a narrower range of temperatures) than seeds in which dormancy has been alleviated (Baskin & Baskin 2014).

Biogeographic Variation in Seed Dormancy

As seed dormancy is driven primarily by environmental factors, it is perhaps unsurprising that studies have shown regional patterns in seed dormancy across all of the world's major terrestrial biomes (Baskin & Baskin 12003*b*; Baskin & Baskin 2014). Seed dormancy is most common in species from ecologically challenging, climatically unpredictable, or highly seasonal regions: the percentage of species with some form of seed dormancy ranges from ca. 50% in tropical rainforests, ca. 57% in tropical semi-evergreen forest, to over 90% in cold deserts (Baskin & Baskin 12003*b*; Baskin & Baskin 2014) and old climatically stable environments such as southwest Australia (Merritt et al. 2007; but see Dayrell et al. 2017). Species with PY are more common in ecosystems with marked wet and dry seasons (e.g. matorral and cold deserts; Rubio de Casas et al. 2017), while species with underdeveloped embryos are more common in mesic environments such as broadleaved evergreen forests (MD) or deciduous forests (MPD) (Baskin & Baskin 2014). PD is well represented in species from most biomes, but subtle differences in germination strategies can occur even between relatively similar ecosystems depending upon their environmental conditions. For example, species from alpine and subarctic habitats most commonly have PD that is alleviated by cold stratification over winter, with germination occurring in early summer when the risk from frost is lowest (Niederfriniger Schlag & Erschbamer 2000; Schwienbacher et al. 2011; Marcante et al. 2012; Körner 2013; Bernareggi et al. 2015; Tudela-Isanta et al. 12018*a*; Tudela-Isanta et al. 12018*b*). However, for populations of the same species distributed across an environmental gradient, germination and dormancy patterns may differ. For example, subarctic populations may be less dormant, germinate more readily under optimal conditions, and may have a warmer suitable temperature range for germination compared to alpine populations (Mondoni et al. 2018). Similar patterns exist in many other bioregions (Baskin & Baskin 2014).

Intra- and Inter-specific Variation in Seed Dormancy

The depth of seed dormancy (or the extent to which germination is inhibited in the absence of appropriate dormancy alleviation conditions) can vary considerably between families, genera, species, and within individuals (Thomas et al. 1979; Langkamp 1987; Baskin & Baskin 2014; Barga et al. 2017; Cross et al. 2018; Seglias et al. 2018). Species within the same family often possess different seed dormancy classes. For example, the Rubiaceae contains species with seeds that are nondormant (*Ochreinauclea missionis*; Jose et al. 2002), PD (*Gardenia ovularis*; Osunkoya & Swanborough 2001), MD (*Coffea arabica*; da Silva et al. 2004), and MPD (*Amaioua corymbosa*; Sautu et al. 2007). Within a single species, seeds generally fall under the same dormancy class, but the proportion of seeds in which dormancy has been induced and the depth of that dormancy may vary on a population or individual plant level, as a result of biogeography, genetic factors, and the environmental conditions experienced during seed development and maturation (Andersson & Milberg 1998; Tieu et al. 12001*b*; Donohue 2009; Bernareggi et al. 2015; Liyanage & Ooi 2015; Liyanage & Ooi 2016). Finally, in some cases, the proportion of dormant seeds may also vary within the same inflorescence (Baskin & Baskin 2014). For example, in many

Figure 4. Internal morphology of a morphologically dormant (MD) seed of *Clematis linearifolia* (Ranunculaceae), a species producing seeds with a small, underdeveloped linear embryo (<1 mm in length; image on the left). The embryos can grow to >5 mm before radicle emergence occurs (image on the right). The embryo requires sufficient time to grow prior to germination, and, as a result, the sowing window must account for the period required for the embryo to reach maturity, which can only occur under specific soil moisture and temperature conditions (Image: A. Fontaine).

species of Asteraceae, the achenes produced by the central disc (tubular) flowers may be more or less dormant than those produced by the peripheral (ligulate) flowers (Marks & Akosim 1984; Brandel 2007).

Identification of Seed Dormancy

Restoration practitioners must be able to correctly assign seed dormancy classes because treatments to alleviate seed dormancy are specific to each class (Silveira 2013; Erickson et al. 2016; Kildisheva et al. 12018*a*; Kildisheva 2019). Applying the wrong treatment can at best result in failure to break dormancy and at worst kill the seeds. In addition, if seeds are broadcast to field sites, sufficient time is needed to ensure dormancy release is followed by favorable soil moisture and temperatures to enable germination to proceed.

By undertaking simple trials (i.e. seed quality, germination, embryo, and imbibition testing) using readily available materials, seeds of most species can be easily assigned to one of the five dormancy classes (Figs. 5 & 6). This information is generally sufficient to inform and facilitate better seed management and restoration planning. In some complex cases, however, subsequent classification of seed dormancy to sub-levels may be needed and can be more involved, requiring a series of experimental studies (Baskin & Baskin 12004*c*; Hilhorst et al. 2010; Hilhorst 2011).

Seed Quality Determination

Seed fill and viability should be assessed prior to beginning a seed dormancy investigation (Dayrell et al. 2017) and should ideally be conducted on representative samples both at the beginning and the conclusion of germination testing. The methods to achieve this include cut testing, x-ray (fill only), and tetrazolium evaluation (Bonner & Karrfalt 2008; Luna et al. 2009). The percentage of unfilled, damaged, embryo-less or nonviable seeds must be reported in order for accurate estimates of percentage of dormant seeds (Fig. 5; see Frischie et al. 2020) for more details.

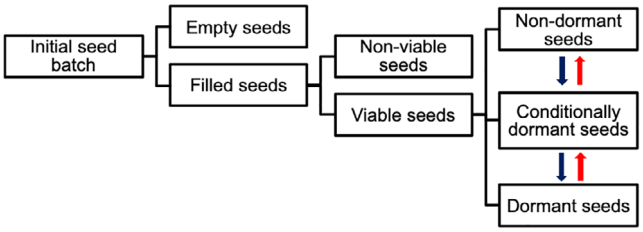

Figure 5. A framework that outlines key factors that should be considered prior to commencing seed dormancy classification. Confounding factors such as empty (embryo-less) seeds and nonviable seeds are discarded (unlike the International Rules for Seed Testing standards [ISTA 2020]) to accurately determine the percentage of dormant seeds. The red arrows indicate the path of dormancy loss whereas the blue arrows indicate the path of secondary dormancy acquisition (modified from Baskin & Baskin 2014).

Germination Testing

The next step in classifying seed dormancy is to establish whether freshly collected seeds (within 2 weeks of seed collection; Baskin & Baskin 12004*a*; Baskin & Baskin 12004*c*; Baskin et al. 2006) germinate readily over a broad range of environmental conditions (Finch-Savage & Leubner-Metzger 2006). Seeds should be incubated on a neutral medium (e.g. moist filter paper or water agar), under a wide range of experimental temperatures that simulate conditions of the natural environment where the species occurs, for at least 4 weeks. The number of germinated seeds should be counted periodically, with germination determined by the protrusion of the radicle from the seed coat, to a length of at least 2 mm. If a large proportion (>75%)

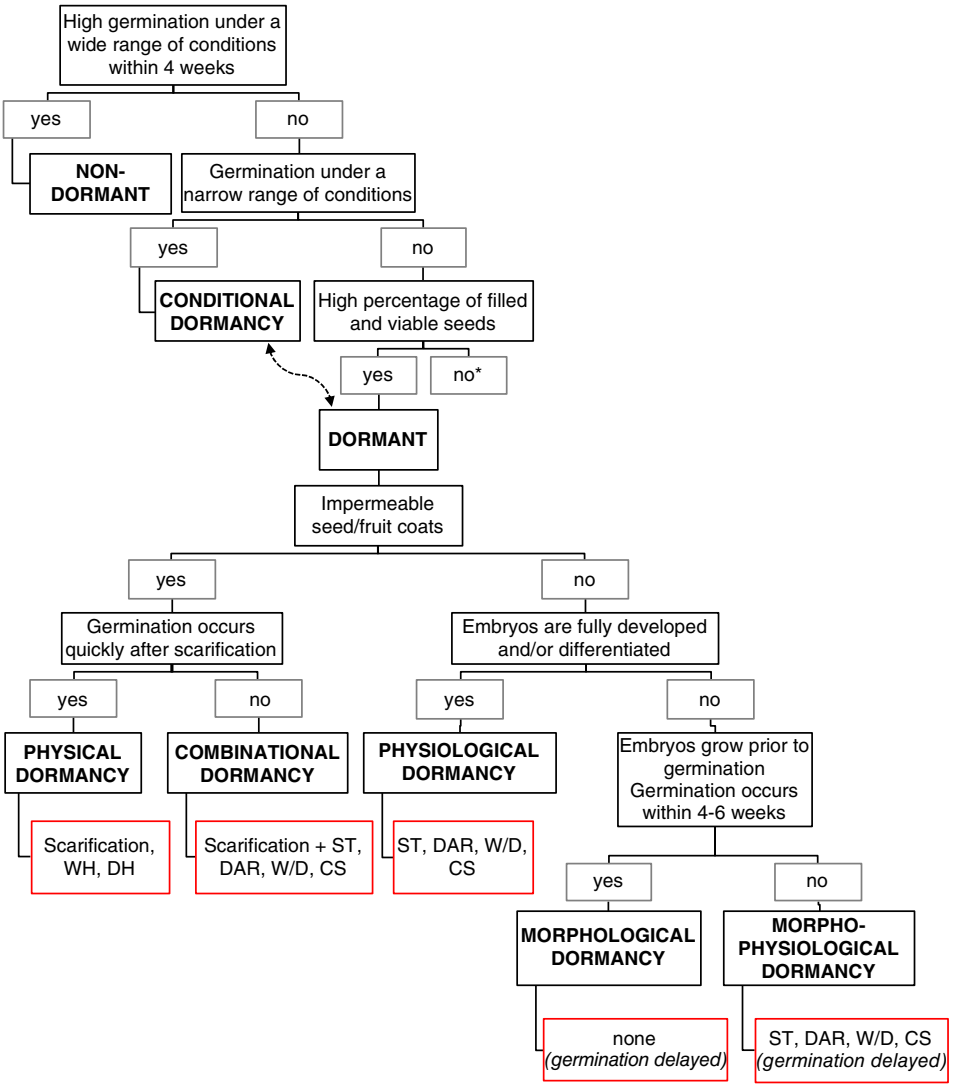

Figure 6. A decision tree for determination of seed dormancy classes following the classification system of (Baskin & Baskin 12004*c*). The first step is to determine whether seeds are nondormant, conditionally dormant, or dormant. Germination assessment should be based on a 4-week period after collection. The asterisk indicates problems with seed quality that preclude accurate dormancy classification. Subsequently, it may be necessary to determine the classes of primary seed dormancy to understand the appropriate action needed for dormancy alleviation (shown in the red boxes), where CS—chemical stimulants (e.g. ethylene, gibberellic acid, karrikinolide), DAR—dry after ripening, DH—dry heat (placement in >90–100°C environment), ST—warm/cold stratification, W/D—wet/dry cycling, WH—wet heat (submergence in 70–90°C water). The dashed line indicates the potential for dormancy cycling; however, this only relates to seeds with a physiological dormancy component (physiological, combinational, and morphophysiological dormancy).

of viable seeds germinate in less than 4 weeks over a wide range of temperatures, they are considered to be nondormant (Erickson et al. 2016). Conversely, if germination is low or does not occur across the tested range of conditions seeds may be dormant.

Imbibition Testing and Scarification

If dormancy is suspected, imbibition testing should be undertaken to determine whether seed/fruit coats are water-permeable (Silveira et al. 2012). Water-impermeable seeds are physically dormant and will require scarification and subsequent germination testing to determine if physiological dormancy is also present. If seeds are able to absorb water but have poor germination, such seeds will require a detailed inspection of embryo development. In such cases, fully developed and/or differentiated embryos indicate physiological dormancy (Fig. 6).

In the case of water-impermeable seed/fruit coats, monitoring germination following scarification is needed to classify seeds as having physical dormancy or combinational dormancy (Fig. 6). Physically dormant seeds will germinate rapidly and to a high extent after scarification. If germination is low even after scarification, this implies poor growth potential of the embryo induced by physiological dormancy; such seeds have combinational (physical + physiological) dormancy (Baskin & Baskin 2014; Kildisheva et al. 12018*a*).

Embryo Measurements

The extend of embryo development in mature seeds can further help identify the dormancy class. Dissecting seeds under a stereomicroscope and measuring embryo:seed length ratio (Forbis 2010; Erickson et al. 2016), is typically sufficient to determine the status of embryo development. If embryos are underdeveloped (length of the embryo increases prior to the point of radicle emergence) and/or undifferentiated (not differentiated into organs; Fig. 6), then monitoring embryo growth inside the seed periodically (e.g. every few days) is required (Baskin & Baskin 2014). If embryo growth leads to germination, seeds are morphologically dormant. Alternatively, when embryo growth is detected but germination remains low within 4 to 6 weeks, this may indicate that seeds have morphophysiological dormancy (Baskin & Baskin 2014; Erickson et al. 2016).

Seed Dormancy Alleviation

Determining the Approach

In the context of restoration, assuming that dormancy loss will occur naturally within the desired timeframe often results in seed losses and establishment failures (Broadhurst et al. 2016; Erickson et al. 2016; Erickson et al. 2017; Kildisheva 2019). Thus, relieving dormancy to promote greater and more predictable germination is generally beneficial, assuming that sowing occurs at an appropriate time to support seedling emergence and survival.

The process of determining the optimal methods for dormancy release should be based on the dormancy class and consider the phenology of the species as well as the environmental conditions experienced by seeds during maturation, dispersal, and germination. Where the environmental conditions for a particular plant population are not known, climate databases like WorldClim (Fick & Hijmans 2017) can be a useful tool.

Existing germination data for the same or related species can also provide valuable clues about potential dormancy behavior and alleviation requirements. For example, species with PY are known in a relatively restricted number (ca. 18) of families (Table 1) and scarification of the water-impermeable seed coat will often enable germination in species that belong to one of these families. Physiological dormancy, however, occurs far more widely across taxa and dormancy alleviation requirements for these species are closely linked to the climatic conditions (Willis et al. 2014; Seglias et al. 2018). Species-specific information, though limited, is available in the published literature (Baskin & Baskin 2014) and on RBG Kew's Seed Information Database (RBG Kew 2018). Related species are a useful, but not infallible, reference, as dormancy alleviation and germination requirements can vary within families, genera, as well as within and between populations and individuals of the same species (Baskin & Baskin 2014).

When little germination information exists for a particular taxon, or when the sequence of conditions needed to relieve dormancy in water-permeable seeds is unclear, the 'move-along' approach may be useful (Baskin & Baskin 12003*a*). This double germination phenology study is simple to carry out, requires a small number of seeds, and can provide key germination information quickly.

In the 'move-along' experiment, freshly collected seeds are placed on agar plates, moist filter paper, or sand and cycled through a series of temperature regimes designed to replicate natural conditions. For temperate species, these conditions would represent the typical length of spring, summer, autumn, and winter seasons. Samples are split into groups, some begin the cycle with the summer and others with winter temperatures, while control samples remain at each temperature throughout the experiment (Baskin & Baskin 12003*a*). The point within the temperature cycle at which dormant seeds germinate indicates whether cold stratification, warm stratification, or a sequence of both is required to break dormancy. The conditions used in the move-along experiment can be modified to fit any bioregion, for example to include periods of dry after-ripening, drying and re-wetting, or be continued through multiple cycles over more than 1 year (Chia et al. 2016; Kildisheva 2019).

Existing Dormancy Alleviation Techniques

Many dormancy alleviation techniques have been developed, with the choice of technique reflecting the class of dormancy and environmental conditions that seed would naturally experience (Table 2). More information is available in the Kew's Technical Information Sheets (Davies et al. 12015*a*; Davies et al. 12015*b*). Whilst these techniques are well established in laboratory or nursery settings, their application and effectiveness in field scenarios and at restoration scales is less understood (Broadhurst et al. 2016). Some treatments can be scaled up (and mechanized)—scarification with sandpaper or a pneumatic scarifier, wet and dry heat, percussion, or acid scarification can be applied to large quantities of seed to break PY (Khadduri & Harrington 2002; Kimura & Islam 2012; Mondoni 2013; Hall et al. 2017; Kildisheva et al. 12018*b*), whilst flash flaming, dry after-ripening, smoke compounds, gibberellic acid, and other chemical stimulants can be applied to physiologically dormant seeds (Erickson et al. 2016; Guzzomi et al. 2016; Erickson et al. 2017; Hall et al. 2017; Lewandrowski et al. 2017). Understanding the scalability of a treatment technique is important to prevent embryo damage and ensure effectiveness. Additionally, the influence of a dormancy alleviation on germination timing must be adequately considered to increase the likelihood of survival following germination.

Reintroduction may be planned to take advantage of natural opportunities for dormancy release, for example by sowing spring germinating species in autumn (Wagner et al. 2011), but this may not be sufficient in all cases (Kildisheva 2019). Creating multiple germination niches at different phases of the restoration process may be an effective approach especially in cases where site conditions are limiting or unpredictable (Davies et al. 2018). By relieving dormancy in only a portion of a seed batch sown onto a site, managers can incorporate additional bet-hedging and ensure that some recruitment occurs within the first growing season, while maintaining the rest of the seeds in a dormant state for potential later recruitment (Kildisheva 2019).

Table 2. Summary of the most commonly used dormancy alleviation techniques based on dormancy class.

Dormancy Class	*Treatment*	*Description*
PY	Scarification	Chip (with scalpel or secateurs), file, sand, abrade, or remove a portion of the seed coat to enable water uptake (imbibition), away from the root axis to avoid damaging the embryo.
	Dry heat	Place seed in an oven (90–100°C for up to 30 minutes, time and temperature vary by species, see Erickson et al. 2016).
	Wet heat	Immerse seed in hot water (70–90°C from 30 seconds to several minutes, time and temperature vary by species, see Erickson et al. 2016).
	Acid scarification	Immerse seed in concentrated sulfuric acid for up to 120 minutes.
	Percussion scarification	Place seeds inside a metal container (adjust the container size based on distance you want the seeds to travel within container). Placed on an industrial paint shaker and run for 3–20 minutes (see Khadduri & Harrington 2002 and Mondoni et al. 2013).
	Pneumatic scarification	Place seeds inside the scarification chamber lined with sandpaper or other abrasive material (e.g. using a Mater Pneumatic Scarifier, PSS2000, OEM, Inc. attached to an air compressor). Adjust the air pressure and scarify seeds for at least 20 seconds, depending on the thickness of the seed coat (see Kildisheva et al. 12018*b*).
PD	Cold stratification	Expose imbibed seed to cold temperatures (<10°C), mimicking winter conditions.
	Warm stratification	Expose imbibed seed to warm temperatures (>20°C), mimicking summer conditions.
	Dry after-ripening	Place dry seed in warm, moderately humid conditions (e.g. 50–60% relative humidity) for several weeks or months, mimicking a natural dry season.
	Mechanical nicking	Remove a portion of the seed coat close to the root tip with a scalpel.
	Flash flaming	Place seeds in a rotating drum with a direct flame for several seconds (see Guzzomi et al. 2016); distance from the flame and the processing duration vary by species.
	Chemical growth stimulants	Use chemicals such as potassium nitrate (KNO_3), gibberellic acid (GA_3), or smoke solutions (e.g. KAR_1 or smoke water) to stimulate germination (see Baskin & Baskin 2014; Erickson et al. 2016).
PY + PD	Combination of the above treatments	Apply multiple treatments to release physical then physiological dormancy.
MD	Provide conditions for embryo development	Place imbibed seeds at a suitable temperature for 4 weeks, using environmental conditions at the time and place of natural dispersal as a guide.
MPD	Combination of PD and MD treatments	Use environmental conditions as a guide to determine the temperature cycles required for both embryo development and physiological dormancy release.

Labeling and Reporting of Seed Dormancy Status and Dormancy Alleviation Treatments

To ensure restoration outcomes meet their objectives and quality standards, it is important to maintain accurate records of the seed dormancy status and germination requirements across seed batches using standardized methods and criteria (Silveira 2013; Frischie et al. 2020). As a minimum, the following information should be reported for each seed batch:

- *Collection site description*, including geographic coordinates, soil type, and vegetation community
- *Collection information*, including the date of seed collection, the number of fruits sampled per individual, the number of individuals sampled, an estimate of population size, and sampling strategy
- *Seed cleaning and quality information*, including any techniques used to clean seeds, the percentages of seed fill, and the number of viable seeds
- *Seed storage information*, including the length and conditions under which seeds where stored
- *Dormancy testing data*, including the results of imbibition testing, the specific environmental conditions, the duration of seed germination experiments, the details of presowing treatments, and the germination results

Conclusions

The success of seed-based restoration efforts relies on the ability of practitioners to accurately predict germination requirements and ensure these are met through natural conditions at the restoration site or appropriate artificial presowing treatments. A thorough understanding of the quality, dormancy status, and germination requirements of the seeds sown is therefore essential. This information can be readily obtained for each seed accession through seed quality assessment and dormancy classification, following a series of standard seed testing steps. Accurate records that include seed collection, quality, cleaning, storage, and dormancy information for each seed batch (maintained from seed collection to seed use) are equally critical to ensuring restoration success. The seed dormancy and germination guidelines outlined in this article should be a standard component of any seed-based restoration planning process and should be considered in conjunction with the 'International principles and standards for native seeds in restoration' (Pedrini & Dixon 2020).

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REVIEW ARTICLE

Seed enhancement: getting seeds restoration-ready

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Seed enhancement technologies such as seed priming and seed coating, developed by the agricultural seed industry, are standard procedures for the majority of crop and horticultural seeds. However, such technologies are only just being evaluated for native plant seeds despite the potential benefits of such treatments for improving restoration effectiveness. Key approaches applicable to native seed include: (1) seed priming, where seeds are hydrated under controlled conditions, and (2) seed coating, in which external materials and compounds are applied onto seeds through a diversity of treatments. These technologies are commonly employed to accelerate and synchronize germination and to improve seed vigor, seedling emergence, establishment, and to facilitate mechanized seed delivery to site, through standardizing seed size and shape. Seed enhancement technologies have now been tested on native seeds to overcome logistical and ecological barriers in restoration. However, further research is needed to extend the application of seed enhancements to a broader array of species, ecosystems, and regions as well as to evaluate new and innovative approaches such as the incorporation of beneficial soil microorganisms and plant growth regulators in the coatings. As techniques in native seed enhancement develop, these approaches need to be capable of being scaled-up to provide the tonnages of seed required for global restoration.

Key words: agglomerates, coating, encrusting, germination, pelleting, priming, seed technology

Implications for Practice

- Seed priming can provide synchronized, rapid, and on-demand germination, establishment, and confer resilience to a variety of stresses, improving plant survival in harsh environments.
- Seed coating technology modifies the shape and size of field-ready seed units, improving delivery to site, especially for small-seeded species, or for seeds with confounding appendages or complex morphology.
- Seed coating and priming can be used to deliver compounds such as germination promoters, protectants, and predator deterrents that have the potential to greatly improve seed emergence and plant establishment.
- Seed coating and priming can be used to broaden potential seed germination response as a bet-hedging strategy to compensate for often extreme spatial and temporal variability in the seedbed microclimate of disturbed systems.

Introduction

Seeds are the most cost-effective option for ecological restoration compared with the planting of seedlings, particularly at larger scales or in highly biodiverse ecosystems (Pérez et al. 2019). However, fewer than 10% of seeds deployed to field-based successfully establish to produce a mature plant (James et al. 2011; Merritt et al. 2011; Ceccon et al. 2016). Given the challenges and cost of procurement and production of native seeds (Merritt & Dixon 2011) and the potential negative impacts of increasing seed collection rates on wild populations (Nevill et al. 2018), such a high failure rate is unsustainable and severely limits the success of seed-based restoration at the scales that are now required (Menz et al. 2013). Thus, there is an emerging need and market demand for techniques and technologies that improve restoration outcomes associated with direct seeding.

The high failure rates in seed-based restoration have been attributed to physiological, logistical, and ecological-environmental factors. These include low seed viability,

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dormancy, limited emergence, challenges in handling and delivery of seed mixes due to variation in seed size and morphology, and variability in the environmental conditions across restoration sites. As such, seed enhancement technologies (in which seeds are artificially treated to promote "germination and establishment on-demand") represent an area where research and development are urgently required to improve the quality, deliverability, and reliability of native seed batches and to confer resilience to environmental stresses, such as moisture or temperature extremes, and ecological challenges (e.g. predation, competition, and disease).

However, seed enhancement technologies have received limited attention in ecological restoration for a variety of reasons including: extensive research and development needed to customize existing crop seed technologies to complex and diverse native seed types, high initial cost of equipment, and hurdles in scaling the enhancement processes. Yet, such technologies are a standard feature in the crop and horticulture seed supply chains as the benefits they provide far outweigh the costs (Pedrini et al. 2017). In recent years, seed enhancement technologies have been developed for ecological restoration with identified potential benefits if the technologies can be optimized and effectively scaled (Madsen et al. 12016*a*; Erickson et al. 2017).

The aim of this review is to present a broad and practical overview of the currently available seed enhancement technologies developed in agriculture (seed priming and seed coating) and provide examples of how such technologies have been applied in the context of ecological restoration.

Seed Priming

Seed priming refers to the controlled hydration of seeds before sowing, where seeds begin the germination process but are redried before germination proceeds to the point of radicle/epicotyl extension (Heydecker & Coolbaer 1977; Bradford 1986). Priming can reduce the variability in seed germination rate within a population, ensuring more uniform and rapid germination and establishment (Taylor et al. 1998; Jisha et al. 2013; Paparella et al. 2015; Bhanuprakash & Yogeesha 2016). It can also confer greater resilience to thermal, moisture, and osmoticum (salt) stresses (Bruggink 2005) and therefore may be beneficial for plant establishment in harsh environments (Kildisheva 2019).

The Priming Process

The physiological processes that occur during seed priming (see Supplement S1) begins with water uptake. Water uptake (or imbibition) is modulated by seed coat permeability and the area of contact with, and hydraulic conductivity of, the growth substrate (Koller & Hadas 1982; Bradford 1995). Seed water uptake can be divided into three phases: (1) imbibition or the physical uptake of water, (2) "activation" of metabolic activity, and (3) embryo and radicle/epicotyl growth and commencement of mitosis (Fig. 1; Taylor et al. 1992; Bradford 1995).

For imbibition to occur, the seed coat must be permeable to water (Kildisheva et al. 2020). For seeds with a water-impermeable seed coat, permeability must first be achieved by the opening of the water gap or through artificial means such as scarification or hot water treatment (Baskin & Baskin 2014). Once seeds are permeable, imbibition generally occurs within hours or days and water uptake subsides at the initiation of metabolic activity (Bradford 1995).

Seeds are tolerant to desiccation during the first two phases of water uptake, but become desiccation sensitive once embryo growth has been initiated (e.g. phase three; Taylor et al. 1992). Thus, effective priming treatments result in imbibition and activation of the germination process up to a point prior to embryo development so that seeds are poised to complete germination, but retain the ability to be dehydrated and stored prior to delivery to the restoration site, without major viability losses. The optimal priming duration can depend on several factors, including priming method, species biology, seed size, dormancy status, and germination speed (Powell et al. 1984; Karssen et al. 1989; Bradford 1995; Bruggink 2005). Similarly, the extent to which germination can visibly occur before seeds become desiccation-sensitive can also vary by species. For example, for most species if priming has induced visible radicle emergence, viability can be assumed to have been negatively impacted (Tarquis & Bradford 1992; McDonald 1998; Bruggink et al. 1999); however, seeds of some desert species can tolerate desiccation even after the radicle has fully emerged and commenced root extension (Gutterman 2002). Seed priming can be accomplished through a number of different means, including hydro-, chemo-, osmo-, and solid matrix priming (Taylor et al. 1992; Bruggink 2005; Paparella et al. 2015).

Hydro-Priming. Hydro-priming refers to the hydration of seeds in pure water, typically in aerated conditions (Fig. 2) and at temperatures considered favorable for germination (Ward & Powell 1983; Coolbear & McGill 1990; Gray et al. 1990; Harris et al. 1999). Because the extent of priming is controlled by treatment duration, hydro-priming is the least precise of the priming techniques and is applied in combination with other treatments (e.g. chemo- or hormone-priming) or immediately before sowing under nursery conditions (e.g. "soaking," Luna et al. 2014).

Chemo- or Hormone-Priming. In chemo-priming or hormone-priming, germination promoters (e.g. cytokinins, jasmonates, gibberellins, and karrikins), inhibitors (e.g. *ABA*), or plant protective compounds (e.g. salicylic acid, fungicides) can be used to improve seed germination of dormant species, control germination timing to optimize recruitment, and protect seeds from biotic and abiotic stresses (Carrow & Duncan 2011; Górnik et al. 2014; Badrakh 2016; Erickson et al. 2017; Call 2018).

Osmo-Priming. Osmo-priming is a widespread priming approach that relies on the use of an osmoticum bathing solution at water potentials below 0 MPa that allows controlled hydration of seed. This is accomplished through the use of salts

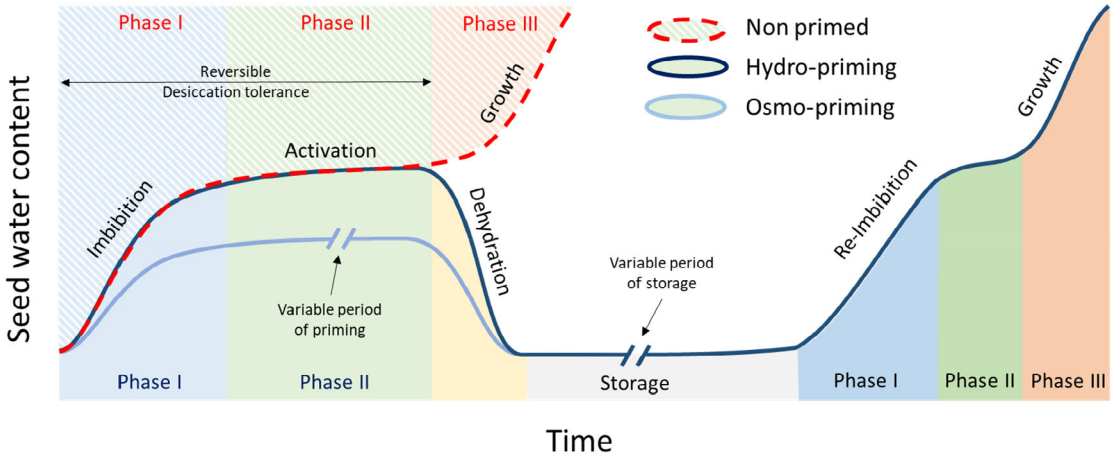

Figure 1. Seed water uptake can be divided into three phases: imbibition, the initiation of germination (activation), and embryo and radicle/epicotyl growth (growth). In seed priming, imbibition is interrupted at the beginning of the growth phase and seed are dried back to be stored.

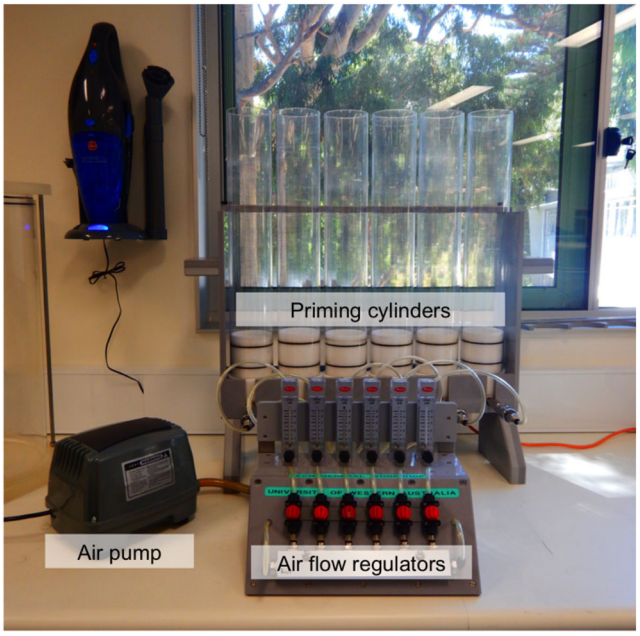

Figure 2. Custom-built seed priming unit used for hydro-, osmo-, and chemo-priming seeds was developed at the University of Western Australia and Kings Park Botanic Garden. This six-cylinder unit is able to treat 1–2 kg of pure seeds of numerous native species and differing morphologies (Erickson et al. 2019; Kildisheva 2019). Cylinders are filled with priming solution and connected to an air pump (Hailea Air Pump, IPX4, Guang Dong, China; flow rate ca. 3–4 L/min) to promote aeration during priming (image modified from Kildisheva 2019).

(e.g. KNO_3, NaCl, $CuSO_4$), polyethylene glycol (PEG), or mannitol ($C_6H_{14}O_6$) (Yadav et al. 2011; Amirkhiz et al. 2012).

Matrix Priming. Solid matrix priming is another approach in which seeds are primed in a solid substrate (e.g. compost, clay, peat, sand, or vermiculite) moistened with water to achieve desired water potentials for priming (Taylor et al. 1988). In some cases, matrix priming can be more effective than osmotic priming (Harman & Taylor 1988; Taylor et al. 1988), presumably because the process is thought to simulate natural seedbed conditions and because oxygen is freely available to seeds throughout the priming duration. Matrix priming has demonstrated positive results by improving germination and emergence of both horticultural and wild plant species (Bosma et al. 2002; Madsen et al. 2018) and has the potential to be combined with other seed technologies.

Seed Priming Materials and Equipment. In the seed industry, hydro-, osmo-, or chemo-priming is accomplished using a number of approaches, such as incubation trays moistened with polyethylene glycol (PEG) or other solutes; incubation inside aerated solution (e.g. PEG, inorganic salts, mannitol) typically inside large upright cylinders; or membrane priming, where PEG and seeds are separated by a semipermeable membrane to improve aeration needed to maintain seed viability (Bruggink 2005). Smaller-scale priming units have been developed for restoration use. For example, Erickson et al. (2019) describe a six-cylinder priming apparatus developed for seed priming for mine site restoration (Fig. 2). The unit can be used for hydro-, osmo-, and chemo-priming and is able to treat 1–2 kg of pure seeds. It has been tested across a range of osmotic potentials, aeration rates, and seed morphologies of different native species—and has proven effective at treatment delivery (Erickson et al. 2019; Kildisheva 2019).

Solid matrix priming can be used to treat large quantities of seed by storage in a matrix media (e.g. peat, vermiculite), typically inside a drum that rotates around a central axis to ensure even moisture distribution (Rowse 1996; Bruggink 2005). Another method, "drum priming," involves mixing seeds with a specific quantity of water to raise the seed moisture content to the desired level, either in a static or rotating drum (Khan 1992; Bruggink 2005).

Seed Priming in Ecological Restoration

Although priming is used extensively in the agricultural industry, examples of its use in ecological restoration with native plant spices remain limited, despite the potential for considerable benefits. Seed priming has been successfully used to stimulate the germination of pioneer tree species, which are commonly used in the tropical forests (Rodrigues et al. 2009). For example, hydro-priming (immersion in water for 16 hours) and osmo-priming (polyethylene glycol-PEG 8000, −0.8 MPa for 56 and 88 hours) improved seedling establishment of a pioneer tree species *Guazuma ulmifolia* (Brancalion & Tay 2010). Priming also induced rapid germination of several tree species (*Albizia saman*, *Cedrela odorata*, *Enterolobium cyclocarpum*, and *Swietenia macrophylla*) native to the tropical semideciduous forest of Veracruz, Mexico. Natural priming (manual seed burial, similar to matrix priming described above) was effective for *A. saman*, *C. odorata*, and *S. macrophylla* while hydro-priming enhanced the performance of *E. cyclocarpum* seeds (Peraza-Villarreal et al. 2018). Hardegree and Van Vactor (2000) reported that matrix priming enhanced total emergence of four North American bunchgrass species (*Elymus elymoides*, *Elymus lanceolatus*, *Poa sandbergii*, and *Pseudoroegneria spicata*) in the field, but the range of effects on germination were contingent upon seed lot, planting date, and soil type.

Priming has been used in combination with other pre-sowing techniques, for example Wagner et al. (2011) investigated the response of 10 difficult-to-establish species from European calcareous grasslands to: osmo-priming (PEG 6000, osmotic potential of −21.0 MPa), osmo-priming combined with gibberellic acid (GA_3), or cold stratification treatments. Germination was enhanced by osmo-priming (*Campanula glomerata, Filipendula vulgaris*, and *Helianthemum nummularium*) and osmo-priming + GA_3 (*Thymus pulegioides*). Interestingly, the addition of GA_3 to the osmo-priming solution promoted germination in suboptimal conditions (e.g. drought, light/dark) or substituted for the temperature fluctuation requirement of some species, thus expanding the germination "niche" of the tested species to a wider range of environmental conditions (Wagner et al. 2011; Lewandrowski et al. 2018; Kildisheva et al. 2019).

The ability to promote more rapid germination across a wider range of conditions may be particularly salient in regions where environmental conditions are highly stochastic, such as drylands (Pedrero-López et al. 2016; Erickson et al. 2017; Kildisheva 2019). Erickson et al. (2017) and Kildisheva et al. (2019) reported on the potential for the integration of seed priming into the restoration tool kit in the context of mine rehabilitation, either alone or in combination with other seed enhancement techniques. Priming with karrikinolide, a smoke-derived germination stimulant, was shown to improve germination and emergence of *Triodia pungens* L. (Erickson et al. 2017; Kildisheva 2019)—a keystone species in the Pilbara bioregion of Western Australia (Nicholas et al. 2009). Priming was particularly effective when combined with seed coating, possibly by acting to expand the capacity of plants to take advantage of available precipitation more effectively and facilitating greater root development to increase survival under increasingly arid conditions (Kildisheva 2019).

Madsen et al. (2018) demonstrated how solid matrix priming (−0.5 to −2.5 MPa for up to 12 days) can be effectively combined with seed coating technologies (e.g. seed "pods") to improve emergence and establishment density of two grass species (*Poa fendleriana* and *P. spicata*) seeded on the Kaibab Plateau in Arizona. Results show that emergence from the primed-seed pods was 66–82% faster than for non-treated seeds. Additionally, the final density of *P. spicata* seedlings originating from primed-seed pods was 2.9- to 3.8-fold higher than non-treated seeds.

Thus, while the evidence of use in restoration remains to be broadly tested, seed priming has the potential to improve restoration outcomes, especially when combined with other seed enhancement technologies. However, effective adoption of priming into restoration practice requires an in-depth understanding of species-specific seed biology (Hardegree 1996), site-dependent recruitment limitations, and seed delivery methods to ensure cost-effective integration (Bujalski & Nienow 1991).

Seed Coating

Seeds of native species vary widely in shape and size, posing challenges for handling and mechanical sowing, such as lack of flowability and bridging (where seed cross-link and block the seed delivery system). By applying external material to the seed, seed batches can become more homogeneous and easier to manipulate in the deployment to the restoration site (Hoose et al. 2019). Moreover, an artificial coating can be loaded with active ingredients that, once released to the seed or in the surrounding soil, protect the seed from pathogens and improve germination, survival, and growth (Taylor et al. 1998; Halmer 2008).

Seed coating has been widely employed by the agricultural industry for decades, but so far, its application to native seeds remains limited to experimental trials. A significant impediment to the implementation of seed coating for ecological restoration using native plant species is the limited access to the expertise and seed coating techniques that are mostly confidentially confined to the agrochemical industry that specializes in seed coating of agricultural and horticultural species (Pedrini et al. 2017).

Seed Coating Types, Materials, and Equipment

Three major seed coatings have been developed for agricultural, forestry, and horticultural species that have relevance to restoration (Fig. 3). These include *film coating*, where a thin layer of material is applied to the seed (less than 5–10% of the weight of the seed); *encrusting*, in which materials that increase the weight and volume of the seed are added but the shape of the original seed is still recognizable; and *pelleting*, in which materials are added to the seed to create an oval-spherical shape where the initial seed shape is indiscernible (Taylor et al. 1998; Halmer 2008). Further variations of seed coating have been developed and adapted in recent years for native plant seeds, such as *agglomerates or conglomerates* (Madsen et al.

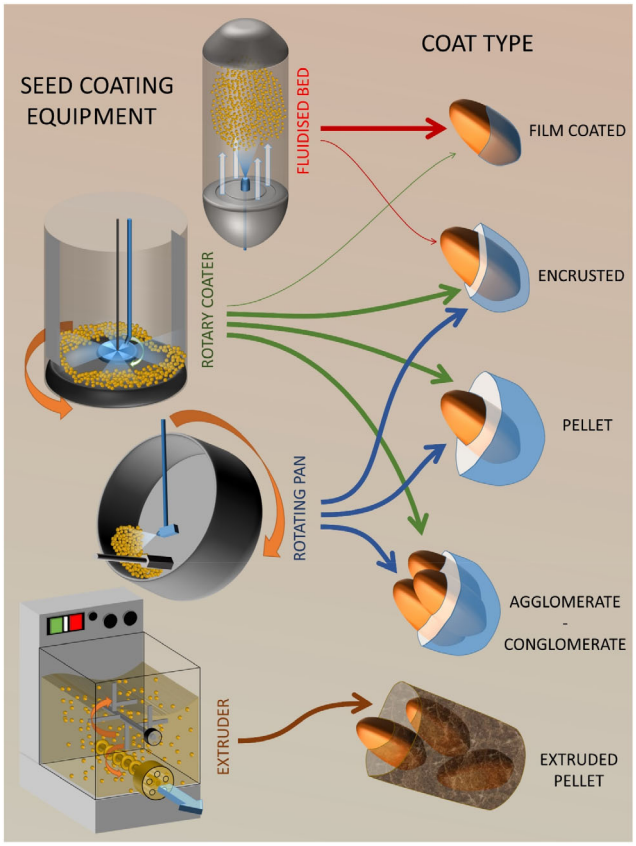

Figure 3. Examples of seed coating equipment and seed coating type. Images adapted from Pedrini et al. 2017.

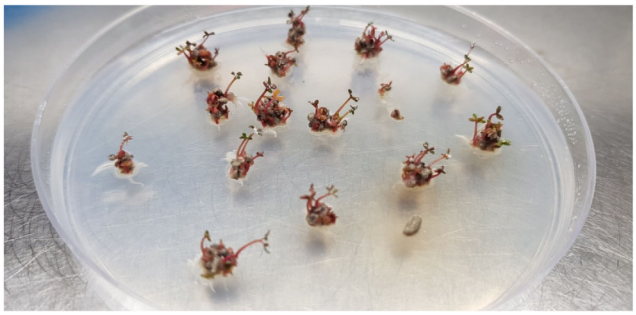

Figure 4. Agglomerates of *Eucalyptus loxophleba* made with diatomaceous earth and polyvinyl alcohol (approx. 5 seeds per pellet).

2012; Hoose et al. 2019), in which multiple seeds are clustered together into a single delivery unit (Fig. 4) and *extruded pellets* (Madsen et al. 12016*b*, 2018, Brown et al. 2019), when seeds are mixed with a relatively large amount of material and then passed through an extruder that forms and cuts the extruded material into desired shapes (usually cylindrical). To avoid confusion, it is important for restoration researchers and practitioners to consistently define the seed coating type according to the currently agreed agricultural definitions and allow for constructive collaborations in the development of this technology with crop and horticulture seed companies and scientists.

In the seed industry, there are three main types of equipment in use (Fig. 3): *fluidized bed*, used for film coating; *rotary coater*, commonly employed on native seeds; and *pan coater*, used on very small seeds (Gregg & Billups 2010; Bennett & Lloyd 2015). Seed agglomerates can be made with either a rotary coater or pan coater. Extruded pellets instead require a specific machine, the *extruder*, that is similar to the ones used by the food industry to make pasta (Watkins 2014; Madsen et al. 2016*b*).

Seed coating is not always feasible on pure seed units for many native species without prior reduction or removal of external structures (Guzzomi et al. 2016; Pedrini et al. 2019), and extensive seed processing is sometimes required (Frischie et al. 2020).

Materials and compounds used to provide for the physical, thermal, and mechanical properties of the seed coating can be broadly divided into two groups: *binders*, usually polymers such as celluloses and gums that adhere to the seed and allow for the retention of other materials, and *fillers*, powdery materials used to increase the volume and weight of the original seed (e.g. clay, lime). A wider range of active ingredients, either biological or chemical, can be incorporated into coatings to improve seed survival (e.g. by protecting from pathogens and predators), aiding in germination (e.g. nutrients, hormones, plant growth promoters, symbionts), and improving stress resistance (e.g. salicylic acid, beneficial microbes) (Taylor et al. 1998; Rocha et al. 2019).

Seed Coating in Ecological Restoration

Seed coating technologies, particularly when combined with beneficial biological and chemical active ingredients or protectants, can play a role in the success of seed-based restoration programs (Table 1) by targeting specific challenges that limit plant recruitment on a site, such as variable soil moisture, low soil nutrients, pests, and diseases (Gornish et al. 2019). For example, the inclusion of a soil surfactant agent in the coating of *Pseudoroegneria spicata* (bluebunch wheatgrass), for post-fire restoration in the northwestern United States, improved seedling emergence and plant survival in water repellent soil (Madsen et al. 2013). Similarly, the inoculation of microorganisms in the coating improved seedling emergence and survival of two species on degraded rangeland in the Qinghai–Tibetan Plateau (Liu et al. 2010), while seeds coated in salicylic acid improved plant survival and growth for native grasses in Australia (Pedrini 2019). Seed predator repellents incorporated in seed coatings reduced seed consumption rates from rodents (Taylor et al. 2020) and improved plant establishment (Pearson et al. 2019).

Seed coating has also been tested as a means of controlling germination timing, for example, Richardson et al. (2019) delayed germination in *P. spicata* by applying abscisic acid (ABA) and delaying germination from late autumn, to spring, when conditions for seedling emergence and plant establishment would be more favorable.

Table 1. Studies on the application of seed coating to native species published in peer-reviewed journals from 2006 to 2020.

Publication	*Ecosystem—Species*	*Coating Type, Machine, and Material*	*Active Ingredients*	*Experiment Results Compared to Uncoated Control*
Taylor et al. (2020)	*Pseudoroegneria spicata*	Encrusting on rotary coater with bentonite clay and polyvinyl alcohol	Nine predator repellents such as peppers and oils.	Lab germination: similar to control. Feeding trial: reduced consumption by rodents in some treatments.
Pearson et al. (2019)	Twelve dominant species in intermountain grasslands of Western Montana (U.S.A.)	Encrusting on rotary coater with bentonite clay and polyvinyl alcohol	Predator repellent chili-pepper (*Capsicum chinense*)	Lab feeding trail: reduced consumption for treated seed in all species. Field plant establishment: variable in the first 3 years, but improved for treated seeds in fourth year.
Hoose et al. (2019)	*Artemisia tridentata* ssp. *wyomingensis*	Conglomeration on rotary coater with mineral soil (azomite), compost, and commercial binder	None	Seed flowability and broadcast delivery improved for treated seeds. Higher seed germination in lab trials but similar seedling emergence in the field.
Richardson et al. (2019)	*P. spicata*	Encrusting on rotary coater with calcium carbonate and commercial binder	Abscisic acid (ABA)	Seed treated with ABA showed a delay in germination tested in the lab.
Madsen et al. (2018)	*Poa fendleriana*, *P. spicata*	Extruded pellets with various clay filler materials, absorbents, bio-stimulants, plant protectants, water	Matrix priming prior extrusion	Seedling emergence improved in one soil type and seedling density improved.
Erickson et al. (2017)	*Tridia pungens*	Encrusting on rotary coater with calcium carbonate and polyvinyl alcohol	Hydro-priming with KAR_1	Improved seedling emergence in rain manipulated shelter.
Madsen et al. (12016*b*)	*A. tridentata* ssp. *wyomingensis*	Extruded pellets with various clay filler materials, absorbents, water	Bio-stimulants, plant protectants	In soil, improved seed emergence at different sowing depth and increased growth.
Williams et al. (2016)	Four plant species native to arid and semiarid regions in western United States	Encrusting on rotary coater with biochar and polyvinyl alcohol	Biochar used as a promoter	Lab germination: neutral or negative impact on all species. Field trial: no difference in plant cover and biomass.
Madsen et al. (2014)	*P. spicata*	Encrusting on rotary coater and extruded pellets with activated carbon, diatomaceous earth, polyvinyl alcohol, and water.	Activated carbon used as herbicide safeners	Encrusted seeds and extruded pellets were protected from pre-emergence herbicide and resulted in higher seedling density, height, and biomass. Best results were given by extrusion.
Rushing et al. (2013)	*Panicum virgatum*	Non-specified coating type in pan coater with commercial coating powder and binder	Commercial herbicide safeners	Field trials: coating with safeners generally did not protect plants from herbicidal injury. Controlled hydration was more effective in delivering protection.
Madsen et al. (2012)	*P. spicata*	Agglomeration on rotary coater with diatomaceous earth and polyvinyl alcohol	None	Improved seedling emergence in clay and sandy soil. Increased aboveground biomass.
Liu et al. (2010)	*Lolium multiflorum, Astragalus sinicus*	Manual mixing of seeds and material with algal powder gums and wheat flour	Biologicals (*Aspergillu*s sp. and *Streptomyces* sp.)	Indoor experiment: seedling emergence improved. Outdoor pot trial: growth improved.
Mangold and Sheley (2007)	*Agropyron cristatum*	Nondisclosed	Mycorrhizal, algae, beneficial *Bacillus* inoculums, vitamins, and growth hormone	In pot trial: seedling emergence reduced while seedling survivorship and biomass were not affected by either seed treatment.
Turner et al. (2006)	Eleven banksia woodland species in South Western Australia	Outsourced, nondisclosed	Outsourced, nondisclosed	Ex situ trial: in 10 species similar seedling emergence, in one reduced emergence. In situ trial: emergence improved in two species, similar for the others.

When agglomeration of multiple seeds per pellet was tested with *P. spicata*, the treatment improved seedling emergence and plant growth in crusting soils (Madsen et al. 2012). This approach also improved the handling and sowing efficiency of a small-seeded species, *Artemisia tridentata*, while increasing germination in laboratory conditions, but the improvement was not detected in field emergence (Hoose et al. 2019). However, Anderson (2020) found in a more extensive field trial across the Great Basin, United States, that the agglomeration technique improved *A. tridentata* seedling emergence and plant establishment in comparison to untreated seed.

Extruded pellets have been tested in dryland systems. For example, extruded pellets made with activated carbon, protected seeds of native grasses (*Elymus elymoides*, *P. spicata*, *Poa secunda*) and a shrub (*A. tridentata*) from pre-emergent herbicide and in some cases promoted higher seedling density, height, and biomass compared to the untreated control (Madsen et al. 2014; Clenet et al. 2019).

Other compounds have also been incorporated to address other specific establishment limitations. When seeds of a keystone shrub, *A. tridentata*, were incorporated into extruded pellets with super-absorbent polymers, seedling emergence was also improved, particularly in crusting soils, presumably resulting from the swelling action of the pellet that elevated the seeds in the soil and created a moisture-rich micro-environment (Madsen et al. 12016*b*).

However, the effectiveness of these techniques can vary between sites, years, species, coating formulations, application approaches, and the plant demographic stage being evaluated (Williams et al. 2016; Davies et al. 2018; Kildisheva et al. 2019).

Furthermore, the scope of existing studies and the probable under-reporting of failed seeding experiments in restoration make it difficult to evaluate the true effectiveness of these technologies. Based on the agricultural seed literature, which includes reports of the negative effects of coating on seed germination and emergence (Pedrini et al. 2017), developing effective techniques may require time and substantial investment. For development efforts to be successful, scientists and practitioners alike need to share failures and challenges associated with seed coating native plant seeds, to help identify the potential limitations and improve the general understanding of the factors underpinning successful coating formulations in relation to specific ecological or logistical constraints.

In a few instances, seed coating was outsourced to private companies and coating specifications not disclosed (Turner et al. 2006; Mangold & Sheley 2007), making it difficult to replicate. Whenever feasible, seed scientists and users should collaborate with organizations and companies that are willing to share material and methods or try to develop seed coating recipes and protocols independently. For example, a recently published open-access tool for developing seed coating protocols (encrusting and pelleting) provides a practical step-by-step guide to develop species-specific seed coating treatments (Pedrini et al. 2018). This tool can be used for the testing of coating methods, materials, and active ingredients, using readily available seed coating equipment and chemical agents.

Economies of Seed Enhancement Technologies for Ecological Restoration

In spite of some evidence of successful application of seed priming and coating to treat seeds of native species, most of this work has been conducted in controlled experimental settings, and the costs and benefits related to the employment and scalability of these technologies have rarely been investigated. A baseline approach to estimate economies of seed coating should compare the cost for each successfully established plant from treated and untreated seeds (Pearson et al. 2019). Additionally, many external variables should be considered beyond the cost of seed coating material and personnel time. For example, if coating improves a seed's ballistic proprieties, allowing for a wider seed broadcast area, it will reduce seeding time and equipment use (e.g. fewer mechanized passes). Such benefits should be accounted for in the cost/benefit evaluations of seed coating in ecological restoration (Hoose et al. 2019) and to facilitate the adoption of seed enhancement technologies at scale.

Is Fast and Synchronized Germination Always Better? Bet-Hedging Strategies With Seed Enhancement Technologies

Soil seed beds (awaiting restoration seeding) can exhibit relatively high spatial variability in soil water availability and temperature (Hardegree et al. 2020) and often have logistical constraints that require seeding well in advance of germination and emergence (Rajagopalan & Lall 1998; Eiswerth & Scott Shonkwiler 2006; Boyd & Lemos 2015; Hardegree et al. 2018). Additionally, many restoration sites present challenging edaphic and environmental complexities that in cases of significant degradation can substantially differ from the natural reference site conditions (Seastedt et al. 2008; Coates et al. 2016). These include human-induced management disturbances, such as mining or grazing, as well as competition from invasive species.

Regardless of the source of the disturbance, natural environmental variability can also impose severe constraints on the timing and location of suitable microsites for establishment in any given year (Hardegree et al. 2016, 2018). Various seed enhancement techniques and technologies can be designed to shift the germination behavior of a given seed population to compensate for environmental changes that have diminished the probability of successful establishment (Angevine & Chabot 1979). However, it may be beneficial to broaden (rather than merely shift) the germination behavior of a desirable species (Madsen et al. 12016*a*; Erickson et al. 2017; Davies et al. 2018; Hardegree et al. 2020). This approach can provide a bet-hedging capability to compensate for both environmental and edaphic variability resulting from ecological disturbance and degradation factors (Davies et al. 2018; Lewandrowski et al. 2018; Kildisheva 2019). Seed priming and coating can be used to accelerate or delay a germination response, narrow or broaden the variability in seed germination rate within a population, or compensate for undesirable site conditions (Hardegree 2002; Madsen et al. 12016*a*; Kildisheva 2019).

Conclusion

The success of large-scale restoration using direct seeding will continue to depend on efficient and effective seed use. Seed enhancement technologies, though in their infancy in ecological restoration, are likely to provide major improvements in field establishment akin to that achieved for crop species. We have described how individual seed treatments can accelerate, delay, or stagger germination and emergence in the field but any or all of these effects need to be tailored to the site and local climatic conditions. These treatments should not be used indiscriminately for the sake of novelty or innovation but need to have proven benefit with deployment able to target specific ecological or logistical limitations and give every seed the optimum chance for germination, emergence, and successful establishment. Thus, the effectiveness of the development and use of seed enhancement technologies is contingent on dedicated research and implementation programs that work to understand and address the species- and site-specific challenges that limit plant recruitment from seed. As such, seed enhancement technologies must become part of a broader restoration strategy that integrates relevant issues of site conditions, species availability, and species performance.

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STRATEGIC ISSUES ARTICLE

Seed use in the field: delivering seeds for restoration success

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Seed delivery to site is a critical step in seed-based restoration programs. Months or years of seed collection, conditioning, storage, and cultivation can be wasted if seeding operations are not carefully planned, well executed, and draw upon best available knowledge and experience. Although diverse restoration scenarios present different challenges and require different approaches, there are common elements that apply to most ecosystems and regions. A seeding plan sets the timeline and details all operations from site treatments through seed delivery and subsequent monitoring. The plan draws on site evaluation data (e.g. topography, hydrology, climate, soil types, weed pressure, reference site characteristics), the ecology and biology of the seed mix components (e.g. germination requirements, seed morphology) and seed quality information (e.g. seed purity, viability, and dormancy). Plan elements include: (1) Site treatments and seedbed preparation to remove undesirable vegetation, including sources in the soil seed bank; change hydrology and soil properties (e.g. stability, water holding capacity, nutrient status); and create favorable conditions for seed germination and establishment. (2) Seeding requirements to prepare seeds for sowing and determine appropriate seeding dates and rates. (3) Seed delivery techniques and equipment for precision seed delivery, including placement of seeds in germination-promotive microsites at the optimal season for germination and establishment. (4) A monitoring program and adaptive management to document initial emergence, seedling establishment, and plant community development and conduct additional sowing or adaptive management interventions, if warranted. (5) Communication of results to inform future seeding decisions and share knowledge for seed-based ecological restoration.

Key words: broadcast seeding, drill seeding, native seed, reference site, seed delivery, site preparation

Implications for Practice

- Site characteristics and the ecology and germination requirements of each species in the seed mix will guide development of the seeding plan that includes site preparation and seeding operations.
- Site preparation practices remove impediments to vegetation establishment and create a germination conducive soil seedbed.
- Seeding equipment and sowing strategies should be selected to provide optimal seed placement for each species in the seed mix. Good seed-to-soil contact is critical for successful seed germination and establishment.
- Monitoring seedling emergence and plant establishment in the first months and years post-seeding assesses seeding success and identifies needs for further treatments (e.g. weed control, seed addition). Continued long-term monitoring identifies the need for active management to ensure a satisfactory restoration trajectory is established.

Introduction

A seeding plan that specifies when, where, and how native seed mixes will be used is the foundation for a successful field sowing and an integral part of the overall restoration plan created during the initial phases of a project (see Erickson & Halford 2020). A seeding plan (Fig. 1) includes data from the site evaluation,

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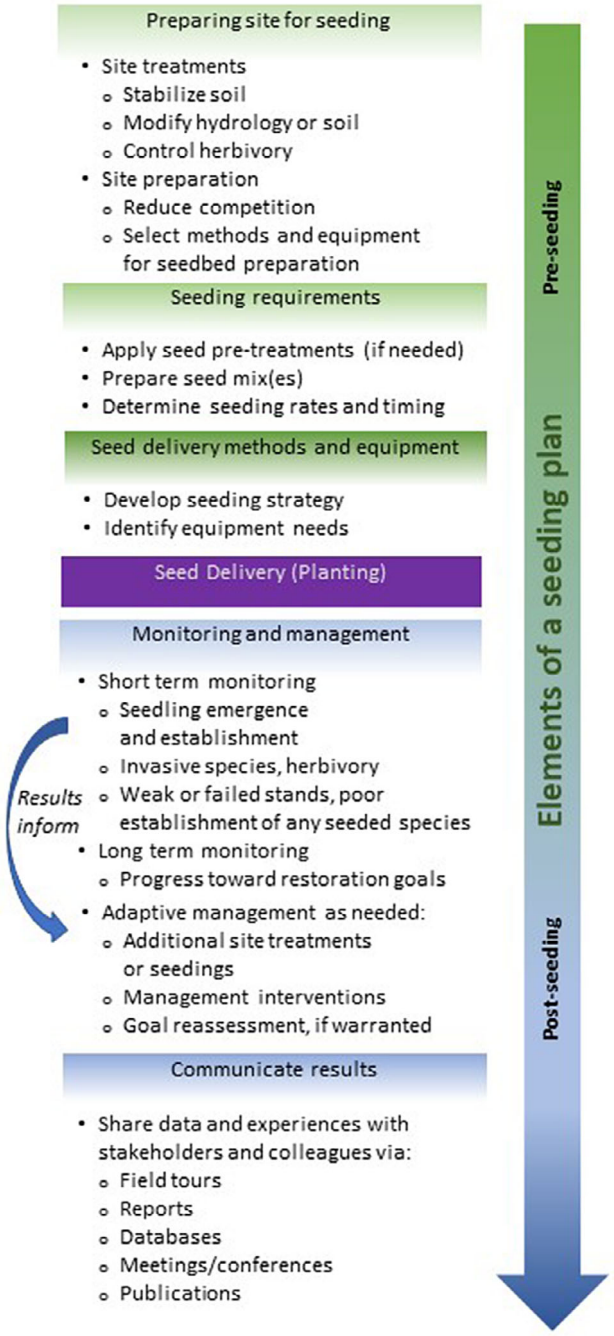

Figure 1 The suggested steps of a seeding plan from site treatments through communication of results.

including climate, hydrologic functioning, surface and slope stability, soil types and condition, native vegetation status, and problematic weed species (Krautzer et al. 2012; Armstrong et al. 2017). This is combined with information about the species to be sown and seed mix quality (Frischie et al. 2020) to provide a detailed timeline of actions required from site preparation through seeding and monitoring. The goal is to provide an optimal germination environment for seeds of each species and establish seedlings at a desired density and species composition (Armstrong et al. 2017). Because hurdles (e.g. funding, resource limitations) or disruptive events (e.g. floods, drought, wildfires, herbivory) could occur, contingency and remediation plans should be considered. Common elements of most seeding plans include:

(1) *Site treatments and seedbed preparation* to stabilize the site, remediate soil problems (e.g. compaction, nutrient levels, hydrological issues such as repellency, etc.), and resolve other problems identified by the site evaluation that might jeopardize seeding success and vegetation development. Operations are then conducted to remove competition, facilitate the seeding operation, and create an appropriate seedbed for the species being sown.
(2) *Seeding requirements* include seed mix preparation and appropriate seeding dates and rates for each species in the mix. Seed pre-treatments to relieve dormancy or seed enhancement to facilitate seeding or improve germination and emergence may be required for some species.
(3) *Seed delivery methods* are identified to efficiently and effectively deliver seeds of each species to appropriate microsites for establishment and growth. Equipment used in agriculture or forestry, implements developed for specific restoration situations, or manual methods can be selected.
(4) *Monitoring and management* to assess initial emergence, seedling establishment, and plant community development and identify the need for additional seeding or adaptive management.
(5) *Communicating results* locally and more broadly to inform future seed-based restoration projects.

Site Treatments and Seedbed Preparation

Site treatments prior to seeding may be required to remove or reduce competition with undesirable species, increase site stability, repair hydrologic function, or reduce nutrient levels (Morgan 2005). Seedbed preparation operations may then be required to produce physical disturbance of the germination substrate and provide establishment niches (Schmiede et al. 2012; Kiss et al. 2020). Tillage, herbicide application, prescribed fire, and other treatments can be used to create seedbed conditions appropriate to the species and seed delivery system (Whisenant 1999; Morgan 2005; Krautzer et al. 2012; Armstrong et al. 2017). These operations are essential for seedling establishment and vegetation development and can take months or even years to produce a soil condition suitable for optimizing germination and plant establishment. Such procedures should be identified early and incorporated in the seeding plan, budget, and scheduling process.

Mechanical Seedbed Preparation

Mechanical methods are frequently employed to prepare sites for seeding. Equipment can be selected to remove surface vegetation, reduce soil compaction, bury weed seeds, reduce erosion,

Figure 2 Mechanical seedbed preparation equipment: (A) Chisel plows are used for conservation tillage to control weeds, increase infiltration, and leave residue on the surface. (B) Mid-size offset disks till friable soils, turn under surface debris, break up surface crusts, and uproot shallow-rooted vegetation. (C) Pipe harrows with spiked pipes can be used on level to rocky and rugged terrain to reduce clods but leaves a rough surface. Photos: H. Wiedemann.

improve infiltration, break up surface crusts or clods, and firm the soil surface for seeding (Figs. 2, 3A & 3B). A number of reviews are available that describe the effectiveness of different plows, disks, harrows, and other implements for creating a suitable tilth for plant establishment (e.g. Munshower 1993; Whisenant 1999; Walker et al. 2004; Krautzer et al. 2012). In cases where the soil seed bank contains undesirable species or soils are fragile, tillage is not advised. Where surface and subsurface soil compaction are extreme, ripping with steel shanks can increase infiltration and improve soil conditions for seedling establishment. Most mechanized equipment, however, cannot be operated in wetlands, on rugged terrain, or in partially intact native plant communities. Alternative methods should be selected for such situations to protect site and operator safety.

Chemical Seedbed Preparation

Where removal of invasive species or existing undesirable vegetation is the primary concern, chemical treatments can be used alone or in combination with other methods. Chemical methods are often less costly than mechanical treatments, generally easily applied, and they can provide an adequate seedbed, reduce erosion potential, create a mulching effect, and be applied on rugged terrain (Whisenant 1999; Armstrong et al. 2017). Disadvantages of herbicide use in site preparation are human health and environmental concerns (impacts on waterways, soil health, and associated ecological processes), production of excessive litter (in some cases), and potential herbicide impacts on seeded species, residual natives, pollinators, and other organisms. Herbicide applicators must be appropriately trained, certified if required, and have access to personal protective equipment.

Other Site Preparation Methods

Topsoil replacement, fertilization, or inoculation may be necessary on some sites such as mine spoils or other severely degraded or soil-less sites to improve organic matter, nutrient status, and soil microbial communities (Munshower 1993). Conversely, on formerly cropped land, steps may be required to reduce nutrient status to favor native species and reduce weed invasions (Kirmer & Tischew 2014; Glenn et al. 2017). Techniques used to reduce nutrient status include soil inversion, deep tillage to mix topsoil with deeper soil layers, or cultivation without fertilization for one or more years. These techniques can also reduce the weed seedbank. Cover crops, nurse crops, or other transition plantings can also be established to improve site conditions and facilitate establishment of target species (Padilla & Pugnaire 2006; Kirmer et al. 2012).

Precision use of fire is employed in some circumstances to enhance natural regeneration in systems where such practices are well understood (Whisenant 1999; Campbell & Hooymans 2016). Planned burns can also be used to facilitate mechanical seedbed preparation and overseeding into existing vegetation (Ryan et al. 2013). However, great caution is required in the application of fire to ensure there is no unintended collateral damage, increased invasive species abundance, or loss of faunal species (Bradshaw et al. 2018).

Seeding Requirements

Seed and Seed Mix Preparation

Concerns when preparing native seed mixtures (Fig. 3) are the presence of seed units (fruits) with complex structures (e.g. florets, appendages) and the high variability in seed size and density that can cause uneven seed mixing and equipment

Figure 3 Seeding operations for converting degraded lands to forests and savannas in Brazil. (A) Seedbed preparation using a harrow. (B) Seedbed prepared with multiple harrow passes to break up clods and loosen the soil. (C, D) Seed mixes of tree, legume, and grass seeds with sand added to improve seed flow. (E) Direct seeding with a unit seeder in a nontillage system. (F) A broadcast spreader sowing native seed in tilled fields. (G) Seeds are hand broadcast in small or difficult to access areas. (H) Monitoring a broadcast seeding to assess biodiversity and development of cover. Photos kindly provided by Instituto Socioambiental, The Seed Path, and Agroicone, Brazil. Photos: (A) M. Ferreira, (B, C, D, F, G) N. Jacobi, (E) A. Canciano, (H) J. Prado.

blockages during seed delivery. Once the seeds required for a project are obtained (see Erickson & Halford 2020), each seed lot should be examined to determine whether further seed cleaning is required to improve seed flowability (Pedrini et al. 2018; Frischie et al. 2020). In addition, seed pre-treatments (e.g. scarification, application of chemical stimulants) may be required to relieve dormancy of some species (Kildisheva et al. 2020). Seed coatings (e.g. pelleting and encrusting) can further improve uniformity of seed size and shape and precision in seed metering and placement in the soil (Pedrini et al. 2020a). Seed coatings can also provide nutrients, inocula, or improved microsite conditions for sown seeds, particularly for seedings on arid lands or degraded soils such as found in post-mined areas.

Seed delivery method and seed and plant characteristics must be considered when creating the seed mix(es) (Erickson & Halford 2020). All species can be mixed together for sowing (Fig. 3), or they can be divided into separate mixes for seeding areas with different site conditions (e.g. soil type, hydrology). Separate seed boxes and seed drops (Fig. 4C) or operations can be used for small seeds that are surface seeded and pressed into the soil and larger seeds that are planted in the soil. Similarly, grasses and other more rapidly developing species can be segregated in separate rows or strips from slower growing forbs and woody species. Seeds that are expensive or in short supply can be selectively seeded in areas most favorable for their establishment.

Seeding Rate

Seeding rates for individual species should be presented as the number or weight of pure live seeds (PLS) sown per area or row length. PLS is based on test results for seed lot purity, viability or germination, and 1,000 seed weight (see Armstrong et al. 2017; Erickson & Halford 2020; Frischie et al. 2020; Pedrini & Dixon 2020). In the absence of seed testing, bulk numbers of seed or bulk seed weight sown per area or row length is used (Goldblum et al. 2013). When mixtures of species are harvested together, the percentage of each species by weight following seed cleaning can be used to calculate approximate seeding rates.

Practitioner knowledge, results of previous restoration projects, and pertinent research outcomes can provide valuable guidance when setting seeding rates for the components of a mixture and the overall seeding rate. Survival percentages, however, are variable, and can be low, particularly on dry or highly disturbed sites (Whisenant 1999; Bainbridge 2007). Seeding rate guidelines are available for some plant communities and individual species (Diboll 2005; Kiehl et al. 2010; Kirmer et al. 2012; USDA NRCS 2019). Where these guidelines exist,

Figure 4 Seeding equipment: (A) Motorized seed mix delivery broadcaster for small-scale projects. This broadcaster provides some degree of seed-to-soil contact but requires a prepared seed bed (Romagnese, Italy). (B) Custom-made precision seeder for delivery of seed mixes used in reconstruction of farmland to the local reference community in southwestern Australia. (C) Minimum-till drills reduce surface disturbance. The triple seed box provides for separate seeding and even flow of large, small, and fluffy seeds. (D) Helicopters can seed irregular areas in rugged terrain and do not require a landing strip. Seed in the rotary spreader is dropped onto a spinner for distribution, but spread is affected by flight elevation, wind, and seed characteristics. (E) Hydroseeding a road cut in Oregon, U.S.A. Photos: (A) S. Pedrini, (C, E) U.S. Forest Service, (D) H. Wiedemann.

they serve as general recommendations and should be modified based on site evaluation, seed lot data, species growth habits, and specific project goals. Cost or availability of some native species can impact their seeding rate. Species richness can be improved in future years by natural dispersal to the restored site, overseeding, or planting seedlings, particularly species required at low densities such as tree species in woodland communities.

Timing of Seed Delivery

Seeding dates are determined by such factors as climate, seed ripening dates, and seed dormancy syndromes (Whisenant 1999; Frischie & Rowe 2011; Hardegree et al. 2016). Seed mixes may be applied on a single date or in sequential operations in subsequent years to mimic natural successional processes, where known. In general, seeding should be undertaken prior to the longest period of favorable growing conditions to maximize first-year growth, thereby enhancing survival and competitive ability with weedy species. Exposure of seeds to environmental conditions required to release dormancy and prepare seed for germination, however, may dictate different sowing dates for some species. For example, most tallgrass prairie species are sown in the autumn for the benefit of exposure to winter conditions that relieve dormancy and result in spring germination concurrent with natural emergence. However, early ripening tallgrass prairie species may require spring sowing and exposure to summer conditions to improve establishment (Frischie & Rowe 2011).

Seeding Depth

Recommendations for seeding depth vary by species and soil type (Whisenant 1999; Hardegree et al. 2016). Small seeds have limited energy reserves and often require exposure to light or specific day lengths as cues for germination (Kirmer et al. 2012). Such seeds should be sown on the soil surface and pressed onto the soil to provide good seed-to-soil contact without being buried too deeply. Small seeds are subject to drying and survival percentages may be low without such contact. Larger seeds with energy reserves sufficient for emergence can be sown at appropriate depths (based on natural emergence depths, if known) where moisture, temperature, nutrient, and microbial conditions may be more favorable (Bond et al. 1999). There is, however, a need for improved knowledge of optimal seeding depths for native species and development of seeding equipment that provides precise depth control (Masarei et al. 2019).

Seed Delivery

Selection of seeding methods and equipment requires consideration of equipment availability, the terrain, other site conditions, and the seed mix. Seeding methods and equipment are reviewed below.

Drill Seeding

Standard agricultural drills can be used to place seed in the soil on well-prepared sites with level terrain when soils are dry or frozen (Fig. 3E). Rugged drills with high clearance are essential for use on rough or rocky terrain (Stevens & Monsen 2004). Minimum-till drills can be used to reduce surface disturbance (Fig. 4C). Drills can be fitted with multiple seed boxes (Fig. 4C) that segregate seeds of different sizes, morphological characteristics, competitive abilities, or growth habits and permit seeding them in separate rows and at different rates and depths, but careful calibration is required to avoid planting seed beyond their emergence capacity. Agitators maintain the seed flow in seed boxes for small seeds, while picker wheels move chaffy seed through seed boxes and reduce bridging in the seed drops (cross-meshing of seed that blocks delivery). Inert carriers (e.g. rice hulls, cracked wheat, vermiculite, clean sand) can be added to maintain seed mixing, improve seed flow, and facilitate seeding species with small or fluffy seeds. Press wheels, chains, imprinter wheels, or other mechanisms can be used to cover seeds in drill furrows or press seed of surface-seeded species into the soil to increase seed-to-soil contact. To improve water availability in arid and semi-arid sites, deep furrow drills or equipment that creates divots, trenches, or other types of depressions can be used to enhance water catchment or trap snow (Bainbridge 2007).

Broadcast Seeding

Broadcast seeding places seed on the soil surface and is used in a variety of seeding scenarios, but it is often the most wasteful of all seed delivery methods. Broadcast seeding is conducted with equipment-mounted seed broadcasters, aerial broadcasting, or hand broadcasting (Fig. 4A–D) (Stevens & Monsen 2004). Precision in seed placement varies with the broadcasting equipment employed and the level of seedbed preparation. Where seed is broadcast on weed-prone or highly erodible sites or in areas where site preparation is otherwise inadequate or impossible, fewer seeds will land in appropriate microsites, and the resulting outcome will be inconsistent across the site. Higher seeding rates and, where feasible, use of a harrow (Figs. 2C, 3A & 3B), roller, rake, or other implement to improve seed-to-soil contact is recommended when broadcasting seed using equipment that does not provide for seed-to-soil contact (Turner et al. 2006).

Ground broadcasters mounted on tractors or utility vehicles are used to seed on level terrain when there is a well-prepared soil seedbed (Fig. 3F). Cultipackers and land imprinters create a pattern of depressions or pits to improve water catchment and can be used on moderate terrain where the soil is not rocky or highly compacted. Seed boxes or broadcasters can be attached to these implements to drop seed to the soil ahead of the implement, which then press it into the soil. Seed dribblers mounted in front of vehicle or tractor tires deliver seed in narrow strips that are pressed into the soil by the rolling tires. Dribblers are especially useful for adding diversity, including woody species, to depauperate natural vegetation or low-diversity seedings. Some ground broadcasters can be mounted on all-terrain vehicles and used in rugged areas that are not accessible to drill seeding equipment (Stevens & Monsen 2004; Campbell & Hooymans 2016).

Aerial broadcasting is used where large areas are to be seeded rapidly and on rugged or mountainous terrain that is not accessible to ground seeding equipment. Aerial broadcasting is accomplished with fixed-wing aircraft, helicopters (Fig. 4D), and, more recently, drones. Drones also have the capacity to map restoration areas to identify optimal microsites for seeding (Andrio 2018).

Hand broadcasting (Fig. 3G) is often the most efficient means of seeding small or fragmented areas with difficult access (e.g. understories, streambanks, wetlands, steep slopes), microsites within larger seedings that require specific seed mixes, or overseeding. Field crews and volunteers can distribute seed manually or with one of the many types of hand-held broadcasters available. However, depending on the ecosystem, species, and site conditions, outcomes can be highly variable.

Seed-Rich Hay Transfer

Transfer of seed-rich hay (Fig. 5) provides a means of capturing multiple species of local ecotypes to restore depleted native or near native grasslands and highly disturbed areas (Kiehl et al. 2010; Kirmer et al. 2012; Pedrini et al. 2020b). Green hay is harvested from appropriate donor sites (Fig. 5A–C) when most target species are mature and transported immediately to the receptor site where it is scattered by hand or with mechanical spreaders (Fig. 5D & 5E) (Kiehl et al. 2010; Scotton et al. 2012*b*). Hay can also be dried and stored until use. Threshing prior to transport reduces volume and transport costs (Scotton & Ševčíková 2017). The hay also provides a mulch that improves microenvironmental conditions, conserves moisture, and reduces seed movement and erosion, though it can also limit seed-to-soil contact. Capturing seed of the full range of species and seed of different ripening stages of indeterminate species can require repeated harvests using a brush harvester or sequential harvests of different areas within the donor site (Pedrini et al. 2020b). Care must be taken to avoid overharvesting and species loss at the donor site (Meissen et al. 2017). Seed of additional local native species not present or not mature on the harvest date(s) can be sown on the restoration site before the hay is distributed or added to the threshed material prior to sowing (Kirmer & Tischew 2014; Baasch et al. 2016).

Hydroseeding

Hydroseeding (Fig. 4E) delivers seeds to sites that are inaccessible to ground seeding equipment or have a high risk of soil erosion (e.g. roadcuts, unstable or steep slopes) (Kirmer et al. 2012; Armstrong et al. 2017). Seed, mulch, fertilizers, soil additives, and an organic adhesive are mixed with water to form a thick slurry that is sprayed over the site with a high-pressure pump (Fig. 4E). Application equipment is mounted on trucks or tractors, but helicopters can be used in areas not accessible to vehicles. Hydromulching (an additional mulch layer applied and

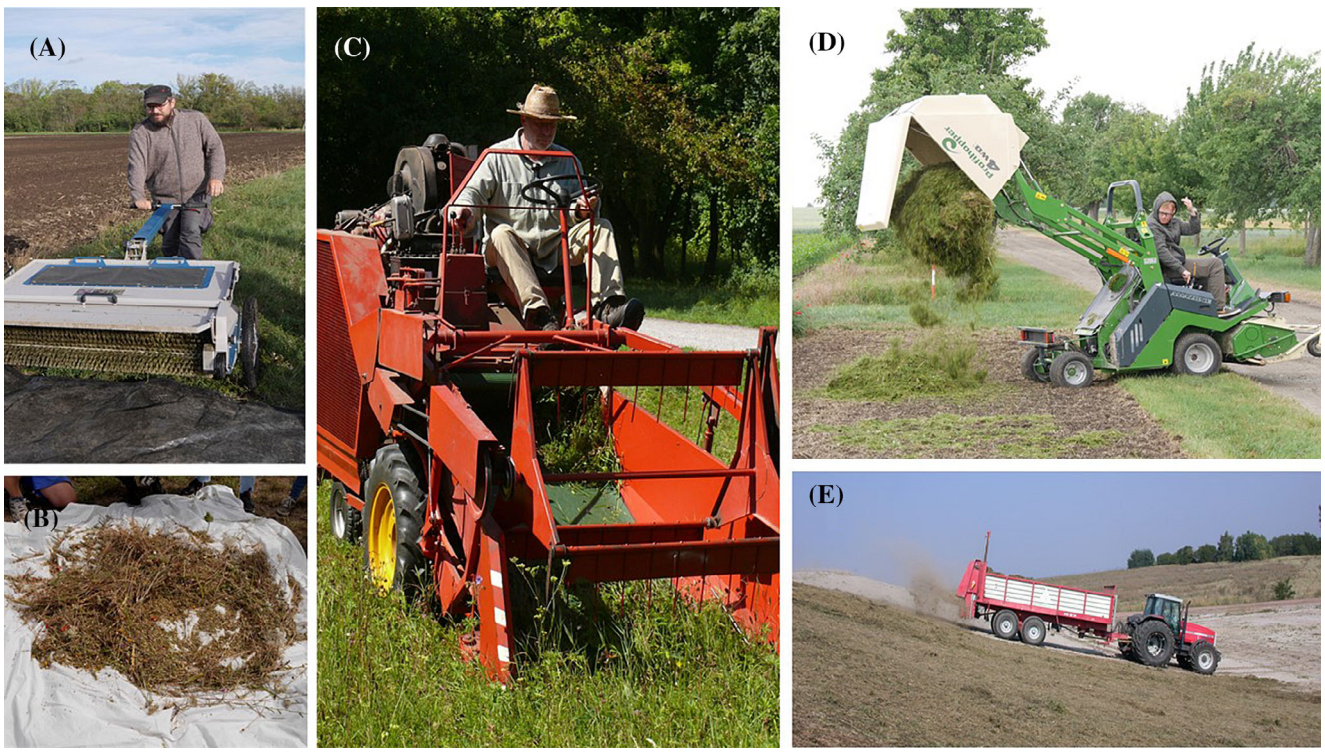

Figure 5 Seed-rich green hay harvested at a suitable donor site in Germany using equipment such as (A, B) a brush-type harvester that collects mature seeds and minimal vegetative material or (C) a plot harvester that produces threshed material. The seed-rich material should be transferred immediately to the receptor site and spread using equipment such as (D) a mower with a bucket spreader or (E) a manure spreader to restore species-rich near native grasslands and field margins. Photos: (A-D) A. Kirmer, (E) S. Mann.

fixed with an organic adhesive) can accelerate germination, protect seedlings from drying and frost, and provide protection against erosion caused by rain (Lee et al. 2018). It is important to spread the mulch evenly, prevent seed damage during application, ensure good seed-to-soil contact, and use appropriate fertilizer and mulch rates to minimize germination inhibition (Armstrong et al. 2017).

Mulching to Enhance Germination

Low or erratic moisture availability can preclude restoration success, especially in arid or seasonally arid landscapes. Organic mulch (e.g. hay, bark) is used in low-precipitation areas to enhance germination and emergence by retaining moisture and moderating the soil surface temperature (Ji & Unger 2001; Kader et al. 2019). Mulch depth and stability, light requirements for germination of seeded and weedy species, and species' responses to potential changes in soil C:N resulting from organic amendments are considerations when selecting mulch type and application depth (Fehmi & Kong 2012; Kirmer et al. 2012). Organic mulches may also reduce moisture availability by wicking moisture from the soil or intercepting moisture from light rains (Jalota & Prihar 1998).

Overseeding, Interseeding, and Gap Seeding

Overseeding, interseeding, and gap seeding involve the addition of seeds to enhance an existing natural community or seeding. These operations can be conducted to increase species diversity, alter community structure across a site, or restore depleted or weed-infested areas (Rayburn & Laca 2013; Silva et al. 2019). Diversification of species-poor native vegetation or seedings is often hampered by microsite limitations (Münzbergová & Herben 2005); disturbance to reduce competition or improve microsite availability is often necessary before species introduction occurs (Schmiede et al. 2012; Baasch et al. 2016; Kiss et al. 2020).

Post-Seeding Monitoring and Management

Short- and long-term monitoring results provide a basis for assessing seeding success and informing future seeding plans. While monitoring can be time and resource intensive, post-seeding monitoring during the first few growing seasons of plant establishment and community development is critical (Scotton et al. 2012*a*). Sequential monitoring during this period can provide management information on: (1) problems encountered (e.g. weeds, herbivory) that require active management interventions; (2) a need for reseeding particular species or areas; (3) the percent of sown seed to emerge and establish; and (4) factors to consider in future seeding plans.

Protocols for monitoring and statistical analyses are available in many ecology books and regional manuals (e.g. Scotton et al. 2012*a*; U.S. Fish and Wildlife Service 2013). For seeding programs, measurements of species composition and abundance, and indices of relevant biodiversity measures (i.e. richness, evenness, etc.) are often used to describe restoration outcomes (Fig. 3H). Additional biodiversity metrics, such as species diversity, reproductive maturity, and functional diversity, can help to determine ecosystem function and trajectory over the long term. Collaboration to include expertise on various taxonomic groups and aspects of community ecology (e.g. wildlife responses to the seeded plant community) can enhance monitoring program effectiveness and interpretation of results.

Communicating Results

Sharing monitoring data, and experiences gained with partners and stakeholders during meetings and field tours, provides an opportunity to acquire and share knowledge and increase support. Reporting results at conferences, through publicly available reports and publications, and via web reporting (such as providing the information for uploading to the International Network for Seed-based Restoration website) increases the availability of restoration outcomes to others working in similar plant communities or with similar seed-based restoration challenges and goals.

Conclusions

Improving the success of seed-based restoration requires effective site preparation, a sound understanding of plant and seed biology, and the use of site- and species-specific seed delivery methods. Today's seeding systems have evolved through innovative modifications of equipment and practices used in agriculture and forestry and novel approaches to meet the challenges of restoring specific disturbance types and improving diversity in highly varied ecosystems. Continued innovation and adaptation of equipment and technologies, extensive research on all aspects of site preparation and seed delivery, and inclusion of practitioner's knowledge are required to optimize existing systems for use with local resources. Careful short- and long-term monitoring is essential to identify and resolve problems, record successes, and contribute data to improve restoration approaches. Sharing outcomes, both positive and negative, is important for improving successful delivery of native seeds and for achieving ecological restoration targets.

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PRACTICAL ARTICLE

International principles and standards for native seeds in ecological restoration

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The growing demand for native seeds in ecological restoration and rehabilitation, whether for mining, forest, or ecosystem restoration, has resulted in a major global industry in the sourcing, supply, and sale of native seeds. However, there are no international guidance documents for ensuring that native seeds have the same standards of quality assurance that are regular practice in the crop and horticultural industries. Using the International Principles and Standards for the Practice of Ecological Restoration as a foundation document, we provide for the first time a synthesis of general practices in the native seed supply chain to derive the Principles and Standards for Native Seeds in Ecological Restoration ("Standards"). These practices and the underpinning science provide the basis for developing quality measures and guidance statements that are adaptable at the local, biome, or national scale. Importantly, these Standards define what is considered native seed in ecological restoration and highlight the differences between native seeds versus seeds of improved genetics. Seed testing approaches are provided within a logical framework that outline the many different dormancy states in native seed that can confound restoration outcomes. A "pro-forma" template for a production label is included as a practical tool that can be customized for local needs and to standardize reporting to end-users on the level of seed quality and germinability to be expected in a native seed batch. These Standards are not intended to be mandatory; however, the guidance statements provide the foundation upon which regulatory approaches can be developed by constituencies and jurisdictions.

Key words: native seed supply chain, pure live seed, seed enhancement, seed provenance, seed quality, seed storage

Introduction

Seed is an underpinning and often limited resource in restoration programs worldwide. The second edition of the International Principles and Standards for the Practice of Ecological Restoration (Gann et al. 2019) highlights how seed is the foundation of many restoration programs. Yet globally there are few countries where there are quality controls on the seed supply chain that guarantee a minimum quality standard (Vogel 2002; Mainz & Wieden 2019). Thus a logical step in building capacity to deliver large-scale, effective, and predictable ecological restoration is formulating a methodological framework for seed quality assurance in the same way that commercial crop seed is assured with internationally accepted rules and testing methodologies (International Seed Testing Association [ISTA] 2019). This is now more critical than ever with the U.N. Decade of Ecosystem Restoration (2021–2030) aiming to restore 350 million hectares globally, which will lead to unprecedented demands for reliable and sustainable supplies of native seed. Thus for suppliers, end-users, practitioners, funders of restoration, and regulatory agencies, having confidence in seed quality is fundamental to achieving local to global restoration success.

For most countries with native seed enterprises or large-scale restoration programs, seed is traded with little consideration of seed quality and viability (Ryan et al. 2008). This has resulted in poor quality and even dead seed entering the seed supply trade. For example, when germination of native seed lots was tested on eight species from different suppliers across Europe, high variability among suppliers was detected, with some batches containing no viable seed (Marin et al. 2017). With such scenarios, if quality is not guaranteed, this ultimately will erode the confidence of buyers and restoration practitioners in the efficacy of native seeds. Such outcomes could seriously undermine the credibility of native seed producers and suppliers, reducing the quantity and diversity of native seed available. This will have consequences in limiting the effectiveness of restoration programs and the aspirational goal of full ecosystem recovery outlined in the International Principles and Standards for the Practice of Ecological Restoration (Gann et al. 2019).

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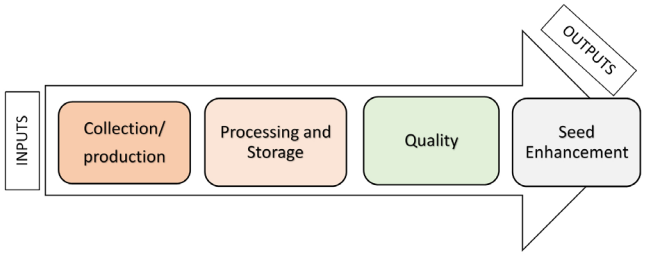

Figure 1. Key elements of the seed supply chain. Each step is outlined in the Standards sections that follow. Seed collection/production: refers to wild and cultivated sources of seed. Processing and storage: optimal treatments and storage conditions to achieve and retain the highest level of seed quality. Quality: is a means to derive a measure of pure live, germinable, and dormant seed. Seed enhancement: all treatments applied to the seed that break dormancy and increase germination (after-ripening, stratification, scarification, chemical agents, and priming) and promote establishment success (including seed coating).

> "This Standard aims to strike a balance between what are reasonable quality expectations and guarantees for the seed user and what is practically achievable and economically viable for seed suppliers."

A key tenet for these, the first International Standards for Native Seed in Ecological Restoration ("Standards"), is to provide the underlying principles (with attendant guidance statements) for stages in the supply of seed from source to restoration site (Fig. 1)—the *seed supply chain*. The seed supply chain provides the logical framework for the key steps with the Standards maximizing outputs and outcomes for production and supply of quality seed (Cross et al. 2020). However, the seed supply chain is only as robust as the weakest link. Ensuring all stages are managed to optimize seed quality in the chain is a key aspect of these Standards where seed suppliers aim for the highest and best seed quality outcomes. Seed quality encompass all the intrinsic attributes of a seed batch that can be tested and given a numeric value, such as purity, viability, germinability, and, if applicable, dormancy state (Frischie et al. 2020).

Who Do These Standards Apply to?

These Standards apply to suppliers/producers, merchants, native seed banks, regulators both government and industry-based, as well as end-users of native seed. It provides commercial confidence in the purchase of native seed while setting appropriate methodological standards for seed testing. These Standards will develop for native seed what has occurred in commercial crop and horticultural seed enterprises, where the "International Rules for Seed Testing" developed by ISTA and the "Rules for Testing Seeds" by AOSA (Association of Official Seed Analysts in the United States) created a commonly agreed and shared principles and methodology platform for seed testing. Consumers were confident that standards and regulation based on the ISTA/AOSA Rules provided a guarantee of quality that then contributed to establishing a sound business footing for development of a global market in seed trade for crops and horticulture. In a similar vein, we envision the Standards as enabling the development of a robust and sustainable native seed industry based on the guiding principles of the International Restoration Standards (Gann et al. 2019). These seed Standards are specifically for wild, nonselected species that are component species of the reference site or community of the restoration program. Cultivars (enhanced genetic materials) of wild species are not covered by the Standards (see next section).

> "This Standards are designed to be accessible and practical for all those involved in the collection, production and use of native seeds, and are relevant at all levels, from indigenous community programs to large-scale commercial native seed enterprises."

What Constitutes a Native Seed for Ecological Restoration Purposes

A seed batch is appropriate for restoration purposes when its genetic diversity, representative of the population of origin, is preserved, as far as practical, throughout the supply chain and deployed on a restoration site of suitable ecological conditions (Erickson & Halford 2020).

Some native seed producers have developed breeding programs from native species and actively select for traits to improve seed farming efficiency, reduce seed cost, improve vigor, and ultimately select varieties that bear little resemblance to the genetic makeup of the original wild population. In some cases a variety was developed starting from a single plant (Native Seed Quality Task Force 2011). Where such varieties are produced, seed standards should follow the ISTA/AOSA Rules and relevant local regulations. Although there are uses for these types of materials in revegetation and rehabilitation programs they are not generally acceptable within the framework of what is considered ecological restoration (Gann et al. 2019).

Though these Standards are applicable to both conservation and restoration programs, due to the small sample size of seed in conservation collections, sampling protocols for conservation species should follow appropriate recommendations in national and international guidelines such as the European Native Seed Conservation Network (ENSCONET 12009*a*, 12009*b*), the Australia FloraBank Guidelines (FloraBank 1999), and the US Seeds of Success program (Bureau of Land Management 2018).

Crop and Native Seed Standards—Are They Different?

There are fundamental points of difference between crop species and wild species. For example, the ISTA/AOSA Rules are designed to provide the testing procedures for cultivated (non-wild sourced) seed of agricultural, forestry, horticultural, and miscellaneous commercial species (flowers, spices, herbs, and medicinal plants). Most species covered by the ISTA/AOSA seed rules are the result of long periods of plant breeding and selection where seed characteristics such as dormancy and seed fill are substantially altered for agronomic reliability and economic reasons. For example, major crop species have low to nil seed dormancy and high degrees of genetic stability to ensure that season-to-

season genetic variation is minimized or eliminated. Agricultural seed supply chains are designed to maintain the genetic purity of specific varieties, by avoiding cross pollination with other cultivars or wild forms and ensuring that seed of different lines are not mixed. This information follows the seed batch through the supply chain to ensure genetic conformity and varietal fidelity (especially for varieties of the same species with no clearly identifiable seed morphological differences).

On the other hand, native seed represents a broad array of genetic diversity indicative of parental diversity and local adaptation in the wild populations. Such traits are important when dealing with climatic gradients and climate change. Genetic traits adapted to local restoration conditions mean there is appropriate genetic heterogeneity that often reflects high levels of phenotypic variation. Genetic monocultures are rare in nature and therefore are rarely valid in ecological restoration programs. Thus, the Standards address the need to incorporate in seed batches the variability inherent in wild populations, with such variability not easily accommodated by existing benchmarks such as the ISTA/AOSA Rules. To guarantee genetic diversity representative of a specific ecotype is correctly represented, information on seed collection from the wild (such as locations, time of collection, collector) should follow the seed batch through the supply chain to the end-user. When native seeds are multiplied through cultivation, multiplication should be performed for a limited number of generations, usually less than five, to avoid the selection of certain traits and consequent reduction of genetic variability (Erickson et al. 2020; Pedrini et al. 12020*b*).

These differences between crop and native seeds, in most cases, would make the application of traditional agricultural regulations and testing methods (ISTA/AOSA) to native seeds unfeasible (see Seed Quality section). While the ISTA Rules are designed to provide uniform seed quality assessment to facilitate the international seed trade, these native seed Standards reflect the local and nuanced nature of wild seed that is usually limited to regional or national seed supply networks with only occasional trans-national or trans-border trade in native seed.

Finally, seed dormancy is a key attribute in native seed that has been removed from seed of most crop species. Dormancy refers to the morphological and physiological status of seed that controls the expression of germination. Suppliers of native seed are encouraged to define the dormancy condition and a dormancy breaking treatment in a seed batch. Such dormancy breaking treatments can be applied by the supplier or recommend to end-users as a necessary step for ensuring successful deployment of germination-capable seed.

Why Labeling of Native Seed Is Important

Labels are the principal means for communicating seed information between seed suppliers and end-users. Labels designed for seed varieties or crops are not suitable for native species as defined in these native seed Standards and have either irrelevant fields or lack fields necessary for ensuring quality in native species destined for ecological restoration programs. The pro-forma provided is structured to reflect an "ideal" and comprehensive label for native seed batches (Fig. 2). Component parts of the pro-forma are outlined in this section, addressing how species, origin, collection, and production should be reported; how quality should be determined; and how information on seed dormancy state, seed enhancement, and seed storage, should be conveyed. The pro-forma is designed to encompass all possible aspects of native seeds. If such a template is adopted by native seed companies or local native seed associations, sections that are not relevant to the kind of product/species (e.g. production, dormancy, enhancement) can be left blank, removed, or customized with site/region/species-specific sections added.

This pro-forma is designed for a seed batch comprising a single species. The label for mixes, where seeds are collected/cultivated separately and then mixed prior to sale, seed source location, batch quality, and treatments should be reported for each species in the mix, along with the weight percentage of each species present in the mix. In cases of grassland restoration, where seed lots are directly harvested as a mix of different species using techniques such as seed stripping, vacuum, and green/dry-hay, the label should report the origin, the list of species present (based on the vegetation survey prior to harvest, seed visual assessment or germination), and, if feasible, an estimated weight percentage of each species. When dealing with such material, refer to the "Practical Handbook for Seed Harvest and Ecological Restoration of Species-Rich Grassland" (Scotton et al. 2012).

Is Certification Necessary for Native Seed?

As the global native seed industry grows to meet restoration demands, certification with appropriate standards that are nationally recognized could be considered. Certification schemes are designed to ensure that processes and products conform to standards and regulations outlined by an association of producers (industry), regulatory bodies (governments), or both. For example in Germany, in response to a European directive (2010/60/UE) that regulates the commercialization of native seeds of grassland species, two certification systems, VWW-Regiosaaten and RegioZert (Mainz & Wieden 2019), were developed by local associations of native seed producers. However, if regulations do not address the complexities and nuances of the native seed supply chain, the development of effective certification schemes and well-structured native seed markets will be hampered. For example, the aforementioned European directive (2010/60/UE) provides derogations to pre-existing legislation that regulates the market for fodder species and, as such, treats native seeds similarly to cultivars and genetically improved varieties, thus limiting its effective applicability to the native seed supply chains (Tischew et al. 2011).

The principles and standards outlined in this document provide the foundation for the next logical step toward developing certification of native seed suppliers and native seed testing laboratories. Such certification approaches may be considered in future editions of these Standards.

Seed Origin, Collection, and Cultivation

A guiding principle in ecological restoration is the use of an appropriate native reference site or ecosystem (see Gann et al.

Figure 2. Pro-formas that can be used as indicated or modified depending on local conditions, for labeling of native seed batches prior to sale. This template is divided in three sections based on the seed supply chain key steps: Seed sourcing/provenance, seed quality testing, and seed enhancement.

2019 for detailed guidance on selecting a native reference). Therefore the genetic composition of a species in the reference is reflected in the restored site to ensure, as far as is practicable, matching of the genetic resource. Seed collection from the wild, or from managed seed production areas (SPAs), should therefore indicate the origin and collection site details. Other factors should be taken into account during seed collection from the wild in order to effectively represent the genetic diversity of

the donor population without harming its reproductive capability (see Pedrini et al. 12020*b*).

As detailed in the Seed Procurement and Planning paper of this special issue (Erickson et al. 2020) and Appendix 1 of Gann et al. (2019) provenance is difficult to predetermine in the absence of detailed genetic, phenotypic, or common or garden studies to guide collection sites of genetically appropriate seed.

Eco-regional approaches that define areas encompassing similar geology, climate, soils, hydrology, and vegetation or other geographic descriptors can guide seed collection and transfer zones. When such information is combined with species-specific ecological and genetic information and local knowledge it is possible to approximate a seed zone as has been done in the United States (Bower et al. 2014) and some European countries (De Vitis et al. 2017) (Fig. 3).

Seed collectors should refer to local or national guidelines for guidance or seek expert opinion as to what constitutes a seed zone/seed transfer zone or provenance relevant to the restoration site in question before undertaking a seed collection program.

Five key classes of seed source type are provided in Gann et al. (2019) and end-users should use this to guide their outplanting requirements. The sourcing classes are: strict local provenancing, relaxed local provenancing, composite provenancing, admixture provenancing, and predictive provenancing.

Guidance Statement 1

1.1 Seed is accompanied by a taxonomically valid species name, according to nationally recognized botanical nomenclatural standards.

1.2 Specify if the seed lot is wild harvested or has been multiplied through cultivation.

1.3 Provide information about the wild seed source, such as: georeferenced location, date of collection, and collector, with this information retained and tracked through the seed supply chain, and provided to the end-user with the seed lot.

1.4 For collections of new species or material of uncertain taxonomic status, herbarium vouchers must be taken with the accession number of the seed collection synonymous or matched to the herbarium voucher number.

Seed Collection From Natural Populations

The global demands for restoration mean that for the present, most seed is sourced from wild stands. In some cases, particularly in the global biodiversity hotspots where less than 30% of the natural vegetation remains, there are even stronger demands for seed for restoration which can result in considerable pressure on the few precious natural ecosystems that remain. Ethical sourcing of wild harvested seed (Nevill et al. 2018) and care with the postharvest handling and management of seed is critical to retain as much viable seed as possible so as to make every seed count in the restoration program.

Guidance Statement 2

2.1 To protect the viability of wild donor populations, no more than 20% of the seed produced in one season should be collected. For annual species, this may be as low as 10%.

2.2 To adequately represent the genetic diversity of the population, seed should be randomly selected from multiple

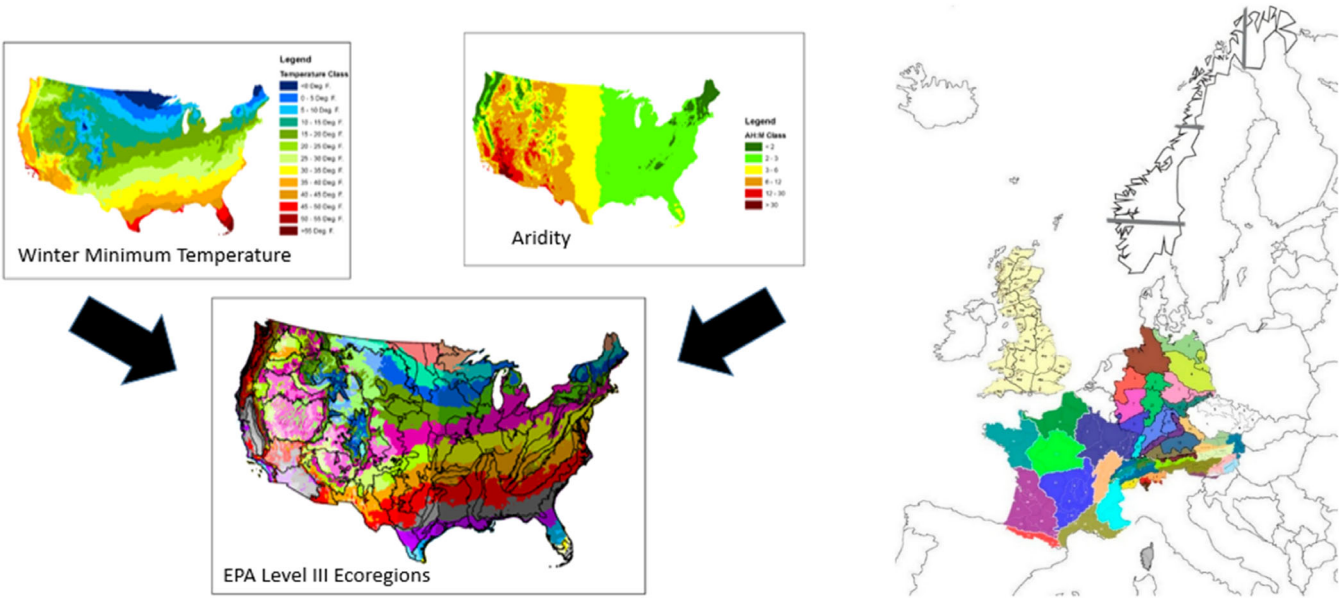

Figure 3. Seed zones in the United States and Europe: Left: U.S. provisional seed zones for native plants (color polygons) are unique, climatically delineated (temperature-aridity) areas nested within EPA Level III Ecoregion boundaries (black lines). The provisional zones can be used to guide seed sourcing decisions when species-specific genetic information is lacking (Bower et al. 2014). Right: National seed zones developed for European countries (De Vitis et al. 2017). Permission provided.

individuals. For large continuous stands, a more systematized approach such as regular sampling along a transect is more appropriate.

2.3 To ensure good seed that is mature and ready for harvest, a small sample is taken and a visual assessment of seed maturity/fill is performed prior to commencing seed collection.

Managed Seed Production

Seed Production Areas (SPAs) include managed wild stands and cultivated fields of native species. Seed produced from SPA require considerations that may be different to those from wild sourced seed such as evidence that genetic fidelity has been retained and there is no induced hybridity or genetic drift through the seed production process.

Guidance Statement 3

3.1 The seed batch from SPAs includes information on:

(a) The number of generations from the original wild seed collection. The number of generations should not exceed *five* before restocking using original, wild sourced genotypes.
(b) The location of cultivation, name of the company/person responsible for the cultivation, and date of harvest is specified.

3.2 Prevent potential hybridization with wild types growing naturally in the region of the SPA by ensuring wild species are beyond the pollination drift to the SPA.

3.3 Prevent interspecific hybridization when related species are in cultivation and ensure that provenance lineages are sustained free of interbreeding.

Note: It is important that seed produced from a SPA has storage, dormancy, and germinability characteristics that are understood as these may vary from those for wild sourced seed.

Seed Processing and Storage

Correct management of seed after wild or field harvest is crucial to ensure that seed quality is maintained to a high standard. The collected material should be visually assessed to ensure that seed are mature, healthy, non-predated, and free from bacterial or fungal infection. For species with sporadic seed production and asynchronous maturation (e.g. tropical forest species), diaspores can be collected early and maintained at appropriate condition to allow for postmaturation. Seed should be transported in a dry and ventilated state to the processing/storage facility.

Seed processing is recommended if the collected/harvested batch contains impurities and inert materials, nonseed material, and seeds of other species. A wide range of seed processing methods and techniques are described in the seed processing and quality essay of this special issue (Frischie et al. 2020). Emerging technologies that show promise for improved seed processing, handling, and germination include flash flaming and acid digestion (Stevens et al. 2015; Guzzomi et al. 2016; Pedrini et al. 2019). Processing should be performed to maximize the batch purity, without degrading seed integrity and viability. Seeds should then be stored at appropriate environmental conditions that maximize seed longevity.

Relative humidity, usually recorded as relative humidity percentage (RH%), is correlated to the seed moisture content. High seed moisture content accelerates the seed aging process and may provide the ideal conditions for fungal contamination leading to seed losses. 15% RH level is generally considered safe for seed storage, and should be adopted for storage of orthodox seeds. Where there is limited knowledge of storage conditions, empirical testing of seed tolerances to 15% RH should be undertaken before commencing long-term storage.

The optimal temperature to ensure seed viability for medium-term storage is 15°C. For long-term storage, seed should (after appropriate drying) be stored at −18°C.

Note: For recalcitrant species, whose seed cannot be dried, medium- or long-term storage is not feasible and seeds should be used shortly after harvest (depending on species this may be weeks to months).

Guidance Statement 4

4.1 Seed management after collection or harvest requires that the seed batch is dried (desiccation sensitive seed will require only moderate drying noting that such seed can be easily killed by drying) as soon as is practicable after collection and is transported dry, cool, and, if necessary, ventilated to prevent condensation, moisture build-up, and molding while being delivered to the seed processing and storage facility.

4.2 Seed processing: If the seed batch contains nonseed material (leaves, flowers, branches, soil, rocks, empty/predated seeds) or seeds of different species, the batch needs to be processed to the highest practicable degree to ensure high seed purity. Seed processing methods and techniques are described in Frischie et al. (2020).

4.3 Seed must be equilibrated to 15% RH and 15°C until seed achieves a moisture content of between 5 and 10%. Seed moisture content is determined using the methods described in De Vitis et al. (2020)

4.4 Once at the desired moisture content, seed can be stored under the same conditions, or stored in air-tight containers at the appropriate storage temperature.

4.5 The relative humidity RH% and temperature of facilities where the seed batch is stored should be monitored and reported on the seed supply pro-forma (Fig. 4)

Seed Quality

The objective of the seed quality assessment is to obtain information concerning the purity, viability, germinability, and, if present, dormancy of a native seed batch. The results of these tests:

- Provide important feedback to the seed supplier (collector–producers) regarding collection and cultivation methods and strategies.

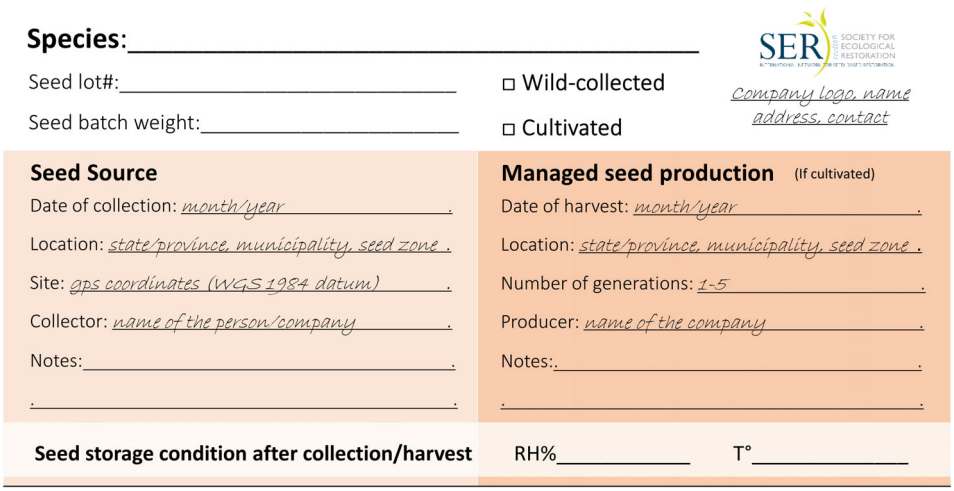

Figure 4. Details of the pro-forma relevant to reporting on seed source, seed cultivation, and seed storage.

- Determine the value of the seed batch as a restoration product.
- Inform the seed user of expected seed performance outcomes.
- Provide the seed user with assurance of the quantum of germinable/viable seed purchased.

Lack of such information may lead the end-user to assume that all the seeds in the batch are viable and readily germinable and therefore overstate the expected restoration outcome.

Seed testing procedures developed for the quality assessment of agricultural varieties are often adaptable for the testing of native seeds. However, due to the potential high variability within a seed batch, and the high diversity of native seed morphology, physiology, desiccation tolerance, and dormancy type, the approaches described in the ISTA International Rules for Seed Testing (ISTA 2019) and AOSA (AOSA 2019) need to be adapted and customized on a species-by-species basis. This will require the development of a seed quality testing protocol for a species or group of species that share similar attributes. However, wild species, in contrast to crop and horticultural varieties, may vary in dormancy state, seed mass, purity, and quality across seasons and across geographic, topographic, and edaphic ranges.

The crop seed testing standards provide species/variety specific thresholds of minimum seed quality and tolerance levels that need to be achieved for a seed batch to be considered acceptable for sale. However, for native seed, quality of different batches of the same species could vary greatly as genetic and environmental variables for wild sourced seed are out of the control of the seed supplier. It is therefore not reasonable to set minimum standard quality requirements; nonetheless quality testing should be performed and results communicated to the seed user.

To guarantee the impartiality of seed testing results, seed quality tests should be performed by independent certified seed testing laboratories. If not available, the seed quality assessment could be performed by the seed supplier with this stated on the supplied seed label (Fig. 6). To create trust in the process of self-testing native seed batches, a licensing system for seed suppliers could be implemented if required.

The following section outlines common seed quality testing methods and a framework for native seed quality testing. The key procedures and analysis required to perform a comprehensive seed quality test are illustrated in Figure 5 and described in the following section.

Guidance Statement 5*

5.1 The sample should be representative of the entire seed batch.

5.2 If the seed batch received seed enhancement treatment (e.g. coating, priming), a seed quality test should be performed on the treated seeds.

5.3 A purity test is undertaken and the percentage of pure seed units (PSUs), inert material, and contaminating (nontarget species) seeds present in the lot are indicated as a weight percentage.

5.4 A viability test is undertaken (where possible) to determine the percentage of PSU that are viable (VSU) and the method used to determine viability indicated.

5.5 A germinability test is undertaken (where possible) that provides the average percentage of PSU that are readily germinable (germinable seed units [GSUs]).

5.6 The difference between the viable seed unit VSU and GSU represents the dormant seed unit (DSU). DSU is the percentage of seed that are viable, but not able to germinate due to dormancy. If dormancy is detected, type of dormancy should be indicated (based on literature or expert opinion) and, where possible, a proven dormancy breaking approach is provided.

5.7 Pure live seed (PLS), pure germinable seeds (PGS), and PDS provide information on the percentage by weight of the seed lot that can be considered viable, germinable, and dormant. PLS, PGS, and PDS can be calculated from the VSU, GSU, and DSU respectively, multiplied by the PSU.

5.8 PDS is the percentage of seed in the batch that are viable, but not able to germinate readily due to dormancy.

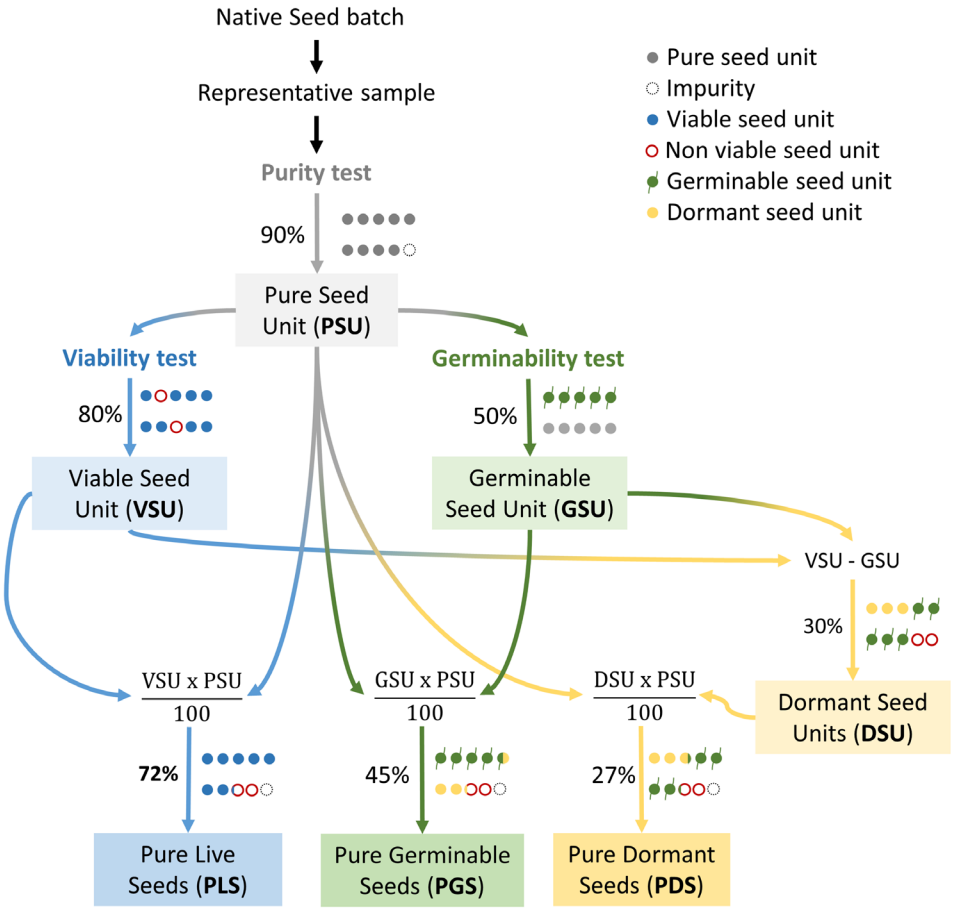

Figure 5. Native seed quality tests and outcomes used to derive key indicators of seed quality—PSU, GSU, DSU, PLS, PGS, and PDS. Between each test, a model seed batch is diagrammatically represented with values indicative of weight percentages. This is one of the approaches used to determine pure live/germinable/dormant seeds. Different approaches are illustrated in the "Alternative Viability Tests Determined by Germinability" section.

* To be read in conjunction with the seed quality flow diagram in Figure 5.

Note: Strict adherence to local phytosanitary guidelines is necessary to avoid the spread of potential pests and diseases including avoiding weed seeds in supplied seed.

Sampling

Seed testing for purity, viability, and germinability analysis requires that an appropriate subsample is taken from a seed batch. Sampling for quality testing is relevant to a seed batch after collection/harvest and prior to field deployment if the seed has been stored for extended periods. Resampling is often required for native species and reflects the uncertainty around storage conditions for many wild species and potential for loss of viability through the storage cycle.

Guidance Statement 6

6.1 Representative samples must be taken from homogenized parts of the seed batch noting that settling may occur during transport and processing.

6.2 For free-flowing seed without confounding chaffy materials, sampling should be taken from representative parts of the storage container. Sampling devices such as Trier devices (that come in a number of forms including single and double sleeved) can be used with large (>5 kg) batches where the diameter of sample holes is 2–3 times that of the largest seed (includes nontarget seed and nonseed residues). *Note*: for small seeds discrete sampling using spatulas taken from spread-out samples may be necessary

6.3 Ensure samples are representative and if visibly non-uniform then resample to ensure uniformity is achieved.

6.4 For seed that has confounding appendages (including chaffy seed) or are large seeded, hand sampling or use of cupped devices may be necessary. Visual inspection is necessary to ensure subsample conformity is achieved.

6.5 For sample containers up to 20 kg, each sample container should be sampled following the AOSA Rules for Testing Seeds or ISTA Sampling Intensity guidelines. *All containers up to six should be sampled randomly from each container. When the number of containers is greater than six, for example, 7–14 containers, six samples should be collected; seven samples from 15 to 24 containers; eight*

samples from 25 to 34 containers …. 15 samples from 95 to 104 containers.

6.6 Samples shall consist of 400, preferably 800, PSUs (see purity section) per sample referred to as the "primary sample." The primary sample is submitted for testing and a sub-set known as the "working sample" undergoes purity, viability, and germinability testing. Seed that has been treated with external coating materials should consist of 1,000–2,000 coated units for germination testing.

6.7 If samples are forwarded to a seed testing facility they must be appropriately sealed to prevent moisture ingress and protected from crushing during transport.

Preparation of Working Samples for Seed Testing. A subsample of the primary seed sample known as a working sample is the seed that is subjected to seed purity testing (see Purity section). This subsample must be taken in such a way as to ensure representativeness. When primary samples are large, mechanical subsamplers or dividers (that include riffle, conical, Boerner divider, and centrifugal devices) are used to ensure an unbiased, representative sample is used (see AOSA Rules section 2 for details of device specifications). Hand-halving where a sample is spread evenly onto a flat surface and leveled then divided in half can also be used if mechanical dividers are not available. Hand mixing and spoon sampling can be applied to small seeds or for seed batches that are small in size.

Recommended Number of PSUs for Testing

Purity Seed Number

Sufficient material is selected to determine the weight of starting material to derive 100–500 PSU. The number of seed units per gram is species dependent and based on size, ease of sorting, degree of purity. Repeat at least three times from additional working samples.

Viability Seed Number

From the purity test, subsample 100 PSU for viability testing.

Germinability Seed Number

From the purity test, four replicates of 25 seeds each are subjected to germination testing usually on agar, moist sand, or germination paper. Very large seed will use less seed such as for many desiccation sensitive species.

1,000 Seed Weight

This value is used in a number of databases and is determined by taking four samples of 50 PSU and then calculating the expected weight of 1,000 seeds.

Note: The Millennium Seed Database (https://data.kew.org/sid/) has the 1,000 seed weights for a wide number of species from many countries. This is a useful guide for understanding seed size for some wild species.

Purity

The purity test is performed on a representative working sample (see Sampling section) to estimate the weight percentage of the batch that is to be considered pure seed. High seed purity can be achieved through careful and informed seed collection or seed farming practices and correct application of seed processing and cleaning techniques. The purity test is performed by separating the sample in the three fractions: PSU, seeds of other species and inert matter.

Pure Seed Units. What is regarded as a PSU varies from species to species. The ISTA Rules provide a list of 63 different seed unit types for almost 450 genera. For example nine different type of achenes, five types of pods, and eight types of spikelets are described. Although the genus of some native species might not be listed, it could be categorized into one of the seed unit types described by ISTA. If none of the available definitions are applicable, a new seed unit type will need to be described.

> "Unlike the ISTA and AOSA guidelines for Pure Seed Units, here we categorize underdeveloped, germinated, infected, abnormal, undersize, or damaged seed incapable of normal germination as inert material."

Guidance Statement 7

7.1 Seeds of the target species that upon visual assessment during the fraction separation appear to be healthy and potentially viable should be considered PSU.

7.2 Seed of other species in the seed batch (other natives, weeds) need to be accounted for and reported. If possible, those seeds need to be evaluated to determine if a potential invasive species is present in the mix. Other species detected should be reported in the *notes* field in the purity section of the pro-forma (Fig. 6)

7.3 Inert matter is accounted for and represents all the components that are not considered seeds or essential to the germination of the target seed such as empty seed units, broken, damaged, underdeveloped, and abnormal seeds, leaf and stem fragments, soil, branches, and any other impurities.

Purity Testing Methods

The results of the purity test are important for the seed collector/producers and provide valuable feedback on the collection and farming methods along with indications for improvement of the seed processing and cleaning phase.

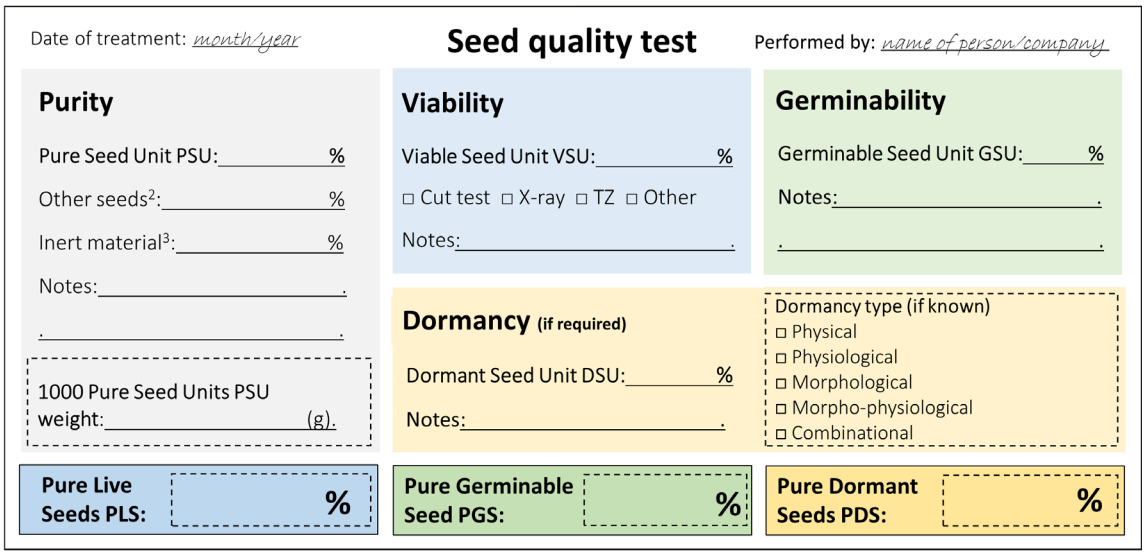

Figure 6. Component of the pro-forma with information regarding the seed quality test. The boxes are completed as required depending upon local circumstances and local technical capability. If a species does not have dormancy, the dormancy section remains blank.

Guidance Statement 8

8.1 Divide the seed sample into three equal fractions either by hand separation, sieves (for filtering material according to size), or use of an air jet (which separates fractions of different density). A dissecting microscope can help with sorting the sample for small or dust-like seeds.

8.2 Assess if seed units are filled or empty by applying pressure to the seed unit using forceps (or for larger seeds between paper or squeezing with fingernails). A diaphanoscope that provides sub-stage illumination or X-rays (see section below) are helpful for determining filled and empty seed units.

8.3 Each fraction must be weighed and presented as a percentage of the total (sum of the three fractions). If seeds of potentially invasive exotic species are detected in the "other seeds fraction" it must be reported.

> "Although useful, the results of the purity test alone do not provide information on the viability/germinability of the Pure Seed Units, and should not be used as a predictor of seed germination outcomes."

Seed Weight Determination

Once PSUs are obtained from the seed batch, the weight of a fixed quantity of seed units (usually a thousand, and known as "thousand seeds weight"; TSW) can be determined. This information is relevant for end-users when composing seed mixes and calibrating seeding rates (Shaw et al. 2020).

Guidance Statement 9

9.1 The TSW of seeds for many native species is available in the "SID" seed database developed by the Millennium Seed Bank (https://data.kew.org/sid/). If information for a species is not available, the TSW can be estimated by recording the weight of four replicates ($R_{1\text{-}4}$) of 50 PSU and applying the following equation:

$$\text{Thousand seeds weight (TSW)} = \left(\frac{R_1 + R_2 + R_3 + R_4}{4}\right) \times 20$$

Viability

Viability tests are performed to determine the percentage of seeds in the batch that are alive and could potentially germinate. Standard methods have been used to estimate seed viability; however, due to the complexity and diversity of native seeds, some methods require careful evaluation and calibration before being reliable for determination of viability.

The most common methods for assessing viability are the cut test, X-ray, and the tetrazolium test. Germinability tests can also be used as a surrogate test for viability; however, it should be combined with a viability test to address if nongerminated seeds are in fact viable, but dormant, or indeed nonviable.

Viable Seed Unit VSU. Viability tests are performed on PSUs obtained from the purity test, and are designed to estimate the percentage of viable seeds units (VSU) present in a pure seed sample. The percentage of VSU can be considered relative to the weight of the sample, assuming that the weight of a viable and nonviable seed unit is equal. This assumption depends upon what is considered a PSU for native seeds (see section on Seed Purity).

Cut Test. This method is a simple and effective way to estimate viability. The seed unit is bisected with a scalpel blade, knife, or other sharp instrument and the internal contents of the seed visually examined. Viable seeds have white and turgid endosperm (no observable shrinkage) with an embryo that exhibits no observable discoloration or shrinkage. If seed internals are missing or appear shriveled, diseased, infected, detached, or abnormal, the seed could be considered nonviable. This test requires a good knowledge of seed morphology and experience in testing the species. Calibration of the technique can be performed by combining with other viability tests or a germinability test. A limitation of this test is that it can overestimate viability for seeds that appear healthy but have lost the ability to germinate (i.e. dead seed).

Guidance Statement 10

10.1 A minimum of 100 seeds, randomly selected from the PSU, should be used to determine viability using the cut test. Seed should be held securely and bisected longitudinally using a sharp blade such as a scalpel. The halves are then inspected (a dissecting microscope is helpful particularly for embryo inspection) for evidence of discoloration or shrinkage in the endosperm or embryo that indicates a nonviable seed. Report Viable Seed Unit (VSU)—Cut Test as a percentage of the PSU.

X-Ray

Evaluation of the X-ray image allows determination of which seed units appear intact and most likely viable. This nonintrusive procedure retains viable seed after imaging that can be combined with other tests for estimating viability and germinability to improve predictive accuracy and calibration. As with the cut test, this method does not indicate actual seed vitality and therefore may potentially overestimate the viability of the seed batch.

Guidance Statement 11

11.1 25–100 randomly sampled PSU (depending on seed size) are placed in the X-ray machine and exposed to X-rays for a duration and intensity sufficient to penetrate the seed external structures (e.g. seed coat, fruit, pericarp, or florets) to enable visualization of internal seed structure. Report Viable Seed Unit (VSU)—X-ray, as a percentage of the PSU.

Tetrazolium Test

The tetrazolium test is considered the most complete of the viability tests, but can be time consuming and requires skilled and trained operators to perform and evaluate correctly based on prior knowledge and experience with the species. This test entails the use of 2,3,5-triphenyl tetrazolium chloride commonly known as tetrazolium (TZ). This compound reacts with the hydrogen ions released by living cells during respiration, forming an insoluble red compound triphenyl foramzan. Formazan is then visible as a red-pink stain in the parts of the seed where the dehydrogenase is active—with the presumption that this reflects cellular vitality. Many seeds might contain dead tissue that will not be colored, forming a "topographic" map of the staining pattern in the seed. It is important in native seed to understand seed morphology of the tested seed so that the staining pattern of the living tissue reflects seed viability.

Guidance Statement 12

A tetrazolium test is performed as follows:

12.1 *Sample size*: this test should be performed on a minimum of 50 seeds randomly sampled from the PSUs.

12.2 *Moistening*: Seed should be moistened by imbibing with water (between wet paper or soaked in water at 20°C for 12–24 hours). For seed with impermeable seed coats, the coat must be pierced/scarified to allow water to enter the seed. Moistening softens the seed unit to facilitate the subsequent tissue exposure and the chemical reaction of TZ.

12.3 *Tissue exposure*: The tissues of the seed should be exposed before TZ staining. Depending on the structure of the seed, this could be performed by cutting (transverse or longitudinal) with a sharp instrument, embryo excision, or complete removal of the seed coat. For more information refer to the ISTA or AOSA Rules in the Tetrazolium testing section.

12.4 Seed is immersed in a 1% aqueous TZ solution at 30°C for 2 to 24 hours depending on the species (usually between 12 and 24 hours). ISTA guidelines provide the specification for TZ test for various crop, tree, and bush species. AOSA provides specification for a geographically limited set of native species at the genus level.

12.5 Evaluation/interpretation of the TZ staining pattern: For some species the viability is assessed by the presence/absence of red-pink staining. However, it is important to understand what are the vital parts of the seed that should be colored (e.g. radicle tip, shoot apex), and to what intensity (red, pink, light pink) is necessary for a seed to be deemed viable. This will require further testing and evaluation in order to describe species-specific staining patterns and corroboration with other tests including correlation to germination testing if the staining pattern is not considered conclusive of viability.

Germinability

Germination is the ultimate expression of a measure of seed viability involving the conversion from a viable, dormant seed to a germinant and ultimately a plant. Germination operates through a dormancy filter that restricts germination to the most favorable period for seedling establishment.

Germination is therefore a significant step in the seed testing protocol as it defines the outcome of a sowing or restoration program and expected plant numbers. Germination testing for most native species requires simple tools—a germination substrate, suitable temperature and moisture, plus an understanding of how to manage and break dormancy (if present). Thus where other tests of viability may be out of reach of the operator

(tetrazolium, X-ray) a germination test is a useful substitute measure particularly for seed with no or low dormancy (see section Alternative Viability Test Determined by Germinability). However if dormancy breaking treatments are not fully understood, germination may significantly underestimate the amount of viable seed.

Refer to Figure 2 that illustrates the logical framework for each of the key steps listed below.

Germinable Seed Unit. The GSU refers to the number of seeds capable of producing a germination outcome (production of the radicle up to full seedling development). To determine the GSU, seed from the PSU fraction is subjected to an appropriate germination test where environmental conditions (temperature, light requirement) are understood and the expected timing for germination known. The number of germinants resulting from the test form the basis of the GSU.

Guidance Statement 13

13.1 Ensure seed for germination testing is sourced from the PSU and is therefore clean, free of inert materials, and, as far as is practicable, reflects intact, turgid seed likely to be capable of germination.
13.2 Using an automated seed counter, weight, or hand counting, dispense four replicates of 25 seeds (from the PSU) into individual germination dishes/containers that have been prepared with water agar or with moistened germination papers, sand, vermiculite, or other support medium. The germination medium remains moist for the duration of the germinability test. To limit desiccation of the germination medium, and limit fungal and bacterial contamination, germination dishes/containers should be sealed.
13.3 Incubate in the dark (or light if required for germination) at a temperature that is optimal for seed germination. Such information is obtained from the literature or online databases. If unavailable, preliminary germination experiment are required at different temperatures and with or without light to determine optimal germination conditions.
13.4 Germination should be recorded when a visible radicle has emerged to a length of 1–2 mm depending upon seed size.

Germination is usually recorded when a radicle protruding from the seed is detected; however, this does not provide information about the health/vigor of the seedling. For most species this would not be an issue, but in cases where abnormal seedlings are common, the test should be continued until normal and abnormal seedlings can be distinguished. Such a test would provide a more reliable estimate of expected seed emergence and seedling establishment in the field.

Note: If testing a seed unit that contains multiple seeds or seed agglomerates (see seed coating) germination is considered successful when at least one radicle emerges from the unit, regardless of the number of actual seeds contained within the unit. Multiple radicle emergence is still recorded as a single germination event.

Dormant Seeds Units

Unlike crops where dormancy has been eliminated or reduced, seed of wild species will possess simple to complex dormancy systems. Resolving whether seed has a dormancy state can be calculated from the PLS percentage (see previous section) and subtracting the PGS. All the values are weight percentages. For example DSU is the weight percentage of seed that are dormant over the entire weight of PSU.

$$\textit{Dormant Seed Unit}\,(DSU\%) = \textit{Viable Seed Units}\,(VSU\%) - \textit{Germinable Seed Units}\,(GSU\%)$$

Once dormancy has been established, the type of dormancy (and therefore an appropriate dormancy breaking treatment) can be determined from the literature or by empirical analysis. See Baskin and Baskin (2014) for details on how to identify seed dormancy condition. A description of the dormancy classes and methods to alleviate dormancy are described in Box 1.

Note: Some wild species such as drupaceous Ericaceae and many Australian Rutaceae, Restionaceae, and dryland Cyperaceae have *deep intractable dormancy* where germination blocks are not easily resolved under laboratory conditions. Such species only respond to germination cues such as smoke application following a period of aging in soil that can be 6 months to 2 years, or, for some species treating with a pulse of dry heat.

Alternative Viability Tests Determined From Germinability

In the previous sections, seed quality tests for viability and germinability are presented sequentially, with the recommendation to perform the tests in that order. However two alternative methods to determine seed viability without performing a full viability test, can be done by using a germinability test.

Germination Followed by Viability Test. The first method can be used if dormancy or dormancy breaking mechanism are unknown or not available (Fig. 8).

Guidance Statement 14

14.1 Perform the germinability test on the PSUs as described in the Germinable Seed Unit section. This test would return a Germinable Seed Unit percentage.
14.2 At the end of the germination experiment, nongerminated seeds that are free of bacterial or fungal contamination and appear filled and healthy should be examined to determine if these seeds are dormant or nonviable by using one of the methods described in the Viability section (cut test, X-ray, or tetrazolium). This test would return a DSU percentage.
14.3 The Viable Seed Unit (VSU) is calculated by adding the GSU to the DSU.

BOX 1 Seed Dormancy

Seed dormancy is the key mechanism whereby seed persist over time and space in such a way that cues germination to only occur when environmental conditions are favorable for germination and seedling establishment. Millennia of human selection have removed dormancy for crop, forestry, and horticultural species with the result that associated seed testing standards may have little relevance to a native seed that may have complex dormancy states. In contrast, wild species can be broadly divided into species that are nondormant or have one of the following five dormancy classes as defined by Baskin and Baskin (2014): (1) *Physical*: seeds possess an impermeable seed coat that prevents moisture reaching the endosperm and embryo. (2) *Physiological*: the balance of seed-based hormones is such that it prevents germination—sometimes referred to as restricting the "push power" of the embryo to grow out of the seed. (3) *Morphological*: the embryo is under-developed at the time of seed dispersal and requires time to grow within the seed usually in response to periods of moisture contact. (4) *Morphophysiological*: The embryo is under-developed and a hormone imbalance inhibits further development and germination. (5) *Combinational*: seeds possess a physical barrier to water uptake and have physiological dormancy.

Dormancy therefore represents a key constraint in the use of seed in restoration programs (Merritt & Dixon 2011). However in deploying dormant seed to site it is critical to understand that dormancy loss and germination stimulation may represent different components as a seed transitions from a quiescent state to a state capable of accepting a germination stimulant such as light, smoke, nitrate, and fluctuating temperatures (Long et al. 2015). For example, for fire stimulated germination, seed may reside in the soil seed bank, cycling in and out of dormancy awaiting a smoke cue that may arise from the passage of a fire. For these species, to field broadcast or use them in nursery propagation without managing the two phases of dormancy and germination stimulation will result in seed where the applied germination stimulant is out of synchrony with the window of dormancy release (Fig. 7).

Germination With an Applied Dormancy Breaking Treatment

The second method can be applied when appropriate dormancy release and environmental constraints on germination are fully understood and able to be applied to the seed (Fig. 9).

Guidance Statement 15

15.1 Germination stimulants (smoke water) or dormancy breaking treatments and compounds (gibberellic acid, nitrate, karrikinolide) at concentrations appropriate for the species are incorporated in the agar or in the water used to moisten the germination substrate. Alternatively, seeds can be soaked in an appropriate dilution of the germination stimulant/dormancy breaker followed by incubation in the germination dish containing the germination substrate of choice.

15.2 Where applicable, ensure seed has been appropriately conditioned (after-ripened, stratified) and/or treated to release physical dormancy (i.e. scarified, boiling treatment).

15.3 The germinability test provides the Viable Seed Unit Percentage.

15.4 If required, a DSU percentage (DSP) can by determined by subtracting the result of this test from the result of the germinability test performed without dormancy breaking.

Note: Where laboratory facilities are not available it is possible to perform a simple cut test on nongerminated seed remaining after a germinability test as an estimate of seed dormancy and viability.

Pure Live Seed

The result of the viability test, expressed as viable seed units (VSU) combined with the result of the purity test (PSU), enables calculation of the weight percentage of PLS using the following equation.

$$\text{Pure Live Seed (PLS\%)} = \frac{\text{Viable Seed Unit (VSU\%)} \times \text{Pure Seed Unit (PSU\%)}}{100}$$

Guidance Statement 16

16.1 PLS percentage is the minimum quality testing requirement and should be reported on the label of the seed batch

The PLSs value is an estimate of the percentage of live seed in the weight of the entire seed batch. For example a 72% PLS for 20 kg of seed means 14.4 kg of seed are considered viable. An additional way to present PLS is by expressing the estimated number of PLS on a per unit weight basis. Both values are particularly useful for seed users when planning seeding operations (Shaw et al. 2020).

Pure Germinable Seeds and Pure Dormant Seeds

Once a germinability test has been conducted the germination outcome (GSU) and dormancy (DSU) is then related to the PSU to derive the PGS and PDS as a weight percentage of the total seed batch weight:

$$\text{Pure germinable seeds (PGS)} = \frac{\text{Germinable seed unit (GSU)} \times \text{Pure seed unit (PSU)}}{100}$$

$$\text{Pure dormant seeds (PDS)} = \frac{\text{Dormant seed unit (DSU)} \times \text{Pure seed unit (PSU)}}{100}$$

The value of PLS, PGS, and PDS should then be reported in the pro-forma (Fig. 6).

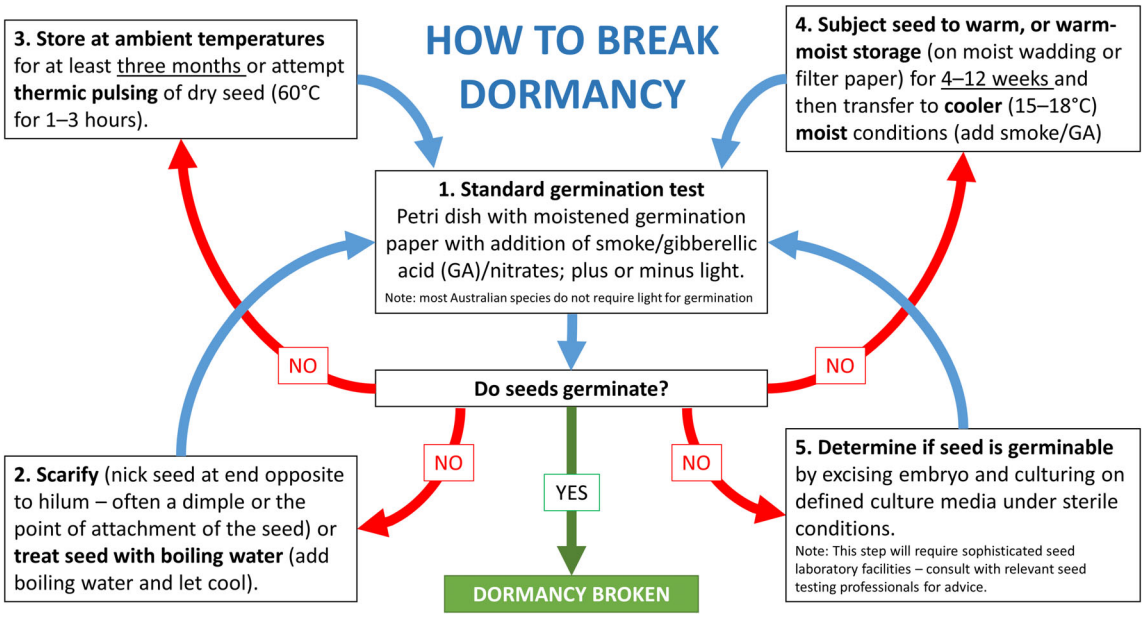

Figure 7. Where the germinability of a species is unknown and problematic, the above illustration provides the logical framework for resolving what seed treatment and procedures are required to release dormancy. Some species with deep intractable dormancy may not have dormancy release procedures known.

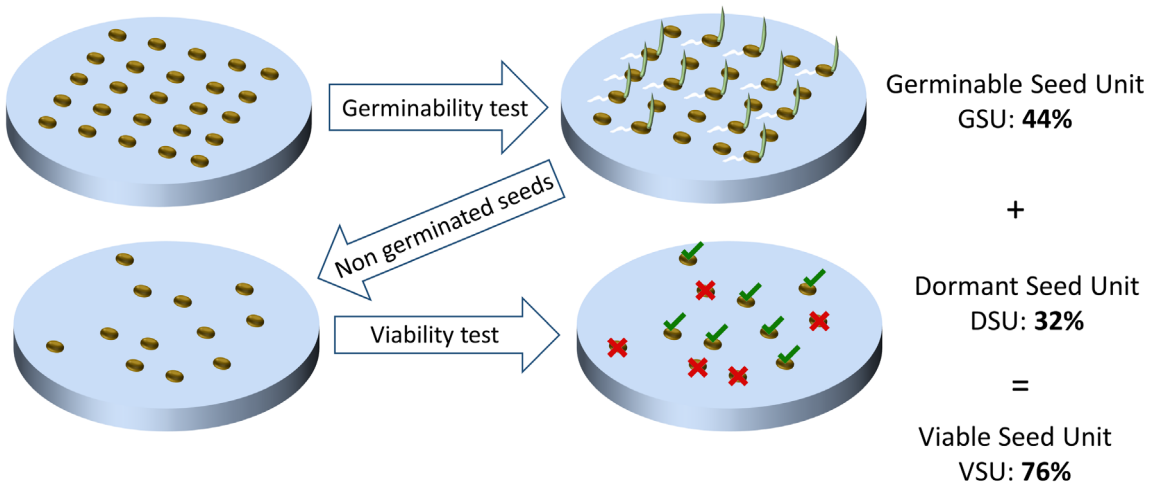

Figure 8. Viability determined by the germinability test when seed dormancy alleviation treatments are not performed or unknown. Seed with a 2 mm protruding radicle, and in the image, a cotyledon, are considered germinated. In the petri dish (bottom right), seed with a green tick are viable, the ones with a red cross are nonviable by the appropriate viability test.

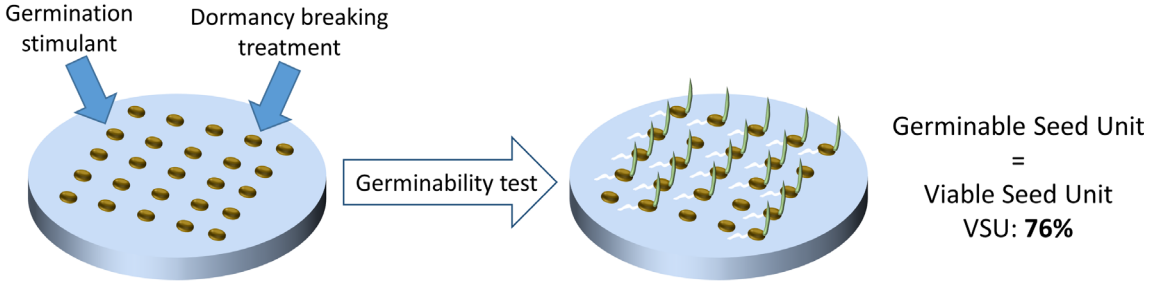

Figure 9. Viability determined by the germinability test where germination stimulants and dormancy breaking treatments were applied. Seed with a 2 mm protruding radicle are considered germinated. If dormancy is removed entirely, the result of the germinability test, GSU, would also provide the result for the viable seed unit VSU.

Seed Enhancement: Dormancy Breaking, Priming, and Seed Coating

The key processes and approaches used in the development and application of enhancement technologies for seed are outlined in Pedrini et al. (2020a). Enhancements can range from simple programmed release of dormancy (scarification, gibberellic acid, etc.) to seed priming and seed coating. Such approaches and technology have only recently been adapted from the agricultural/horticultural sector to the native seed industry. Additional emerging technologies involving novel extruded composites with embedded seed or seed agglomerations are under development for particular restoration applications (Fig. 10).

Though seed enhancements are in their infancy in the native seed industry, the rapid rise in global demand for native seed to meet restoration targets is driving an interest for improving efficiencies in the deployment of seed in restoration programs. Seed enhancements have the potential to meet many of these efficiency goals. The following guidance statements, drawn in part from experiences with the crop/horticultural seed enhancement industries provide a sound foundation for ensuring seed purchasers can have confidence that "enhanced" seed will deliver restoration improvements.

Guidance Statement 17

17.1 If seed enhancements are undertaken by seed suppliers, the treatment applied (dormancy release, germination stimulation, priming, coating) should be indicated. The type of treatment used should be appropriate to the species and site.

17.2 Seed enhancement should be reported if the entire seed batch is treated. If enhancement is performed just on a sample to assess viability, it should not be indicated in this section, but reported in the notes of the viability/germinability section.

17.3 Date of seed treatment should be reported, and, if known, shelf-life of the enhanced seeds should be specified.

17.4 If a combination of treatments is applied, all treatments should be reported.

17.5 Germination/growth enhancements, anti-predation, insecticidal and anti-microbial agents, biologicals (beneficial bacteria and fungi) incorporated in the seed or in the seed coat should be specified, and the concentration of the compound reported.

17.6 When biocidal chemicals are incorporated into enhanced seed, seed suppliers must adhere to national pesticide standards in their use and provide the required legal labeling and handling instructions.

Dormancy Release

If seed dormancy is known, treatment can be applied to the seed batch to remove or alleviate the dormancy, to ensure the seed is readily germinable. There are many different approaches depending on type of dormancy (Kildisheva et al. 2020). If the seed batch has been treated to release dormancy and stimulate germination, it should be reported in the pro-forma.

Guidance Statement 18

18.1 For after-ripening and stratification indicate the duration of treatment and the conditions applied (temperature, humidity).

18.2 For scarification the method used, such as acid, dry heat, boiling water, abrasion, percussion, pneumatic should be reported.

18.3 If chemicals amendments have been used during the process, the name of the compound, the concentration at which it was used, and the delivery methods employed (e.g. imbibition) should also be specified.

Seed Priming

Seed priming consists of controlled hydration of seeds that is stopped prior to the onset of irreversible germination and, after drying, the seed retains viability. In other words, germination is taken to a stage where it is reversible. Seed priming

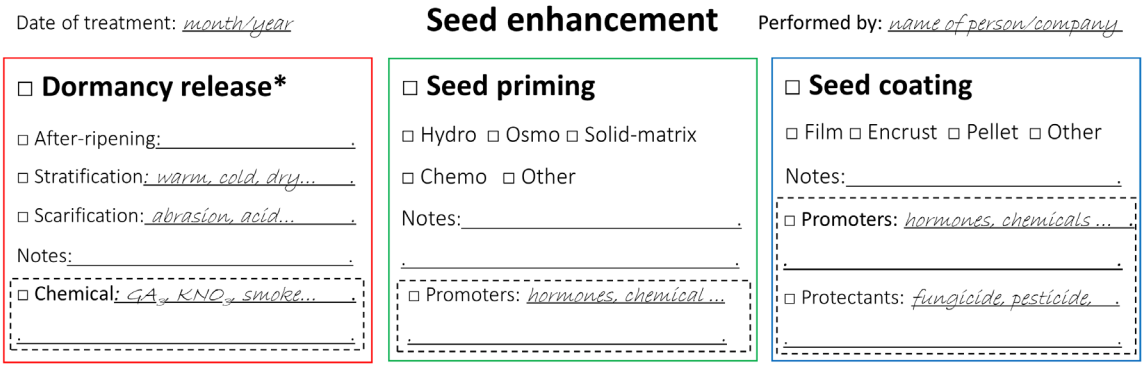

Figure 10. Component of the pro-forma for reporting on seed enhancement treatments. The boxes are filled as required. If compounds such as chemical promoters/protectants (pesticide) or hormones are used, they are reported in the dashed line boxes in each of the relevant sections. When multiple treatments are applied to a seed batch, these should be reported.

is known to improve germination speed and synchronicity and seedling vigor. The process can also be used to deliver potentially beneficial compounds such as germination promoters (e.g. smoke and smoke compounds) and hormones (GA3, salicylic acid). A potential drawback of seed priming is the reduced shelf-life of seed after treatment, compared to untreated seed.

Guidance Statement 19

19.1 Specify the type of seed priming used.
19.2 Duration of treatment, condition (temperature, water potential), and equipment used are reported.
19.3 If promoters are deliver to seed via priming, the type of promoter used (chemical or hormone) and its concentration are reported.

Seed Coating

Seed coating is the practice of applying external material to seed in order to deliver beneficial active ingredients (protectants or germination promoters) and to regularize seed shape and size to improve seed handling and flowability. Usually seed coating is performed on single seeds (singulation); however, in some circumstances multiple seeds pellets (agglomerates) can be produced. In this case the pelleted units should be treated during seed quality evaluation as single seed units, however, the average number of seed per agglomerate should be reported.

Guidance Statement 20

20.1 Indicate the type of seed coating applied.
20.2 Coating materials (binder and filler) and coating equipment used are indicated in the notes.
20.3 If promoters and protectants (fungicide, pesticide) are used they are reported and the concentration provided.
20.4 For coated seed, where many seeds are present in a unit (agglomerates) indicate the mean number of seeds in each unit.

How the Native Seed Standards Can Be Used

The Standards provide a practical, step-by-step guide for ensuring each step in the native seed supply chain is robust and evidence-based. The pro-forma provided (Fig. 2), if adopted, will result in the development of a robust seed supply

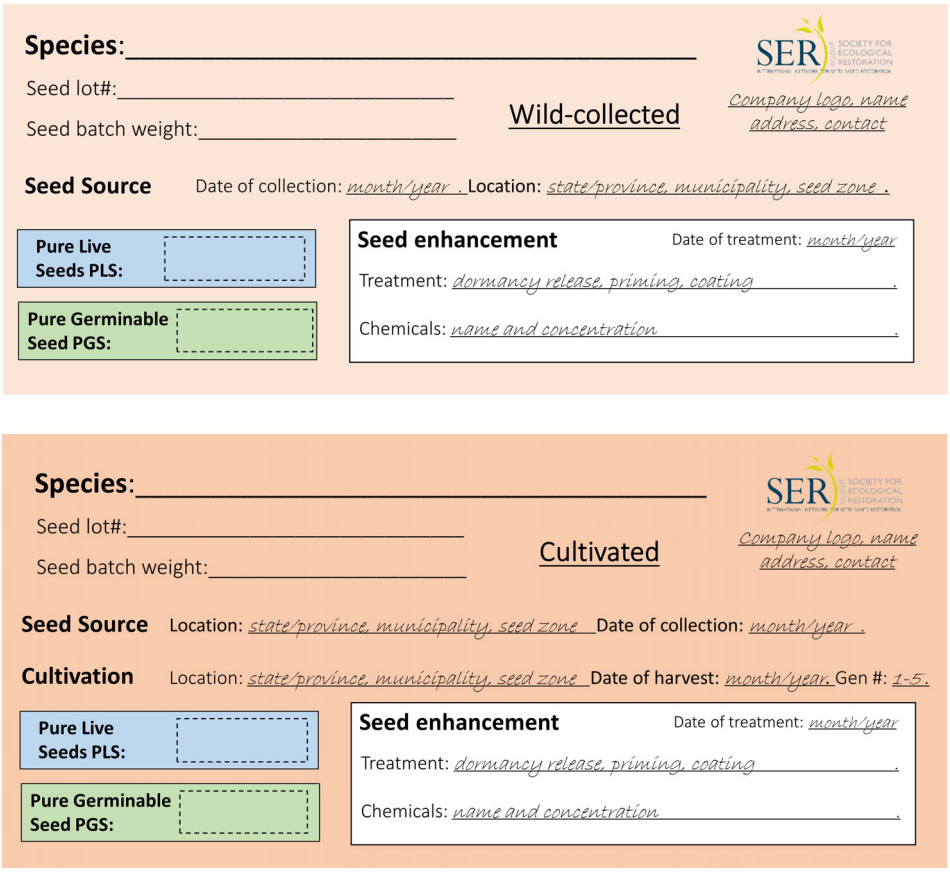

Figure 11. Example of labels that are applicable to point-of-sale release of native seed. The top label is for seed directly harvested in the wild, the bottom label for seeds derived from managed seed production areas.

Mix name: reference ecosystem/community .

Total Seed mix weight: 5 kg . Date of preparation: month/year

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Species name	Batch #	Cultivated	Seed source	Collection/ Harvest date	Enhancement*	PLS%	% on total weigh	Weight (g)
Species 1	#	Y	state/province, municipality, seed zone	month/year	Y	80	40	2,000
Species 2	#	Y	state/province, municipality, seed zone	month/year	N	60	30	1,500
Species 3	#	N	state/province, municipality, seed zone	month/year	N	75	15	750
Species 4	#	Y	state/province, municipality, seed zone	month/year	N	90	10	500
Species 5	#	N	state/province, municipality, seed zone	month/year	Y	45	5	250

*enhancement: If yes specify kind of enhancement and if potentially harmful chemicals are present

Species 1 : Seed coating- encrusting with natural predator deterrent (Chili powder)

Species 5: Hot water treatment to release dormancy

Figure 12. Label example for a supplied seed mixture.

management system and in combination with online databases, will allow tracking of seed batches through the supply chain while ensuring that necessary and relevant information is provided and accurately reported to the end-user.

However, in most cases, it would be unreasonable to expect the pro-forma to be provided with each bag of the seed batch, especially if seeds are sold in mixes, as is often the case.

Alternative abbreviated labels are provided that are relevant to single species seed bags (Fig. 11) and for mixes (Fig. 12).

Conclusion

These Standards represents a practical tool for improving the reliability of each step in the native seed supply chain. They aim to strike a balance between what are reasonable quality expectations and guarantees for the seed end-user and what is practically achievable and economically viable for seed suppliers. It is expected that the 20 Guidance Statements that form the basis for these Standards will be modified and additional Statements provided in future editions of the Standards. We welcome input on improvements, amendments, and additions to these Guidance Statements.

This document, and the labeling specifications and statements within, are nonbinding, but provide clear guidance applicable to global biomes and different socio-economic scenarios. It is designed to be accessible and practical for all those involved in the collection, production, and use of native seeds. A practical benefit of the Standards is the provision of an "industry-ready" label (the "pro-forma") that provides a level of consistency in what a seed user/purchaser can expect from a native seed batch.

Importantly these Standards guide the users through the qualities and characteristics of native seed that that are often very different to the standards developed for crop, forestry, horticultural, and pasture species.

Regional and local adaptation of the standards will be required in many instances to reflect the qualities and characteristics of species, local demand for native seeds, the structure of the native seed market, and the regulatory environment. If regulations or guidelines on native seeds are not present, this standard is an ideal template upon which to develop a regulatory framework. These Standards can be used to inform regulators on the distinctiveness of native seed and encourage regulatory updates to ensure the sound and sustainable development of the native seed in concert with effective future ecological restoration industries.

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