

## 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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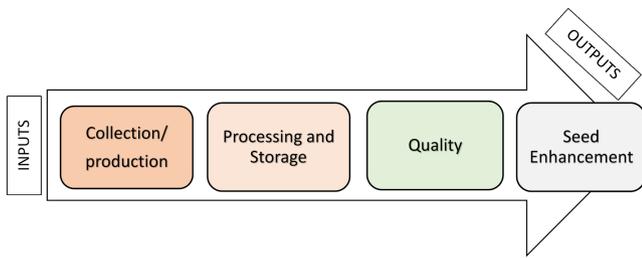


Figure 1. Key elements of the seed supply chain. Each step is outlined in the Standards sections that follow. Seed collection/production: refers to wild and cultivated sources of seed. Processing and storage: optimal treatments and storage conditions to achieve and retain the highest level of seed quality. 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. 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.

#### 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 et al. 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 12009a, 12009b), 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. 2020b).

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, VWV-Regiosaat 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.

Species: \_\_\_\_\_

Seed lot#: \_\_\_\_\_

Seed batch weight: \_\_\_\_\_

Wild-collected

Cultivated



<p><b>Seed Source</b></p> <p>Date of collection: <i>month/year</i> _____.</p> <p>Location: <i>state/province, municipality, seed zone</i> _____.</p> <p>Site: <i>gps coordinates (WGS 1984 datum)</i> _____.</p> <p>Collector: <i>name of the person/company</i> _____.</p> <p>Notes: _____.</p>	<p><b>Managed seed production</b> (If cultivated)</p> <p>Date of harvest: <i>month/year</i> _____.</p> <p>Location: <i>state/province, municipality, seed zone</i> _____.</p> <p>Number of generations: <i>1-5</i> _____.</p> <p>Producer: <i>name of the company</i> _____.</p> <p>Notes: _____.</p>
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Seed storage condition after collection/harvest RH% \_\_\_\_\_ T° \_\_\_\_\_

Date of treatment: <i>month/year</i> _____			<b>Seed quality test</b>			Performed by: <i>name of person/company</i> _____		
<p><b>Purity</b></p> <p>Pure Seed Unit PSU: _____ %</p> <p>Other seeds<sup>2</sup>: _____ %</p> <p>Inert material<sup>3</sup>: _____ %</p> <p>Notes: _____.</p>	<p><b>Viability</b></p> <p>Viable Seed Unit VSU: _____ %</p> <p><input type="checkbox"/> Cut test <input type="checkbox"/> X-ray <input type="checkbox"/> TZ <input type="checkbox"/> Other</p> <p>Notes: _____.</p>	<p><b>Germinability</b></p> <p>Germinable Seed Unit GSU: _____ %</p> <p>Notes: _____.</p>	<p><b>Dormancy (if required)</b></p> <p>Dormant Seed Unit DSU: _____ %</p> <p>Notes: _____.</p> <p>Dormancy type (if known)</p> <p><input type="checkbox"/> Physical</p> <p><input type="checkbox"/> Physiological</p> <p><input type="checkbox"/> Morphological</p> <p><input type="checkbox"/> Morpho-physiological</p> <p><input type="checkbox"/> Combinational</p>			<p><b>Pure Live Seeds PLS:</b> _____ %</p>		
<p>1000 Pure Seed Units PSU weight: _____ (g).</p>		<p><b>Pure Germinable Seed PGS:</b> _____ %</p>						

Date of treatment: *month/year* \_\_\_\_\_ **Seed enhancement** Performed by: *name of person/company* \_\_\_\_\_

<p><input type="checkbox"/> <b>Dormancy release*</b></p> <p><input type="checkbox"/> After-ripening: _____.</p> <p><input type="checkbox"/> Stratification: <i>warm, cold, dry...</i> _____.</p> <p><input type="checkbox"/> Scarification: <i>abrasion, acid...</i> _____.</p> <p>Notes: _____.</p> <p><input type="checkbox"/> Chemical: <i>GA, KNO<sub>3</sub>, smoke...</i> _____.</p>	<p><input type="checkbox"/> <b>Seed priming</b></p> <p><input type="checkbox"/> Hydro <input type="checkbox"/> Osmo <input type="checkbox"/> Solid-matrix</p> <p><input type="checkbox"/> Chemo <input type="checkbox"/> Other</p> <p>Notes: _____.</p> <p><input type="checkbox"/> Promoters: <i>hormones, chemical...</i> _____.</p>	<p><input type="checkbox"/> <b>Seed coating</b></p> <p><input type="checkbox"/> Film <input type="checkbox"/> Encrust <input type="checkbox"/> Pellet <input type="checkbox"/> Other</p> <p>Notes: _____.</p> <p><input type="checkbox"/> Promoters: <i>hormones, chemicals...</i> _____.</p> <p><input type="checkbox"/> Protectants: <i>fungicide, pesticide...</i> _____.</p>
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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. 2020b).

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 out-planting 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

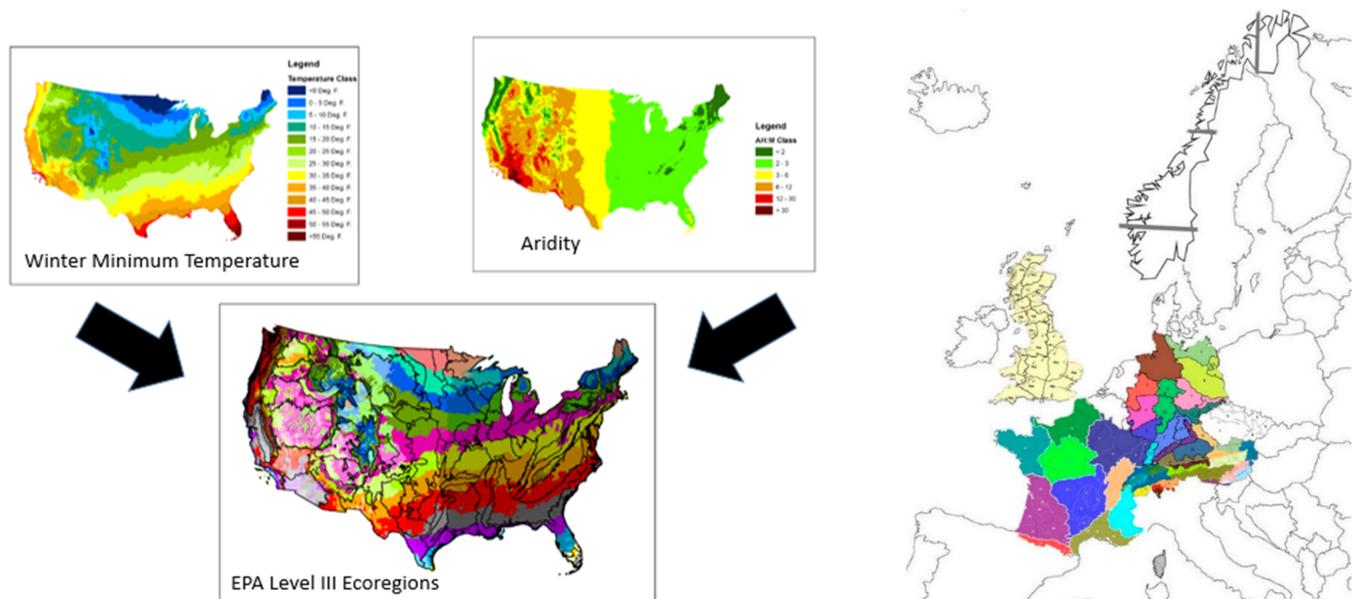


Figure 3. Seed zones in the United States and Europe: Left: U.S. provisional seed zones for native plants (color polygons) are unique, climatically delineated (temperature-aridity) areas nested within EPA Level III Ecoregion boundaries (black lines). The provisional zones can be used to guide seed sourcing 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.

<b>Species:</b> _____		 <i>Company logo, name</i> <i>address, contact</i>
Seed lot#: _____	<input type="checkbox"/> Wild-collected	
Seed batch weight: _____	<input type="checkbox"/> Cultivated	
<b>Seed Source</b> Date of collection: <i>month/year</i> _____ Location: <i>state/province, municipality, seed zone</i> _____ Site: <i>gps coordinates (WGS 1984 datum)</i> _____ Collector: <i>name of the person/company</i> _____ Notes: _____ _____		<b>Managed seed production</b> (If cultivated) Date of harvest: <i>month/year</i> _____ Location: <i>state/province, municipality, seed zone</i> _____ Number of generations: <i>1-5</i> _____ Producer: <i>name of the company</i> _____ Notes: _____ _____
<b>Seed storage condition after collection/harvest</b>		RH% _____ T° _____

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.

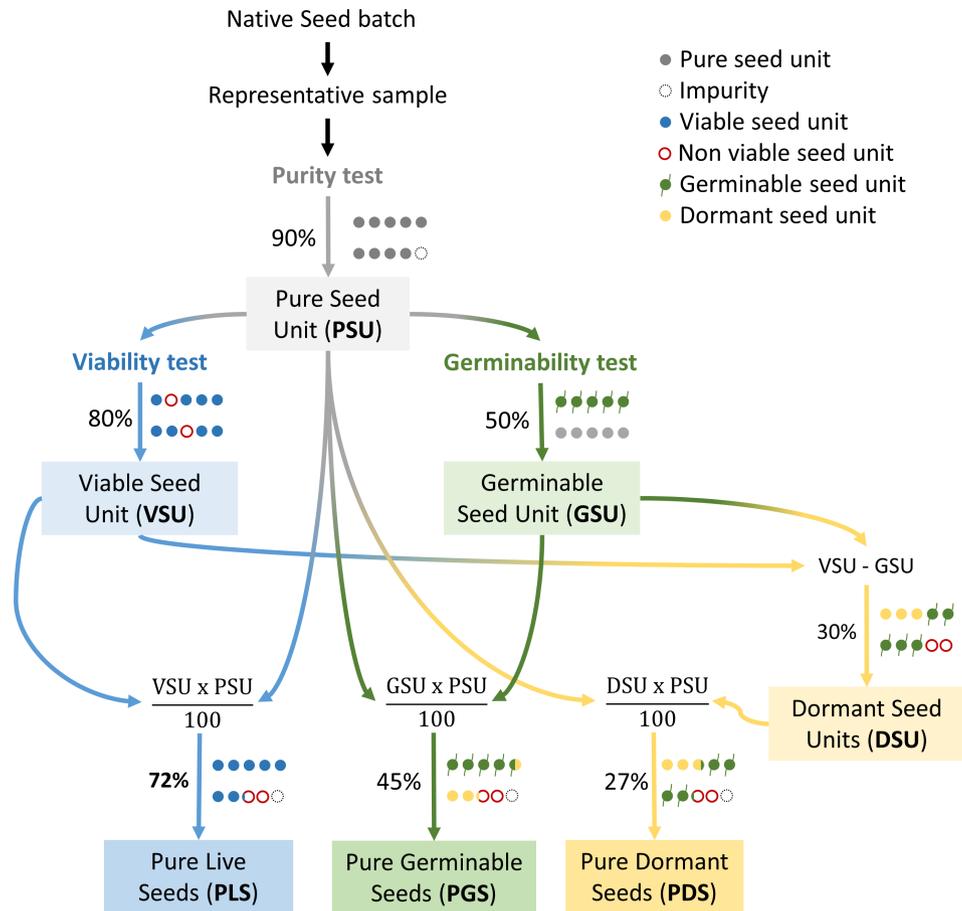


Figure 5. Native seed quality tests and outcomes used to derive key indicators of seed quality—PSU, GSU, DSU, PLS, PGS, and PDS. Between each test, a model seed batch is diagrammatically represented with values indicative of weight percentages. This is one of the approaches used to determine pure live/germinable/dormant 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.

Date of treatment: <u>month/year</u>		<b>Seed quality test</b>	Performed by: <u>name of person/company</u>
<b>Purity</b> Pure Seed Unit PSU: _____ % Other seeds <sup>2</sup> : _____ % Inert material <sup>3</sup> : _____ % Notes: _____ _____ 1000 Pure Seed Units PSU weight: _____ (g).	<b>Viability</b> Viable Seed Unit VSU: _____ % <input type="checkbox"/> Cut test <input type="checkbox"/> X-ray <input type="checkbox"/> TZ <input type="checkbox"/> Other Notes: _____ _____	<b>Germinability</b> Germinable Seed Unit GSU: _____ % Notes: _____ _____	
	<b>Dormancy (if required)</b> Dormant Seed Unit DSU: _____ % Notes: _____ _____	Dormancy type (if known) <input type="checkbox"/> Physical <input type="checkbox"/> Physiological <input type="checkbox"/> Morphological <input type="checkbox"/> Morpho-physiological <input type="checkbox"/> Combinational	
<b>Pure Live Seeds PLS:</b> _____ %	<b>Pure Germinable Seed PGS:</b> _____ %	<b>Pure Dormant Seeds PDS:</b> _____ %	

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.

### 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 ( $R_{1-4}$ ) 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 noninvasive 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 formazan. 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.

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

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 be 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.

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.

**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:

Pure germinable seeds (PGS)

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

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).

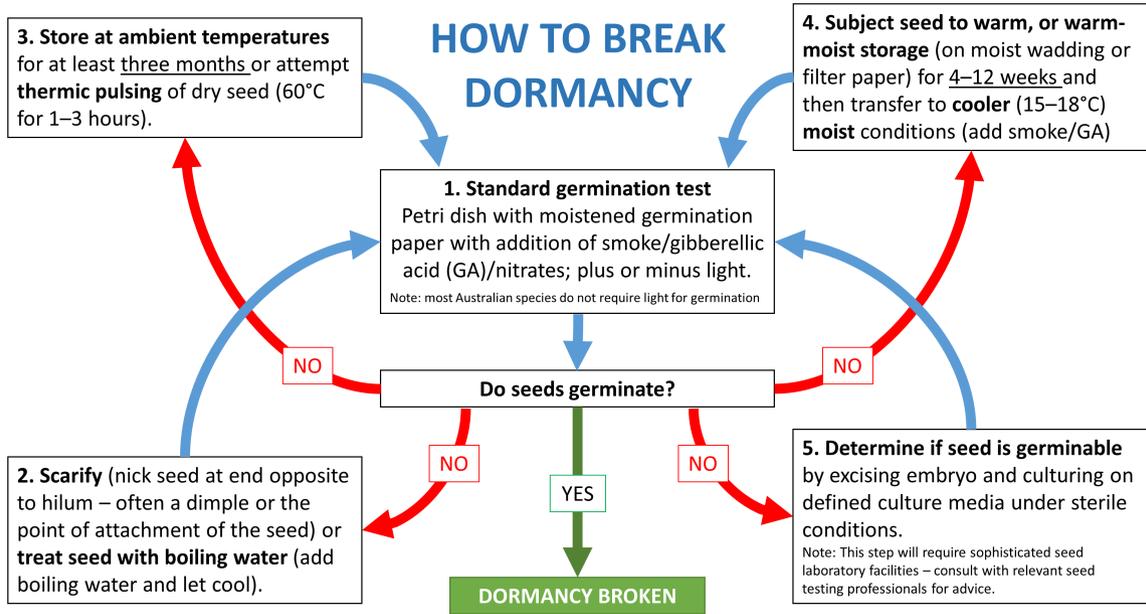


Figure 7. Where the germinability of a species is unknown and problematic, the above illustration provides the logical framework for resolving what seed treatment and procedures are required to release dormancy. Some species with deep intractable dormancy may not have dormancy release procedures known.

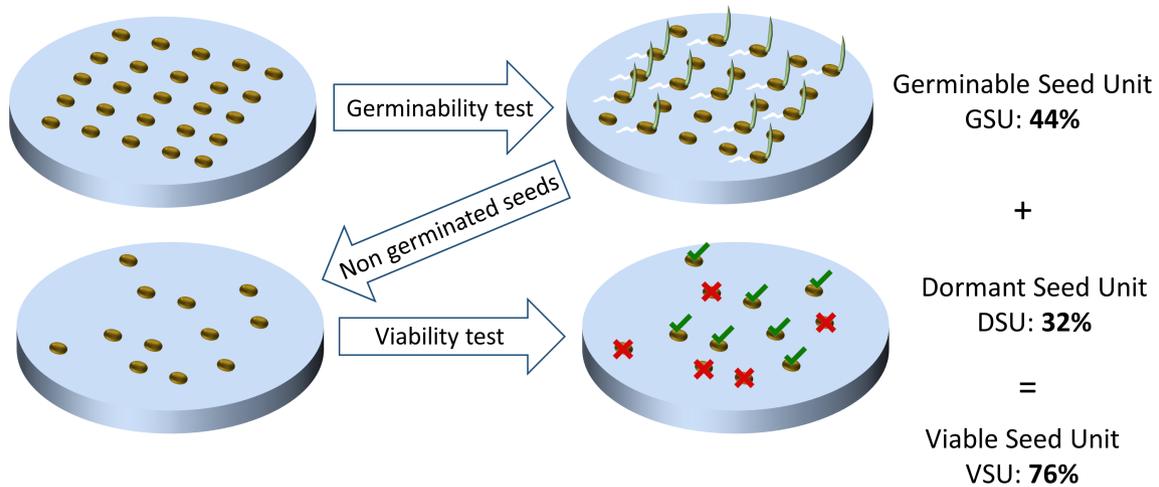


Figure 8. Viability determined by the germinability test when seed dormancy alleviation treatments are not performed or unknown. Seed with a 2 mm protruding radicle, and in the image, a cotyledon, are considered germinated. In the petri dish (bottom right), seed with a green tick are viable, the ones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

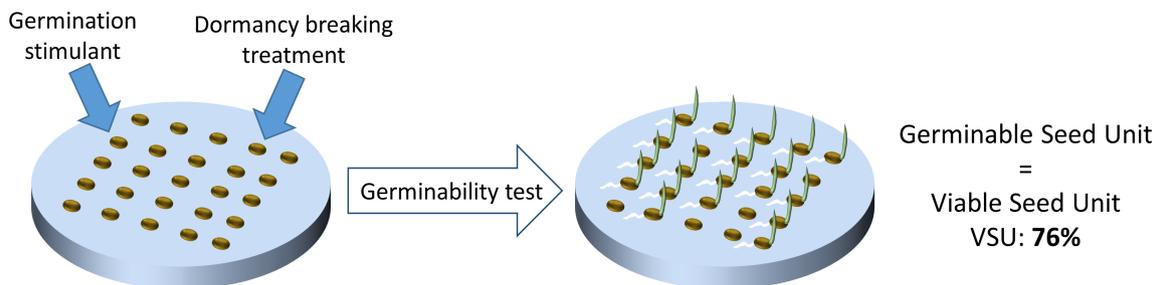


Figure 9. Viability determined by the germinability test where germination stimulants and dormancy breaking treatments were applied. Seed with a 2 mm protruding radicle are considered germinated. If dormancy is removed entirely, the result of the germinability test, GSU, would also provide the result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

Date of treatment: *month/year*

**Seed enhancement**

Performed by: *name of person/company*

<input type="checkbox"/> <b>Dormancy release*</b> <input type="checkbox"/> After-ripening: _____ <input type="checkbox"/> Stratification: <i>warm, cold, dry...</i> <input type="checkbox"/> Scarification: <i>abrasion, acid...</i> Notes: _____ <div style="border: 1px dashed black; padding: 2px;"> <input type="checkbox"/> Chemical: <i>GA, KNO<sub>3</sub>, smoke...</i> </div>	<input type="checkbox"/> <b>Seed priming</b> <input type="checkbox"/> Hydro <input type="checkbox"/> Osmo <input type="checkbox"/> Solid-matrix <input type="checkbox"/> Chemo <input type="checkbox"/> Other Notes: _____ <div style="border: 1px dashed black; padding: 2px;"> <input type="checkbox"/> Promoters: <i>hormones, chemical...</i> </div>	<input type="checkbox"/> <b>Seed coating</b> <input type="checkbox"/> Film <input type="checkbox"/> Encrust <input type="checkbox"/> Pellet <input type="checkbox"/> Other Notes: _____ <div style="border: 1px dashed black; padding: 2px;"> <input type="checkbox"/> Promoters: <i>hormones, chemicals...</i> </div> <div style="border: 1px dashed black; padding: 2px;"> <input type="checkbox"/> Protectants: <i>fungicide, pesticide...</i> </div>
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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 delivered 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

The figure shows two examples of seed labels. The top label is for 'Wild-collected' seeds and the bottom label is for 'Cultivated' seeds. Both labels include the following fields:

- Species:** \_\_\_\_\_
- Seed lot#:** \_\_\_\_\_
- Seed batch weight:** \_\_\_\_\_
- Seed Source:** Date of collection: *month/year* . Location: *state/province, municipality, seed zone* .
- Seed enhancement:** Date of treatment: *month/year* . Treatment: *dormancy release, priming, coating* . Chemicals: *name and concentration* .
- Pure Live Seeds PLS:** [ ]
- Pure Germinable Seed PGS:** [ ]
- Company logo, name, address, contact:** \_\_\_\_\_

The labels also feature the SER logo (Society for Ecological Restoration) and checkboxes for 'Pure Live Seeds PLS' and 'Pure Germinable Seed PGS'.

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: 5kg

Date of preparation: month/year

*Company logo, name  
address, contact*

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