Title: Ensuring seed quality in ecological restoration: Native seed cleaning and testing

Running head: Native seed cleaning and quality testing

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Abstract

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

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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.

Keywords: germination, native seed industry, purity, quality standards, seed conditioning, viability

Implications:

- The diversity of morphological and physiological traits among seeds of native species requires a variety of seed cleaning techniques and equipment to achieve optimal seed lot quality while maintaining representative genetic diversity.
- Seed testing provides assurance about the value of a seed lot and is important for calculating seeding rates for ecological restoration. Despite this, standardized rules for native seed testing are lacking for many species, countries, and ecosystems.
- The seed biology and uses of native species are distinct from those of agricultural species and cleaning and testing methods should be adapted for native seeds.

- As an alternative when third-party seed testing services are not available, testing should be performed by the seed supplier.

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, FAO REDD+ are working to reverse degradation globally (Food and Agriculture Organization of the United Nations n.d.; Plant for the Planet n.d.; United Nations n.d.). 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 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 non-viable seeds from a seed lot (or batch) (Houseal 2007; Bartow 2015; Bonner et al. 2008). 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) (Fig. 2) (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) (Fig. 1, Fig. 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 (Fig. 1, Fig. 2) (Center for Plant Conservation n.d.; 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 (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 labelling 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), 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) (Pedrini & Dixon 2020; Ryan et al. 2008). 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 & 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 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 pure seed units. 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 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 be soaked in a solution of 2,3,5-triphenyl tetrazolium chloride (usually between 1 and 4 %) for 2 to 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 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, electrical conductivity test (EC) test (Marin et al. 2018) and low-tech 'pop' test (Tilley et al. 2011) may be used in some cases (Supplement 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 (Pedrini & Dixon 2020; ENSCONET 2009). 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 n.d.; 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) are 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 four to six 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 n.d.; 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 n.d.). 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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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.

190x190mm (267 x 267 DPI)

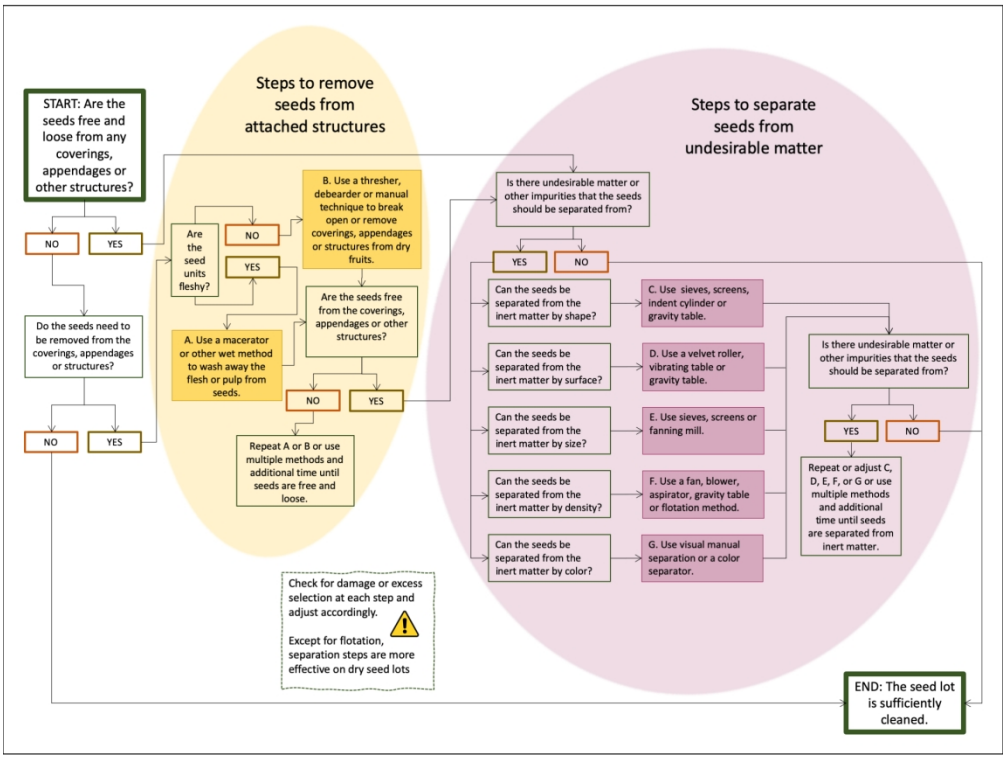

Figure 2. Flowchart showing the steps and processes for cleaning native seed lots.

355x266mm (143 x 143 DPI)

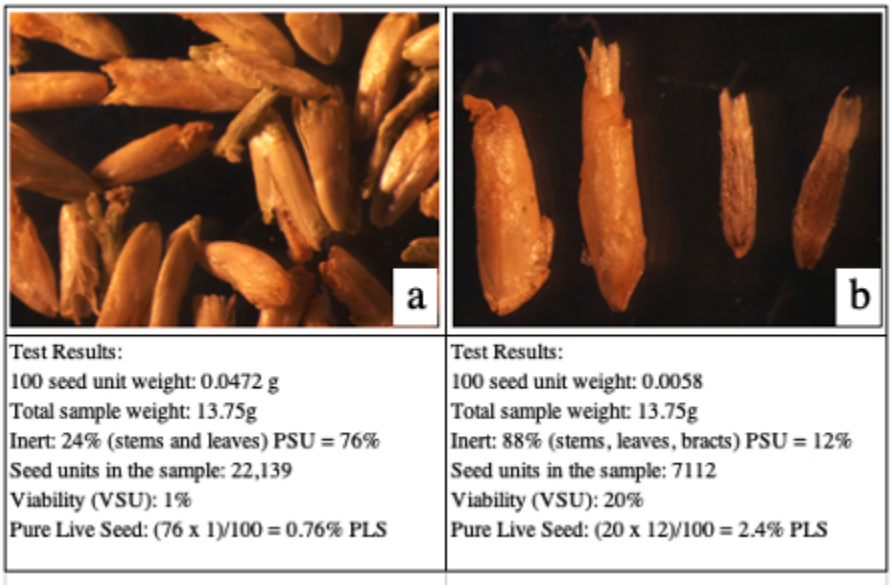

Figure 3. A comparison of different definitions of Pure Seed Unit (PSU). Intact seed heads (a) and individual achenes (b) from a seed sample of Gutierrizia microcephala (Asteraceae). One can see the substantial difference in the purity and viability results depending on the pure seed unit definition. 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.

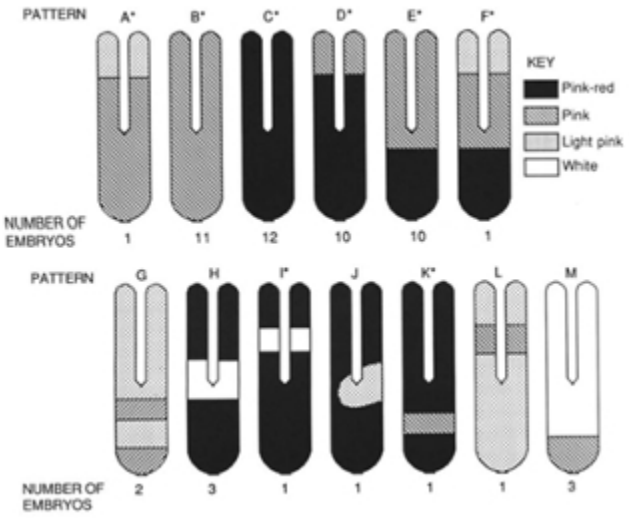

Figure 4. Tetrazolium (TZ) staining patterns in excised embryos of the native Australian species Geleznowia verrucosa (Rutaceae) showing a large variation in embryo staining in wild collected seed (Paynter & Dixon 1990). In this case, the staining patterns were then correlated with in vitro growth of another batch of extracted, non-stained embryos. Patterns A, B, C, D, E, F, I and K were considered viable. Patterns G, H, J, L and M were non-viable.

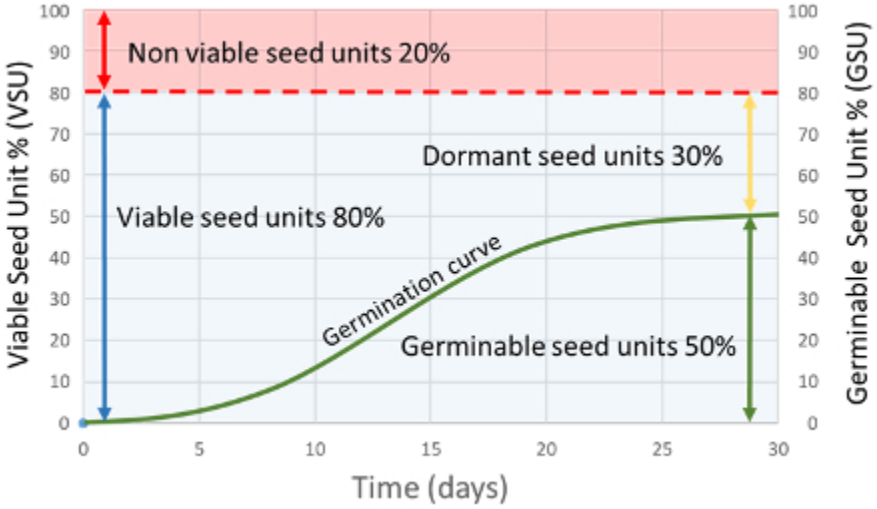

Figure 5. Theoretical representation to show how a germination test combined with a viability test is used to determine the fraction of the seed lot that can be considered viable (blue), germinable (green), dormant (yellow) and non-viable (red).