Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

from Tree Seed Working Group Newsbulletin #50 Dec 2009

Quick Tests

Most people generally think of estimating the ability of seedlots to produce plants through germination tests, but a variety of quick tests are available. Are they useful and for what? Here is my perspective. If you have additional information, please consider submitting an article for our next Newsbulletin.

It should be clear that germination tests provide an estimate of germinability using a specific dormancy-breaking pretreatment and specific germination conditions. Quick tests on the other hand estimate viability, or proportion of living seeds, in a sample independant of the pretreatment or environmental conditions provided. The viability will always be higher or equal to the germinability of a seedlot . The difference is generally attributed to dormancy – seeds that are viable , but cannot germinate with the pretreatment and conditions provided. The advantages of quick tests is obviously the reduced time needed to get results, but they also provide an estimate of the maximum potential germination of a seedlot. The disadvantage is that actual nursery germination may differ greatly due to dormancy (and how well our pretreatments overcome dormancy) and the environmental conditions of the nursery.

I will discuss four quick test methods in this article i) Hydrogen Peroxide ii) Tetrazolium iii) X-ray and iv) Cutting tests. At our facility we have some, but limited experience with the first two, use x-rays as more of a documentation of seed anatomy, and cannot imagine working efficiently at our facility without cutting tests. The first three techniques (plus a modification of the x-ray test) are well described with many references in a publication from Dr. Carole Leadem "Quick Tests for Tree Seed Viability" from 1984 which can be found and downloaded at: http://www.for.gov.bc.ca/hfd/pubs/Docs/Mr/Lmr018.htm

I won't review the methodology, but try and summarize some of the highlights of the methods in the Table below and provide some comments on each method.

METHOD	Time	Seed Cutting Method	Chemicals Required
Hydrogen Peroxide	7 days	Clipping at radical end avoiding radicle	Hydrogen Peroxide (1%)
Tetrazolium	8 to 24 hours	Cut seed parallel to embryo	Tetrazolium Chloride (1%)
X-ray	Instantaneous to weeks	NONE	NONE
X-Ray Contrast	Instantaneous to weeks	NONE	Trichloromethane (Chloroform)
Cutting	Instantaneous to days	Bisect seed in two equal parts longitudinally	NONE

HYDROGEN PEROXIDE The hydrogen peroxide (H2O2) test is relatively simple to perform and assess, relatively quick and is the only test that actually measures growth to estimate viability. As with all of the quick tests requiring some form of seed cutting, the smaller the seed the more challenging it is to perform the cut properly. This is even more important with the H2O2 test as any damage to the radical can impact its growth and the reliability of the estimate . In Leadem (1984) references are provided on the observation that hydrogen peroxide can substitute for stratification and the explanation generally offered is that this is due to the resulting increased oxygen concentration. I am unaware of recent studies substantiating this and although some BC nurseries use hydrogen peroxide as a sterilant of surface borne contaminants (most prominently *Fusarium* spp.), there really isn't a clean comparison between unstratified + H2O2 treated seed and stratified seed. It may be a useful tool (last resort) when there is no time for stratification, but in general it would be a much more expensive treatment than cold stratification.

TETRAZOLIUM The Tetrazolium (TZ) test is probably the most well known quick test and is used extensively for a wide variety of agronomic crops, especially grasses. For species with deep dormancy (requiring long pretreatments) the TZ test is often used in seed valuation in Europe. There is also general support for this method as both AOSA and ISTA have guidebooks dedicated to TZ testing, although emphasis is on agronomic crops. Test methodology is relatively simple, but it is important that the seed is cut parallel to the embryo so that staining intensity is related to embryo 'health ' and not the distance the stain must travel. Interpretation can be difficult due to improper cuts, difficulty in classifying partially stained embryos and the complication of recently damaged seeds showing greater staining intensity (Leadem 1984).
I don't see TZ testing as a very useful predictive tool to estimate viability % given the time required, variability exhibited , subjectivity in interpreting the partially stained embryos and the fact that the condition of the nutritive tissue is totally ignored. I do think that it can play a key role in providing information of a general nature as to whether seeds are alive (viable) or dead due to some type of insult. An actual example relates to some hemlock seed that was stratifying in a fridge at a nursery. The fridge 'failed' and the imbibed seeds were exposed to subfreezing temperatures of -7°C. The question was are the seed still viable or do I need to stratify a new quantity of seed? A great use for TZ testing, and yes the seed was still viable and was able to produce a crop. Noland and Mohammed (1997) advocated the use of fluorescein diacetate as an alternate viability stain for tree roots and seeds.

X-RAY At our facility, x-rays are used as a means of documenting seed anatomy when seedlots receive their initial suite of tests prior to being placed into long-term storage. We x-ray the fourth germination replicate (100 seeds) as a standard part of new seedlot testing. We do not classify individual seeds, but use the x-ray as a possible means of explaining poor seedlot performance and assessing the probability of upgrading the seedlot using more modern processing methods than were originally used. Characteristics that are easily seen and considered useful are extent of mechanical damage (indicating PRE-VAC may be an efficient upgrading technique), proportion of insect-filled seeds (most commonly *Megastigmus* spp.), proportion and degree of embryo immaturity and proportion of empty seeds remaining. I have not used any contrast agents and cannot comment on their operational use. The one limitation of x-ray analysis is that the properties of imbibed seeds are more difficult to assess and these tests are best performed on dry (<10% moisture content) seeds.

CUTTING I saved the best for last – cutting tests. We don't routinely use these as a final seedlot test reported to clients, but they play a key role in pre-collection evaluations (to assist in

quantifying embryo maturity and megagametophyte condition); evaluations on cone receipt (to identify problems and provide a 'ballpark' estimate of viable seed yield) ; at seed extraction (to confirm viable seed are not retained in cones before discarding) and during various processing phases (to ensure viable seed is not being discarded). In final cleaning cutting tests are the main criteria used to determine which seed fractions are retained, discarded or should be considered as another seedlot with reduced viability. The advantage of cutting tests is that results are available instantaneously following seed bisection and decisions can be made immediately or information forwarded to clients. The disadvantages is that it is difficult to produce a rigorous set of interpretative instructions or a photo manual due to the large number of species and conditions in which the test is performed under. At our facility we often use the phrase 'Art and Science" in relation to cone and seed processing and this is an appropriate description of cutting test methodology. The relevant **variables** in a cut test are discussed below.

Several items in a cutting test can vary depending on the seed development phase, moisture content, proportion of questionable seed, and any ***a priori*** information regarding collection or post-collection handling. Cutting tests can be viewed as having a supporting and decision-making role rather than a quantitative one in seedlot construction. Sample size can be adjusted in relation to the specific situation, but a minimum sample at final cleaning is 5 replicates of ten seeds. Sample sizes can be increased if unusual characteristics are seen or there is a larger occurrence of immaturity, megametophyte discolouration or deterioration. The classification and any photo guide can be difficult as the observed characteristics can be strongly influenced by moisture content of the material, ability to accurately bisect the seed, magnification, and even type of light source used. Cutting tests can be performed in the field using a 10X to 15X hand lens, but best performed under a dissecting microscope with a ring light that can provide a magnification up to 30X.

Any seed bisection provides some information on the internal contents of a seed, but a longitudinal section through the thinnest dimension provides the most embryo and megagametophyte tissue to base your classification upon. If the product appears to have a high proportion of viable seed (or obviously deteriorated seeds) then cutting tests based on dry seed (<10% moisture content) may suffice, but to identify more subtle forms of deterioration we generally consider imbibed cutting tests to be superior. Dry seeds are more difficult to cut, but allow decisions to be made immediately. Duration of imbibition can vary by species or sample condition with some samples requiring 48 or more hours required to aid in seed characterization.

A simplified version of a cutting test flowchart can be found on page 8 of the Seed handling guidebook (http://www.for.gov.bc.ca/hti/publications/misc/seed_handling_guidebook_hi.pdf) indicating one of many possible roadmaps in characterizing bisected seeds. Cutting tests are similar to other viability tests in that it is generally easy to identify viable seeds and non-viable seeds, but the seeds in the grey-zone in between can be extremely difficult to classify. A simplification of the individual seed assessments would be a quick assignment of rotten (or severely deteriorated) seeds; immature seeds and empty seeds to their respective categories – that's the easy part. The remaining classification is based on the appearance of the embryo in terms of consistency and colour (which can be quite variable between and within species) and the megagametophyte consistency and colour which should be firm and light cream to white in imbibed seed. Moisture content plays a key role in assessing colour differences and is probably the largest obstacle to standardizing the tests. An example would be that the colour and degree of shrinkage in viable dry seeds would be indications of a problem if seen in an imbibed seed.

A further optional step in confirming viability estimates is to put the cut seeds into a covered germination dish for several days to observe if any photrophic behaviour such as greening of the embryo or physical bending toward light occurs. These have been referred to as incubation tests, although even less documentation is available regarding these compared to cutting tests. They are an additional tool that can be incorporated into the quick test to try and maximize information obtained.

All of these viability tests require some amount of calibration to make them useful for tree seeds which generally have higher levels of variability compared to other plants. These quick viability tests are extremely valuable in making decisions from when to collect to what fractions to combine in a seedlot. I'm still convinced that even with its unstandardized shortcomings the cutting test is still the most useful viability estimation tool for efficient operational decision-making. This review has made me re-consider the usage of some of these other viability tests and that we should have a certain degree of competence in all of them as they can be useful additions to our suite of diagnostic tools. I'm very curious how other facilities approach estimating viability under operational conditions and hope others will contribute on this subject in the future. No one said excellence was going to be easy!

REFERENCES

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