The Tetrazolium Estimated Viability Test for Seeds of Native Plants[1]

Victor Vankus[2]

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Abstract--The demand for seed and planting stock of native species has grown substantially over the past several years. The demand is driven by federal and state agencies, conservation groups and the public that want to use the plants for a wide range of land management applications. Seed producers and nurseries need clean, viable, high quality seed of native species. Seed testing is an important component in the production process of these seeds and test results can be used to manage a variety of activities. One of the tests commonly used to evaluate seed viability of native plant species is the tetrazolium or TZ test. The TZ test is accurate and can be conducted within a few days by an experienced analyst.

INTRODUCTION

The demand for seed and planting stock of native species has grown substantially over the past several years. The demand is driven by federal and state agencies, conservation groups and the public that want to use the plants for a wide range of land management applications. In the context of this paper, native plants can be defined as those plants endemic to a particular area that have not been traditionally used by land managers or produced by the seed and plant production industries for timber, ornamental and other uses. This would include grasses, wildflowers, forbs, wetland and many hardwood tree and shrub species. In order to meet the demand for these species, more seed companies and nurseries are working with these plants than ever before. Seed producers and nurseries need clean, viable, high quality seed of these species. Seed testing is an important component in the process of producing high quality seeds and plants. Test results can be used to manage a variety of activities from seed production and processing to nursery production and commercial sales. One test commonly used to evaluate seed viability is the tetrazolium or TZ test.

THE TETRAZOLIUM ESTIMATED VIABILITY TEST

The TZ test was developed in Germany in the early 1940's by Georg Lakon and introduced in the United States after World War II. The use of the test has increased and expanded since then because it can be completed quickly, usually within a few hours. This is a faster method of determining seed viability than a standard germination test and TZ results are commonly used in place of germination test results. The procedure is also used to determine the viability of ungerminated seed at the end of a germination test.

The TZ test measures the activity of the dehydrogenase enzymes used in the respiration process. Respiration is the cellular process of breaking down sugars to produce energy, carbon dioxide, and water, using oxygen. The enzymes react with substrates releasing hydrogen ions to the soluble tetrazolium chloride salt solution. The salt solution is reduced by the hydrogen ions. The colorless TZ salt solution is changed into an insoluble reddish compound called formazan. If the embryo and possibly endosperm or secondary nutrient reserve or storage tissues are actively respiring, formazan will be present and the tissues will stain red. Seed viability is determined by evaluating the amount of area stained, the intensity of staining, the pattern of staining and by evaluating other critical characteristics including turgidity, presence and damage of essential structures, abnormalities, and pathogen presence.

The estimated viability of a seed lot is based on the number of seeds per testing sample that display the required staining, structural and other characteristics necessary to classify a seed as viable. A viable seed is considered to be capable of producing a normal seedling under favorable conditions. Classifications of normal or abnormal, healthy or damaged are elaborated on in much detail in seed testing TZ handbooks.

Table 1 outlines the basic procedure and materials required to conduct TZ tests.

Table 1. The Tetrazolium test procedure and materials required.

Step and Description		Materials Required
Preconditioning.	Imbibing seed on a wet substrate or soaking in water. This makes it easier, to cut the seed in the preparation step and it will facilitate the staining process.	Preconditioning and staining require beakers, watch glasses, petri dishes paper towels, germination blotters and filter paper.
Preparation for staining.	In order for the TZ solution to come into contact with the tissues inside of the seed coat, the seed will usually need to be cut or pierced.	Tools to cut, pierce and crack seeds: razor blades, needles, clippers and tweezers.
Staining.	The tissue of the seed must be exposed to the TZ solution for some minimum amount of time at a set temperature.	Tetrazolium solution consisting of 2,3,5-triphenyl tetrazolium chloride, a buffer additive to balance the pH and water. Solution should be stored in an amber bottle designed for light sensitive material.
Post staining.	The seed may require another cut or removal of the seed coat to observe vital tissues that need to be analyzed during the evaluation phase.	Temperature control units. An oven cabinet or germinator that can the maintain 20^o to 40^oC. for staining and a refrigerator to store the TZ solution in or to hold stained seed for an extended period of time.
Evaluation.	This step can be quite complex and is beyond the scope of this paper but it basically consists of looking at the embryo and the other tissue to see if it has stained.	Evaluation tools. Light, magnification, handbooks.

The International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) have TZ testing handbooks. The 1985 ISTA handbook is a little hard to use but the information is thorough. The proposed 1998 AOSA publication (an update of the 1970 AOSA publication) will have introductory pages with general information and basic instructions and then specific procedures listed by family for approximately 300 genera in over 90 families. Most families will have one set of procedures listed for preparation, staining and evaluation.

Some families with a large number of diverse genera will have two or three sets of procedures listed. The handbooks are a guide that contain procedures that can be used by analysts with limited experience. The directions will establish satisfactory test conditions that will allow an analyst to evaluate good staining results on viable seed. Once an analyst is familiar with a particular method or has worked with a particular genera or family for some time and is familiar with the intricacies of preparation and evaluation, strict adherence to the guidelines may not be necessary to produce reliable, accurate results. These are reference books, not rule books.

The most important requirements to performing accurate, reliable tests are training and practice! It is much easier for an analyst to learn how to conduct a TZ test if they have access to the handbooks and if they can receive training from experienced analysts. The USDA Forest Service, National Tree Seed Laboratory and the Oregon state seed laboratory in Corvallis, Oregon routinely provide training on conducting TZ tests and are familiar with native plant species. There are many state and commercial seed laboratories that conduct TZ tests but most do not provide training. Most seed laboratories focus primarily on testing agricultural species and do not have the money, personnel, time or experience with native plants required to develop and teach a workshop that covers this subject.

Testing genera that do not have procedures established requires a bit of investigation by the analyst. The analyst should determine whether or not there are procedures developed for other genera in the family with a similar seed morphology. If there are, it is likely that the same procedures can be used. If there isn't any information on the family or if there are not any genera with a similar seed morphology, there are likely to be genera of another family that do have similar morphological characteristics and these procedures may be used as a starting point. It is a good idea when testing seed of a new species or genera, to test a seed sample of known viability. This will allow the analyst to see what healthy seed look like when stained and will eliminate some of the uncertainty each analyst has when testing a new species for the first time. Using seed lots of a known viability is also useful when training new analysts. As with any type of seed testing procedure, the results of the test are only as good as the sample taken and sent to the lab. Proper sampling procedures must be followed to obtain a representative sample.

A few of the companies that sell chemicals (including TZ chloride) and laboratory supplies and the contact information for the seed testing associations are listed in Table 2. The cost of equipment and supplies are listed in Appendix A.

Table 2. Sources of chemicals and supplies for TZ testing.

Fischer Scientific	800-766-7000
Aldrich	800-558-9160
Baxter	800-328-9696
Sigma	800-325-3010
Hoffman Manufacturing Inc.	800-692-5962
Seedburo	800-281-5779
Carolina Biological Supply	800-334-5551

Association of Official Seed Analysts
201 North 8th Street, Suite 400
PO Box 81152
Lincoln, NE 68501-1152
402/476-3852 TEL
402/476-6547 FAX

International Seed Testing Association
PO Box 412
8046 Zurich
CH-Switzerland
41-1-371-34-27 FAX

Conducting TZ tests is time consuming and requires a variety of materials and expertise. Unless a large number of samples will be tested, it is more cost effective to have tests conducted at a seed testing laboratory. Some of the public and privately owned laboratories that conduct TZ tests on some types of native plant species (as opposed to agricultural species) include those listed in Table 3.

Table 3. Seed testing laboratories that conduct TZ tests.

State Laboratories	California, Colorado, Idaho, Montana, Nebraska, New Mexico, North Dakota, Oregon and Utah
Private Laboratories	Seed Testing of America (STA) in Longmont, CO, Ransom Seed

	in Carpinteria, CA
Federal Laboratories	USDA Forest Service, National Tree Seed Laboratory Dry Branch, GA

TETRAZOLIUM AND GERMINATION TESTING OF NATIVE PLANTS

The results of a TZ test are based on live seed as opposed to the germination test which is a measure of viability based on the actual number of germinants under a defined set of test conditions. A germination test can provide accurate, reliable data on many native plant species. Two common problems with using a germination test, though, are that there may not be any published information on how to germinate a species and that successful germination may require a lengthy stratification or pretreatment period to overcome dormancy.

A dormant seed can not germinate even if there are otherwise favorable germination conditions. Dormancy can either be structural or chemical. A seed coat that blocks the uptake of water is considered a structural barrier. Chemical or physiological dormancy means that there is a chemical pathway that is blocked by an inhibitor or that may need to be established to allow the development or transport of essential chemical compounds or nutrients that are necessary to initiate germination. Breaking or overcoming physiological dormancy can take many months of stratification or conditioning. Completing this process however does not guarantee that all of the dormant seed will germinate.

Dormancy and environmental test conditions that can influence the results of a germination test do not usually affect a TZ test. Although dominant seed can be difficult to stain, an experienced analyst can determine the viability of a dormant seed lot.

The germination test and the TZ test both provide information about a seed lot that a grower or land manager can use. Sometimes, depending on the species, the information from either one of the tests is enough to base a decision on. Other times it isn't and it can be beneficial to have results of both types of tests. While the two tests will produce similar end results, a TZ test is not designed to determine the degree of dormancy present in a seed lot. This kind of information is produced by a germination test. For this reason, some seed laboratories, Ransom Seed Laboratory for example, will not conduct a TZ test alone. Determining which test will provide the data required depends on the species and on how the test results will be used. Ideally, the TZ test procedure will be used as part of a germination test to determine the number of potential germinants and the number of dormant seed. The two tests together also provide the best information on the type of damage that may have caused a seed lot to deteriorate.

TETRAZOLIUM TEST RESULT APPLICATION

Tetrazolium test results can be used throughout the process of producing seed and plants. Tables 4 and 5 list the applications and the advantages and disadvantages of tetrazolium testing. Since so many native species have seed that are difficult to germinate, using a TZ test is the best alternative at this time. The TZ test is a tool. Like any tool, it has an application that it was designed for and it has its limitations. A TZ test can provide fast, reliable information on the viability of a seed lot. The test can take from a few hours to a few days, but this is much faster than a standard germination test. The TZ test also requires specialized training and experience. An analyst unfamiliar with TZ testing has to have knowledge of seed morphology and have the ability and time necessary to learn how to evaluate or "read" a stain. The cost of the test and interpreting the results are also considerations.

Table 4. Tetrazolium Test Result Applications

Seed Harvest.	If seed collection of a particular species is time consuming or costly, a TZ test on a small collection will provide more information on viability than a cut test. This will prevent wasting time and money on seed that is not worth collecting.
Processing	A TZ test can be used to determine if a seed lot was damaged during seed cleaning, reducing viability.
Production	Land managers and nurseries often need to sow or plant before germination test data is available. Tetrazolium test results can be used to determine the number of seeds that need to be planted to produce the required number of plants.
Commerce	Tetrazolium tests provide a measure of quality upon which seed trade can be based. At the July 1997 meeting of the Association of Official Seed Certifying Agencies (AOSCA), participants relayed information indicating that prices for some native plant seed lots are determined by the field results (Karrfalt, 1997). Using TZ test results may help reduce some of the uncertainty present in this arrangement. Some states allow labeling and certification of some seed lots based on TZ tests.

Table 5. Advantages and Disadvantages of Tetrazolium Testing.

Advantages	Disadvantages
-Rapid estimate of seed viability	-Requires specialized training and experience
-Dormancy not generally a factor	-Labor intensive
-Detection of seed vigor	-May not detect minor damage
-Reliable backup for germination tests	-May not detect disease or fungal infection or chemical or fungicide damage
-Best when used as part of a germination test	-Test result interpretation and application
	-Cost

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[2]*USDA Forest Service, National Tree Seed Laboratory, Dry Branch GA 31020.*

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Appendix A. Equipment and Supplies Price List

		Laboratory set-up	Minimum to conduct
2,3,5-Triphenyl Tetrazolium chloride	10 gram bottle, cs of six	$270	$270
Buffer chemicals			
KH_2PO_4(Potassium Phosphate Monobasic)	500 grams	$40	$40
$Na_2HP0_4 \cdot 7H_20$ (Sodium Phosphate Dibasic Heptahydrate)	500 grams	$37	$37
Beam balance		$130	$130
Amber bottle	1 gallon	$10	$10
Glycerol	1 liter	$100	
Gibberellic Acid	1 gr not less than 90%	$20	
pH Tester		$60	
Razor blades	cs of 100	$7	$7
Clippers	set of two	$20	$20
Tweezers		$24	$24
Beakers	assorted sizes	$200	
Beveled watch glasses	cs of twelve	$50	
Petri dishes	cs of 500	$130	
Blotters	per 1000	$70	$70
Hand lens		$20	$20
Magnification Scope w/ light		$1000	$1000
Incubator	desk size	$800	
ISTA 1985 TZ Handbook		$30	$30
AOSA 1998 TZ Handbook		$50	....
	Approximately	$3100	$1650

This price list provides an approximate equipment and supply cost for the fewest items necessary to conduct a TZ test and for a small laboratory-type operation. The most costly item not included in a minimum set-up is the incubator. Tetrazolium tests can be conducted at room temperature but the seeds will take longer to stain and the temperature can not always be controlled. An estimate of $800 is on the low side for an incubator. Most incubators are larger than desk size and cost between $1500 to $3000.

The other major cost to consider is labor. An experienced analyst can prepare and evaluate a TZ test on 200 seeds in roughly 45 minutes. Some species take less time, others more.

Most seed laboratories charge between $35 and $65 per lot. Some labs have one set fee for all TZ tests while others set the price depending on the species to be tested. Forty-two tests at $40 each is approximately $1700. Seventy-eight tests at $40 each is approximately $3100. Most seed processing companies and nurseries do not conduct their own TZ tests. It is usually more cost effective to send the few samples each year that need to be tested in this manner to a seed lab.