- Different plant families may have embryos that require a specialized evaluation criterion. For example, unstained radicle tips are generally interpreted differently in grasses than in legumes.

- Abnormal staining patterns and TZ hue may be symptomatic of thermal or mechanical damage. Seed may be damaged by excessive heat or cold, or improper handling in the field, conditioning plant, or warehouse. Examples are: early freeze when seed has high moisture levels, overheating in windrows, mechanical damage during cleaning, improper storage conditions or damage during transport. Such damage may be evident in a TZ test as a watery red color, purplish red, or especially dark red and flaccid tissue.

- Some types of abnormal seedlings will not be detected in a TZ test. For example, seedlings may develop stunted roots in a germination test, even though radicles of embryos of seeds from the same sample stain normally in TZ. For this reason, paired TZ and germination tests should be conducted whenever possible.

15.1.3.1 False positives -

If seeds are over-stained, a false positive evaluation may result. This often occurs within the Poaceae family. Other tissues or other organisms (e.g. fungi or bacteria) can settle onto the cut surface of the seed and create a normal red staining appearance. With some seeds, the embryo and endosperm must both stain (eg. carrots and onions). If the embryo stains, butthe endosperm does not (or vice versa), the seed may have low vigor, produce an abnormal seedling, or be dormant. Interpretation of the staining pattern of both the embryo and nutritive tissue is critical.

15.1.3.2 False negatives -

Seeds that have deep dormancy may not stain at all because of low respiration levels. The seed will only stain in certain stages of physiological development. The analyst must be aware of dormancy within species, ways to overcome or break dormancy, and how to interpret a ‘no stain’ or ‘light stain’ in this situation.

Artifact damage may also give a false negative evaluation. Keep cutting implements sharp to avoid this problem. This damage often shows up as a white coloration over the cut surface of the seed and masks the red coloration below. This frequently occurs on seed that have a hard, brittle seed coat or floral parts. Artifact damage may also manifest itself as dark red to black coloration on the cut surface of the seed. This is like a ‘bruise’ and occurs on seed with softer tissues.

Embryos with chlorophyll can be difficult to interpret. The presence of chlorophyll is not alone an indicator of viability. If the green chlorophyll is metabolically active, there is no red coloration (e.g. *Acer*). If the tissue texture is firm (turgid), these seeds may be considered viable even though there is no stain. Green embryos in Chenopodiaceae are nonviable regardless of turgidity. In many legumes (Fabaceae), the green embryos indicate immature seeds. The texture of the immature seeds is usually soft (flaccid), and the cotyledons do not expand as they imbibe. These seeds are considered non-viable. In some species, both the green coloration of the chlorophyll and the red of the TZ stain may be present, giving a brownish color or tint (e.g. *Phlox*, *Kochia*). Consult the family pages for more specific evaluation criteria. Because of the difficulty in interpreting the TZ stain in seeds with green coloration from chlorophyll, a germination test is recommended.

15.1.3.3 Multiple seed units and multiple embryos -

Some species have seed units that produce multiple seedlings. These may be multiples of seeds or fruits that are attached together, such as beets, dogwood, little burnet, yellow poplar and buffalograss. Another kind of multiple is a seed that has more than one embryo within one true seed. Examples of species that can sometimes produce more than one embryo per seed are bluegrass, New Zealand spinach, and pine. The seed unit of either type is considered viable if at least one normally stained embryo is present. The tetrazolium viability is reported as the percentage of viable seed units.

Additionally, the analyst may be asked to report the total percentage or number of viable seeds or embryos in a given number of seed units and the number of seed units with two or more viable embryos. This way, customers can determine the actual number of 'total germinants' that they will have in the seed lot. (This is especially important for bare-root nursery production.)

example:

Cornus amomum (silky dogwood)
Tetrazolium test = 75% of seed units were viable.
Additional information:
Seed units of silky dogwood may contain two seeds. 10% of the seed units in this seed lot have a second live seed. There are 85 live seed per 100 seed units for this seed lot.

15.1.3.4 Immature seed

Immature seeds lack sufficient differentiation and/or development to germinate and produce a plant. These symptoms may indicate nonviability. Refer to the individual genus or family page for additional guidance.

Indications of immaturity can include:

- green embryos
- shrunken or smaller than average seeds
- seeds with undifferentiated essential stuctures or essential structures lacking

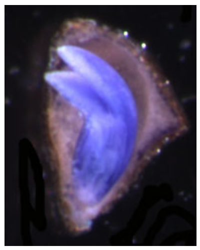
Immaturity in grasses:

- shrunken endosperm
- embryo appears as a solid undifferentiated mass of stained tissue
- separation of scutellum from endosperm

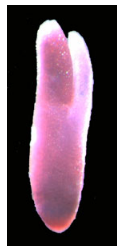
15.1.3.5 Pigmented embryos

Some species or varieties have naturally pigmented embryos that change the appearance of stained seed. These purple pigments are normal and not an indication of seed damage or decay. If the entire embryo is pigmented, the difference between stained and unstained tissue is much more subtle than usual. If the pigments are in spots on the embryo, the pigmented tissue will appear as muddy darkened red spots next to brighter red unpigmented tissue after staining.

Nierembergia 'Purple Robe'



before staining



after staining

Aster novae-angliae

before staining

after staining

15.2 TZ as a vigor test

A vigor test is an indicator of how well the seed may perform under a wide range of field conditions. Vigor is associated with seed age, hardiness, soundness, and health and is a measure of the physiological condition of the seed. Vigor tests can include growth measurements, stress tests, and biochemical tests.

The tetrazolium test is a 'biochemical vigor test'. At the same time the viability is being evaluated, the TZ test can be used secondarily as a vigor test. The intensity of the staining reaction is used as a basis of seed vigor. Older seeds tend to stain a much darker red, take up little or no stain, have a mottled staining pattern, or have less tissue turgor. More vigorous seeds tend to stain pink to red and show turgid, firm tissue.

Four categories used to estimate vigor as the seed is being evaluated for germination:
1) high vigor 2) medium vigor 3) low vigor 4) non-germinable

Seeds are placed in the respective categories based on: intensity of staining, location of deteriorated and/or dead tissue, amount of dead or dying tissue, and the development of the embryo. For details, refer to the AOSA *Seed Vigor Testing Handbook, Contribution No.32* or the ISTA *Handbook of Vigor Test Methods.*

PART II. TETRAZOLIUM TESTING PROCEDURES

USING THE HANDBOOK AS A REFERENCE GUIDE

Part II of the AOSA *Tetrazolium Testing Handbook, Contribution No. 29* describes the procedures for TZ testing by plant family. Taxonomic names and groupings of agricultural crops, vegetables, flowers, trees and shrubs, native species and weed seeds used in this handbook adhere to guidelines described in the *Uniform Classification of Weed and Crop Seeds, Contribution No. 25.* A list of genera included under each family heading is given. Some families have diverse genera with varying embryonic morphology and require more than one TZ procedure. These families are broken down into sub-groupings within a family (e.g. Fabaceae). The genera listed for each family are only select examples. Additional genera, that are not included within this handbook, exist for each family. The TZ testing procedures for various genera in a particular family are generally similar, if not the same.

This handbook is a reference and guide for all seed analysts who perform tetrazolium testing. An analyst, depending on his or her level of TZ testing experience, may choose to exercise some latitude with time, temperature, and solution concentration parameters when following the TZ procedures. Notes and methodologies submitted by experienced analysts have been included in the procedures for some families. Feel free to incorporate your own special notes on the procedure pages. With the long range goal of standardization of methods in mind, the TZ procedures in this document should be used as a 'baseline' of information and periodically updated as research and technology demands.

The intent of this handbook is that it be dynamic with continual improvements through future revisions. TZ procedures for families that analysts have had little previous experience with can be modified and enhanced over time by further study, including: testing data, experimentation, statistical analysis, and germination and dormancy data. Seed analysts and other users must take it upon themselves to take notes and make comments, conduct experiments, and suggest changes. This will ensure that this document will be concise and current with each revision.

HANDBOOK CONTENTS

Part I - Principles of Tetrazolium Testing
An introduction and overview into the principles involved with tetrazolium testing.

Part II - Tetrazolium Testing Procedures
Procedures on how to conduct and evaluate TZ tests on various plant families (methodology and explanation).

Illustrations
Illustrations in Part II of this handbook represent: 1) external/internal seed morphology; 2) diagrammatic preparation techniques in cutting or piercing the seed; and 3) embryonic and other vital tissues assessed in the TZ staining evaluation. Most evaluation illustrations are of intact embryos even though some embryos are bisected for evaluation or during preparation for staining.

Appendix
The Material Safety Data Sheet (MSDS) for 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) is provided.

Glossary
A glossary of terms and definitions is listed to assist the analyst in the terminology of seeds and TZ testing.

Index

A cross-referenced inventory is provided of genera to the proper plant family. (For associated common names, see the *Uniform Classification of Weed and Crop Seeds, Contribution No. 25.*)

Laboratory References

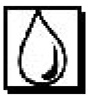
Seed laboratories that submitted or contributed to the Handbook's TZ testing procedures and the appropriate contact information.

Bibliography

A tetrazolium reference resource is listed in the back of the handbook. All pertinent TZ references have been consolidated for inclusion here. Some of the articles, notes, and texts relate closely to procedural TZ methods for specific seeds, while others may be more general or specialized in nature. This should serve as a valuable tetrazolium testing resource to a seed analyst.

SYMBOLS AND ABBREVIATIONS

Icons



PRECONDITIONING:
Seed moistening step prior to preparation and staining.

PREPARATION AND STAINING:
Methods and procedures of tetrazolium treatment

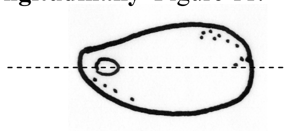
EVALUATION:
Physical staining patterns and evaluation of seed soundness to determine seed viability.

Illustration labels

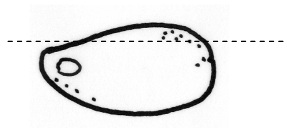
Seed structure	Label
Carpel	Carp
Caryopsis	Cary
Cotyledon	Cot
Embryo	Emb
Endocarp	Encp
Endosperm	End
Fruit wall	FW
Hilum	Hil
Hypocotyl	Hyp
Female gametophyte	FG
Membrane	Mem
Pericarp	Pcrp
Perisperm	Pspm
Plumule	Plu
Radicle	Rad
Scutellum	Scut
Seed coat	SC

TETRAZOLIUM SEED PREPARATION METHODS AND TECHNIQUES

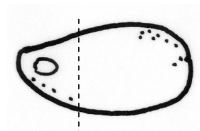
Bisect cut longitudinally Figure 11.



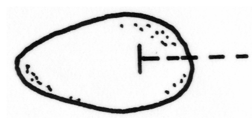
Off-center cut longitudinally Figure 12.



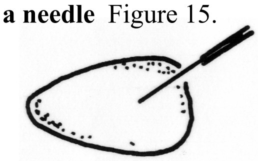
Cut laterally Figure 13.



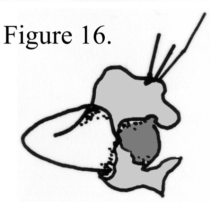
Superficial (cut or nick) Figure 14.



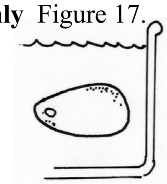
Pierce seed with a needle Figure 15.



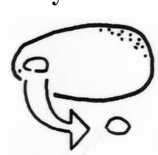
Remove seed coat Figure 16.



Preconditioning only Figure 17.



Excise (cut or tease) embryo from seed Figure 18.



No preconditioning or preparation Figure 19.