Seed quality studies of native shrubs
by Gerhard Peter Weber

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
Three forb end eight shrub species native to the Rocky Mountain and Northern Great Plains regions were evaluated for seed viability with 2,3,5-triphenyl-2H-tetrazolium chloride (TZ). Specific staining techniques were developed for each species. Treatments include presoaks, seed coat puncture, or removal, and seed bisection. Species tested and their viability values were Achillea millefolium L., (yarrow) 86%; Linum lewisii (Pursh), (Lewis flax) 93%; RatTbida columnifera (Nutt.) Wooten and Standley, (prairie coneflower) 72%; Amelanchier alnifolia (Nutt.) Nutt., (serviceberry) 84%; Amorpha fruticosa L., (indigobush) 94%; Artemisia tridentata Nutt., (big sage-brush) 66%; Ceretojdes lanata (Pursh) Howell, (winterfat) 91%; Prunus virginiana L., (chokecherry) 98%; Purshia tridentata (Pursh) DC., (antelope bitterbrush) 100%; Rhus trilobata Nutt., (skunkbush sumac) 91%; and Symphoricarpos albus (L.) Blake, (snowberry) 68%.

Skunkbush sumac (Rhus trilobata Nutt.) and serviceberry (Amelanchier alnifolia Nutt.) are native shrubs extensively distributed in the western United States, and have achieved importance for their use in revegetation of disturbed lands. Standard germination tests were performed on each species according to methods outlined in the literature. Seed viability was determined with triphenyl tetrazolium chloride (TZ). Results indicated special techniques would be required to affect the rapid germination which is needed in current seed testing programs. Both species have hard or impermeable seed coats and embryo dormancy normally overcome by cold stratification or fall sowing. Results confirm that skunkbush sumac germination is promoted by 75 minutes acid scarification and that KNO3 or GA produce no additional response. Acid scarification for 30 minutes and a mixture of thiourea (TU) and benzyladenine (BA) as a moistening agent for the media was beneficial to serviceberry germination. Analysis predicted maximum germination to occur at 100 ppm BA and 100 mM TU. An interaction of BA and TU on germination was observed at the lower concentrations tested.

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature Gerhard Peter Weber

Date September 2, 1980

SEED QUALITY STUDIES OF NATIVE SHRUBS

AND FORBS

by

GERHARD PETER WEBER

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Agronomy

Approved:

Loren E. Wiesner
Chairman, Graduate Committee

Hayden Ferguson
Head, Major Department

Michael Malone
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August, 1980

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. Loren E. Wiesner for serving as my major professor and for giving freely of his advice, guidance and friendship; Dr. Ronald Lockerman for his candid advice and for help with the preparation of this thesis; Dr. Jarvis Brown and Mr. Lee Hart for their help and cooperation with the completion of my degree program; and Dr. Richard Lund for helping with the statistical analysis of the data presented in Chapter II of this thesis.

I also thank my wife, Debby, for giving her support and for showing the patience and perseverance necessary when one is married to a graduate student.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Three forb and eight shrub species native to the Rocky Mountain and Northern Great Plains regions were evaluated for seed viability with 2,3,5-triphenyl-2H-tetrazolium chloride (TZ). Specific staining techniques were developed for each species. Treatments include presoaks, seed coat puncture, or removal, and seed bisection. Species tested and their viability values were *Achillea millefolium* L., (yarrow) 86%; *Linum lewisii* (Pursh), (Lewis flax) 93%; *Ratibida columnifera* (Nutt.) Wooten and Standley, (prairie coneflower) 72%; *Amelanchier alnifolia* (Nutt.) Nutt., (serviceberry) 84%; *Amorpha fruticosa* L., (indigobush) 94%; *Artemisia tridentata* Nutt., (big sagebrush) 66%; *Ceretoides lanata* (Pursh) Howell, (winterfat) 91%; *Prunus virginiana* L., (chokecherry) 98%; *Purshia tridentata* (Pursh) DC., (antelope bitterbrush) 100%; *Rhus trilobata* Nutt., (skunkbush sumac) 91%; and *Symphoricarpos albus* (L.) Blake, (snowberry) 68%.

Skunkbush sumac (*Rhus trilobata* Nutt.) and serviceberry (*Amelanchier alnifolia* Nutt.) are native shrubs extensively distributed in the western United States, and have achieved importance for their use in revegetation of disturbed lands. Standard germination tests were performed on each species according to methods outlined in the literature. Seed viability was determined with triphenyl tetrazolium chloride (TZ). Results indicated special techniques would be required to affect the rapid germination which is needed in current seed testing programs. Both species have hard or impermeable seed coats and embryo dormancy normally overcome by cold stratification or fall sowing. Results confirm that skunkbush sumac germination is promoted by 75 minutes acid scarification and that KNO_3 or GA produce no additional response. Acid scarification for 30 minutes and a mixture of thiourea (TU) and benzyladenine (BA) as a moistening agent for the media was beneficial to serviceberry germination. Analysis predicted maximum germination to occur at 100 ppm BA and 100 mM TU. An interaction of BA and TU on germination was observed at the lower concentrations tested.

LITERATURE REVIEW

Seed Viability as Determined by Tetrazolium

Historical

Seed production and marketing depends upon the accurate assessment of seed quality, specifically purity and germination. Whereas, the former may be evaluated in a few minutes, the latter may require a time range of two weeks to several months. Decisions regarding the processing and marketing of a particular seed lot may often be delayed pending the results of germination tests. For this reason rapid methods of assessing seed viability have been studied since the early 1900's (17, 35).

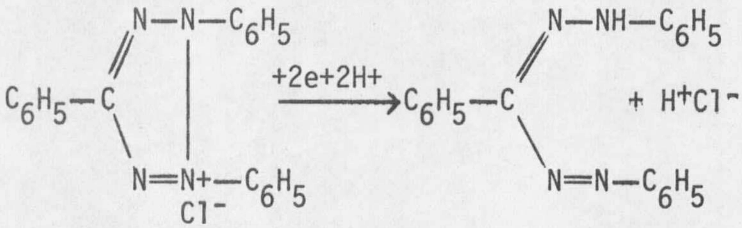
These early investigations concentrated on chemically analyzing ground and pulverized groups of seeds. Unfortunately, the localized defects of individual seeds such as fractures or dead tissues escaped detection with these methods (69). One method was based on the theory that nonviable seeds were more permeable than live seeds and would readily lose electrolytes to a water solution (43). Measurements of the electrical conductivity of the leachate would vary directly with seed viability. This technique has recently been more successfully applied to determinations of seed vigor (35). Another method (16), involved measurements of the relative quantities of heat produced by a seed sample. High heat production was related to extensive microbial activity, whereas low heat was thought to indicate poor viability and vigor.

Viability assessment based upon the evaluation of individual seeds was eventually recognized as being superior to group evaluations (69). The techniques used were based on vital staining; that is, the ability of some materials to differentially stain live and dead tissue. Moore (69) credits Dr. Turina of Yugoslavia with the development of vital staining techniques and Dr. Neljubow of Russia for applying the use of indigo carmine, a tontoxic dye, to this technique. He found that this dye would penetrate dead tissue more readily than healthy tissues. Evaluations of viability were based on the relative proportions of colored and uncolored tissues. However, vital staining based on dyes was found to be of limited value.

Hasagawa is credited (69) with the development of a vital staining procedure based on the seed's metabolic processes. With the application of selenium and tellurium salts, enzymatic activity within the seed would affect a color change. As described by others (83, 17, 69, 12) work with these techniques was taken up by the German scientist, Lakon, who developed the topographical viability test. This test stressed the reaction of specific seed tissues and resulted in considerable accuracy in seed viability assessment. The toxic selenium salts were soon abandoned by Lakon in favor of nontoxic tetrazolium salts which functioned in the same manner. He found that when the colorless tetrazolium solution came in contact with live seed tissue it was reduced to an insoluble red pigment; whereas, nonliving tissue did not stain. Viability was then estimated by evaluation of the extent and location of stained embryo tissue.

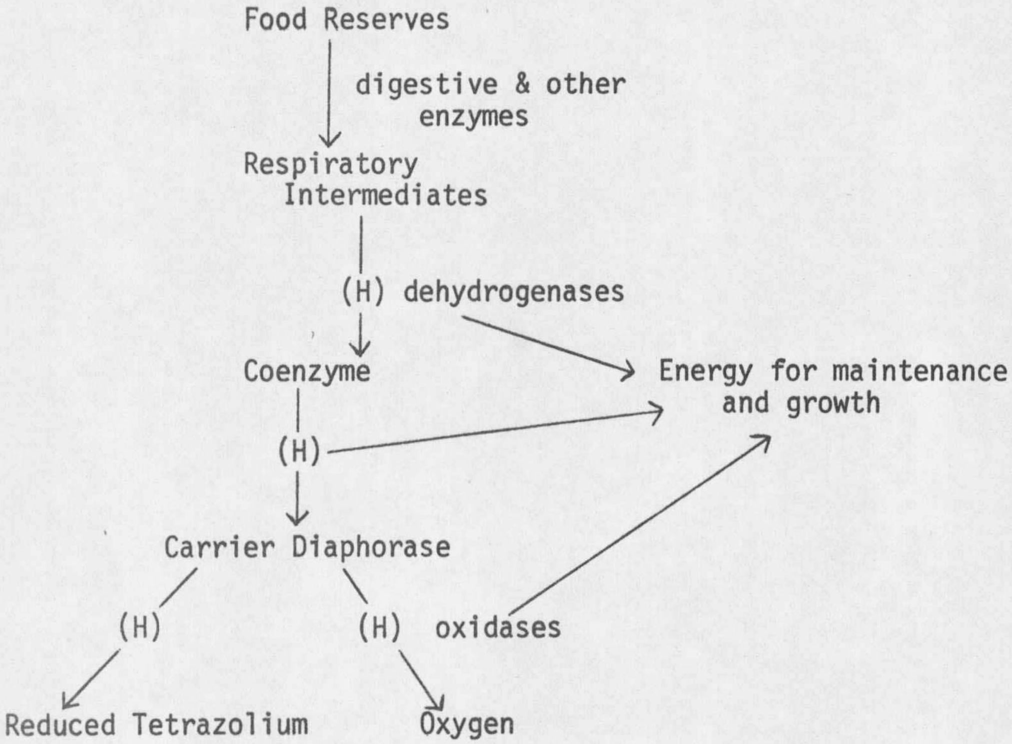
Specific Mode of Action

Of the several types of tetrazolium salts, the 2,3,5-triphenyl tetrazolium chloride derivative, now commonly known as TTC or TZ, is best suited for use in the topographical test (17, 35). Tetrazolium chloride occurs as a white to pale yellow crystalline powder, readily water soluble and darkens on exposure to light. In the presence of viable tissue the colorless TZ solutions forms the insoluble red triphenyl formazan according to the following reaction (83):



Colorless Red Color

Detailed studies (51, 83, 84) have indicated that one or more of the dehydrogenase enzyme systems appears to be involved in the reduction reation. Specifically, the reduction of TZ in corn embryo tissues is catalized by diphosphopyridine nucleotide (DPN)-linked dehydrogenases, particularly the malic and alcohol systems, and is mediated by diaphorase (84). This sequence may be summarized in the following way (85):



The ability to measure dehydrogenase activity is of particular significance. These enzymes are of a delicate nature and are responsible for the maintenance of energy without which the embryo could not remain alive (85). Therefore, the loss of active dehydrogenases probably indicates loss of germinating ability (51). Measurement of other enzymes have not provided consistent results. For example, the hydrolytic enzymes responsible for food mobilization and other respiratory enzymes such as catalase and peroxidase are of a more stable nature and have been shown to exist in dead seeds (85).

Other advantages of tetrazolium include its nontoxicity and that it is one of the few organic compounds which is colored in the reduced state (83). The insolubility of the reduction product is important. Since the reaction occurs within cells and the pigment is nondiffusable, there is a sharp delineation between viable and nonviable tissue (17). Finally, it has been shown through extensive testing that loss of dehydrogenase enzyme activity tends to parallel loss in seed viability (85). This characteristic coupled with a thorough knowlege of seed structure, makes possible accurate viability assessment in much shorter times than similar results obtained with germination tests.

The Seed

A true seed is a fertilized mature ovule containing the embryonic plant, stored nutrients and the integument(s) differentiated as the protective seed coat or testa (25, 57, 62). The term "seed" is usually applied to the unit of dissemination. Many dispersal units which are often referred to as seeds are not true seeds but single, sometimes two to several seeded fruits. The pericarp remains and may even fuse to the testa, as in cereal grains (57, 8). Physiologically and biochemically, these dispersal units should be considered as seeds (8, 95).

The seeds of Angiosperms develop as a result of a process called "double fertilization" (62). The embryo is derived from the fertilization of the egg cell by one of the male nuclei from the pollen tube. The "second" fertilization is the fusion of a male pollen nuclei with two mother plant polar nuclei. This fertilization results in the development of the endosperm which may persist as a storage organ, or degenerate and remain rudimentary, possibly fused to the seed or fruit coat (62). The testa or true seed coat comprising the embryo envelope is derived from one or both integuments of the ovule. At times the testa may be derived from tissues other than that of the integuments, e.g., the nucellus, the endosperm or rarely the chalaza (8, 62). The testa is usually a hard coat. Its physiological importance is derived from an often fatty or waxy cuticle, and one or more layers of thickened protective cells (8, 62). These features are responsible for variable degrees of impermeability to water and/or gases and consequently exert some regulatory influence over the respiration of the embryo (8, 62). The features of the testa in some species are apparently lacking, the outer covering being derived from extra-ovular tissue is called a pericarp. Seeds with this structure are actually fruits, for example sunflowers (62).

Germination

Germination, as defined by Bewley and Black (8) consists of those processes which begin with water uptake and successfully terminate with the emergence of the radicle or hypocotyl through the seed coverings. This definition is deceptively simple, as the physiological events leading to the protrusion of some part of the embryo through the seed coat are not well understood. Additionally, where germination ends and growth begins is difficult to delineate. Emergence of the radicle may indicate that germination has occurred but this may already be considered a part of growth (62). Whether this growth is a result of cell expansion, cell division or both is still unresolved (7). Probably the fundamental processes which cause germination are different from those of growth (8, 62, 80).

A seed must be in a favorable environment for germination to occur. Specifically, there must be adequate moisture, suitable temperatures, a correct ambient gas composition, and in certain photoblastic species, a requirement for light (62, 8). A seed which is unable to germinate due to a lack of one or all of these environmental factors is termed quiescent (95, 62, 80).

Dormancy

Dormancy describes the condition in which an otherwise viable seed fails to germinate under environmental conditions suitable for germination (95, 62), and is considered advantageous in adopting the growth