

SEED SCIENCE
Horticulture and Crop Science 420

M. B. McDonald
312B Kottman, 292-9003

SYLLABUS
Autumn Quarter, Every Year

I. TEXTS: *Seed Science and Technology*, L. O. Copeland and M. B. McDonald, 2001, Kluwer Press, 4th edition, 409 pp.

II. WEBSITE: Class content for this course can be accessed on OSU's CARMEN server. To login, use your OSU Internet Username (lastname.#) and its password (same as you use for accessing OSU e-mail and the one you used to enroll at the registrar's site).

III. GUEST SPEAKERS:

Andrew Evans, Seed testing and seed testing organizations
Dr. David Tay, Ornamental Plant Germplasm Center and the National Plant Germplasm System
John Armstrong, Ohio Seed Improvement Association and seed certification
Mike Gahn/Dennis Wickham, Pioneer Seed Company and the seed industry

IV. CLASS SCHEDULE:	Lecture: M W F	9:00 a.m.	334 KH
	Laboratory:	ARR	321 KH

IV. INSTRUCTOR HOURS:	M W F	10:00 a.m.	312B KH
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V. GRADE DISTRIBUTION:	Mid-term I	20%
	Mid-term II	20%
	Final	30%
	Laboratory	30%

VI. HOLIDAYS:	Veteran's Day
	Columbus Day

VII. COURSE SYLLABUS:

General Objectives

The student will be able to:

- Describe the physiological and morphological processes of seed development as modified by the environment from anthesis to maturity.
- Apply introductory principles of biochemistry and physiology to the processes controlling seed viability.
- List those seed harvesting and conditioning factors that contribute to seed deterioration and vigor.
- Diagram the handling of seeds from the producer to the consumer including testing, regulatory, and marketing practices.
- Identify and use seed conditioning equipment.

Discussion Topics and Instructional Objectives

- I. Seed Formation and Development. The student will, given the required information
- a) identify economically important crop and weed seeds
 - b) identify seed morphological structures and detail their development

- II. Seed Chemistry. The student will, given the required information
- identify the chemical structure of seed carbohydrates, lipids and proteins
 - describe the importance of seed carbohydrates, lipids and proteins as storage reserves
 - describe the types of chemical compounds found in seeds
 - list the factors that influence the chemical composition of seeds
- III. Seed Germination. The student will, given the required information
- outline the differences between seed germination and deterioration
 - identify the difference between hypogeal and epigeal germination
 - list the environmental factors required for seed germination
 - list the stages of seed germination
 - identify the chemicals that promote seed germination
 - list the factors that influence imbibition
 - describe the roles of carbohydrates, lipid and protein metabolism during germination
 - describe the roles of endogenous hormones and their interactions on seed germination
- IV. Seed Dormancy. The student will, given the required information
- identify those environmental, physical and physiological factors that cause seed dormancy
 - list those preharvest factors that influence hard seed coat dormancy
 - describe the difference between scarification and stratification
 - describe the roles of endogenous hormones and their interactions on seed dormancy
- V. Seed Vigor. The student will, given the required information
- list those vigor tests, their advantages and disadvantages, that are currently being used in seed testing
 - list those pre- and post-harvest factors that are most influential in seed vigor expression
 - list those crops that are most likely to suffer seed vigor loss
 - name several methods to stimulate seed vigor
- VI. Seed Longevity and Deterioration. The student will, given the required information
- name crop and weed species that are noted for their short- or long-lived seed
 - detail the influence of temperature and relative humidity, independently and jointly on preserving seed viability in storage
 - identify the appearance of the symptoms of seed deterioration
 - identify the endogenous physiological and biochemical mechanisms of seed deterioration
- VII. Seed Testing. The student will, given the required information
- outline the four components of a purity evaluation
 - define a noxious weed
 - explain the “normal seedling” concept in interpreting laboratory germination tests
 - describe several techniques of distinguishing varieties in a seed testing laboratory
- VIII. Seed Testing Organizations. The student will, given the required information
- name and list functions of seed testing organizations
 - describe the differences between breeder, foundation, registered, and certified seed
 - explain the advantages and disadvantages of seed certification
- IX. Seed Production. The student will, given the required information
- describe the importance of equilibrium moisture content and its influence on seed drying
 - describe grass seed production of warm and cool season grasses
 - describe hybrid seed production
- X. Seed Marketing. The student will, given the required information
- describe how varieties are developed and become eligible for certification
 - list the principles of the Plant Variety Protection Act

- c) outline the advantages and disadvantages of growing seed under contract
- d) describe the role of the Federal Seed Act in seed marketing

VIII. ACADEMIC MISCONDUCT: Students are trusted to act in good faith in exams, laboratory projects, and essays. If we personally determine that students have breached that trust, we will report this through appropriate channels to the University Committee on Academic Misconduct. The OSU Student Handbook thoroughly covers the subject of academic misconduct and its treatment, if students require further information. Academic misconduct will not be tolerated.

IX. DISABILITY ACCOMODATIONS. Students with chronic disabilities are encouraged to inform the instructor before or immediately at the start of the term. The instructor will work with the student and the Office of Disability Services to provide appropriate accommodations. No special accommodations will be made for students who do not inform the instructor in a timely fashion, or who do not involve the Office of Disability Services. Temporary disabilities will be accommodated at the instructor's discretion.

Laboratory

The laboratory is an arranged exercise. A Teaching Assistant will assist you with the exercises. There will be a dedicated lecture that will explain all of the objectives of this laboratory exercise. Andy Evans, Registered Seed Technologist, 321 KH, will also be present to answer any questions you may have.

SEED QUALITY TESTING LABORATORY EXERCISE

Miller B. McDonald
Seed Biology Program
Department of Horticulture and Crop Science
The Ohio State University

INSTRUCTIONAL OBJECTIVES

Upon completion of this exercise, the student should be able to

1. Understand the role and importance of the standard germination, tetrazolium and accelerated aging vigor tests in quantifying seed quality.
2. Identify and define the following conditions encountered in germination testing: normal seedlings, abnormal seedlings, firm seeds, and hard seeds.
3. Conduct a standard germination test using established equipment and protocols.
4. Distinguish between first and final counts and the objectives of each.
5. Use the Association of Official Seed Analysts Rules for Testing Seeds in conducting standard germination tests.
6. Evaluate nongerminated seeds for dormancy using the Tetrazolium (TZ) test.
7. Conduct a TZ test and make correct evaluations of test results.
8. List the distinctions between seed viability and germination testing.
9. Define and contrast seed vigor with the standard germination and TZ tests.
10. Perform an accelerated aging test using protocols from the International Seed Testing Association Vigour Test Handbook.
11. List standardization problems associated with seed vigor tests.

Germination Testing

Why test seeds for germination? This question is seldom asked because there is an intuitive answer: "Because we have to know how the seeds will perform in the field or appropriate planting environment." But, there are even more subtle rationales than this. Seeds are tested for germination because a seed lot is composed of a population of individual seed units; each possessing its own distinct capability to produce a mature plant. A seed germination test is an analytical procedure to evaluate seed viability and germination under standardized conditions. It enables the seed producer to determine and compare the quality of a seed lot before it is marketed to the consumer. Thus, we test seeds for germination not only to determine how they will perform in the planting environment, but also to determine the planting value of a seed lot, its storage potential, to satisfy labeling laws, and to provide for standardized marketing of seed lots.

On the one hand, the purpose of a germination test is to provide an indication of the plant producing ability of the seed under favorable conditions. On the other hand, germination test results must be reproducible among seed testing laboratories in order that the quality of the seed can be assessed by differing individuals - a process permitting interstate and global shipment of seed lots and allowing verification of results reported on seed labels. While the standard germination test provides an indication of seed viability, it is also designed to be reproducible (a process called standardization). This is reflected in the Association of Official Seed Analysts (2000) definition of seed germination that states "germination is the emergence and development from the seed embryo of those essential structures which, for the kinds of seed in question, are indicative of the ability to produce a normal plant under favorable conditions." Note that seeds are tested under favorable conditions; conditions seldom encountered in the field. While this process often leads to an overestimation of field emergence, it does focus on test repeatability since these favorable conditions presumably lead to an optimization and standardization of conditions so that the maximum germination percentage is obtained and can be consistently reproduced.

Germination testing is conducted under standardized conditions that are defined by the Rules for Testing Seeds (AOSA 2000). The Rules provide specific testing requirements such as substrata, temperature, test duration, additional directions and seedling evaluation criteria for the species being tested. Without question, the Rules represent the single most important contribution to standardization of seed testing and are an invaluable asset to the seed analyst in the conduct and interpretation of a germination test.

Source of Seeds for Germination. The first and most critical question when germinating seeds is what kind of germination methods will be used? This question is answered following a purity test. At this time, the purity test results identify what seed kind is being evaluated using scientific nomenclature. Both scientific and common names are used throughout the germination tables. Appropriately, the scientific nomenclature is provided first and the kinds of seeds are listed according to alphabetical priority in the tables using this nomenclature. The answer to the original question concerning what kind of test procedure to use in the germination test is determined at the time of the identification of the pure seed sample.

Seed Counting and Spacing. Counting of seeds for a germination test can be accomplished by hand, by a counting board, or with a vacuum seed counter. Counting boards generally are used for counting large seeds such as corn, beans, peas, etc. A counting board consists of two perforated wooden or plastic boards approximately the size of the planting substratum with 25, 50 or 100 holes that are slightly larger than the seeds to be counted. The two perforated boards are offset enough to permit the holes of the top board to rest on the solid portion of the bottom board. Seeds are placed on the board and scattered until one seed fills each hole. Excess seeds are removed by tilting the counting board and allowing the seeds to slide or roll off into a suitable container. The counting board is placed directly over the planting substratum, the top board moved until the holes of each

perforated board are aligned. At that point, the evenly spaced and counted seeds fall through the bottom board onto the planting substratum.

Vacuum counters are generally preferred for small, free-flowing, smooth seeds although they can be used for large seeds with similar properties provided sufficient vacuum is available. The heads are generally metal (brass or aluminum) or plastic. The face of the counting head contains a ridge around most of the periphery to retain seeds and usually 50 to 100 evenly spaced holes. Seeds to be counted are placed on the counting head, the vacuum turned on, and the seeds spread over the head surface. As this occurs, the suction of the vacuum retains a single seed over each hole. Excess seeds are poured off the head. The counting head is then positioned over the substratum, the vacuum turned off and the evenly spaced and counted seeds drop on the planting substratum.

The proper spacing of seeds is important in germination testing to reduce potential disease problems associated with microorganism infestations and to permit certain seeds that increase dramatically in size following imbibition appropriate room to expand. As a general rule, the distance between seeds should not be less than 1.5 to 5 times the width or diameter of the seed being tested.

Substrata. The general requirements of any germination substratum are that it provide adequate moisture and aeration for the germinating seeds, is nontoxic to germinating seedlings and is relatively free of fungi and other microorganisms. The most popular germination substrata are blotters and towels although sand or soil, filter paper, and creped cellulose paper (Kimpak) can also be used.

Germination blotters placed in plastic boxes are used primarily with small seeds and those requiring light. The method for adding water (or KNO_3 solution) to blotters (as well as paper towels) is very important to insure an even and thorough uptake of water or solution by the substrate. The blotter (paper towels) should be placed into the liquid and allowed to absorb as much water as possible. The liquid should never be added directly to the blotters in the germination dishes unless a precise delivery system is used that insures equal amounts of water and sufficient time for the blotters (towels) to uniformly absorb the water. When the blotter (towels) is saturated with water prior to the germination test, the excess water is allowed to drain prior to use. Tests should be monitored daily and water added when necessary. A "rule of thumb" is that the substratum is too wet if, by pressing the substratum with the thumb, a film of water surrounds the thumb. Fungal problems are less pronounced in blotters kept slightly on the dry side, particularly when germinating seeds at higher temperatures.

Rolled towel germination tests are usually placed in the vertical position. A properly rolled paper towel test will be loose enough to permit normal expansion of seeds and seedlings throughout the test period. A tightly rolled towel can cause abnormal seedling development, encourage the spread of fungi, and make unrolling difficult because of the potential for seedling breakage. A loosely rolled towel can allow seed movement, inadequate seed/towel contact for normal imbibition, and more rapid evaporation of water from the paper towels. Drying can be avoided by covering paper towels with plastic bags or placing them in plastic boxes.

Temperature. The ability of a seed to germinate rapidly is largely dependent on temperature. For this reason, temperature guidelines are precise and must be maintained by specialized germination equipment. In the Rules, temperatures are presented as constant temperatures expressed as a single temperature for 24 hours (e.g., 20°C) or alternating temperatures expressed by two temperatures (e.g., $15\text{-}25^\circ\text{C}$) separated by a dash. For alternating temperatures, the first temperature is for 16 hours and the second for 8 hours per day.

Light. Light is known to break dormancy of some seeds culminating in enhanced germination and improved seedling development (in order to evaluate essential structures with greater certainty) in others. When light is required, seeds should be illuminated for at least 8 hours in the 24-hour cycle. In those instances when light and alternating temperatures occur

simultaneously, the light period should be given during the high temperature cycle. All seeds requiring light should be germinated on top of the specified substratum.

Duration of Test. During the germination test, one (first count) or more may be necessary. The first count, which is approximate and can deviate one to three days from the specified time, serves a number of important functions. First, it enables the recording of the number of seedlings that germinated rapidly providing the student a view of the rate of germination of the seed lot. Second, the seedlings that have been classified as germinable can be removed from the substratum, thus conserving substratum moisture and reducing crowding of rapidly growing seedlings. Third, the first count ensures that the student evaluates the moisture status of the substratum and makes appropriate adjustments. Fourth, the disease status of the seedlings can be studied. First counts have a place in providing students additional information concerning the germination capability of the seed lot that the final count cannot supply. The final count identifies the time at which the germination test is terminated. It is generally considered a time when all seeds have had sufficient time to express germination capability.

Evaluation of Seedlings. Throughout the test duration, seedlings are evaluated for normal or abnormal classification. Normal seedlings are defined by the Rules as "seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable conditions." Abnormal seedlings are defined as "all seedlings that can not be classified as normal seedlings." Only normal seedlings are considered germinable and reported in the percent germination. Normal seedlings have a well-balanced symmetrical growth pattern of all their essential parts.

Without question, the ability of a seed analyst to discriminate and classify normal and abnormal seedlings is one of the most subjective aspects of seed testing requiring constant education and training to assure uniformity in interpretations. The criteria for normal seedlings are set forth in AOSA Handbook 35, Seedling Evaluation Handbook. In addition, line drawings depicting differences between normal and abnormal seedlings are provided to enhance the ability of a student to differentiate these specific classes and serve as a guide in discriminating among questionable seedlings. This Handbook is available for your perusal.

Provide the following information for the germination test your used for your seeds:

Kind of Seed:

Seeds per Test/Number of Replicates:

Substrata:

Temperature of Test:

First count days:

Final count days:

Additional Germination Procedures:

Percentage Normal Seedlings

Percentage Abnormal Seedlings:

Percentage Dead Seeds:

Percentage Dormant Seeds:

Other Seeds:

Answer the following questions:

1. What is the difference between seed viability and seed germination? How does the AOSA definition of seed germination generally contrast with a plant physiologist's definition? Which definition do you prefer and why?

2. What testing mechanism would you develop or employ to ensure that germination testing laboratories are achieving standardized germination results for the same seed lot?
3. Why are seeds used in a germination test taken only from the pure seed portion obtained in a purity analysis?
4. Define "pure live seed" and describe why it is important to understand this parameter .
5. The environmental conditions that can be controlled and altered by the seed analyst during a standard germination test include moisture, aeration, temperature, light, and substratum. Of these five factors, which do you think is most critical in maintaining an optimum seed germination test and why?
6. Distinguish between hard and swollen seeds. How can you often tell whether a seed is dead and not dormant simply by examining it on a paper towel or blotter?

Tetrazolium Testing

Germination testing does not always provide an accurate assessment of the plant producing ability of a seed lot. In many cases, seeds may be alive, but fail to germinate because of intense dormancy at the time the germination test is conducted. This dormancy, however, may be short-lived, and those seeds that did not germinate may produce seedlings at the time of planting. Seed viability can be defined as "the capacity of a seed to germinate under favorable conditions in the absence of dormancy." Note that this definition is distinguished from germination, which assesses the ability of a seed to germinate under favorable conditions. Thus, the difference between a viability and germination test is represented by the percentage of dormant seeds.

Consult the AOSA Tetrazolium Testing Handbook for appropriate TZ procedures for your crop. If none exist, develop them using similar descriptive protocols in the TZ Handbook. Since many seeds are neither completely alive nor completely dead, knowledge of the relationships of seed structures to seedling structures is necessary to interpret the importance of unstained seed tissues. For example, to the beginning practitioner of TZ testing, the intensity of red (formazan) color formation that is a consequence of respiration is indicative of the quality of the seed lots. While this may be true in some instances, it is not always so. For example, a particular seed may be completely stained by TZ with the exception of a small necrotic lesion on the radicle tip. Even though the rest of the seed is completely viable, since the seed is incapable of producing a root, it would be considered nongerminable. This example illustrates that it is not the intensity of staining, but what is stained that is important. Thus, one must be familiar with the function of the various seed parts in making a correct TZ test.

Often speed is of great concern when trying to determine seed quality. The Tetrazolium (TZ) test is one of the most widely used and helpful seed quality tests. The TZ test distinguishes between viable and dead tissues of the embryo on the basis of their relative respiration rate in the hydrated state. Although many enzymes are active during respiration, the test specifically utilizes the activity of dehydrogenase enzymes as an index of the respiration rate and seed viability. Dehydrogenase enzymes react with substrates and release hydrogen ions to the oxidized, colorless, tetrazolium salt solution that is changed into red formazan as it is reduced by hydrogen ions. Seed quality is interpreted according to the topographical staining pattern of the embryo and the intensity of the coloration.

The TZ test is often called a topographical test because the pattern, or topography, of staining is an important aspect of interpretation. Some seeds are neither completely alive nor completely dead. The staining pattern reveals the live and dead areas of the embryo and enables the student to determine if seeds have the capacity to produce normal seedlings. The cell division areas of the embryo are most critical during germination, and if they are unstained, or abnormally stained, a seed's germination potential is weakened.

The TZ procedure may be different for different seed types, but the following four steps are generally recognized when conducting a TZ test: preconditioning, seed preparation, staining, and evaluation. The AOSA Tetrazolium Testing Handbook (2000) should be consulted whenever questions concerning TZ methodology arise.

Preconditioning. During preconditioning, seeds are hydrated by placing them in water or between wet substrata such as blotters or paper towels. As the seeds take up water, dehydrogenase enzymes become active which later react with TZ to indicate viability. Preconditioning is generally conducted under temperatures favorable for germination.

Seed Preparation. During seed preparation, seeds are either cut or pierced to facilitate entry of the TZ solution into the embryo. Not all seeds require special preparation. For instance, many dicot seeds such as beans, peas, vetch, and small legumes are placed directly into TZ without piercing or cutting. Such seeds are predominantly embryo and readily absorb TZ. Those seeds not absorbing TZ, from a species known to possess hard seeds, are listed as hard during the evaluation process, just as is done during a germination test.

Staining. During staining, seeds are placed in a TZ solution (usually 1.0 or 0.1 %) and placed in an oven at about 38°C to hasten the staining reaction. Respiring embryonic tissue will stain; however, storage tissue such as the endosperm of grasses will not stain. The general rule is: Use 0.1% TZ when the seed is bisected through the embryo before it is put in TZ; use 1.0% TZ when the seed is bisected laterally or diagonally, or pierced, or when no preliminary incisions are needed as for legumes. The length of the staining period varies with species. If seeds are kept too long in the TZ solution, they will become overstained, making evaluations difficult.

Evaluation. Evaluation requires the most experience. Beginning seed analysts should compare TZ and germination results until they are confident in their ability to read TZ tests. Some seeds (such as grasses) may be placed in a clearing solution (such as 85% lactic acid) in order to clearly see through the lemma and palea to evaluate the stain.

Provide the following information on the TZ testing protocol you used for your seeds:

Preconditioning Protocol -

Seed Preparation -

Staining Protocol -

Evaluation Criteria - (You may wish to provide drawings)

After staining, make your evaluations of seed quality on the following table:

Crop	TZ Interpretation	
	% Germination	% Nongerminable
Seed lot A		
Seed lot B		

Answer the following questions:

1. Can the TZ test distinguish between dormant and non-dormant seeds? Explain.
2. Besides color intensity, what other seed features must be monitored for an accurate TZ test result?
3. What do you think would happen if you stained your crop seeds with either 1.0% or 0.1% TZ solution instead of the concentration you used?
4. Are your TZ results the same as the percent germination if the seed lots studied were not dormant? Why?

Seed Vigor Testing

While the standard germination test remains the seed industry's prime method for identifying the quality of a seed lot, in reality, germination results often overestimate actual field emergence. The major reason for this overestimation is that germination tests are conducted under favorable conditions that are seldom encountered in the field. In order to provide a more accurate appraisal of seed quality as it relates to field emergence, the concept of seed vigor was established. Seed vigor is defined in the AOSA Seed Vigor Testing Handbook as "those seed properties that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions." It is well established that declines in seed vigor precede those observed in germination as seed deterioration progresses. This phenomenon underscores the importance of using seed vigor tests as a more sensitive measure of seed quality and plant emergence capability of a seed lot. Many seed vigor tests have been developed and proposed. In this laboratory, we will consider only the accelerated aging test. However, you should consult your text to be aware of the other types of vigor tests, their strengths and limitations.

Accelerated Aging Test. Prior to germination, seeds are placed in an accelerated aging chamber that provides a relative humidity of near 100% at 40 to 45°C for 48 to 96 hours depending on the crop. Following the stress period, the seeds are removed and germinated according to the criteria for the standard germination test. This test has been successful in predicting the storability of seed lots.

Conduct the accelerated aging test according to the protocols described in the International Seed Testing Association Vigour Testing Handbook. Record your results in the following table:

Vigor Test	% Germination	Vigor Classification (High/Low)
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Accelerated Aging Test

Crop

Lot 1

Lot 2

Answer the following questions:

1. List three standardization problems associated with the accelerated aging test and explain each.
2. Many people believe that an accelerated aging test is of value only for those species that experience hot/warm stress such as in the humid tropics. Do you agree with this philosophy and why or why not?

3. What other approaches to an accelerated aging test can be used to retard rapid seed moisture uptake for small-seeded crops under a 100% relative humidity environment?
4. Would you consider the accelerated aging test to be applicable to all seed kinds or are there other types of seeds where it may not be of value? Explain your answer.
5. Does seed moisture content influence accelerated aging vigor test results? How would you address this issue?
6. If you had to select one vigor test to recommend to a farmer or seed company, which would you select and why?

Include all data and answers to these questions in your lab exercise. The lab report is due at the end of the Quarter.