Tests for Germination In the Laboratory

VERA L. COLBRY, THOMAS F. SWOFFORD, AND ROBERT P. MOORE

WHATSOEVER a man soweth, that shall he also reap applies to the seeds you plant for crops, gardens, lawns, and forests. Sowing seeds that will not grow or are low in viability is a loss of money and time.

To save both, we have the laboratory germination test.

It is designed to indicate as closely as possible the proportion of the seeds that can be expected to sprout and develop into strong plants in the field, garden, and forest.

Experience has taught us that the mere fact a seed will absorb water, swell, and send out a tiny root does not guarantee that it will continue to grow and develop into a plant. It may have only enough vigor to form a root, or it may start to form a shoot and then die. It may even grow into a seedling, but one so weak that it cannot establish itself in the soil and continue to develop into a strong plant. So many hazards are encountered in establishing plants in nurseries, fields, gardens, and lawns that it is only commonsense to plant seeds that will have a good chance of survival.

AGRICULTURAL, vegetable, flower, and herb seeds grown for seeding in the United States are regularly tested for germination by private, commercial, State, and Federal laboratories.

The term "agricultural seeds" denotes the kinds that are planted as field crops or lawns and are not ordinarily considered as vegetables. Herb seeds are those of plants, such as sage and dill, that are grown mostly for food seasonings.

To comply with State laws and interstate and import provisions of the Federal Seed Act, agricultural and vegetable seeds ordinarily are tested before being placed on the market. They also may be tested later by seed law enforcement laboratories. Standardized procedures are necessary to assure that results of the different laboratories are uniform.

Flower and herb seeds usually need not be labeled to meet minimum germination standards. Many kinds of them are tested nevertheless.

The adoption of uniform rules for testing these seeds has been slow, partly because economically they are less necessary than agricultural and vegetable seeds. Testing them has been done mostly in laboratories that serve producers and distributors.

Flower and herb seeds and several new kinds of agricultural and vegetable seeds were added in 1959 to the rules for testing seeds of the Association of Official Seed Analysts. We have standardized germination procedures for 172 kinds of agricultural seeds, 60 kinds of vegetable seeds, 145 kinds of flower seeds, and 18 kinds of herbs.

Uniform germination results cannot be expected unless the analysts follow precise laboratory procedures, among them such operations as subdividing the sample to be used, unbiased selection of the seeds tested, the use of a standard number of seeds for the test (400 is the usual number), adequate spacing of the seeds on the germination medium, and correct regulation of moisture of the substratum, which is the material or medium on which the seeds are placed.

THE EQUIPMENT and substrata must provide and maintain throughout the test period the conditions of moisture, temperatures, aeration, and light to induce the various kinds of seeds to sprout.

An adequate space under controlled

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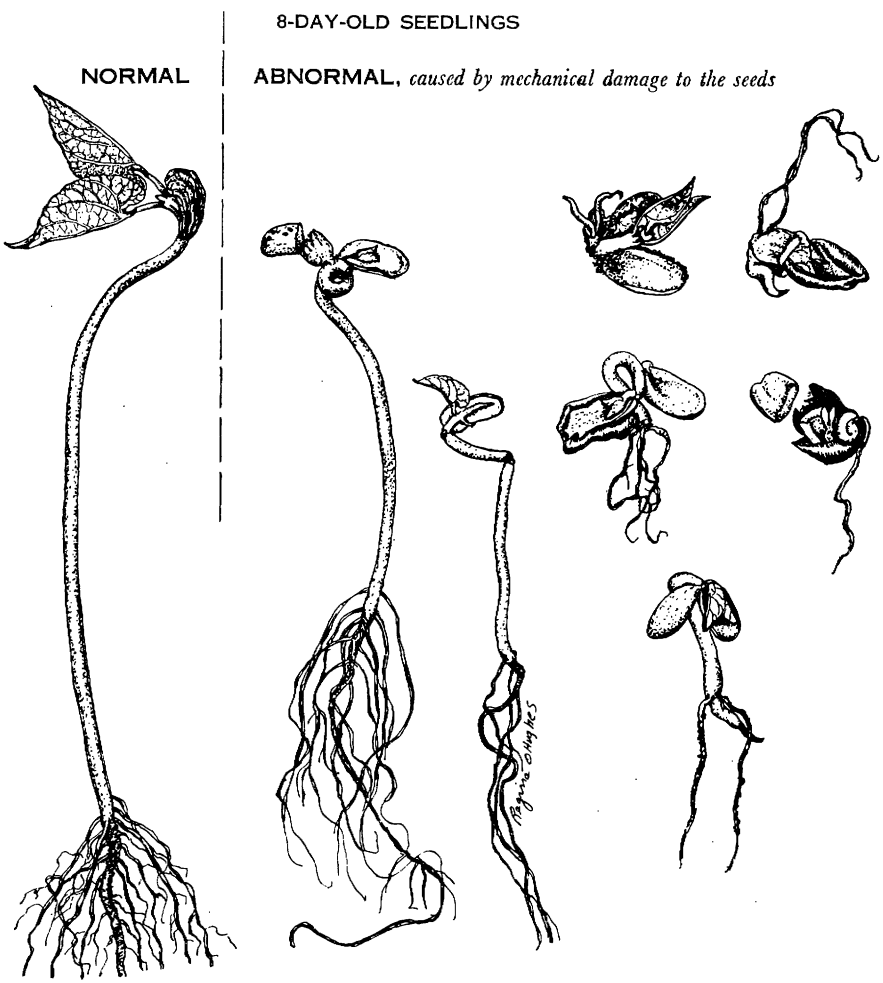
Seedling Classification of Garden Bean (Phaseolus vulgaris)



conditions also must be provided for conducting sand or soil tests. The amount depends on the kinds of seeds tested in the laboratory and any problems the samples present in evaluation of seedlings that make it necessary to test them in soil or sand.

The usual type of germinator in the United States is the insulated cabinet or sometimes a room equipped with movable shelves or trays, on which the tests are placed. The temperature is controlled by a regulated balance between refrigerated water and heating coils, both controlled by thermostats. Light comes through glass walls or doors and from electric lights.

Most of the testing is done on nontoxic substrata such as blotters, towels, or filter paper, which are used alone or enclosed in glass petri dishes or other containers. This type of test saves germinator space and is easy to do.

Sand or soil must be sterilized before use as germination media to destroy fungi, bacteria, and weed seeds. Ex-

panded mica and granulated peat moss, alone or mixed with sand or soil, are used in some laboratories, especially for tests that require long prechilling or test periods.

ADEQUATE MOISTURE must be supplied to seeds throughout the test period.

Except for a few kinds adapted to growth in water, the moisture in the substrata should never be so much that a visible film of water surrounds the seeds. Excessive moisture can cause restriction of respiration (the intake of oxygen and the giving off of carbon dioxide by the seed) and stop germination of the seed. It also can cause certain types of abnormal development, such as lack of root hairs and transparent or "glassy" seedlings.

The temperature must be controlled accurately. Some kinds of seeds germinate over a fairly wide range of temperature, but others sprout promptly only in certain narrow ranges. Seeds germinate generally at temperatures to which they have been acclimated in the place they were produced. For instance, seeds of crops grown in the Southern States germinate well under warm, alternating temperatures; seeds of crops grown in Northern States germinate well in cool temperatures.

Most nondormant seeds are tested at temperatures of 59° to 86° F. A few southern crops require temperatures as high as 95°. The particular temperature under which seeds are placed depends on the kind. Some are placed at constant temperatures, usually 59° or 68°. Others are subjected to temperature alternations during the course of the test period. That is, they are placed at a low temperature during the night (16 hours) and at a higher temperature during the day (8 hours). The most common alternation is 68° at night and 86° during the day.

Light is supplied for a few hours daily to most of the grasses and to some of the vegetable, flower, and herb seeds, whether they are dormant or not. This is because light has a stimulating effect on the germination of many seeds of these kinds, especially when they are freshly harvested, and seedlings exposed to light during part of their test period are easier to evaluate because they are not too pale.

BREAKING the dormancy is a big problem in inducing seeds to sprout. Seed analysts consider dormant seeds as those that are potentially viable but do not sprout promptly when placed under favorable temperature conditions unless they have been subjected to some special treatment. Seeds that have been domesticated for long periods generally germinate readily. They comprise such crops as beans, corn, wheat, rye, peas, and onions. Range grasses are hard to germinate.

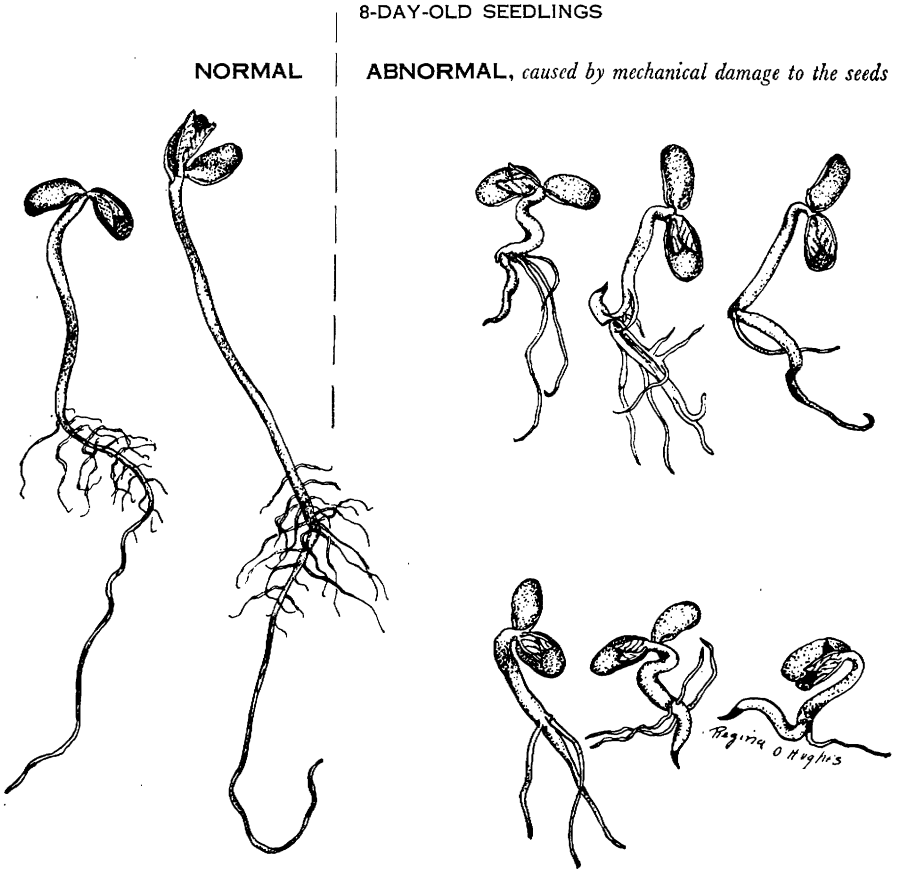
Laboratory methods for overcoming dormancy have been restricted as far as possible to techniques that are practical, rapid, and easily performed, will not require excessive equipment, and will approximate the field performance of the seeds.

Sometimes several treatments are necessary. The analyst draws on his experience as to the requirements of the seeds usually grown or received for test in a certain area and as to the age of the seed. If information is lacking about the previous history of samples, he often has to conduct a double test—one under usual conditions and one under conditions specified for dormant seeds.

Commonly used treatments for overcoming dormancy are prechilling, the use of low-high alternating temperatures during the test period, moistening the substratum with a dilute solution of potassium nitrate, and predrying.

Hard seeds, which do not absorb water during the test period because of impermeable seedcoats, is a type of dormancy that occurs mainly in legumes. No attempt is made in the laboratory to overcome this condition. The hard seeds are reported and shown on the label when the seeds are offered for sale. These seeds will soften

Seedling Classification of Soybean (Glycine max)



gradually and absorb water. They may have considerable field value.

Most of the kinds commonly tested can be removed from test 7 to 14 days from the time they are planted. Grasses as a group require longer periods—21, 28, or even 35 days. If seeds are dormant, the test period is extended to include the time allowed for pretreatments to overcome dormancy. That usually is 3 to 7 days or, for a few kinds of seeds, 2 or 3 weeks.

EVALUATION of the sprouted seeds is just as important as inducing the seeds to sprout.

The germination analyst reports as normal only the seedlings that will continue to develop into strong plants under favorable field conditions. All badly broken, weak, and obviously malformed seedlings are considered as abnormal and are not included in the germination percentage.

Some samples may have up to 30 percent of abnormal seedlings. Correct seedling evaluation therefore can be a critical part of the germination test. Sprouts must not be counted as normal and discarded until they have grown large enough for the analyst to observe whether their essential seedling parts are present.

Guides for seedling classification are

given in the rules for seed testing and in U.S. Department of Agriculture Handbook No. 30. They include detailed descriptions and photographs of normal and abnormal seedlings of most of the kinds tested commercially.

Because evaluation of seedlings is based on their estimated field performance, testing in sterilized soil or sand is a recommended procedure for seeds that may produce seedlings difficult to evaluate when they are grown on artificial media.

Abnormal seedlings may be due to several causes, the recognition and reporting of which may help the seedgrower, merchant, and processor to avoid future losses.

Perhaps the most serious abnormalities are the ones caused by mechanical damage to the seeds, insect infestation, decay of seedling parts because of certain disease organisms, injury from various chemicals, and frost damage.

Mechanical injury is any sort of breakage to the seed, usually caused by threshing operations, cleaning, or scarification processes (the abrasion of the seedcoats to reduce the percentage of hard seeds in such seeds as clovers).

The injury may be externally visible, or it may be internal and may not be discovered until the seeds have germinated. Some kinds of seeds are more susceptible to injury of this type than others because of the size and location of the tiny embryonic plant within the seed. Beans, peas, soybeans, clovers, alfalfa, and some of the cereals are particularly susceptible to mechanical breakage of embryo parts. Seed analysts have also detected similar damage in seeds such as garden spinach and some severely milled grass and flower seeds.

Insect infestation occurs oftenest in such seeds as vetch, field pea, some of the clovers, alfalfa, and cowpea and sometimes in stored seeds, such as wheat. The analyst has the problem of classifying seedlings that are partly injured by insects or weakened because most of the stored food in the seed has been eaten.

Some seedlings show the result of exposure of the seeds to chemicals—notably the overtreatment of seeds with fungicides.

Injury from treatment or accidental exposure to insecticides and herbicides, such as DDT and 2,4-D, has also been observed. The seedling symptoms of both types of injury are much the same—a thickening of the root and lower stem region often so extensive that the seedlings grow just a little.

Frost can cause serious damage to seedlings, especially in grains grown in cold climates. Some years certain samples of northern-grown oats contain seeds that produce a high percentage of frost-damaged seedlings, which exhibit a weakness in the lower shoot region.

Decay of seedling parts during the test period may indicate the presence of serious diseases, which may attack the plants in the field. This problem in seedling evaluation has not been solved entirely. Research into the development and adoption of laboratory techniques for the detection of serious seedborne diseases may help answer it.

The cause for some types of seedling abnormalities has not been established. For instance, certain samples of lettuce produce weakened seedlings that have dark places on the tiny leaves and a general shortening of the sprouts. Such seedlings die or do not grow strongly. They may reflect a physiological weakness within the seeds.

A seedling is indeed a miraculous organism. As it develops from a tiny, embryonic plant within the resting seed, it reveals the secrets which were hidden beneath its protective seed covering. The seed analyst can then convey these secrets to the ultimate planter of the seeds.

TESTING seed of trees for germination is a specialized procedure whose objectives, problems, and techniques differ from those encountered in the testing of other types of seeds.

Seedling Classification of Oat (Avena sativa)

Tree seed is unlike many other seeds in that a fresh supply cannot always be expected each year. It is grown under uncontrolled environmental conditions. Because of extremes of climate and attacks by insects and fungi, the interval between crops of tree seeds in commercial quantities varies with kinds and may range from once every year to once in 8 years. The storage of tree seed therefore becomes necessary.

Results of the germination test can indicate the temperature and moisture limits for successful storage of each kind and whether the original levels of quality are being maintained.

Expanding nursery production and the development in recent years of direct seeding of areas to be converted into forests have created an increasing demand for tree seed. Since the collection season is short and the seed is

perishable, the production of large amounts of high-quality seed requires that it be processed as quickly as possible. The germination test can indicate the need for changes in methods of collecting cones and fruits, temporary storage, and design of processing machinery to prevent injury to the seed.

Seedlings must have proper spacing in nursery beds if they are to become straight and strong. The nursery manager therefore must know how many seeds to sow so as to grow seedlings within the desired density range. Data from germination tests and related information will enable him to calculate the required sowing rate.

Georgia, South Carolina, New York, and South Dakota have set up standards for certification of tree seeds. Pennsylvania, Massachusetts, and Michigan have adopted laws on labeling seed as to quality. Germination tests reveal whether a seed lot fulfills the minimum requirements for certification.

As for pelleted seed of trees, the germination test can determine whether the sticker material and the repellent chemicals used in pelleting are harmful to the seed. Special germination testing techniques usually are needed for that.

The International Seed Testing Association has set up testing rules for 96 different species of trees. A committee of the Association of Official Seed Analysts started working in 1958 on testing rules for tree and shrub seeds of the United States. The Northwest Forest Tree Seed Committee, Corvallis, Oreg., in 1959 proposed testing rules for seeds of 23 tree species in the Pacific Northwest. The Region 8 Tree Seed Testing Laboratory, Macon, Ga., in 1959 proposed testing rules for seed of 20 tree species in the Eastern and Southern States.

Delay in the adoption of uniform rules is due probably to the difficulty encountered in inducing certain kinds of tree seeds to germinate. As research progresses toward the solution of these problems, the probability of general agreement on testing rules will increase.

Dormancy is the condition that prevents germination even when light, moisture, aeration, and temperature are satisfactory. It may be a hereditary characteristic or it may be induced during extraction or storage. Some kinds of trees always have dormant seeds, others almost never, and still others only occasionally. Dormancy may result from impervious seedcoats, which prevent absorption of water and oxygen, or from the condition of the seed parts inside the seedcoat.

The geographical source of the seed has little apparent relationship to dormancy in most kinds of trees. Some that grow throughout a wide range of climatic conditions are the exceptions, however. For example, eastern white pine and Scotch pine usually produce dormant seeds in the southern part of the range, but in the northern part of the range their seeds may or may not be dormant.

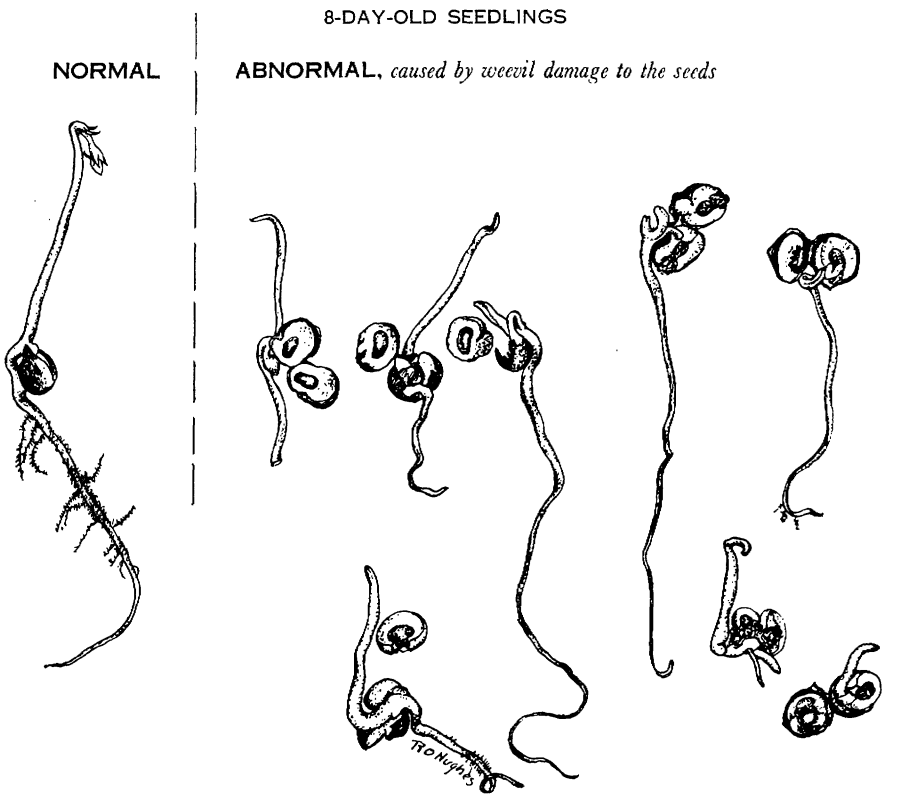
The quality evaluation of dormant tree seeds can be made only after a proper pregermination treatment.

Two germination tests are required for species that have dormant seeds occasionally. Upon the basis of comparative tests made before and after pregermination treatment, the presence of seed dormancy can be determined and a decision be made as to the best test method and field treatment.

When dormancy is due to impervious seedcoats, pregermination treatment of tree seed may be acid or mechanical scarification, or soaking in water or in solvents, such as ether, alcohol, or acetone. If a chemical in the seedcoat prevents germination, as in baldcypress, soaking in a solvent will remove the inhibitor and soften the seedcoat.

Methods used to overcome dormancy due to the internal condition of the seeds are temporary storage under cold, moist conditions; temporary storage in a warm, moist condition, followed by cold, moist conditions; temporary storage in cracked ice; alternating the temperatures during testing; and cold, dry storage.

Seedling Classification of Field Pea (Pisum sativum arvense)



Even the most rapidly germinating tree seeds require a longer testing period than most agricultural seeds. When severe dormancy is encountered, the time required for pregermination treatment and the germination test may be several months. Research has been undertaken to develop faster methods of breaking dormancy. Of course, any method developed for laboratory use must also be practical for use in the field with large masses of seeds.

The excised-embryo method of determining the germinative capacity of tree seeds is useful for many kinds that exhibit dormancy. An approximate measure of germination may be obtained in a week or 10 days instead of months required to complete regular pregermination treatments and testing. The embryo is removed from all enclosing seed structures and placed upon the germination medium. Seeds with extremely hard coats must be cracked before the embryo can be removed. Seeds with soft to moderately hard coats are soaked in water 1 to 4 days to facilitate removal of the embryo. Scarification with acid before soaking in water helps soften impervious coats.

Germination is indicated when growth starts or the embryo remains white and firm at the end of the test. Nonviable embryos become discolored and soon deteriorate. Since embryos only are used in this test, a correction for empty seed or discarded shriveled embryos in the original seed must be

made. This method of germination testing is limited to kinds of tree seed whose embryos can be removed easily.

A NEW TYPE of test of seed quality involving the differential chemical staining of strong, weak, and dead tissues has come into use. Trained analysts can use it and get rapid, informative, and encouraging results. Of the chemicals tried, tetrazolium salts are the most promising and widely used.

Testing with tetrazolium (TZ) is based on the principle that respiration processes within living tissues release hydrogen, which combines with the colorless tetrazolium solution and produces a red pigment. Strong, healthy tissues develop a normal red stain. Aged tissues reveal a pale or mottled stain. Dead tissues remain white. Staining, however, is only one part of a chemical seed test. Factors other than death prevent the germination of many seeds.

Tetrazolium testing was started in 1941 by the late Georg Lakon of Germany, who used it as a substitute for a poisonous selenium salt that was used in studies of seed. Tetrazolium became available in the United States about 4 years later. It is now used in many countries and States. In the United States, it is largely used as a rapid, nonofficial testing supplement to standard methods. A few laboratories make a specialty of chemical seed testing.

The TZ test makes it possible to determine the potential viability and vigor ratings within 15 minutes to 24 hours. It also provides a different approach to testing and new insights into seed evaluation. It can reveal causes for seed troubles that may remain concealed or uncertain in growth tests. These informative insights make the test useful in predicting or diagnosing reasons for failures of germination due to storage, laboratory, or field conditions. The TZ test is especially useful in evaluating dormant seeds at harvest or seeds that require long or uncertain testing periods otherwise.

Another good opportunity to use TZ testing is in the evaluation of seeds that remain firm at the end of growth tests. Producers of seed of corn and sorghum use the test before processing to evaluate the seriousness of injury by early frosts.

TZ testing is not infallible and is not a final answer to all testing problems. It is merely another useful tool. The test can only reveal. The accuracy of results depends on the qualifications of the analysts who interpret it.

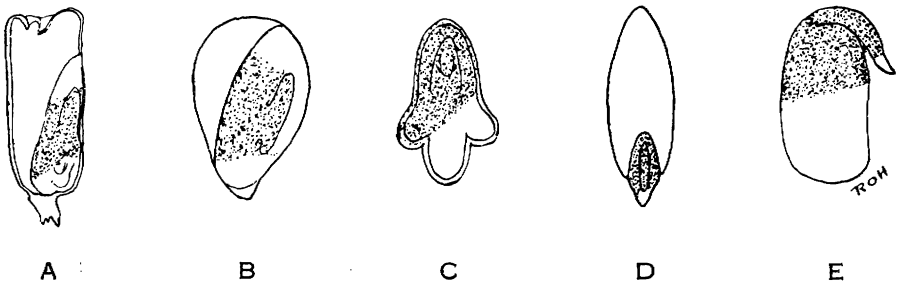
One objection to the widespread use of TZ tests in trade channels is that a uniform system of standards is yet to be established and coordinated for most kinds of seed. Furthermore, unless TZ testing is approved as official for given kinds of seeds, the results are not acceptable in labeling for legal requirements. Another objection is the extra time needed to prepare and examine individual seeds.

Another consideration is that TZ tests reveal potential germination percentages based strictly on internal seed condition, but growth tests reveal a combined performance of seed quality and a given growing condition. Germination evaluations from the two approaches to testing are often similar, especially if disease-susceptible seed lots are treated with a fungicide prior to growth testing under favorable conditions. TZ tests may not detect recent applications of toxic levels of fungicides.

When TZ tests are properly evaluated, the limitations usually are not so serious as they appear at first, especially if they are evaluated along with weaknesses of any other single type of test. The limitations should not discourage a person from taking full advantage of the potential usefulness of TZ testing for vigor and viability.

A knowledge of internal embryo parts as they relate to the development of seedling structures is essential for a proper evaluation of TZ tests. Most agricultural seeds are characterized by one cotyledon, or seed leaf, as in

Viability of Embryos



In one test of quality, a colorless solution produces a red pigment in strong, healthy tissues. In these drawings the shaded parts represent the red staining and show the live cells in embryos. a, corn; b, sorghum; c, wheat—cut surface of upper half of embryo; d, bluegrass—noncut surface; e, soybean—noncut surface.

grasses, or by two cotyledons, as in legumes. A knowledge of embryo structures of the two groups is a guide for understanding many kinds of seeds.

Testing techniques require that certain basic procedures be followed. Seeds should first be softened in water to activate enzyme systems, and to promote clean cutting and uniform absorption of tetrazolium. Seeds with fragile structures, such as snap beans, should be preconditioned first by slow absorption of water from a moist medium to avoid critical fracturing.

Seeds are prepared for absorption of tetrazolium by methods appropriate for the kind involved. Corn and large-seeded grasses are cut longitudinally through the center of germs to expose embryo leaves and root buds. Fine-seeded grasses are punctured or cut crosswise immediately back of the germ, or under it. Seeds of legumes and other crops, which absorb tetrazolium through seedcoats, are stained without previous physical alteration.

A satisfactory testing solution consists of 0.5 gram of 2,3,5-tripheynl tetrazolium chloride in 200 cubic centimeters of water.

Staining should occur in darkness and at temperatures near 70° to 90° F. Approximate staining times at 86° are: Corn and other cut seeds, 2 to 4 hours; cotton, peanuts, and other seeds with exposed noncut embryos, 4 to 6 hours; beans, grasses, and other crops in seedcoats, 6 to 18 hours.

When adequate staining has occurred, the solution should be discarded, and the seeds covered with water and refrigerated at 40° to 50° until analyzed. Seeds should be kept moist until evaluated.

SATISFACTORY interpretation of potential germination can best be acquired by an inexperienced analyst by comparing results from growth tests with observations from TZ tests.

Evaluation for vigor and viability requires observation of individual structures of each embryo and the relating of the condition of the decisive parts to the potential formation of normal or acceptable seedlings. Embryos should be observed individually, preferably under 5 to 10 × magnification, for the presence, extent, and seriousness of trouble areas. Variations in color patterns, texture, bruises, fractures, abnormal structures, infected areas, and insect infestation are all of potential importance. Firm, hard, or dormant seeds react normally to TZ staining and may be either germinative or nongerminative.

It is informative to classify individual germinative seeds within a lot on the basis of 1 to 5 and nongerminative 6

to 8. The physical condition of the embryo is used for these ratings. The percentage of seed in each class establishes different levels of vigor which is useful in predicting relative storage life of seed lots and response to adverse germinating conditions.

Advanced degrees of a localized or general aging, revealed as pale-red, mottled, necrotic, or flaccid tissues, cause more difficulty in interpretation than most other seed conditions. Interpretations of aging symptoms can be mastered with experience, and with this mastery will come a deeper appreciation of the gradual process of aging and the formation and enlargement of necrotic areas that tend to lead first to a nongerminative condition and later to complete death.

The TZ test has given us many insights into the mysteries of seeds. We expect to get more as its techniques are refined to reveal the causes of weaknesses of seeds and failures in germination. The advantages commend its use by agencies that need nonofficial evaluations quickly.

VERA L. COLBRY *is a seed technologist in the Seed Branch, Grain Division, Agricultural Marketing Service, Agricultural Research Center, Beltsville, Md. She holds degrees from Oregon State Agricultural College and George Washington University. She has worked in the field of seed technology since 1931.*

THOMAS F. SWOFFORD *is in charge of the Region 8 Tree Seed Testing Laboratory, Macon, Ga. Other assignments have been administrative positions at various national forests in the South and Pacific Northwest. Mr. Swofford is a graduate in forestry of the University of Washington and Yale.*

ROBERT P. MOORE *is professor of research, crop stands, North Carolina Agricultural Experiment Station, Raleigh. He was formerly a teacher of field crop courses at the Oklahoma State University and the University of Tennessee; a graduate assistant with the Ohio Seed Improvement Association, Columbus; and Director in charge of the North Carolina Seed Improvement Association.*

Ways To Test Seeds For Moisture

LAWRENCE ZELENY

THE MOISTURE in a seed has a strong bearing on the length of time it remains viable. Seeds may sprout or molds may develop at high levels of moisture, and the seeds may lose viability in a few days.

At the ordinary temperatures, if the relative humidity of the air around the seeds is more than 75 percent, the seeds are likely to support the growth of molds to the extent that they should not be stored even for a short time.

The moisture content of seeds in equilibrium with this critical relative humidity varies among different kinds. For the various cereal grains, an atmospheric relative humidity of 75 percent corresponds to moisture contents in the range of 13.5 to 15 percent. Seeds high in oil usually have a lower moisture content at this humidity.

Moisture levels below those that cause actual sprouting or mold development may still be high enough to support fairly active physiological activity within the living seeds. Such activity will result in time in premature weakening and loss of viability.

Within certain limits, the lower the moisture content of any kind of seed, the greater the time it will maintain viability. The optimum moisture level for the storage of many kinds appears to be between 6 and 8 percent. Excessively low moisture may cause injury to the embryo. Complete dehydration no doubt would destroy the life of the embryo. Unless artificial drying with heat is employed, however, excessive dryness in seeds is rarely a practical problem.