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Laboratory Determination of Seed Viability

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LABORATORY DETERMINATION OF SEED VIABILITY

One of the primary functions of a seed laboratory is that of determining seed viability. Viability implies ability to grow or to germinate when the proper environmental conditions are provided. In many botanical text-books the term "germination" is defined as "sprouting" which means the enlargement and elongation of one or more parts of the embryo to such an extent that it protrudes from the seed. The definition referred to does not necessarily carry the implication that sprouting must continue until a new plant is established. The criteria of growth in plants are cell division, cell enlargement and cell differentiation; but there is nothing in the definition of germination to imply that growth must continue indefinitely following its early manifestations. It is evident to all seed technologists that although germination is the final evidence of seed viability, yet one cannot in truth be too arbitrary about classifying a seed as non-viable simply because he is unable to make it sprout. He may not have provided the conditions that permit the embryo to undergo all the necessary changes incident to growth. On the other hand there are certain evidences of dead seeds which are reliable and cannot be ignored or questioned.

The seed technologist, if he would fulfil his responsibility, which is that of furnishing some measure of seed quality to the buyer, seller and planter of seeds, must set up some standard other than sprouting by which he can measure seed viability in terms of germinated, dormant and impermeable seeds. It is the purpose of this paper to suggest standards which have had a high degree of validity in the measurement of seed viability.

RULES FOR SEED TESTING

The Association of Official Seed Analysts in its "Rules and Regulations for Seed Testing" (13) has attempted to define germination as follows: "A seed will be considered to have germinated when it has developed into a normal seedling which might be expected to continue its development in soil under favourable conditions." The Rules and Regulations under the Federal Seed Act (14) state "A seed shall be considered to have germinated when it has developed into a normal seedling. Broken seedlings and weak, malformed and obviously abnormal seedlings shall not be considered to have germinated." The first definition attempts to define normal seedlings, and the second one attempts to classify what may be considered abnormal seedlings. It may make some difference in the final interpretation whether one's goal is to determine how many seedlings are **normal** or how many are **not normal**. Stated in another way, it may make a difference whether we seek to (1) consider all seedlings normal unless we can prove them abnormal, or (2) consider all seedlings abnormal unless they can be proved normal.

It is necessary at the outset that the point of view of the analyst be clearly understood in this problem of seed germination and that, if possible, a common point of view be adopted. The point of view adhered to in this paper is expressed in a proposed definition (9) which is as follows: "A seed will be considered to have germinated when it has developed those structures which are commonly recognized in different varieties, species or families of plants as essential in normal seedlings. Broken, malformed or other seedlings which are known to be incapable of producing plants in laboratory soil or sand under favourable conditions will be considered not to have germinated." On the basis of this latter definition a given type of seedling would be considered normal until it was demonstrated to be abnormal.

There are at least two reasons for the point of view which is represented in the proposed definition. The first is that uniform interpretation is difficult as has been shown repeatedly in referee tests, and some reasonable effort must be made to make uniform interpretation easier. A second reason is that the great majority of differences in interpretation occur among those questionable seedlings which one analyst may call normal and another one abnormal. In the small-seeded grasses, legumes and other groups of seeds the tremendous number of seeds per ounce or pound together with the common rates of seeding make it unnecessary to be too arbitrary and technical as to what constitutes a normal seedling. A difference of 10 or 15 percent in laboratory germination by two laboratories on the same lot of seed will not mean the difference between success and failure to obtain a stand. Yet this difference of 10 or 15 percent in normal sprouts, which about represents the extremes as reported by seed laboratories, occurs in the area of questionable seedlings. The emphasis placed on such seedlings is out of all proportion to the practical effect it has on field stands, on the movement of such seed in commerce or on the welfare of many people whose livelihood is represented by the transactions that have taken place. These small differences can only be adjusted as we cease to overestimate their importance.

ESSENTIAL STRUCTURE OF NORMAL SEEDLINGS

A seedling for the purposes of seed technology may be defined as a young plant that is still dependent on the food in the seed for its continued growth. It is impossible to determine the exact stage at which every seedling ceases to be a seedling and becomes a plant, but it is possible on the basis of observation and experimentation to determine the essential structures of a seedling.

In the monocotyledons of which grasses constitute the largest group the first structure to appear is the coleorhiza which encloses the radicle as in corn, timothy and many other grasses. The radicle is known as the primary root. In some of the grasses, such as barley and wheat, two or three roots may emerge at about the same time. The second structure to emerge is the coleoptile which encloses the plumule or primary stem. In corn seedlings the secondary roots from the region where the plumule and radicle join emerge early in the life of the seedling and constitute the root system on which the early life of the plant depends. For most of the grasses it is common to count as normal seedlings all those that possess a radicle and a plumule neither of which is abnormal in appearance. Root hair development on the radicle and continued elongation of both plumule and radicle are additional indications of normal seedlings. Corn seedlings should not be classed as normal until the secondary root system has appeared in the form of one or more secondary roots.

Two other members of the monocotyledons commonly tested in a seed laboratory are onion and asparagus. The radicle emerges first in both these seeds in normal seedlings, and the plumule develops slowly. In some lots of onion seed few to many seeds may develop no radicle, only a plumule followed by the development of adventitious roots. Such seedlings probably should not be classed as normal, although some may be able to live and grow because the secondary root system is the one on which the future growth of the plant depends.

Among the dicotyledons the first structure to emerge is the radicle, after which the hypocotyl (epicotyl in peas) elongates, the cotyledons usually separate from the seed coat

and the plumule finally appears from between the cotyledons. Both the radicle and plumule are essential structures in normal seedlings of the dicotyledons, and at least one cotyledon, possibly both, should remain attached to the hypocotyl.

ABNORMAL SEEDLINGS

Abnormal seedlings are somewhat difficult to determine, although the type of medium in which the seeds are germinated is of some importance in seedling classification. Seeds germinated between or on top of blotters are subjected to no resistance as the parts of the seedlings emerge. In nature some resistance is offered which is probably of importance. Seeds germinated on top of blotters vary considerably in their response. Flax, timothy, red top, bluegrass, rye-grass, fescue grasses, orchard grass and many other small-seeded grasses respond well on top of moist blotters. Corn seeds do not germinate uniformly on top of blotters unless the embryo is placed next to the moist substrate. Seeds of wheat, oats, barley and rye respond best if covered unless a saturated atmosphere is provided. There is considerable evidence (1) that the embryo of grass seeds is superior as an absorptive organ to the endosperm, and among the large seeds it is important that the embryo be in contact with moisture. Under the conditions which have been discussed it is possible to determine those seedlings that have no radicle, no plumule and, in corn, those that have no secondary roots. It is also possible to determine if either the plumule or radicle or both are incapable of further development, although corn seedlings with a poorly developed radicle but a vigorous secondary root-system are capable of normal development.

Seeds of clover, alfalfa, crucifers, buckwheat, onion, beans, peas and most other seeds need to be covered either by a blotter fold or by rolling in paper towels. Under such conditions it usually is possible to select those seedlings that are bright, clear and have normal, rapid-growing structures It is also possible to observe those with the radicle broken from the cotyledons, and twisted, watery, malformed and obviously abnormal sprouts that soon cease further growth. Seedlings with a badly split radicle or a radicle that has ceased to elongate belong in the class of abnormal seedlings.

Among the many kinds of beans it is important to keep the seeds in test long enough to observe those in which the plumule fails to develop normally and those in which the cotyledons break from the hypocotyl. Such seedlings are at least slower in development than those without such a handicap and in general should be considered as abnormal. Seedlings with one cotyledon and a normal plumule may rightfully be classed as normal.

There are frequently a number of seedlings in a sample which are neither clearly normal nor abnormal; and in blotters the classification is difficult, especially when saprophytic fungi are abundant. This class of seedlings is responsible for most of the differences of opinion that exist among seed analysts.

There is considerable evidence and more is being accumulated that a laboratory medium which offers some resistance to the emergence of seedlings is preferable to blotters or towels for many kinds of seeds. It is believed that sand or soil if properly used prevents the emergence of most abnormal seedlings and thus eliminates much of the difficulty involved in seedling classification. If a seedling emerges through a layer of sand or soil it should at least be considered as having passed the first requirement of a normal seedling. If no plumule develops or if the root is absent or shows signs of being unable to continue

its development, then the seedling should be classed as abnormal. Non-resistance of the seedling to removal from the medium should not be used as a criterion for abnormality because, in soil especially, it is difficult to have the top layer above the seeds uniformly compact. Furthermore, seeds do not all emerge at the same time and those which emerge first have a time advantage for the development of roots.

Root discolouration, which has been considered as evidence of abnormal seedlings, is difficult to evaluate. This condition occurs more frequently in soil than in sand, and since autoclaved soil and possibly electric pasteurized soil contains some living organisms, root discolouration may be caused by such organisms. Furthermore, soils that are heated to a temperature of 70°C. or higher may well develop toxic substances that cause abnormal root discolouration. Soybean seedlings frequently show root discolouration in soil but not in sand. Samples of sweet clover frequently show root discolouration in autoclaved soil. When such conditions appear a test in sand is desirable. Seedlings of sorghum and cereals sometimes fail to develop more than the first leaf, especially when organisms are present and cause seedling blight. In such cases the base of the stem is rotted and the seedlings are abnormal.

KINDS OF MEDIA FOR GERMINATION

When seed testing was first undertaken the germination tests were made largely between folds of blotting paper or on top of moist blotters. It is probable that the large majority of tests made at the present time are in blotters because of (1) low cost; (2) small space required per sample; and (3) rapidity with which samples can be prepared and counted. For many kinds of seed it is doubtful if seed laboratories will find it practicable in the near future to use any substitute for blotters as the substrate. Clover, alfalfa, timothy and many of the small vegetable crop seeds can be handled rapidly and efficiently in blotters. Furthermore, hard seeds can be easily determined in blotters, whereas in sand or soil such determinations are impossible for the small-sized legume seeds. Small-seeded grasses that require special treatment for dormancy can be handled best in petri dishes with filter papers, or on blotters equipped with wicks. Sterile sand or soil may also be used in petri dishes.

On the other hand it is now recognized by many seed analysts that uniform evaluation of seedlings in blotters and towels is almost impossible for many kinds of seeds, and that frequent comparisons between blotters and either sand or soil is a necessary requirement. Experience has shown that it is much easier to measure normal seedlings of corn, soybeans, garden and lima beans, peas, cucurbits, sorghum and many other crops in sand or soil than in blotters. A medium which offers some resistance to the emergence of seedlings assists the analyst in his evaluation; yet it is important that the medium be one which permits the maximum emergence and development of normal seedlings.

Referee tests conducted by the Sand Test Sub-committee of the Research Committee of the Association of Official Seed Analysts (11) have indicated that sand is often superior to blotters in that more uniform results are obtained. Referee tests in Region No. 2 (12) for the Research Committee referred to, have shown that as experience has been gained in the use of sand, the results obtained by different analysts with uniform lots of seed are less variable than those from blotter tests.

Munn (6), Goss (4), Towers (16), and Fuhr (3) have all compared soil and blotters for germination tests and have emphasized that soil tests in the laboratory are essential. Fuhr (3) also compared soil and sand and concluded that soil was preferable because it was the more natural medium. She noted, however, that sand enabled one to see diseased conditions more clearly and to determine broken sprouts of clovers and flax more readily.

Porter (7) found that autoclaved soil, consisting of equal parts compost and sand, furnished a satisfactory medium for the germination of barley seeds and that the germination in such soil represented field germination more closely than did germination on moist blotters. The same author (5) tested soybeans in blotters, towels, sand, soil and the field. In 1937 the highest correlation, ·72 was between laboratory sand and field soil. Porter, Hendershott and Davis (10) found that in 4 cases out of 20, oats germinated higher in field tests than in laboratory soil, and in two cases out of five, field germination of wheat was higher than in laboratory soil. Erickson and Porter (2) compared the germination of 50 samples of soybeans in soil, towels, sand and the field. Several of the samples germinated significantly higher in the field than in laboratory soil. It is to be expected that a laboratory test would provide conditions more nearly optimum than the field, hence any medium which gives a significantly lower germination than occurs in the field should be questionable for general use.

Thompson (15) tested seeds of 10 lots of seed from 10 different crops in blotters, sand and soil. After a statistical analysis of all the data, she concluded that in general sand was preferable to soil in that the percentage of normal seedlings was higher and the variations between replicates of each lot were more nearly normal.

There are four factors which have a bearing on the relative merits of sand and soil for laboratory tests. The first factor is the reaction of the medium to heat and pressure for devitalizing the organisms which are present. Soil is a much more complicated biological, chemical and physical medium than sand; hence heat may be expected to change it from a natural to an **unnatural medium.** Washed, screened sand can be heated with limited danger of changing its physical or chemical condition. The possibility of producing toxic substances by heat is much less in sand than in soil. The second factor is that saprophytic and parasitic fungi seem to spread less rapidly in semi-sterile sand than in semi-sterile soil. Porter (8) reported limited tests which indicated that sand is a satisfactory medium to use for seeds which are heavily infested with either parasitic or saprophytic fungi. Such organisms on blotters so over-run the seeds that interpretation of normal sprouts is difficult. If the seeds are properly spaced in sand there is very little spread of fungi from one seed to another. The third factor is that the physical and chemical condition of sand in different localities should be much less variable than soil. Furthermore, sand can be screened, washed with hydrochloric acid and clean water and prepared in a standard manner. It is probable that a fairly uniform sand medium could be prepared for use by all seed laboratories. The fourth factor is that the roots of seedlings grown in sand are more easily observed for normality and for the presence of organisms than when grown in soil. A final consideration is that in general a higher percentage of normal seedlings is obtained in sand than in soil. Laboratory tests should measure maximum viability; hence sand should receive serious consideration.

There are instances where the germination of seeds is equal in sand and soil, especially for lots with a high viability. Soil also has a particular value as a medium for the detection of seedling blights of wheat, oats and barley. Natural soil is necessary for the measurement of the resistance of corn to species of Pythium. The selection of a laboratory medium, therefore, is dependent on the object of the test to be made.