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Use of Stress Tests To Determine the Storage and Field Stand Potential of Seeds

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USE OF STRESS TESTS TO DETERMINE THE STORAGE AND FIELD STAND POTENTIAL OF SEEDS

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A stress test may be defined as the application of unfavorable environmental conditions to the seed in order to determine a specific performance characteristic. For example, the two stress tests we will consider here are the accelerated aging test and the cold test. In the accelerated aging test high temperatures and relative humidities are used to accelerate deterioration processes and this then enables us to predict the storage potential of several lots of a given seed kind with a few days. In the cold test, on the other hand, cool temperatures and moist soil are used to determine the ability of the seed to emerge under unfavorable field conditions.

Now with your permission let us think in terms of measuring storage potential with the accelerated aging test and measuring storage potential with the accelerated aging test and measuring the field stand potential with the cold test.

First, let us consider storage potential and the accelerated aging test.

Accelerated Aging

After production and processing, a primary concern of the seedsman is maintenance of the seed in a good viable condition until they are purchased by the farmer. Seedsmen frequently have the unhappy experience that some lots of seed of apparently good viability rapidly decline in germination during storage, while other lots of equal viability hold up well. Similar differential responses in field emergence among lots of equal viability are also frequently encountered. In other words, a high germination percentage does not necessarily indicate that a seed lot will store safely or that it will produce a satisfactory field stand.

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Those of you associated with quality control programs have probably been faced with the difficult task of determining which lots from among many available should be marketed first and which lots should be held for possible carry-over if the market is not strong, or as a hedge against shortages the next year. Germination percentages of the lots provide some useful information for making this decision if there is a considerable range in germination among the lots. This is seldom the case, so the seedsman randomly selects for possible carry-over, and he often finds the next season that several of them did not carry-over very well. The failure of a seed lot of apparent good germination to maintain that germination in storage at a retail outlet or at the wholesaler constitutes a serious problem - one that can be most damaging to the seedsman's profits and reputation.

The solution to problems related to storability of seed lots lies in the development of a test - other than the standard germination test - which will differentiate among seed lots with respect to storage potential. A test that is attracting attention in the seed industry is the accelerated aging test developed here at the Seed Technology Laboratory. This technique is based on the hypothesis that the degree of deterioration is variable among seed lots within a given seed kind, even though deterioration has not progressed to the point where it has affected germination. Therefore, by subjecting small samples of various lots of the same seed kind and approximately the same current germination to an extreme environmental condition, i.e., high temperature and relative humidity, the process of deterioration can be accelerated to the extent that within a few days low vigor lots are drastically reduced in germinative capacity while high vigor lots are relatively unaffected. Thus, accelerating the physiological aging processes permits evaluation or prediction of the long term storage potential of seed lots within a few days.

Environemental conditions and period of exposure required to obtain maximum differences in response among seed lots vary with the kind of seed. In general, the most satisfactory conditions are 100% relative humidity, temperatures of 35° to 45°C., and exposure periods of 2 to 8 days.

The effectiveness of accelerated aging as an assay of the storage potential of seed lots is evaluated by comparison of the germination percentages after accelerated aging with those of the same lots after intervals of storage.

Equipment

The accelerated aging chamber we are using was made from an old, water cooled germinator with 2 1/2" insulated walls and door. It has inside dimensions of 26" width, 24" height, and 20" depth. All water tubing and other internal equipment - except tray racks - were removed from the germinator. All gaskets, holes, etc., were resealed with 3M weather strip adhesive (black). A water reservoir approximately 1 1/2" deep is maintained in the bottom of the chamber. In the water reservoir is a flexible, immersion heating rod. Temperature is controlled by a thermister temperature controller equipped with a general purpose thermister probe.

A plexiglass chamber was designed as an insert or liner for the chamber to maintain a more constant temperature and relative humidity. The plexiglass liner fits on a rack about 5 inches from the bottom of the chamber and 2-4 inches from the side, walls, back and ceiling. The liner has a water reservoir, glides for 2 trays, and a removable access panel in the front. The top rack of the plexiglass liner is covered with blotters to collect any condensation dripping from the top (actually , very little condensation forms on the top or walls of the liner). The bottom rack is used for holding the samples of seed as a set distance of 3 inches from the surface of the water. A long stem dial thermometer is inserted through the front access panel of the liner, and a sensitive glass mercury thermometer is attached to the sample tray placed in the liner.

In operation, water is placed in the bottom of the chamber and plexiglass liner. The temperature controller is energized and adjusted to the desired temperature setting with the glass thermometer inside the liner. After the desired temperature is achieved, the tray is removed and samples to be tested are prepared and placed in small wire screen baskets on the tray. The tray is placed in the liner and accelerated aging begun. After accelerated aging for the desired number of hours or days the samples are removed and standard germination tests are made on the samples. Typical data are given in Table 1.

Table 1. Germination percentages of corn, tall fescue, garden bean, crimson clover and onion seed lots after accelerated aging and intervals in open storage.

Lot No.	Init. Germ.	Accelerated Aging	Open Storage (Months) 4	6	12
		Corn			
1	100	94[a]		100	98
2	100	92		99	98
3	92	44		90	74
4	84	22		94	78
5	96	19		96	22
		Tall Fescue			
1	95	94[b]		96	93
2	92	83		92	52
3	87	80		90	48
4	88	56		73	24
		Garden Beans			
1	97	95[c]		97	94
2	97	35		97	93
3	77	51		77	71
4	87	7		85	35
		Crimson Clover			
1	94	90[d]		96	92
2	94	74		93	86
3	78	52		81	60
4	88	80		92	86

[a] Accelerated aging at 42° C. - 100% r.h./144 hrs.

[b] Accelerated aging at 40° C. - 100% r.h./84 hrs.

[c] Accelerated aging at 42° C. - 100% r.h./72 hrs.

[d] Accelerated aging at 40° C. - 100% r.h./72 hrs.

Table 1 continued.

Lot No.	Init. Germ.	Accelerated Aging	Open Storage (Months) 4	6	12
		Onion			
1	93	93	91		
2	93	46	31		
3	88	76	72		
4	95	67	49		

e Accelerated aging at 40°C. - 100% r.h./120 hrs.

Cold Test

Favorable environmental conditions are used in conducting germination tests, with conditions of temperature and moisture as near optimum as possible. The use of these conditions gives a measure of the maximum germination of a seed lot but does not give a measure of its germination under adverse conditions which often occur in the field. In cold tests, seeds are subjected to cool, wet conditions in order to obtain some measure of the ability of seed lots to emerge under unfavorable conditions encountered during a cool, wet spring. For example, corn may be planted and immediately followed by a "cold front" that causes cool, wet conditions to prevail for several days or weeks. The emergence period may be 2 or 3 weeks. Under these conditions, only vigorous, sound, well treated, seeds are capable of emergence while the weak (but still germinable in germination test) and unsound seeds often decay due to soil organisms before more favorable conditions are again established. Cool, wet soil is unfavorable for both seedling emergence and soil pathogens, but these conditions are relatively less favorable for germination than for the soil pathogens.

Generally Pythium species are the most important soil organisms in cold tests. These species are usually the first invaders of seed planted in moist field soil. Probably the most frequent species identified is Pythium debaryanum. In seed with pericarp injuries, Pythium invasion occurs immediately after planting. Other organisms that cause stand reduction are Penicillium, Rhizoctonia,

and Fusarium.

Uses of the Cold Test

Most of the larger corn seed companies are using the cold test to:

1. Determine the quality of carry-over seed.
2. Evaluate seed treatments
3. Test the effects of processing methods on the seed.
4. Evaluate vigor in parent stocks.
5. Compare the resistance of inbreds and single crosses.
6. Evaluate the adequacy of storage conditions with respect to seed deterioration.
7. Determine the extent of frost injury and immaturity and its effect on seed vigor.

Factors that Affect Cold Test Results

Many interrelated factors are known to influence the results obtained with the cold test. The test is sometimes difficult to standardize between laboratories. Some of the factors that contribute to lack of standardization are:

1. Different soils used in cold test. Soil used in various laboratories give different results due to variation in population and activity of soil-borne organisms.
2. Different species or strains of organisms exhibit various degrees of virulence.
3. Type of storage of soils used in cold tests may be a source of error.
4. Difference in cold test methods. Extremes in moisture content, length of cold period and temperature during cold period may cause varying results.

The lack of standardization, however, need not limit the usefulness of the cold test as a quality control tool. As long as one method is consistantly followed comparisons can be made between lots, varieties, and years. If one test method is used on one lot and another method on another lot, however, the results could be misleading. Thus, each laboratory or company must employ the test method that provides the most information about the quality of their seed. This may often require a more vigorous type cold test for early season varieties while a progressively less severe test may be more realistic for varieties planted later in the season. The severity of the cold test could be regulated simply by varying the temperature and test period.

The methods employed in the Seed Technology Laboratory are:

1. The soil (top soil) is obtained from a field where the seed kind to be planted has been grown the previous year.

2. Next we bring the soil in and screen it through round hole screen to remove crop residue, gravel, lumps of soil, etc.

3. The screened soil is then mixed with builders sand in a 1 part sand to a 1 part soil ratio in a small concrete mixer.

4. 1500 grams of the sand-soil mixture are placed in plastic crispers. The soil is leveled, then the seed are placed uniformly over the soil. The seed are then covered with an additional 1000 grams of the sand-soil mixture. The soil is then packed lightly by pressing with a flat board and adjusted to 60% saturation.

5. The containers are then placed at 50°F. for a specific period of time depending on the crop. We have found that 7 days for corn and 5 days for cotton, soybeans, and sorghum give satisfactory results.

6. After the chilling period the containers are transferred to 86°F. till seedlings are evaluated (usually 3-4 days after chilling).

7. The emerged seedlings are then classified as normal or abnormal.

When considering the cold test as a tool in quality control, some important factors that effect cold test results should be known:

Seed Maturity - Generally, the difference between germination percent and cold test results is greatest for the most immature seed lots. Maturity of seed has only a minor influence on seedling emergence under regular germination test and fails to indicate the ability of seed to germinate in cold, wet soil. Also, the greater mechanical injury associated with immaturity contributes to low seedling emergence in cold test.

Seed Permeability - This is associated with seed coat injury and deterioration of seed. The possible explanations for the low germination as seed permeability increases are (1) materials that diffuse out of seed serve as food for the organisms responsible for reduction in cold test and (2) (2) pathogens can more easily invade the permeable seed.

Artificial drying - Corn seeds artificially dried at $110^{o}F$. are more susceptible to invasion by soil pathogens than ears dried in an open room. Artificial drying causes an increase in the amount of blistering of the seed coat, this is especially true of immature seed. In addition to seed coat injury, a physiological difference exists between artificially and naturally dried seed that affects their susceptibility to soil pathogens. So a combination of seed coat injury and physiological effects seems to be the main factors contributing to seedling reduction in cold test.

Mechanical damage (pericarp injury) - Injury of the seed coat is one of the greatest contributors to reduced emergence in cold test. It should be remembered that a sound intact seed coat is one of the most effective barriers to soil pathogen. Any broken area in the seed coat is an excellent location for entrance by soil pathogens. Experiments with corn have shown that severe crown injury causes the greatest reduction in cold test. Seed with pericarp injury at the crown or over the plumule (embryo shoot) are more susceptible to soil pathogens than seed with injury over the radicle (embryo root). Crown injury also tends to increase with the size of the kernels and more frequently

in "flats than "rounds". However, injury over the embryo (germ) occurs more frequently in "rounds".

Other crops receiving mechanical injury, such as soybeans, will also tend to give low emergence in cold test, but unlike corn, injury in soybeans is often of sufficient magnitude to cause fracturing of embryo parts that cause poor emergence in germination tests as well as cold test. Therefore, soybeans with appreciable injury often give equal emergence under both germination and cold test methods. However, if the sample has also been deteriorated due to inadequate storage or other causes, then one could expect the germination test to give higher emergence than the cold test.

Age of seed (physiological deterioration) - In general one can expect a reduction in cold test as the period of storage or carry-over increases . The rate of decline in cold test will depend on the history of the seed (maturation, harvest method , processing) prior to storage as well as actual conditions during storage. It should be emphasized that seeds at maturation are at their peak in quality, after which the seeds take only one path -- toward lower quality and death. Seedsmen can only hope to slow down this process. Likewise, it should be remembered that appreciable deterioration can occur and yet not be evident in the germination test and this is one of the factors that contributes to the usefulness of the cold test. To detect physiological deterioration with the cold test, seeds should be untreated.

Genetic resistance - Certain corn inbreds and single crosses show different resistance to adverse planting conditions. Likewise, some inbreds and single crosses consistently give higher emergence in cold tests. In the production of crosses, the degree of seedling reduction in cold test or adverse field conditions depends largely on the resistance of the female parent. Therefore, in single crosses an inbred with known resistance to seedling decay should be used as the female parent. To a degree, the pollinator parent also has some influence on the resistance of its progency; however, it is difficult to make any prediction from its resistance rating as an inbred. Due to difference in combining ability, the actual comparative resistance of hybrid seed of any genetic composition must be determined by cold test. It should be pointed out that differences among seed

lots may be due to environmental conditions during the growing season. Also inbreds grown by different seed producers give wide differences in cold test results, due largely to differences in seed coat injury.

Fungicide treatment - Since the cold test measures the extent of interaction of the seed with soil pathogens, any protective barrier around the seed from these decay organisms have a significant influence on cold test results. For this reason, the cold test has been widely used to determine the effectiveness of various seed treatment materials. In general, fungicide treatment greatly increases emergence over similar untreated samples under cold test conditions. The degree of increase in seedling emergence by treatment will depend on the crop, the amount of mechanical injury and deterioration. The effects of harvesting and processing injuries can be minimized with a good seed treatment. Any open breaks in the pericarp in which treatment prevents or delays entrance of organisms will certainly result in higher seedling emergence under adverse planting conditions. High quality, sound seed exhibits little or no increase in seedling emergence for treatment, while low quality seed usually shows considerable emergence increase. Maturation studies have shown that treated seeds gave better emergence than untreated seeds of comparable maturities, however, seed treatment does not compensate for lack of maturity. Severely damaged soybeans show less response to seed treatment. Obviously if the embryo parts are fractured or broken, no amount of treatment can increase seedling emergence.