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Seed vigor tests have been developed to evaluate the relative ability of seed lots to produce stands of seedlings in the field. Some workers have suggested the possibility of differentiating vigor levels by germinating seeds under osmotic stress, but have not conducted field trials to evaluate the usefulness of the test. This study was conducted to 1) develop an osmotic stress vigor test for wheat seed, 2) evaluate the effectiveness of this test in ranking seed lots for field emergence, and 3) compare the osmotic stress vigor test with other vigor tests.

The effects of osmotic potential and temperature on germination of wheat seeds of different vigor levels were investigated. The conditions resulting in greatest differentiation of vigor levels were selected for an osmotic stress vigor test. The osmotic stress test and six other germination and vigor tests were compared for effectiveness in predicting seedling emergence in three field trials of 16 artificially aged and 19 naturally aged seed lots.

Reduced osmotic potentials lowered the germination rate of low vigor seeds more than high vigor seeds, but total germination remained the same. The conditions selected for the osmotic stress vigor test were a sand substrate with 50 mL PEG 8000 solution at -0.5 MPa, with a 10-day germination period in the dark at 20° C. Under adverse field conditions, the osmotic stress test was significantly correlated with field emergence of artificially aged ($r = .85$) and naturally aged seeds ($r = .62$). However, the predictive value of the osmotic stress test was lower than some of the other vigor tests, especially accelerated aging and the 4-day sand test. The vigor test rankings varied somewhat under more favorable field conditions. These results indicate the necessity of including several vigor tests and field planting dates when evaluating a specific vigor test. Research should continue to determine the potential of the osmotic stress test and four-day sand tests for application to other seed kinds.

OSMOTIC STRESS VIGOR TEST FOR WHEAT SEED

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INTRODUCTION

It is common knowledge that seedlots which attain high germination percentages in standard germination tests frequently fail to perform well in the field. The discrepancy between germination test scores and field performance has been attributed to differences in seed vigor not detected by germination tests. Seed vigor has been studied and attempts to quantify it have been made for over 40 years. The Association of Official Seed Analysts (AOSA) has recognized seven vigor tests in three categories which can be useful for assessing seed vigor. First, there are tests that attempt to measure some aspect of seedling growth such as rate-of-growth (seedling growth rate) or structural condition of the seedling (seedling vigor classification). Second, there are tests which measure some biochemical aspect of seeds such as dehydrogenase activity (tetrazolium) or membrane permeability (conductivity). Third, there are tests which expose seeds or seedlings to an environmental stress and seed performance is measured. High temperatures and high humidity (accelerated aging) and cold temperatures and seed pathogens (cold test) are such environmental stress tests (AOSA, 1983).

Many other vigor tests have been proposed and among these is the osmotic stess test. Several authors have studied osmotic stress for differentiating cultivars or species for drought resistance (Lafond and Baker, 1986; Masiunas and Carpenter, 1984; El-Sharkawi

and Springuel, 1977). But few have studied osmotic stress as a vigor test.

Parmar and Moore (1966 and 1968) were perhaps the first to use osmotic stress to differentiate seed vigor levels. They noted that germination under osmotic stress was slower for low vigor than for high vigor seeds of corn (*Zea mays* L.). They did not, however, conduct field trials to evaluate the usefulness of the test. They compared the osmotic stress test with topographical tetrazolium and not against other accepted vigor tests. They only tested two seed lots.

Hadas (1977a) suggested that osmotic stress may be an effective vigor test for chickpea (*Cicer arietinum* L.) seeds. In his study, however, there was no indication that more than one seedlot was used or that seed lots of varying vigor were tested. He did not correlate laboratory results with field performance nor did he compare the osmotic stress test with accepted vigor tests. He presented no data to support the theory that the osmotic stress test can differentiate seed lots of high and low vigor.

Van de Venter (1988) compared an osmotic stress test with four other stress tests on corn, but did not find any advantage to using osmotic stress for ranking six seed lots of varying vigor. He correlated laboratory stress tests with laboratory germination and did not conduct field trials so that the relative effectiveness of the stress tests for predicting field performance could not be determined.

Than (1986) used artificially and naturally aged wheat (*Triticum aestivum* L.) seed in an osmotic stress test. Naturally

aged seed germination at 21 days, 20°C and -0.6 MPa osmotic potential had a correlation coefficient of 0.61 with the only field planting. These data indicated some potential for using osmotic stress as a vigor test. Than did not, however, plant in more than one field condition and he did not evaluate the osmotic stress vigor test in relation to other accepted vigor tests.

The objectives of this study were 1) to develop an osmotic stress vigor test for wheat,
2) evaluate its effectiveness for ranking seed lots in relation to field emergence and 3) to compare this osmotic stress test with other vigor tests for relative ability to correlate with field performance.

LITERATURE REVIEW

Need for Seed Vigor Tests

European and American seed testing workers have been getting the highest potential germination from most domesticated crops for many years. But there was a controversy 40 years ago between the European school of thought and the American about whether the american germination test was actually discriminating between lots of varying vigor or whether it was giving the true maximum germination potential of all seed lots. Franck (1950) broached this controversy at the 1950 International Seed Testing Association (ISTA) Congress and asked for standardization to facilitate understanding and interpretation of seed test results across national borders. In the United States, Porter (1944) had recognized that the U.S. concept of seed germination was actually like the European vigor tests and he acknowledged that a germination test should be done under optimum conditions which ease standardization. A standard germination test was needed as a benchmark for marketing, buying and planting considerations and Porter believed that vigor tests could be used in addition to standard germination to predict seed performance under unfavorable conditions.

Goss (1933) perhaps best encapsulated the pursuit of better vigor knowledge with his question:

> Is it not reasonable to expect that the condition of storage or age which proved fatal to one-third of the seed lot has left its degenerating influence upon many of the remaining seed?...that the vigor of the 62% which germinated has been impaired.

His question forms the basis for modern vigor testing. All vigor tests try to assess or measure some aspect of decreased metabolism (AOSA, 1983).

What is vigor? Since the 1950 ISTA Congress, committees have been trying to define it. Finally, in 1977, ISTA accepted a nine paragraph definition of which the following is the first paragraph:

> Seed vigor is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence (Perry, 1978).

In 1980, the Association of Official Seed Analysts accepted this simpler but analogous definition:

> Seed vigor comprises those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions (MacDonald, 1980).

Knowledge of seed vigor is considered valuable for many reasons. It is desired by farmers, seedsmen, and researchers. Farmers recognize seed vigor information as being important in making decisions regarding the cost of seeds, earliness of planting, quantity of seeds to plant, and the anticipated uniformity of stand.

Farmers can use seed vigor knowledge to great advantage in high value crops like lettuce and celery, or in crops like soybeans and corn. Seedsmen, those who produce and market seeds for planting purposes, can use vigor information to monitor the phases of seed

production which can have an adverse effect on seed quality. Seed quality can be affected by insect and disease control measures, harvest practices (time of harvest and combine settings), conditioning operations (cleaner settings, dryer temperature and rate of dry down). "Vigor tests have become a routine part of opening some new seed companies" for the purpose of detecting points in the process where seeds are damaged (AOSA, 1983).

Seedsmen would like to use vigor information as a competitive tool in marketing seed. Researchers would like to use vigor knowledge for many reasons. Plant breeders can use it to develop cultivars with improved seed performance. Agronomists can use a temperature-based vigor test to identify different rates of emergence and to identify cold hardiness. Effects of seed size on seed performance also can be studied with vigor tests (Burris et al., 1971; Edwards and Hartwig, 1971; Payne and Koszykowski, 1979).

As summarized by Grabe (1973), there are genetic and environmental components of seed vigor. The genetic part represents the maximum potential seed vigor and is determined by hybridization and variety development. But many things can happen to the seed which cause it to perform below its maximum potential, these things come from the seed's environment. Weather, mechanical damage, and storage conditions can all reduce the performance of seeds. The environmental component can be further broken down to factors like seed size, maturity, chemical composition, pathological organisms, and deterioration. Most vigor tests target one of these components and measure it to give a quantified estimate of vigor.

The major emphasis of vigor tests, however, has been on measuring the process or extent of seed deterioration. "Because a vigor test is a more sensitive index of seed quality than the germination test, any of the events which precede the loss of germination could serve as vigor tests" (AOSA 1983).

Current Vigor Tests

In the United States there are currently seven officially recognized vigor tests: accelerated aging, cold, conductivity, cool, seedling growth rate, seedling vigor classification and tetrazolium (AOSA, 1983). Vigor tests may be direct or indirect (Isely, 1957). Direct tests are those in which an environmental stress expected in the field is reproduced in the laboratory and the percentage and rate of seedling emergence is recorded, eg. Hiltner brick grit test and the cold test (ISTA, 1987). Indirect tests are those which measure a specific physiological component of seeds. The conductivity test is an indirect test because it measures cell leakage.

AOSA (1983) provided three categories to organize thinking about major vigor tests. Firstly, there are seedling growth and evaluation tests which include the seedling vigor classification test which is similar to the standard germination test. The only difference between the two tests is that normal seedlings are further classified as "strong" and "weak." This test is recognized for cotton, garden beans, peanuts and soybeans. Also included is the seedling growth rate test that seeks to quantify the speed of seedling development and relate it to vigor. The linear growth of

seedlings may be measured at the end of the test period, or seedling dry weight might be measured. This test is recognized for corn and soybeans.

Secondly, there are stress tests. Stress tests are designed to impose an environmental hazard on the seeds as they are germinating or just prior to germination. Among these are a) the accelerated aging test which stresses the seed prior to germination by subjecting the seed to elevated temperatures (40 to 45°C) and high relative humidity (near 100%). The stress is applied for a few days and then the seed is germinated under standard conditions. Accelerated aging is considered a simulation of natural aging and weaker seedlots are distinguished by their lower performance after aging than the stronger lots. Accelerated aging is used on bean, corn, cotton, pea, peanut, pepper, soybean and wheat. Tao (1980) reported test result repeatability in a nationwide refereed accelerated aging vigor test for soybeans.

Also the cold test mimicks a cold soil environment by planting the seed in non-sterile soil and holding the seed at 10° C for 10 days during which time pathological organisms may gain more advantage in weaker seeds than in stronger ones. After the cold period, the seeds are allowed to germinate at optimum temperatures. Grabe (1972) suggested that field emergence of soybeans could be more accurately predicted by the cold test than by the standard germination test. The cold test has been used routinely on field corn and sweet corn.

The cool germination test is a less severe test than the cold test and does not use soil. It was developed for the more temperature-sensitive cotton seed.

Thirdly, there are biochemical tests. The most prominent chemical test for seed vigor came about as a spinoff from the search for a rapid test of viability. The tetrazolium (TZ) viability test, developed by the efforts of G. Lakon, H. Bulat, R.P. Moore and others (ISTA, 1985), has also been used successfully as a vigor test. The chemical principle involved is the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) or -bromide (TTB) to a stable red colored formazan by the hydrogen ions evolved from the seed's respiratory processes. The evaluation of the seed's vigor (or viability) is accomplished by observing the relative staining patterns of the seed's tissues. Experienced analysts can detect low vigor seeds which result from heat damage, frost damage, pre-sprouting, immaturity and other causes. The TZ vigor test is officially recognized for use on corn, cotton, soybeans, and wheat.

The other recognized biochemical test is the conductivity test. First used as a vigor test on peas by Matthews and Bradnock (1967) the conductivity test has recently been evaluated by the AOSA in a blind study of different conductivity methods. Testing corn, soybeans and peanuts, the study concluded that the bulk conductivity method appears to be repeatable across laboratories for soybeans, but the results for the other two crops contained too much variation and a recommendation could not be given. The other methods studied were the dynamic bulk conductivity and the single seed conductivity

methods (Reusche, 1987). The conductivity test is recognized for wrinkled-seeded garden peas and soybeans.

These seven tests are the ones which have qualified as reasonably practical and economic vigor tests. Many attempts have been made to quantify vigor. Some of the seed aspects studied have been glutamic acid decarboxylase activity (GADA) (Linko and Sogn, 1960; Grabe, 1964), adenosine triphosphate (ATP) content (Ching, 1973; Yaklich et al., 1979) and seed respiration (Woodstock and Combs, 1965; Woodstock and Grabe, 1967). But these tests, although reliable, are yet too sophisticated or too expensive to be adopted in seed testing laboratories. Even though the seven tests recognized by AOSA are available, they are all tested on only one or a few crop species and none have achieved repeatability across laboratories. Generally, "a laboratory can standardize test procedures sufficiently to repeat its own vigor test results within acceptable limits, but standardization has not yet reached the point where seed testing laboratories can consistently reproduce the results of other laboratories" (AOSA, 1983).

The Ideal Vigor Test

Certain principles guide researchers who are trying to develop new vigor tests.

> A practical seed vigor test should provide good reproducibility of test results which can be easily interpreted and which provide a good indication of field performance potential. It is desirable also to have tests which can be conducted in a reasonable length of time which do not require expensive equipment or extensive training to conduct and which, therefore, would be relatively simple and inexpensive (AOSA, 1983).
>
> The object of the vigor test is to identify seed lots which are capable of rapid and uniform seedling emergence in the field, and seed lots with high emergence ability under unfavorable environmental conditions (Perry, 1978).

With these guidelines of the ideal vigor test in mind the ISTA Vigor Test Committee (ISTA, 1986) has set three objectives for itself. First, to introduce new and alternative methods and assess whether reliable results can be acheived. Second, to improve precision of conductivity and accelerated aging test methods. And third, make minor amendments on the Handbook of Vigour Test Methods.

In accord with objective number one, the object of this study has been to investigate the feasibility of developing a practical vigor test based on subjecting seeds to osmotic stress with polyethylene glycol (PEG) solutions. The next section reviews the evidence which supports a potential osmotic stress vigor test.

Potential for an Osmotic Stress Vigor Test

Water Potential Relations and Osmoticants

A basic knowledge of water potential is necessary to understand the role of osmoticants in water stress experiments. Water potential is directly related to the diffusivity of water. Pure water

has a water potential of 0.0 megapascals (MPa), theoretically. It diffuses towards areas of negative water potential. In an osmometer on one side of a membrane there may be pure water and on the other side a solution of a certain concentration. The solution, because it contains a solute, has a negative water potential and the pure water will diffuse across the membrane into the solution, thereby raising the water potential of the solution until the water potentials of the two liquids are equal. Equilibrium is achieved because there are at least two components of water potential which can perfectly balance each other creating a zero water potential. In the simple osmometer example water potential has only two components: water potential (WP) = osmotic potential (OP) + pressure (P). As pure water diffuses into the solution the OP of the solution becomes less negative until all the pure water has diffused across the membrane or, if the osmometer has a physical restriction which limits the size of the chamber the solution can fill, then the pure water will move into the solution until the solution quantity exerts a certain force on its restricting walls. This force is the P of the solution and it increases positively until it is equal to but opposite in magnitude to the OP. The WP is then equal to zero and the osmometer is in equilibrium so that no net diffusion takes place.

Water potential in soil systems has another component, matric potential. Matric potential is created by the tenacity with which water molecules adsorb to hydrophilic surfaces in the soil (eg. colloids such as protein, starch and clay) (Salisbury and Ross, 1985).

In a soil system, seeds compete for moisture with at least two components of soil water potential, matric and osmotic potential (pressure is equal to atmospheric pressure in this system and is essentially zero). At first, the seed has a tremendous pull on available moisture. Some dry seeds may have a water potential as low as -100.0 MPa (Shaykewich and Williams, 1971) whereas a clay soil at permanent wilting point has a water potential of only -1.5 MPa (Brady, 1984). But as the seed imbibes water and its water potential becomes less negative, competition for moisture with the surrounding soil becomes greater. At some point, the seed either has enough moisture to germinate or it does not (Hunter and Erickson, 1952).

The purpose of some matric stress and osmotic stress studies has been to manipulate the water potential of the system surrounding the seed so that only more drought resistant species or varieties will germinate or so that only higher vigor seeds will germinate. For convenience, osmotic potential has been used to try to mimic matric potential in its effect on seed germination. Normally in soils, matric potential is the more negative component of water potential, because soil solutions are usually dilute. But the osmotic potential of solutions is easier to control in the laboratory than matric potentials. Various authors have studied several osmoticants for suitability of imitating the effect of matric potential on seed germination.

Prior to Uhvits (1946) some authors had experimented with alkali salts, certain fertilizers, and sodium chloride (NaCl) as os-

moticants to determine their effect on germinating seeds and early seedling growth. Uhvits (1946) studied the effect of reduced osmotic potential and salt concentration on germinating alfalfa seeds. She sought information which could be used to develop a test for screening salt tolerant or drought resistant varieties. Comparing NaCl and mannitol, she showed decreases in alfalfa germination rate as osmotic potential decreased. Her main experiment was complete in 10 days and she recognized that, given enough time, relatively high germination would be obtained with mannitol solutions of -1.2 and -1.5 MPa. She noted that "differences in response to the two substrates at isomotic concentrations suggests a toxic effect of NaCl."

Wiggans and Gardner (1959) tested glucose, sucrose, D-mannitol, NaCl, and polyvinylpyrrollidone (PVP) solutions for simulating drouth conditions on radish and sorghum. They suggested that glucose, sucrose and D-mannitol solutions decreased germination percentage, but their final count at 5 days leaves room for doubt whether the seeds had finished germinating. They found NaCl and PVP solutions to be apparently toxic to seeds.

Lagerwerf et al. (1961) tested 13 osmoticants and concluded that after purification, polyethylene glycol Molecular Weight (M.W.) 20,000 was the most satisfactory for controlling osmotic potential.

Collis-George and Sands (1962) studied NaCl, glycerol and cadmium sulfate. Glycerol replaced mannitol in their experiments because microorganisms quickly develop in the prescence of sugars and they did not want to use antibiotics in the solution because of the effect of reducing the osmotic potential.

Manohar and Heydecker (1964) noted that polyethylene glycol (PEG) of M.W. 4,000 or more appeared to possess osmotic properties with no evidence of toxicity in contrast to previously used osmoticants.

Manohar (1966) compared NaCl, mannitol and PEG and came to the conclusion that

1) percentage germination of seed in contact with an aqueous solution depends on a) the extent of differential permeability of the seed coat to the solute and b) whether the solute is toxic; 2) PEG on MW 4,000 and higher was a suitable osmoticant for "Meteor" peas; 3) solutions of NaCl, glycerol and mannitol may enter the seed through the micropyle and therefore their influence on germination should not be ascribed to osmotic potential; and 4) high concentrations of NaCl seem to be toxic to germinating peas.

Parmar and Moore (1968) compared Carbowax PEG 6000, mannitol, and NaCl. PEG gave the clearest reduction in germination over osmotic potentials of 0 to -1.0 MPa.

Tadmor et al. (1969) could see no consistent differences between PEG (MW 1540) and D-mannitol solutions. They chose D-mannitol for their experiments, but gave no rationale.

Kaufmann and Ross (1970) found sucrose to be inadequate for simulating soil water stress. Wheat seeds germinated at -1.5 MPa in sucrose, but not at all in soil or in PEG solution at the same water potential.

McWilliam and Phillips (1971) used PEG of MW 20,000. Williams and Shaykewich (1969) evalutated PEG 20,000 and PEG 6,000 for os-

motic control of soil water matric potential and found no difference. Comparing PEG MW 20,000 and MW 6,000, Hadas (1976) found practically no differences in water uptake nor in germination time nor in final germination percentage. Hadas, therefore, used PEG MW 6,000 for subsequent experiments because its lower viscosity was desirable. One of the common trade names for PEG is Carbowax 6000, made by Union Carbide. Sometime before Michel (1983) the product was renamed Carbowax 8000. No changes in the product itself were made.

In a more recent comparison of osmoticants, Roundy et al., (1985) found toxicity effects on two grass species from NaCl, $NaSO_4$, and CaCl, but not with PEG 6000 solutions.

In summary, for simply reducing osmotic potential, "sucrose or mannitol solutions can be used, but substances such as PEG have the advantage of not penetrating the tissue or being changed metabolically by the tissue as easily" (Salisbury and Ross, 1985).

Osmotic and Matric Stress Tests

A number of experiments have been conducted using osmotic stress to try to differentiate between varieties for drought resistance and cold hardiness. Soil matric stress and osmotic stress have been compared for equivalency of effect on seed germination and temperature x osmotic stress interactions have been noted.

Helmerick and Pfeifer (1954) examined D-mannitol, and hexanhydric alcohol as osmoticants in an osmotic stress test for varietal differences in wheat and found that D-mannitol showed good germina-

tion differences at -0.7 to -1.0 MPa at the seven-day count. They inferred that these results displayed varietal differences. They did not give standard germination results for the two seedlots studied, but large differences in field emergence counts suggest that there could have been vigor differences not related to variety.

Rodger et al., (1957) used NaCl and sucrose to successfully differentiate between eight varieties of alfalfa of varying winter-hardiness. Dotzenko and Dean (1959) supported Rodger et al., (1957) and Uhvits (1946) by reporting that osmotic potentials of -0.7 and -1.2 MPa did distinguish between six alfalfa varieties, indicating that ability to germinate at low osmotic potentials is heritable.

Eslick and Vogel (1959) studied the effect of reduced matric potentials on 22 grasses and eleven legumes to screen for drought resistant species. Evans and Stickler (1961) used D-mannitol and showed good differences in germination response between four grain sorghum varieties. Gul and Allan (1976) suggested that a soil matric stress test could be used to select wheat lines which have more rapid emergence under lower soil water potentials.

Ashraf and Abu-Shakra (1978), using mannitol solutions from 0 to -1.8 MPa osmotic potential, found that total germination was not lowered for four wheat cultivars at two alternating temperature regimes. Rate of root growth, speed of germination and respiration rates were inversely related to moisture stress. Significant differences were found among the parameters studied suggesting that simulating drought with osmotic solutions can be used in selecting drought resistant cultivars.

A well designed study by Lafond and Baker (1986) revealed that the relative germination performance of nine wheat culitvars was not affected by either temperature or osmotic moisture stress. Increasing moisture stress simply increased median germination time. Their data suggest that a speed-of-germination test at a convenient temperature and carefully chosen counting intervals with or without osmotic moisture stress, would be an adequate technique for screening wheat genotypes for variability in germination response. No advantage was gained by using reduced osmotic potentials.

It is generally accepted that soil matric stress and osmotic stress have different action on seeds. But it is also accepted that the germination response of seeds under osmotic stress may be similar to those under matric stress.

Collis-George and Sands (1962) studied NaCl, glycerol, and cadmium sulfate as osmoticants to simulate reduced matric potentials and concluded that they do not mimic matric stress. Their conclusion was that a permanent semi-permeable membrane system would be needed in the seed and seedling which would always exclude the solute. Such a membrane sysetem apparently does not exist.

McWilliam and Phillips (1971) found that matric and osmotic potentials may be equivalent in their effect on seed germination in some species where soil moisture diffusivity and seed-soil contact are not limiting, but some seed coats (*Phalaris*) are very resistant to absorption of soil water and the equivalence between osmotic and matric potential breaks down.

The results of Sharma (1973) support McWilliam and Phillips (1971) in the hypothesis that, when seed/soil contact and water flow properties of the soil are non-limiting, PEG-induced osmotic stress is equivalent to matric stress. PEG solutions were satisfactory media for studying drought stress on seed germination, but mannitol and NaCl were suspected of entering the seed and NaCl probably was toxic to the seeds.

Lindstrom et al., (1976) showed that soil matric stresses produced in the laboratory could be used to predict wheat emergence in the field when similar temperature and moisture conditions were encountered.

Somers (1983) successfully used PEG solutions to screen sunflower cultivars for improved emergence during moisture stress.

Generally, although osmotically generated water stress is not regarded as equivalent to matric water stress, it has been shown that the ranking of different seed lots is similar under the two situations. It seems reasonable, therefore, to regard germination in solutions of reduced osmotic potential as a simulated drought condition.

Interactions between temperature and osmotic potential are significant and affect test results. When a researcher first becomes interested in using osmotic stress as a variety-screening technique or as a vigor test one of the first questions that arises is, "at what temperature are differences greatest at a given reduced osmotic potential?" Previous researchers have partially answered this question. Generally, the germination response will be different at each

different combination of temperature and osmotic potential. Germination response follows a continuum from low germination to high germination. The selection of a temperature and an osmotic potential, therefore, is based on other factors such as the time desired to complete the test and the spread and location of the germination response between high and low vigor seed. For example, does the high vigor lot germinate at 90 or 40% when the test is finished and does the low vigor lot germinate at 70 or 20%?

Tadmor et al. (1969), using mannitol solutions of 0 to -1.5 MPa noted that rate of germination for wheat, barley, and three range species was greatly affected by temperature at all osmotic potentials. However, final germination was not significantly affected by water potentials down to -0.8 MPa at temperatures of 4, 10, 15, 20 and 25°C. They did see an approximately 20% reduction in final germination at -1.5 and 25°C, but they used only one seedlot of one cultivar.

Kaufman and Ross (1970) found that wheat germinates well at -0.8 MPa, but not at all at -1.5 MPa and temperature had little effect on final germination.

El-Sharkawi and Springuel (1977) simulated reduced matric potential and observed interaction with temperature using PEG solutions. Germination parameters examined included plumule emergence and elongation as well as radicle emergence. Wheat, barley, and sorghum were studied and the seeds responded differently to reduced osmotic potential. Plumule emergence was generally more sensitive

to reduced osmotic potential than radicle emergence and this is in agreement with Parmar and Moore (1968).

Masiunas and Carpenter (1984) found that radicle growth was inhibited by decreasing osmotic potentials (-0.3, -0.8, -1.0 MPa) and that PEG concentration and temperature interacted.

Osmotic Stress Vigor Tests

There have been numerous experiments which used osmotic stress to test for species and varietal differences, but only a few have suggested its use as a vigor test.

Parmar and Moore (1966) were perhaps the first to suggest such an application. Using a -1.0 MPa PEG solution they showed good differences in final germination between one high and one low vigor corn seed lot. They suggested additional studies of osmotic stress tests with PEG for possible standardization as a vigor test. In their subsequent publication (Parmar and Moore, 1968), trends were noted indicating more adverse effects of decreasing osmotic potential on the low than on the high energy lot, and on the shoot than on primary root elongation. Correlation studies indicated that shoot growth alone was an acceptable measure of evaluation for simulated influence of drought conditions.

When Hadas (1977b) studied water uptake and germination rate of chickpea and pea seeds under changing matric and osmotic potentials with PEG solutions, he found that the final water uptake and germination were the same in all cases for a given water potential. Germination rates decreased with decreasing water potential. Refer-

ring especially to mechanical stresses, Hadas noted that at lower water potentials seeds can overcome only small normal stresses. With this in mind it seemed reasonable to subject seeds of high and low vigor to lower water potentials and observe whether high vigor seeds outperform, and thereby can be distinguished from, low vigor seeds (Hadas, 1977a).

Recently Than (1986) used reduced osmotic potentials and was able to differentiate between wheat seed lots of varying vigor levels. In contrast Van de Venter (1988) used a -0.7 MPa PEG solution to test for a differential response between high and low vigor corn seedlots compared to responses under favorable conditions and he found no difference, i.e. the favorable conditions ranked the seedlots from high to low vigor as well as or better that the osmotic stress test.

This study builds on the work of Than (1986) and attempts to answer the question whether the reduced osmotic potential of PEG solutions provide a better vigor test for wheat seed than vigor tests currently in use.

OSMOTIC STRESS VIGOR TEST FOR WHEAT SEED

ABSTRACT

Seed vigor tests have been developed to evaluate the relative ability of seed lots to produce stands of seedlings in the field. Some workers have suggested the possibility of differentiating vigor levels by germinating seeds under osmotic stress, but have not conducted field trials to evaluate the usefulness of the test. This study was conducted to 1) develop an osmotic stress vigor test for wheat seed, 2) evaluate the effectiveness of this test in ranking seed lots for field emergence, and 3) compare the osmotic stress vigor test with other vigor tests.

The effects of osmotic potential and temperature on germination of wheat seeds of different vigor levels were investigated. The conditions resulting in greatest differentiation of vigor levels were selected for an osmotic stress vigor test. The osmotic stress test and six other germination and vigor tests were compared for effectiveness in predicting seedling emergence in three field trials of 16 artificially aged and 19 naturally aged seed lots.

Reduced osmotic potentials lowered the germination rate of low vigor seeds more than high vigor seeds, but total germination remained the same. The conditions selected for the osmotic stress vigor test were a sand substrate with 50 mL PEG 8000 solution at -0.5 MPa, with a 10-day germination period in the dark at 20°C. Under adverse field conditions, the osmotic stress test was significantly correlated with field emergence of artificially aged ($r = .85$) and naturally aged seeds ($r = .62$). However, the predictive

value of the osmotic stress test was lower than some of the other vigor tests, especially accelerated aging and the four-day sand test. The vigor test rankings varied somewhat under more favorable field conditions. These results indicate the necessity of including several vigor tests and field planting dates when evaluating a specific vigor test. Research should continue to determine potential of the osmotic stress test and four-day sand tests for application to other seed kinds.

INTRODUCTION

Seed vigor tests have been developed to evaluate the relative ability of seed lots to produce stands of seedlings in the field. A number of these vigor tests, current and proposed, are based on the imposition of stress environmental conditions on the seeds prior to or during germination. These stresses may simulate the stresses encountered in the field or they may be artificial. Examples of these stresses include cold wet soil (Isely, 1950), layers of ground brick (Hiltner and Ihssen, 1911), accelerated aging under high temperature and humidity (Delouche, 1965), ammonium chloride (Vanderlip et al., 1973), cool temperature (McCarter and Roncadori, 1971), and high temperature (Caldwell, 1960).

Some workers have suggested the possibility of differentiating vigor levels by germinating seeds under osmotic stress. Parmar and Moore (1968) found that germination of corn (*Zea mays* L.) seeds in solutions of Carbowax 6000 had more adverse effects on low vigor seeds than on those of high vigor. Hadas (1977) suggested that osmotic stress may be an effective seed vigor test, but presented no data to support that theory. Van de Venter (1988) found that the ranking of corn seed lots of differing vigor levels was similar whether germination was conducted in water or in a solution with an osmotic potential of -0.7 MPa. None of these workers, however, conducted field trials to evaluate the usefulness of the test.

Than (1986) was able to differentiate between vigor levels in wheat (*Triticum aestivum* L.) by germinating seed lots at a reduced osmotic potential. Also, germination percentages at -0.6 MPa were significantly correlated with seedling emergence in a field trial.

The objectives of this study were to 1) develop an osmotic stress vigor test for wheat seed, 2) evaluate the effectiveness of this test in ranking seed lots for field emergence, and 3) compare the osmotic stress vigor test with other vigor tests.

MATERIALS AND METHODS

Seed Lots

Both artificially and naturally aged seed lots were used for these studies. To obtain artificially aged seed, Foundation seed of 'Stephens' soft white winter wheat was raised to 155 g H_2O kg^{-1} fw moisture content (w.b) by adding misted water in a cement mixer. After 4 d equilibration at 10°C, the seed was placed in 16 sealed 950-mL glass jars at 30°C. One jar was removed at approximately 3-d intervals for 50 d. After each aging treatment, seed lots were spread out to dry to approximately 120 g H_2O kg^{-1} fw at room temperature and humidity. The seed lots were then returned to the sealed glass jars and stored at 5°C for the duration of the experiments.

For naturally aged seed, 19 seed lots of 'Nugaines' soft white winter, 'Wanser' hard red winter, and 'Moro' soft white winter club wheat were supplied by the Washington State Agricultural Experiment Station at Pullman. The seed lots were grown in 1980, 1983 and 1984 at different locations in Washington and stored under different environmental conditions until 1985. They were then stored at 5°C until the completion of this study.

Development of an Osmotic Stress Vigor Test

Artificially aged seed lots were used to determine the test variables that most clearly differentiate seed vigor levels on the basis of total germination and rate of germination. One high (non-aged) and one low (aged 50 days) vigor seed lot were germinated in

the dark at 0.0, -0.4, -0.5 and -0.6 MPa osmotic potential at 15, 20, and 25°C. Twenty-five undamaged seed were placed on top of 100 g grade 20 silica sand (Unimin Corp., Emmett, ID 83617) and covered with 210 g sand in 11 x 11 x 3.5 cm plastic germination boxes. This quantity of sand provided approximately 1 cm of sand above the seed. Fifty mL of distilled water or polyethylene glycol (PEG) (Carbowax PEG 8000, Union Carbide Corp.) solution were added to each box. PEG solutions of desired osmotic potentials were prepared according to the procedure of Michel (1983). Osmotic potential was measured by a Wescor 5100C Vapor Pressure Osmometer (Wescor Inc., Logan, UT 84321) and adjustments in amounts of PEG or distilled water were made as needed. The lid was replaced and the box enclosed in plastic bags. Coleoptile emergence was recorded daily for the first 2 wk after initial emergence and then periodically until germination was complete. For this experiment only, germination data were adjusted to a live seed basis.

Field Emergence Trials

Emergence trials were conducted at Hyslop Crop Science Field Laboratory, Corvallis, Oregon. The soil is a Woodburn silt loam (fine silty, mixed, mesic Aquultic Argixeroll). Planting dates were 30 Sep., 20 Oct., and 5 Nov. 1987. One hundred seeds of the 16 artificially aged and 19 naturally aged wheat seed lots were planted in 6-m rows at a depth of 4.4 cm. The plot area was irrigated with 2.5 cm water on 22 October. Soil temperature at 4.4 cm was recorded daily by an OmniData Digital Recorder (Omnidata International Inc.,

Logan, Utah 84321). Preliminary and final emergence counts were made approximately 12 and 40 d after planting each of the three trials.

Laboratory Tests

All seed lots were subjected to six additional germination and vigor tests for comparison with the osmotic stress vigor test in ability to predict field emergence.

Accelerated Aging

An accelerated aging chamber was built from hardboard and pine with dimensions of 31 x 57 x 86 cm and placed inside a germination cabinet. A fan circulated air internally over a water dish. Temperature was controlled at 41.0 + 0.5°C and relative humidity was maintained near 100% at all times. The tray method of McDonald and Phaneendranath (AOSA, 1983) was used with 11 x 11 x 3.5-cm germination boxes and 10 x 10 x 1.7-cm wire trays (Hoffman Manufacturing Co., Albany, OR 97321). Forty mL of distilled water were added to the box and 2 g seed were spread on the wire mesh tray. The lids were replaced on the boxes and one replication at a time was incubated 72 h. Forty seeds from each tray were then germinated according to AOSA rules (AOSA, 1981).

Four-Day Sand

Forty seeds were planted with the sand substrate method described above. Fifty mL distilled water was added and seeds were

germinated at 20°C in the dark. Coleoptile emergence above sand was recorded at 4 d.

Germination

Seed lots were germinated in rolled paper towels at 20°C for 7 d (AOSA, 1981). Forty seeds were used per replication.

Seedling Growth Rate

Dry weight per normal seedling was determined on the same samples used for the standard germination test and expressed as mg per normal seedling (AOSA, 1983).

Osmotic Stress

Forty seeds were planted with the sand method described above. Fifty mL -0.5 MPa PEG solution was added to each box. The seeds were placed in dark 20°C germinators and coleoptile emergence above sand was recorded at 10 d.

Dehydrogenase Activity

A 500-mg sample of seed was ground 1 min to pass through a 20-mesh screen. Then 200 mg flour was soaked in 1.5 mL 1.0% triphenyl tetrazolium chloride solution (0.1 M phosphate buffer, pH 7.0) at 35°C for 2 h. The samples were centrifuged, decanted, and 5 mL acetone added to extract the formazan at room temperature for 16 h. Absorbance of the solution was measured in a spectrophotometer (Spectronic 20) at 520 nm and expressed on a per gram per hour basis (Sorger-Domenigg et al., 1955).

Glutamic Acid Decarboxylase Activity

Glutamic acid decarboxylase activity (GADA) was measured using a Gilson Differential Respirometer Model GRP14. Ground seed (0.5 g) was placed in reaction flasks and 2.5 mL of 0.75 M glutamic acid solution (0.067 M phosphate buffer, pH 5.8) was added. The flasks were equilibrated 10 min at 30°C before closing the system. Readings of uL gas produced were taken at 30 min (Linko and Sogn, 1960). All determinations were corrected to standard temperature and atmospheric pressure (Umbreit et al., 1972) and for carbon dioxide solubility in water (Gregory and Winter, 1965).

Experimental Design and Data Analysis

Laboratory tests and field trials were replicated four times in randomized block designs and the data were subjected to analysis of variance. Means of laboratory tests were correlated with field emergence in simple and multiple linear regression. Geometric means were used to combine selected vigor tests for correlation with field emergence.

RESULTS

Development of an Osmotic Stress Vigor Test

Preliminary tests indicated that sand was a superior substrate to blotters for maintaining osmotic potentials because of better seed-moisture contact and control of molds without fungicide. These experiments indicated that there was no critical osmotic potential that would prevent low vigor seed from germinating while allowing high vigor seed to do so. Subsequent efforts were directed toward determining osmotic potentials and temperatures that would differentiate vigor levels on the basis of germination rate.

Initial germination of low-vigor seeds tended to be slower than that of high vigor seeds at each water potential at 20°C, although the only significant difference ($P < 0.05$) occurred at 0.0 MPa after 4 days (Fig. 1). With reduced water potentials, low vigor seeds were most clearly differentiated from high vigor seeds after 10 d at -0.5 MPa and 20°C, although with only a low probability level ($P < 0.24$).

Total germination at 20°C occurred after approximately 7, 15, 18 and 30 d at 0.0, -0.4, -0.5 and -0.6 MPa, respectively. There was very little difference in the number of days required for low and high vigor seed to reach total germination.

The relationships between vigor levels, germination and osmotic potential at 15 and 25°C were similar to those at 20°C (data not shown), but differentiation of vigor levels was not as great. Therefore, the conditions selected for the osmotic stress vigor test

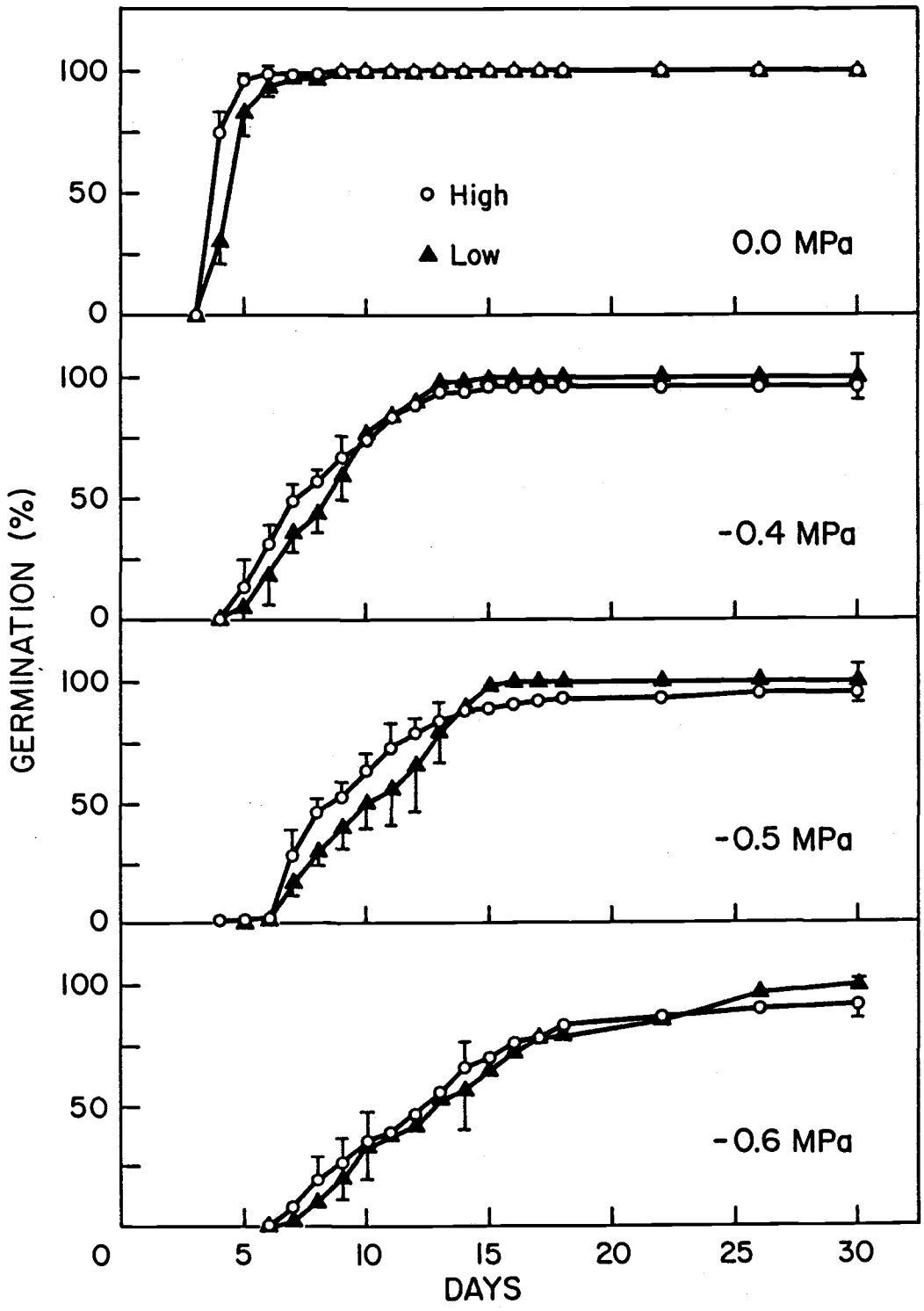

Fig. 1. Effects of reduced osmotic potentials on germination of high and low vigor wheat seed lots in sand at 20°C. Bars indicate SE.

were a sand substrate with PEG 8000 solution at -0.5 MPa, with a 10-d germination period in the dark at 20°C.

Differences in germination rate of high vigor and low vigor samples were not as great at reduced water potentials as in distilled water. Therefore, a germination test conducted for 4 d in sand moistened with distilled water was included in subsequent trials which compared the osmotic stress and other vigor tests with field emergence.

Field Emergence Trials

The 35 wheat seed lots selected for this study exhibited laboratory germination percentages of 85 to 100, well within the range of marketable seed quality. Differences in seed vigor and field environments, however, resulted in a range of field emergence percentages of 43 to 93 for individual lots (Tables 1 and 2). The average emergence of naturally aged seed was 60, 78 and 79% for the 30 September, 20 October and 5 November plantings, respectively (Table 1), while the emergence of artificially aged seed was similar at 59, 83 and 72% for the three planting dates (Table 2).

Seedling emergence was influenced by differences in the soil environment between the three planting dates. The first 2 wk after the 30 September planting had mean daily temperatures in the range of 20.0 to 14.5°C, and moisture was scarce and unevenly distributed. The first 2 wk after the 20 October planting had mean daily temperatures in the range of 15.5 to 10.0°C. The soil was unusually dry so 2.5 cm of water was applied to the entire experiment on 22 October.

Table 1. Performance of 19 naturally aged wheat seed lots in seven vigor tests and three field emergence trials.

Lot	Cultivar	Germination and vigor tests							Planting date					
									Early		Optimum		Late	
		Standard germination	Accelerated aging	Four-day sand	Osmotic stress	Dehydrogenase activity	Growth rate	GADA	First count†	Final count‡	First count	Final count	First count	Final count
		%				absorbance	mg/sdlng	µL/g/h	%					
1	Nugaines	100	57	51	76	0.29	9.1	310	24	73	65	81	17	82
2		93	46	39	59	0.39	9.0	296	20	58	52	67	12	70
3		100	93	77	88	0.34	9.6	318	15	65	71	82	46	88
4		99	96	76	84	0.40	9.8	316	15	62	67	84	41	87
5		99	94	90	63	0.48	11.2	320	18	62	73	89	48	86
6		97	92	79	73	0.55	11.0	340	16	67	76	82	47	89
7		98	91	89	68	0.82	10.5	346	13	64	80	89	60	91
8	Wanser	97	61	71	83	0.22	9.5	299	19	61	75	81	36	77
9		94	51	51	66	0.15	9.7	241	15	59	59	74	20	75
10		97	92	89	91	0.78	10.5	338	15	62	69	76	60	82
11		97	87	85	86	0.76	11.0	337	23	62	62	79	65	88
12		99	85	82	80	0.57	11.1	296	23	64	65	77	63	86
13		99	89	88	75	0.91	11.3	392	24	57	57	73	70	90
14	Moro	89	16	22	51	0.14	8.4	281	11	48	33	53	4	44
15		87	24	37	66	0.17	9.7	247	16	43	51	67	21	53
16		92	81	66	73	0.16	10.6	306	23	56	78	85	33	78
17		97	86	76	76	0.33	11.5	376	21	59	73	86	32	73
18		93	82	72	70	0.25	10.8	351	15	56	79	83	36	73
19		93	87	74	69	0.26	10.1	377	13	54	75	83	55	81
Mean		96	74	69	74	0.42	10.2	320	18	60	66	78	40	79
LSD (0.05)		4	8	14	14	0.18	0.9	34	NS	11	18	9	14	7
CV (%)		3	7	15	13	30	6	7	54	13	19	8	25	6

†Seedling emergence after approximately 12 days.
‡Seedling emergence after approximately 40 days.

Table 2. Performance of 16 artificially aged Stephens wheat seed lots in seven vigor tests and three field emergence trials.

Lot	Germination and vigor tests							Planting date					
								Early		Optimum		Late	
	Standard germination	Accelerated aging	Four-day sand	Osmotic stress	Dehydrogenase activity	Growth rate	GADA	First[†] count	Final[‡] count	First count	Final count	First count	Final count
	%				absorbance	mg/sdlng	µL/g/h	%					
1	95	84	88	66	0.39	13.9	313	13	68	86	87	68	93
2	98	92	91	73	0.38	12.8	310	22	69	86	89	76	89
3	98	80	90	77	0.27	12.6	299	14	67	78	83	64	89
4	95	76	86	76	0.20	13.4	309	13	66	86	87	67	87
5	96	78	83	71	0.14	13.0	306	16	58	81	88	49	85
6	98	69	75	71	0.10	13.2	289	19	58	83	85	34	77
7	94	75	71	62	0.13	12.8	298	13	53	81	83	33	74
8	95	64	73	65	0.09	12.6	285	13	63	73	81	33	80
9	96	65	80	66	0.09	12.7	268	18	58	79	85	26	77
10	95	53	60	67	0.10	12.0	261	13	57	74	85	19	69
11	91	43	56	64	0.12	12.4	241	19	58	80	84	14	62
12	95	38	51	63	0.10	12.3	244	14	62	76	83	8	58
13	92	40	60	60	0.11	12.1	261	12	52	74	84	8	58
14	93	38	59	48	0.09	12.1	255	14	58	71	82	11	54
15	88	37	41	46	0.07	11.4	232	12	53	66	73	5	49
16	85	34	38	53	0.07	12.3	208	12	48	65	74	5	49
Mean	94	60	69	64	0.15	12.6	274	15	59	77	83	33	72
LSD (0.05)	6	14	13	11	0.10	1.1	32	NS	12	9	6	15	10
CV (%)	4	16	13	12	46	6	8	53	15	8	5	32	9

[†]Seedling emergence after approximately 12 days.
[‡]Seedling emergence after approximately 40 days.

The first 2 wk after the 5 November planting had cool temperatures with the daily average in the range of 13.3 to 6.5°C and the soil was wet.

Under the relatively cold wet field conditions of the third planting, the osmotic stress test was significantly correlated with field emergence of naturally aged ($r = 0.62$, Table 3) and artificially aged ($r = 0.85$, Table 4) seeds. Correlation coefficients were also significant, but lower for the other planting dates. However, the predictive value of the osmotic stress test was lower than some of the other tests, especially artificial aging and the four-day sand test.

Vigor tests varied in their ability to rank seed lot emergence in each of the six field experiments (two sets of seed lots at three planting dates). When the vigor tests were ranked according to correlation coefficients, the four-day sand test ranked among the top three tests six times; accelerated aging four times; germination test, four times; GADA, two times; and osmotic stress, dehydrogenase activity and seedling growth rate, one time each.

An attempt was made to include the osmotic stress test with other vigor tests in a multiple regression model to improve the predictive ability over the best single tests. However, the large amount of collinearity among the several vigor tests would have made the model invalid (Steiner et al., 1989) and the use of multiple regression was abandoned.

A prediction model was then developed by calculating the geometric mean of the osmotic stress and four-day sand tests (Steiner

Table 3. Simple correlation coefficients (r) between seven vigor tests and field emergence for 19 naturally aged seed lots of three wheat varieties.

Test	Planting date						Germination and vigor tests					
	Early		Optimum		Late							
	First count	Final count	First count	Final count	First count	Final count	GADA	Growth rate	Osmotic stress	Dehydrogenase activity	Standard germination	Four-day sand
Accelerated aging	0.17†	0.56	0.76	0.81	0.81	0.88	0.70	0.73	0.61	0.58	0.71	0.94
Four-day sand	0.21	0.51	0.72	0.76	0.91	0.86	0.67	0.79	0.63	0.69	0.71	
Standard germination	0.37	0.87	0.44	0.59	0.55	0.83	0.42	0.38	0.61	0.55		
Dehydrogenase activity	0.23	0.37	0.15	0.22	0.79	0.64	0.55	0.55	0.38			
Osmotic stress	0.30	0.51	0.45	0.47	0.58	0.62	0.29	0.33				
Growth rate	0.38	0.19	0.55	0.60	0.73	0.56	0.60					
GADA	0.15	0.23	0.48	0.48	0.62	0.51						

† Correlation coefficients greater than or equal to 0.46 and 0.58 are significant at p = .05 and .01 respectively.

Table 4. Simple correlation coefficients (r) between seven vigor tests and field emergence for 16 artificially aged seedlots of Stephens wheat.

Test	Planting date						Germination and vigor tests					
	Early		Optimum		Late							
	First count	Final count	First count	Final count	First count	Final count	GADA	Growth rate	Osmotic stress	Dehydrogenase activity	Standard germination	Four-day sand
Accelerated aging	0.39†	0.69	0.81	0.69	0.94	0.96	0.94	0.77	0.78	0.74	0.74	0.94
Four-day sand	0.40	0.76	0.83	0.79	0.92	0.96	0.94	0.76	0.82	0.71	0.83	
Standard germination	0.48	0.72	0.74	0.81	0.66	0.78	0.81	0.52	0.77	0.46		
Dehydrogenase activity	0.29	0.78	0.67	0.55	0.86	0.73	0.68	0.61	0.53			
Osmotic stress	0.42	0.67	0.79	0.76	0.77	0.85	0.75	0.66				
Growth rate	0.26	0.57	0.83	0.66	0.76	0.81	0.76					
GADA	0.26	0.70	0.83	0.77	0.90	0.93						

† Correlation coefficients greater than or equal to 0.50 and 0.62 are significant at p = .05 and .01 respectively.

et al., 1989). The geometric mean did not increase the predictive value over that of either accelerated aging or the four-day sand test. Other vigor test combinations were evaluated, but little additional predictive value was gained.

DISCUSSION

Although the potential of osmotic stress vigor tests is frequently mentioned in the literature, the present work and that of Than (1986) are believed to be the first reports on the relationship between osmotic stress vigor tests and seedling emergence in the field.

Since much of the world's wheat is grown in marginal rainfall areas, imposition of a moisture stress during germination would seem to be a realistic basis for measuring wheat seed vigor. This was not borne out in the present study, however, since the lowest correlations were obtained from the first planting date when soil moisture was too low for maximum seedling emergence. Other factors may have prevented seedling emergence then since the seeds lay in the soil for 22 d before irrigation water was applied to promote germination and emergence. Additional field trials under known moisture levels would be needed to relate the effects of moisture stress on germination under laboratory and field conditions.

The vigor test described here is actually a rate of germination test under osmotic stress. A vigor test based on total germination would be more convenient, although more time consuming, than one based on rate of germination. However, it was not possible to find an osmotic potential that would allow high vigor seeds to germinate while preventing low vigor seeds from doing so.

It was originally hypothesized that osmotic stress would accentuate the differences in seed vigor levels. In reality, the differ-

ences between vigor levels were greater when seeds were germinated in water. Parmar and Moore (1966, 1968) and Than (1986) also found this to be true. Furthermore, the laboratory test based on 4 d germination in water was more highly correlated with field emergence than the osmotic stress test.

The relationship between vigor tests and field emergence was generally higher in the artificially aged lots, possibly because of a wider range of vigor levels between these lots. The naturally aged lots were 3 to 7 y old at the start of the field experiments and this set of seed lots lacked a non-aged lot which would have widened the range of vigor levels. Artificially aged seed lots are useful in the initial development of vigor tests because differences in vigor due to deterioration are not confounded with variation due to cultivar differences, seed size, growing environment and other factors. Subsequent testing must include many commercial lots and many field environments.

Unsuccessful attempts were made to combine the osmotic stress test with other vigor tests to develop an improved vigor index. This is in contrast with the work of Steiner et al. (1989) who developed improved multiple predictors of wheat emergence through multiple regression and calculation of geometric means. This serves to illustrate the important role that the nature of seed lots and field conditions play in evaluating the effectiveness of vigor tests.

A major advantage of the osmotic stress and four-day sand tests is their extreme simplicity in comparison to the more difficult procedures, specialized equipment and additional labor required for

many vigor tests. Also, the sand substrate is more desirable than blotters in that it is not necessary to discriminate between coleoptile lengths when making seedling counts, and mold problems are greatly reduced.

In the present study, no single vigor test consistently ranked both artificially and naturally aged seed lots for seedling emergence over the three planting dates. Overall, the accelerated aging and four-day sand tests were more successful than the other five tests. The results of the osmotic stress test and four-day sand tests were encouraging and research should continue to determine their potential as practical vigor tests for other seed kinds.

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APPENDIX

Appendix Table 1. Treatments used to produce 16 artificially aged lots of Stephens wheat.

Lot	Days at 15.5% moisture and 10°C †	Days at 15.5% moisture and 30°C
1	0	0
2	4	0
3	4	10
4	4	15
5	4	20
6	4	25
7	4	28
8	4	30
9	4	32
10	4	34
11	4	36
12	4	38
13	4	40
14	4	42
15	4	46
16	4	50

† Equilibration time after raising moisture content of bulk seed.

Appendix Table 2. History of naturally aged seedlots.

Seedlot	Variety	Year produced	Location†	
			Grown	Stored
1	Nugaines	1980	Pullman	Lind
2	Nugaines	1980	Pullman	Spillman
3	Nugaines	1983	Pullman	Spillman
4	Nugaines	1983	Pullman	Lind
5	Nugaines	1984	Lind	Lind
6	Nugaines	1984	Pullman	Lind
7	Nugaines	1984	Pullman	Spillman
8	Wanser	1980	Pullman	Lind
9	Wanser	1980	Pullman	Spillman
10	Wanser	1983	Lind	Lind
11	Wanser	1983	Pullman	Lind
12	Wanser	1983	Pullman	Spillman
13	Wanser	1984	Lind	Lind
14	Moro	1980	Pullman	Lind
15	Moro	1983	Lind	Lind
16	Moro	1983	Pullman	Spillman
17	Moro	1984	Lind	Lind
18	Moro	1984	Pullman	Lind
19	Moro	1984	Pullman	Spillman

† Naturally aged seedlots were supplied from the Washington State Agricultural Experiment Station.

Appendix Table 3. Field trial planting schedule, irrigation, rainfall and soil temperature data for 1987.

Month	Day	Planting number	Precipitation †	Mean daily temperature ‡
			cm	°C
September	30	1		na
October	1			na
	2			na
	3			na
	4			19.0
	5			19.0
	6			20.0
	7			19.0
	8			18.0
	9			15.5
	10			18.0
	11			18.0
	12			16.5
	13			15.5
	14			14.5
	15			13.5
	16			13.5
	17			14.0
	18			13.5
	19			14.5
	20	2		15.5
	21			13.5
	22		2.5_i	12.5
	23			11.5
	24			11.3
	25			10.5
	26			12.0
	27			10.3
	28			10.0
	29			11.5
	30			13.0
	31		0.69	14.5
November	1		0.71	13.8
	2		0.25	13.0
	3			11.5
	4			11.0
	5	3		9.0
	6		0.05	10.0
	7			10.5
	8			11.0
	9		0.25	11.3

Appendix Table 3. (Continued)

Month	Day	Planting number	Precipitation †	Mean daily temperature ‡
			cm	°C
	10			10.3
	11		0.25	10.3
	12		2.74	11.3
	13		1.27	13.3
	14		0.28	9.0
	15		0.08	8.0
	16		1.37	6.5
	17		0.18	8.5
	18			6.8
	19			7.0
	20			7.0
	21		0.15	8.0
	22		0.61	8.0
	23		0.30	6.5
	24		0.66	7.5
	25		0.64	5.8
	26			6.0
	27			4.5
	28			4.5
	29			4.0
	30		0.10	3.5
December	1		1.50	7.5
	2		2.29	7.3
	3		7.29	9.0
	4		2.46	8.5
	5		0.20	7.5
	6		1.80	8.5
	7		2.31	6.5
	8		0.20	6.0
	9		2.36	6.5
	10		2.59	9.5
	11		0.56	5.0
	12		0.20	3.5
	13			2.5
	14		0.03	1.5
	15		0.13	1.5

† Precipitation occurred as rain except for October 22 where the subsript "i" denotes irrigation.

‡ Temperature was measured at seed depth in soil (4.4 cm).

Appendix Table 4. Germination of Stephens wheat seedlots of high vigor and low vigor (artificially aged) under four levels of osmotic stress at 15°C.

Day	Osmotic potential (MPa)											
	Control			-0.4			-0.5			-0.6		
	Seed lot		Diff	Seed lot		Diff	Seed lot		Diff	Seed lot		Diff
	2	16	(2-16)	2	16	(2-16)	2	16	(2-16)	2	16	(2-16)
	%											
5	13†	0	13	0	0	0	0	0	0	0	0	0
6	81	25	56	0	0	0	0	0	0	0	0	0
7	99	72	27	7	0	7	0	0	0	0	0	0
8	100	95	5	21	10	11	0	0	0	0	0	0
9	100	99	1	32	20	12	0	0	0	0	0	0
10	100	100	0	43	30	13	3	1	2	0	0	0
11	100	100	0	50	45	5	4	2	2	0	0	0
12	100	100	0	60	54	6	13	11	2	3	0	3
13	100	100	0	70	64	6	23	18	5	8	2	6
14	100	100	0	75	71	4	32	25	7	17	7	10
15	100	100	0	84	80	4	38	40	- 2	28	20	8
16	100	100	0	89	88	1	47	45	2	36	28	8
17	100	100	0	92	94	- 2	53	52	1	45	36	9
18	100	100	0	97	95	2	65	54	11	49	39	10
19	100	100	0	97	100	- 3	66	64	2	56	46	10
20	100	100	0	97	100	- 3	71	64	7	61	48	13
22	100	100	0	98	100	- 2	80	71	9	70	61	9
24	100	100	0	98	100	- 2	86	80	6	75	67	8
28	100	100	0	98	100	- 2	89	86	3	82	72	10
30	100	100	0	98	100	- 2	93	89	4	83	75	8
34	100	100	0	98	100	- 2	93	95	- 2	86	83	3
38	100	100	0	98	100	- 2	93	100	- 7	88	87	1
42	100	100	0	98	100	- 2	96	100	- 4	89	90	- 1
46	100	100	0	99	100	- 1	96	100	- 4	92	92	0
50	100	100	0	99	100	- 1	97	100	- 3	93	92	1
54	100	100	0	99	100	- 1	97	100	- 3	93	92	1

†Germination percentages adjusted to a live seed basis.

Appendix Table 5. Germination of Stephens wheat seed lots of high vigor and low vigor (artificially aged) under four levels of osmotic stress at 25°C.

Day	Osmotic potential (MPa)											
	Control			-0.4			-0.5			-0.6		
	Seed lot		Diff	Seed lot		Diff	Seed lot		Diff	Seed lot		Diff
	2	16	(2-16)	2	16	(2-16)	2	16	(2-16)	2	16	(2-16)
	%											
3	91†	40	51	0	0	0	0	0	0	0	0	0
4	100	89	11	29	15	14	7	1	6	0	0	0
5	100	94	6	65	48	17	39	26	13	18	5	13
6	100	99	1	82	66	16	64	49	15	48	23	25
7	100	99	1	91	84	7	71	66	5	63	50	13
8	100	100	0	99	90	9	81	79	2	72	68	4
9	100	100	0	100	91	9	91	93	- 2	81	78	3
10	100	100	0	100	94	6	96	95	1	90	88	2
11	100	100	0	100	94	6	98	99	- 1	96	93	3
12	100	100	0	100	95	5	98	100	- 2	100	98	2
13	100	100	0	100	95	5	99	100	- 1	100	99	1
14	100	100	0	100	95	5	99	100	- 1	100	99	1
15	100	100	0	100	95	5	99	100	- 1	100	100	0
16	100	100	0	100	96	4	99	100	- 1	100	100	0
18	100	100	0	100	98	2	99	100	- 1	100	100	0
22	100	100	0	100	98	2	99	100	- 1	100	100	0

†Germination percentages adjusted to a live seed basis.