

AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF SEED DETERIORATION ON GERMINATION OF
WHEAT UNDER ENVIRONMENTAL STRESSES

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Abstract Approved: _____
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Germinating seeds often encounter stress conditions of temperature, moisture and oxygen in the field. Germination tests conducted under these stress conditions in the laboratory might be methods of differentiating high and low vigor seeds.

The objectives of this study were to (1) determine if these stress conditions reduce germination performance of low vigor seeds more than high vigor seeds, and (2) determine the potential for using one or more of these stress environmental conditions as the basis of a vigor test to predict relative field performance of wheat seed.

Seed lots of varying levels of deterioration were produced by artificial aging of 'Malcolm' wheat (Triticum aestivum L.). Germination tests were conducted at temperatures of 5, 10, 15, 20, 25, and 30°C; water potentials of 0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, and -1.4 MPa; and oxygen levels of 2, 4, 8, 12, 16% and

air (21%). The water and oxygen stresses were applied at six temperatures. Laboratory germination results were compared to field emergence percentages of artificially and naturally aged seed lots.

Germination percentage and rate of germination of low vigor seeds were depressed more than that of high vigor seeds at all water potentials and temperatures. At 20°C, for example, germination percentage of high, medium and low vigor seed lots at -0.6 MPa were 76, 48 and 29% respectively, compared to nearly 100% at 0, -0.2 and -0.4 MPa. Similar relationships existed at the other temperatures.

Germination of low vigor seed generally declined with each reduction of oxygen level while that of high vigor seed remained nearly constant. The germination differential between high and low vigor seed lots widened to as much as 30% in 2% oxygen at 30°C.

Twenty-four naturally-aged seed lots representing six varieties and four production years were evaluated for germination under water stress and field emergence. Correlation coefficients between germination at -0.6 MPa and field emergence were 0.61** and 0.59** for untreated and Arasan-treated seeds, respectively.

It is clear from these studies that environmental stresses reduce the germination of low vigor seeds more than that of high vigor seeds. A vigor test based on one

or more of these stresses has potential for being a practical and realistic method of predicting the relative field performance of wheat seed lots.

Effect of Seed Deterioration on Germination of
Wheat under Environmental Stresses

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Effect of Seed Deterioration on Germination of Wheat under Environmental Stresses

INTRODUCTION

Germination tests conducted by seed testing laboratories are conducted under optimum conditions of temperature, moisture and oxygen to allow the highest possible percentage of viable seeds to germinate. Deteriorated seeds (low vigor) are often able to germinate under these favorable conditions but fail to do so in the field. This is one of the reasons a germination test usually overestimates the stand-producing ability of deteriorated seed lots.

Vigor tests have been developed to provide a more realistic evaluation of a seed lot's ability to germinate and produce a stand of seedlings in the field. A number of these vigor tests, current and proposed, are based on the ability of seeds to germinate under stress environmental conditions. These stresses may simulate the stresses encountered in the field or they may be artificial.

It would seem that germination under sub-optimum conditions of temperature, water and oxygen might be realistic methods of differentiating high and low vigor seeds. These conditions are normally encountered in the soil and are easily reproduced and maintained in the laboratory. The seeds can be subjected to one or more of

these stresses and their response measured in terms of percentage or rate of germination.

Two questions were proposed to be answered in this study. First, do these three stress conditions reduce germination performance of low vigor seeds more than high vigor seeds? Secondly, if the answer to the first question is positive, could germination under one or more of these stress environmental conditions be the basis of a vigor test to predict relative field performance?

Wheat was chosen as the test species for this study. The effects of stress conditions of temperature, water and oxygen were determined on artificially aged seeds. The most promising test conditions were then evaluated for potential as a vigor test on naturally aged wheat seed lots.

The results of this study are presented in the form of a manuscript.

REVIEW OF LITERATURE

Seed Vigor

Differences in seed vigor were observed by the first seed analysts and observations about "germination energy" were reported by Nobbe (1876) in his "Handbuch der samenkunde."

The International Seed Testing Association (ISTA) definition of seed vigor adopted in 1977 is that "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).

The Association of Official Seed Analysts (AOSA) definition of seed vigor adopted in 1980 is that "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 (McDonald, 1980). This definition is similar to the one adopted by the ISTA.

The assessment of seed vigor has many important implications for the seed industry and seed consumers. Seed producers can use seed vigor information to monitor seed quality during the various conditioning phases of seed production.

The germination test is universally accepted and used as a seed quality test. However, germination methods are based on optimum levels of oxygen, moisture and temperature, while field conditions are never optimum. Actual field emergence is often less than that predicted by laboratory germination. Most definitions of seed vigor emphasize the relationship between seed vigor and field performance, and many studies have demonstrated that this association exists. Consequently, the ultimate value of any vigor test may be its ability to predict field performance (AOSA, 1983).

Many vigor tests have been suggested; however, only a few have attained acceptance by seed analysts and seed organizations (AOSA, 1983; Perry, 1981). These are grouped in the seed vigor testing handbooks (AOSA, 1983; Perry, 1981).

Seedling growth and evaluation tests include the seedling vigor classification and seedling growth rate tests. Stress tests include the accelerated aging test, cold test and cool germination tests. Biochemical tests include the tetrazolium and conductivity tests. Other vigor tests include speed of germination, brick grit, osmotic stress, respiration, glutamic acid decarboxylase activity (GADA) and adenosine triphosphate (ATP) tests.

The point at which the seed achieves maximum dry weight, called physiological maturity, confers the seed

with its greatest potential for maximum germination and seed vigor (Delouche, 1974).

Seed deterioration usually starts at the time seeds attain physiological maturity and proceeds at a rate that is greatly influenced by inheritance, traumas, seed moisture content and temperature. The final and most disastrous consequence of deterioration is loss of the capacity for germination, which in seed testing practice means the failure of the seed to reproduce a normal seedling.

Seed vigor and seed deterioration are reciprocal, dimensioned "properties" of a seed or seed lot. Vigor decreases as the level of deterioration increases (AOSA, 1983).

Artificial Aging

During dry storage a seed sample gradually loses viability until after a time, which varies from species to species, all germination capacity is lost. Haferkamp, Smith and Nilan (1953) have shown that this decrease in germination capacity is a function of the age of the seeds. Roberts et al. (1967) have demonstrated increasing damage to chromosome structure in the embryo cell during the period of dry storage; the extent of such damage can be a reliable index of the age of the seeds.

When seeds are maintained experimentally under conditions of high humidity and temperature, the rate of aging is greatly accelerated, and viability falls rapidly with increased storage time (Roberts et al., 1967; Roberts and Abdalla, 1968).

A yellow double-cross hybrid of Zea mays L., var. 'Say' seeds was artificially aged with a seed moisture of 14% at 40°C. The germination capacity fell between 12 and 20 days, and by 28 days the seeds were completely non-viable (Berjak and Villiers, 1972).

Accelerated aging was initially developed as a test to estimate the longevity of seed in warehouse storage. Subsequent studies have verified the accuracy of this test in predicting the life span of a number of different species under a range of storage conditions (Delouche, 1965; Delouche and Helmer, 1967; Delouche and Baskin, 1973).

Baskin (1970) proposed using the accelerated aging test to predict stand establishment of peanut. Other studies have shown that this vigor test functions equally well in forecasting stand establishment of cotton (Bishnoi and Delouche, 1975), bean (Roos and Manalo, 1971), and soybean (Byrd and Delouche, 1971; Tekrony and Egli, 1977).

The controlled deterioration test, in which seeds are subjected to precise periods of aging at high temperature and raised seed moisture content, has been developed as a

vigor test for small seeded vegetable species (Mathews, 1980; Powell and Mathews, 1981). The test reveals differences in the vigor of seed lots which are reflected in their field emergence (Mathews, 1980).

The effect of fusaric acid (FA), potassium and sodium was tested on artificially aged wheat seeds. The treatments were unable to stimulate germination significantly, contrary to the results found in the naturally aged wheat (Petruzelli and Carella, 1983).

Seed Germination

Seeds need water, oxygen and temperature to germinate. Although optimum levels of these conditions give highest germination, optimum conditions for all three are never found in nature. Numerous studies have been done to determine optimum levels of these, and a few studies have been done to determine the effects of stress conditions on seed germination.

Temperature Stress

Although a substantial body of empirical information concerning the temperatures at which seeds will germinate has now been accumulated (Mayer and Poljakoff-Mayber, 1975), little attempt has been made to determine what prevents the germination of seeds such as tobacco and cucumber at temperatures around 10°C (Simon et al.,

1976). Germination entails a variety of biophysical and biochemical processes from the initial imbibition of water and the re-establishment of membrane integrity to the activation of numerous enzymes and metabolic pathways (Mayer and Shain, 1974), and finally the elongation of the root which ruptures the testa. Simon et al. (1976) stated that it is not easy to identify the particular point at which low temperature prevents germination. Moreover, this may not be the same in all cold-sensitive seeds.

Wilson and Hottes (1927) reported that as an average, a temperature of 15°C was more nearly optimum for complete germination of wheat than higher temperatures, although 20° and 30°C gave more rapid germination and 30°C made the wheat more susceptible to attack by mold.

Temperature requirements for germination of wheat are reported as: minimum, 3.5 to 5.5°C; optimum, 20 to 25°C; and maximum, 35°C (Peterson, 1965).

Optimal temperature for germination of maize is reported to be in the range of 32 to 35°C (Mayer and Poljakoff-Mayber, 1975). The temperatures of 5-15°C are detrimental to the germination of maize, with the degree of sensitivity varying between cultivars (Cal and Obendorf, 1972).

Germination and coleoptile growth of nine seed lots of maize were studied at constant temperatures of 13° and 25°C and also at 7°C (7 days) followed by 25°C.

Relative germination response to sub-optimal temperatures reflected relative differences in germination rate at 25°C. Low temperatures appeared to have a differential effect on germination rate and coleoptile growth (Van de Venter and Grobbelaar, 1985).

Temperature is often the main environmental factor governing rate of seed germination (Garcia-Huidobro et al., 1982) and it also has a major effect on time required for germination (Woods and McDonald, 1971).

Woods and McDonald (1971) reported the germination of Lotus corniculatus was delayed by temperatures lower than 15°C, and delayed and reduced by temperatures of 30°C or higher.

Germination of several cool and warm-season grasses was better at alternating temperatures than at constant temperature (McElgunn, 1974; Harty and Butler, 1975; Stubbendieck and McCully, 1972). However, some range grasses were reported to have similar germination under alternating and constant temperatures (Ellern and Tadmor, 1967; Young et al., 1981).

Time to reach 50% final germination (Gt 50), decreased with increasing temperature, and the corrected germination rate index (CGRI) of all the species under study increased as temperature increased (Hsu et al., 1985).

Lafond and Baker (1986), from their 2-year field study of nine spring wheat cultivars, found that the reciprocal of median germination time, a measure of rate of germination, was linearly related to temperature.

Effect on Emergence

Soil temperature is an important factor in seedling emergence. Allan et al. (1962) showed that cold soil temperatures greatly reduce coleoptile length and, in turn, lessen the ability of seedlings to emerge in fall-sown wheat.

Morrison et al. (1981) reported an increase in emergence time of winter wheat with a decrease in temperature.

Another study conducted by Dubetz et al. (1962) showed that the percent emergence of wheat was not affected at four different constant temperatures (6°, 13°, 18° and 24°C), but the rate of emergence was highest at 24°C compared to the other three temperatures.

Hopper et al. (1979) demonstrated, using soybean cultivars, that time required for 50% germination decreased (18.8 - 4.0 days) as the temperature increased from 10° to 35°C. Emergence (50%) from a sand-soil-peat mixture was more rapid (19.8 to 6.3 days) as the simulated data was pushed towards the warmer regime.

Variety Difference in Emergence

Helmerick and Pfeifer (1954), using mannitol solutions, demonstrated varietal differences in wheat for germination and seedling growth under moisture stress.

Morrison et al. (1981) found significant differences among four soft red winter wheat cultivars for percentage emergence and mean rate of emergence under laboratory conditions.

Cultivars showed different emergence rate indices in simulated field conditions in soybean (Hopper et al., 1979) and field conditions in wheat (Lafond and Baker, 1986; and Sunderman, 1964). Austenson (1973) observed yield variations in wheat, dependent on field emergence.

Water Stress

Different crop species and osmotic substances have been used in studies concerning the effects of water stress on seed germination. McGinnes (1960) found that as water stress increased, the germination rates of range grasses were reduced.

The rate of germination is affected as long as water potential is higher than a critical value that is species-specific (Pisum stivum and Vicia sativa) (Hadas and Russo, 1974).

At osmotic potentials below 15 atm, using solutions of carbowax, mannitol, glucose, sucrose, NaCl, and

CaCl₂, Taylor (1965) found no significant differences in germination of radish seed.

Some of the osmotic substrates of low molecular weights may enter germinating seeds and may induce effects, including toxicity, that are more complex than simple drought (Ayers, 1952; Collis-George et al., 1962; Kaufmann et al., 1970; Manohar, 1966).

Water stress conditions can be simulated in laboratory tests by using polyethylene glycol solutions of different water potential levels to study the effect of reduced water potential on germination (Manohar, 1966; Michel and El-Sharkawi, 1970).

High molecular weight PEG 4000 or more is a satisfactory compound for imposing true drought (Manohar, 1966; Parmar and Moore, 1968). McWilliam and Phillips (1971) presented direct evidence on equivalence of osmotic and matric potential simulated by polyethylene glycol (20,000 mol wt) for two pasture species and concluded that equivalence holds only under special circumstances.

Soil moisture potential has two contributing components: osmotic potential of the soil solution and the matric potential at which soil water is held. The effect of osmotic potential is similar to the matric potential (Parmar and Moore, 1968; Kaufmann and Ross, 1970; McWilliams and Phillips, 1971; Sharma, 1973).

Parmar and Moore (1968) found that increased osmotic pressure levels progressively delayed and reduced germination of corn. Reducing water potential had more adverse effects on the low than on the high vigor seed lot, and on the shoot than on the primary root elongation.

The water potentials of solutions of PEG 6000 (Michel and Kaufmann, 1973) and PEG 8000, both in the absence and presence of other solutes (Michel, 1983) have been evaluated. Osmotic potential (ψ) of PEG solutions was curvilinearly related to concentration. At a given concentration, osmotic potential increased linearly with temperature.

Literature dealing with interaction of temperature and water stress on seed germination is rather scarce, despite ample information about single factor effects on germination. The few studies on temperature and water potential interaction effects (Kaufmann and Ross, 1970) indicated no significant effect of this interaction in wheat; wheat germinated well at all temperatures (15° , 25° , 35°C) over a range of water potentials in the soil of 0 to -8 bars. At -14.9 bars, no germination occurred in the soil.

Final germination percentage was dependent on the temperature and osmotic potential interaction in all range species. Wheat and barley were almost independent of osmotic potential (OP) and temperature effects except at the highest OP (15 atm) at low temperature (4°C) (Tadmor et al., 1969).

Conclusions about the effects of water stress at one temperature are not valid at other temperatures if a water stress-temperatures interaction exists.

Water potential effects on germination and emergence of plant species, in general, increase as temperature deviates from the optimum (McGinnies, 1960). That is, under high moisture stress, seeds germinate more rapidly at optimum temperature than at other temperatures, although exceptions are apparent. For example, hypocotyl growth of cotton was highly sensitive to water stress at 37.8°C, which is near the maximum for growth (40°C) for this species, but this interaction was absent at lower temperatures, including the minimum for growth (Wanjura and Buxton, 1972).

Morrison et al. (1981) found a highly significant interaction for temperature and moisture tension for the percent emergence and emergence rate of soft red winter wheat. They reported the best combination of 3.9°C and 3 atm tension to distinguish cultivars for emergence.

In order to germinate, crop seeds have to attain a specific moisture content and all crop seeds germinate in a shorter time at high soil moisture than at low soil moisture (Doneen and MacGillivray, 1943). This minimum moisture content was approximately 30.5% for corn, 26.5% for rice, 50.0% for soybean and 31.0% for sugar beet seeds (Hunter and Erickson, 1952).

Rate of emergence is an important character for stand establishment in much of the semiarid wheat belt. Gul and Allan (1976) conclude from a study of 93 wheat lines that the time required for emergence nearly doubled for each decrease of water potential of -4 bars within the range of -2.2 to -14.4 bars. Total stand, coleoptile length, seedling height and root weight were similarly progressively reduced as water potential decreased.

Read and Beaton (1963) investigated the effect of moisture stress (0.4-60 bars) and temperature (6 to 27°C) on the germination of wheat. They observed that low temperature and decreasing soil moisture levels lowered the rate of germination significantly. No significant effect was found on total germination.

Seed germination of four wheat cultivars was compared under suboptimal temperatures and various levels of simulated moisture stress (Ashraf and Abu-Shakra, 1978). Total germination was not affected by moisture stress levels up to 12 atm, but was significantly reduced at 15 and 18 atm osmotic tensions. Rate of root growth, speed of germination and respiration rates were inversely related to moisture stress (Ashraf and Abu-Shakra, 1978).

McWilliam and Phillips (1971) found that germination of ryegrass and Phalaris declines with decreasing water potentials. This decline was more pronounced in the case of Phalaris where germination was prevented below 6 bars.

Lindstrom et al. (1976) reported nearly complete emergence (80%) of "McCall" and "Nugaines" wheats at soil water potentials as low as -10 bars. The rate of emergence was noticeably reduced as the potential decreased from -4.0 bars, and the lowest water potential at which seedlings emerged was -14.5 bars.

Oxygen Stress

Air is composed of about 20% oxygen, 0.03% carbon dioxide, and about 80% nitrogen gas. Carbon dioxide concentrations higher than 0.03% retard germination, while nitrogen gas has no influence. Respiration increases sharply during seed germination. Since respiration is essentially an oxidative process, an adequate supply of oxygen must be available. If the oxygen concentration is reduced substantially below that of air, germination of most seeds is retarded. However, there are some notable exceptions; rice and other aquatic plants can germinate even in the complete absence of oxygen, although the seedlings are weak and abnormal. Generally, seeds of plants that grow on well-drained soil have a higher percentage germination under normal O₂ concentrations than with reduced O₂ levels (Heichel and Day, 1972).

Although most species germinate best in the oxygen concentrations of air, Cattail (*Typha latifolia*) and Bermuda grass (*Cynodon dactylon*) germination are aided by

oxygen concentrations below that of air (Morinaga, 1926). On the other hand, seeds of carrot, curley dock, sunflower, cocklebur and various cereals germinate better under oxygen concentrations above that of air (Albaum et al., 1942; Barton, 1941; Morinaga, 1926). The influence of carbon dioxide on seed germination is usually opposite to that of oxygen. Most seeds fail to germinate if the CO_2 partial pressure is increased over the 0.03% of air; however, a decrease usually does not hinder germination. Several seeds reportedly have a minimum CO_2 requirement, notably seeds of lettuce and timothy (Mayer and Poljakoff-Mayber, 1975; Thornton, 1936).

In many seeds, the availability of oxygen for seed germination is linked with dormancy and for example, dormant cereals such as wild oat and barley can be induced to germinate at higher oxygen tensions (Crocker and Barton, 1953). However, the germination of a number of aquatic plants, such as Zizania aquatica (Simpson, 1966) is enhanced by low levels of oxygen.

The breaking of seed dormancy in rice (Roberts, 1961) by higher oxygen tensions has been reported.

Takahashi (1985) showed that high oxygen tension inhibits seed germination for a certain period after harvest in Japonica rice, whereas Indica rice cultivars are not inhibited by oxygen at any stage.

Maximum germination of submerged Typha seed was achieved when O_2 concentration in water was between 2.3 and 4.3 mg L^{-1} . At both lower or higher O_2 concentrations, germination was reduced (Bonnewell et al., 1983).

Seed germination and seedling growth require the use of metabolic energy acquired from respiration. Increased germination rate of several species has been achieved by increasing the oxygen concentration (Edwards, 1973). Concentration of oxygen greater than 21% stimulated germination rate of sweet pepper at 25°C but inhibited germination rate at 15°C. At 10% oxygen concentration, germination rates were reduced at both temperatures (Watkins et al., 1983).

Germination of Strelitzia juncea seed was increasingly stimulated by increasing the oxygen concentration in the incubation atmosphere. Maximum stimulation was obtained in an initial oxygen concentration of 100% (Ybema et al., 1984).

Effect of Germination under Stress Environmental Conditions on Seeds of Low Vigor

Vigor tests have been developed to provide a more relative evaluation of a seed lot's ability to germinate and produce a stand of seedlings in the field. A number of these vigor tests, current and proposed, are based on the ability of seeds to germinate under stress

environmental conditions. These stresses may simulate the stresses encountered in the field or they may be artificial.

Thus, the corn (Zea mays L.) cold test (Isely, 1950) is based on the ability of seeds to resist attack by soil-borne microorganisms under the cold, wet conditions corn seeds are often exposed to in the field. The brick grit test evaluates the ability of cereal seeds to germinate and emerge from beneath a layer of ground brick (Hiltner and Ihssen, 1911). The accelerated aging test is also classified as a stress test, but in this case the stress is applied prior to germination and germination is evaluated under optimum conditions (Delouche, 1965). Germination of sorghum (Sorghum vulgare L.) seed in dilute solutions of ammonium chloride gave good correlation with field performance (Vanderlip et al., 1973).

Ability of seeds to germinate under drought conditions has been proposed as a possible vigor test (Heydecker, 1960). Heydecker (1967) demonstrated that germination of year-old pea (Pisum sativum L.) seeds was reduced by a water stress level that did not injure fresh seed. Parmar and Moore (1968) found that germination of corn seed in solutions of Carbowax 6000 had more adverse effects on low vigor seeds than those of

high vigor. Hadas (1977) showed the same to be true in chickpeas (Cicer arietinum L.).

The cool test for cotton (Gossypium spp.) is based on the ability of cotton seed to germinate under cooler than optimum temperatures (McCarter and Roncadori, 1971). High temperature has been proposed as a stress condition for evaluating vigor in pea seed (Caldwell, 1960).

Pollock et al. (1969) described procedures and equipment for controlling moisture, temperature and oxygen levels simultaneously for measuring the response of seedlots to stress during germination.

Stress tests and other vigor test procedures have been reviewed by the Seed Vigor Committee of the Association of Official Seed Analysts (1983).

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Germinating seeds often encounter stress conditions of temperature, moisture and oxygen in the field. Germination tests conducted under these stress conditions in the laboratory might be a realistic method of differentiating high and low vigor seeds.

The objectives of this study were to (1) determine if these stress conditions reduce germination performance of low vigor seeds more than high vigor seeds, and (2) determine the potential for using one or more of these stress environmental conditions as the basis of a vigor test to predict relative field performance of wheat seed.

Seed lots of varying levels of deterioration were produced by artificial aging of 'Malcolm' wheat (Triticum aestivum L.). Germination tests were conducted at temperatures of 5, 10, 15, 20, 25 and 30°C; water potentials of 0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, and -1.4 MPa; and oxygen levels of 2, 4, 8, 12 and 16% and air (21%). The water and oxygen stresses were applied at six temperatures. Laboratory germination results were compared to field emergence percentages of artificially and naturally aged seed lots.

Additional index words: Triticum aestivum L., seed vigor, water stress, oxygen stress, temperature stress.

Effect of Seed Deterioration on Germination
of Wheat under Environmental Stresses

INTRODUCTION

Germination tests conducted by seed testing laboratories are conducted under optimum conditions of temperature, moisture and oxygen to allow the highest possible percentage of viable seeds to germinate. Deteriorated seeds (low vigor) are often able to germinate under these favorable conditions, but fail to do so in the field. This is one of the reasons a germination test usually overestimates the stand-producing ability of deteriorated seed lots.

Vigor tests have been developed to provide a more effective evaluation of a seed lot's ability to germinate and produce a stand of seedlings in the field. A number of these vigor tests, current and proposed, are based on the ability of seeds to germinate under stress environmental conditions. These stresses may simulate the stresses encountered in the field or they may be artificial.

Thus, the corn (Zea mays L.) cold test (Isely, 1950) is based on the ability of seeds to resist attack by soil-borne microorganisms under the cold, wet conditions corn seeds are often exposed to in the field. The brick grit test evaluates the ability of cereal seeds to

germinate and emerge from beneath a layer of ground brick (Hiltner and Ihssen, 1911). The accelerated aging test is also classified as a stress test, but in this case the stress is applied prior to germination and germination is evaluated under optimum conditions (Delouche, 1965). Germination of sorghum (Sorghum vulgare L.) seed in dilute solutions of ammonium chloride gave good correlation with field performance (Vanderlip et al., 1973).

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The cool test for cotton (Gossypium spp.) is based on the ability of cotton seed to germinate under cooler than optimum temperatures (McCarter and Roncadori, 1971). High temperature has been proposed as a stress condition for evaluating vigor in pea seed (Caldwell, 1960).

Pollock et al. (1969) described procedures and equipment for controlling moisture, temperature and oxygen

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Stress tests and other vigor test procedures have been reviewed by the Seed Vigor Committee of the Association of Official Seed Analysts (1983).

It would seem that germination under sub-optimum conditions of temperature, water and oxygen might be methods of differentiating high and low vigor seeds. These conditions are normally encountered in the soil and are easily reproduced and maintained in the laboratory. The seeds can be subjected to one or more of these stresses and their response measured in terms of percentage or rate of germination.

Two questions were proposed to be answered in this study. First, do these three stress conditions reduce germination performance of low vigor seeds more than high vigor seeds? Secondly, if the answer to the first question is positive, could germination under one or more of these stress environmental conditions be the basis of a vigor test to predict relative field performance?

Wheat (Triticum aestivum L.) was chosen as the test species for this study. The effects of stress conditions of temperature, water and oxygen were determined on artificially aged seeds. The most promising test conditions were then evaluated for potential as a vigor test on naturally aged wheat seed lots.

MATERIALS AND METHODS

Seed Lots

Twenty-seven kg 'Malcolm' soft white winter wheat seed produced in 1985 was adjusted to 15.5% moisture by addition of water while the seed was tumbled in a cement mixer. The seed was then placed in 30 sealed 950 mL jars and stored at 30°C in a seed germinator. One jar was removed approximately every 2 days and the seed was spread out to dry at room temperature. The seed lots were rated for vigor on the basis of their germination, seedling growth rate and field emergence. Having identified 30 seed lots of varying vigor, three lots were selected to represent high (aged 0 days), medium (aged 32 days), and low (aged 42 days) vigor levels. The germination percentages of these lots after artificial aging were 97, 94 and 86%. Studies were then carried out to determine the effects of temperature, water and oxygen stress on germination of seed at different vigor levels.

Germination under Moisture and Temperature Stress

A series of solutions was prepared with polyethylene glycol 8000 (PEG 8000) to establish water potentials of 0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.2, and -1.4 MPa. PEG 8000 solutions were prepared according to the procedures of Michael and Kaufman (1983). Osmotic potentials were

measured with a vapor pressure osmometer model 5100cc (Wescor, Inc., Logan Utah), and adjustments in amounts of PEG or deionized water were made as needed.

Fifty Arasan-treated seeds from the three artificially aged lots were placed between paper substrata in 12 x 12 x 7 cm clear plastic germination boxes. The substrata consisted of one germination blotter at the bottom, one heavy-weight germination towel in the middle and one blotter on the top. Twenty-five mL PEG 8000 solution were added to the substrata. Then the seeds were placed underneath the top blotter and on top of the heavy-weight germination towel, which prevented root penetration. The boxes were sealed in 16.5 x 17.8 cm Ziploc plastic bags to eliminate moisture loss. The seeds were placed in dark germinators at 5, 10, 15, 20, 25 and 30°C for 21 days. The experimental design was a randomized complete block with three replications.

Daily germination counts were made after the beginning of germination until 21 days. Seeds were counted as germinated when the shoot length equalled the length of the seed.

A Germination Rate Index (GRI) was calculated for each treatment by the formula of Maguire (1962):

$$\text{GRI} = \frac{\text{no. normal seedlings}}{\text{days of first count}} + \dots + \frac{\text{no. normal seedlings}}{\text{days of final count}}$$

With this formula, a high index value indicates a faster germination rate than a low index value.

GRI ratios were calculated to compare the relative effects of water stress on the three lots. GRI ratios were calculated by dividing the GRI at each water potential by the GRI at 0 MPa (water).

Germination under Oxygen Stress

Twenty-five Arasan-treated seeds from the three lots were placed on top of moist blotters in 9-cm plastic petri dishes. Plastic collars 15 mm in height were placed in the dishes to raise the lids to permit free gas exchange. Six petri dishes were placed in 3800 mL glass jars which were modified to serve as oxygen chambers. The jar lids were fitted with #7 two-hole rubber stoppers with 7-mm glass tubes. The tubes were sealed with rubber septa. The jars were flushed for 15 min with O_2 - N_2 mixtures containing 2, 4, 8, 12 and 16% O_2 , with air (21% O_2) as the control. Flushing was repeated daily. The gas mixture was introduced through hypodermic needles inserted through the septa. The percentages of O_2 and N_2 were regulated with a Matheson Flowmeter Model 7351 H (Matheson Gas Products, Inc.) and monitored with a Percent Oxygen Monitor Model 74223 (Bio-Tek Instruments, Inc., Burlington, VT).

Germination counts were made daily after the start of germination. Germination percentage, Germination Rate Indices (GRI) and GRI ratios were calculated as described.

Correlation of Germination under Water Stress with Field Emergence of Artificially Aged Seed

The germination percentage of six additional artificially-aged seed lots of varying degrees of deterioration were determined at water potentials of 0, -0.4 and -0.6 MPa at 20°C following the procedure described. These lots had been aged for 5, 17, 25, 34, 38 and 44 days. The lots were planted on 2 November 1985 and 27 March 1986 at the Hyslop Crop Science Field Laboratory on Woodburn silt loam (fine-silty, mixed mesic Aquultic Argixeroll) soil.

One hundred seeds of each lot were planted in 3 m rows at a depth of 5 cm in a randomized complete block design with four replications. Final germination counts were made after 47 days in the fall and 22 days in the spring planting. Correlation coefficients were calculated to determine the degree of association between laboratory and field germination percentages.

Correlation of Germination under Water Stress with Field Emergence of Naturally Aged Seed

Twenty-four naturally aged wheat seed lots were furnished by Dr. James Maguire of Washington State University. These lots represented the cultivars 'Kharkov,' 'Paha,' 'Nugaines,' 'Wanser,' 'Luke,' and 'Moro.' They were produced in 1980, 1982, 1983 and 1984

in Lind, WA and stored at Spillman, WA until 1985. Each lot was germinated in the laboratory at a water potential of -0.6 MPa by the procedure described. The lots were planted at Hyslop Farm on 16 October 1986. One hundred seeds of each lot were planted in 6 m rows at a depth of 5 cm in a randomized complete block design with four replications. Final germination counts were made after 20 days. Correlation coefficients were calculated to determine the degree of association between laboratory and field germination percentages.

RESULTS AND DISCUSSION

Germination under Moisture Stress

The effects of reduced water potential on the germination percentages of high, medium and low vigor seed lots are shown in Figure 1. Since the viability of the low vigor lot was lower than the medium and high vigor lots, unequal numbers of viable seeds were planted from each seed lot at each test condition. Therefore, the germination percentages in Figure 1 are presented as percentages of the number of viable seeds planted per lot, with germination in water = 100%.

No germination occurred at water potentials of -1.0 MPa or lower. The lowest water potential at which 50% or more of the high vigor seeds germinated depended on the temperature. Germination occurred at a water potential of -0.6 MPa at the optimum temperatures of 15 and 20°C, while only minimal germination occurred at higher and lower temperatures. Germination occurred at -0.4 MPa at 5, 10 and 25°C, but only at -0.2 MPa at 30°C. This interaction of water potential and temperature was previously demonstrated by El-Sharkawi and Springuel (1977).

Germination percentage of low vigor seeds was depressed more than that of high vigor seeds at all water potentials and temperatures. At each temperature, the greatest differential between the germination of high and

low vigor seed occurred at the highest water potential that caused a significant reduction in germination. At 20°C, for example, germination percentages of high, medium and low vigor lots at -0.6 MPa were 76, 48 and 29%, respectively, compared to nearly 100% at 0, -0.2 and -0.4 MPa. Similar relationships existed at the other temperatures.

Effects of temperature and water stress on germination rates were reflected in differences in the Germination Rate Indices (GRI) shown in Table 1. The GRI was lower at the lower temperatures and at reduced water potentials. A reduction in growth rate occurred before germination percentage was affected. At 20°C, for example, the GRI of high vigor seed at -0.4 MPa was half that at 0 MPa, but the germination percentage remained at nearly 100%.

It is difficult to determine the relative reduction in germination rate by reduced water potential by examining the GRIs because the size of the index is influenced greatly by temperature. Therefore, GRI ratios were calculated to indicate the relative reductions in germination speed on a percentage basis. The ratios are shown on the right-hand side of Table 1. The GRI ratios are most meaningful at those water potentials that allowed 50% or more of the high vigor seeds to germinate. At

lower water potentials, the germination figures are too small to furnish reliable GRIs.

The GRI ratios of low vigor seed were lower than those of high vigor seed at each water potential at all temperatures. At 20°C and -0.6 MPa, for example, the germination rate of high vigor seed was 26% of that in water, while the germination rate of low vigor seed was only 9% of that in water. The germination rate of medium vigor seed was 16%.

It is clear that reduced water potential lowers both the germination percentage and germination rate of low vigor seeds more than those of high vigor seeds. Without further study it is difficult to determine which parameter is more precise in differentiating between seed vigor levels. However, determination of germination percentage is far less time consuming than making the daily counts to calculate the rate of germination. It appears at this point that germination at a reduced water potential satisfactorily differentiates seed lots on the basis of vigor and that it can be done by presenting the data graphically as in Figure 1. On that basis, the best differentiation of vigor levels occurred at 20°C at a water potential of -0.6 MPa. This combination of temperature and water potential was selected for further evaluation in additional artificially and naturally aged seed lots.

Germination under Temperature Stress

The effects of temperature on the germination percentages of wheat seed in the absence of water stress are shown in Table 2. For the high and low vigor lots, the maximum germination occurred at 15°C, with slightly lower germination at the upper and lower ends of the temperature range. Germination percentage of the medium and low vigor lots was depressed more than that of the high vigor lot at 30°C.

GRI ratios were calculated with the GRI at 20°C considered as 100% (Table 3). GRI ratios at 30°C were 1.43, 1.11 and 1.23 for the high, medium and low vigor lots, respectively, indicating that germination rates of medium and low vigor seeds were depressed more than those of high vigor seeds. In general, however, the germination performance of low vigor seed was not depressed to an appreciable degree at 30°C, and temperature alone does not appear to be an effective stress factor for distinguishing levels of wheat seed deterioration. In contrast, high temperatures effectively differentiated vigor levels of pea seed, another species that germinates well at cool temperatures (Caldwell, 1960). Caution would also have to be exercised in employing high temperature as a stress test for wheat vigor because of the danger of confounding the effects with those of high temperature dormancy at temperatures over 25°C.

Correlation of Germination under Water Stress with Field Emergence of Artificially Aged Seed

Six additional seed lots were selected from the original 30 artificially aged lots and germinated at -0.4 and -0.6 MPa at 20°C. The germination percentages were compared with germination percentages from fall 1985 and spring 1986 field plantings (Figure 2).

A water potential of -0.4 MPa was not low enough to reduce germination percentage of low vigor seed to any great extent, and the correlation with field germination was not significant. Germination at -0.6 MPa, however, produced correlation coefficients of .92** and .96** with spring and fall field trials, respectively. Germination at -0.6 MPa was thus successful in differentiating vigor levels of these additional artificially aged seed lots. The next step was to evaluate the use of this test on naturally aged seeds of several varieties and from different years of production.

Correlation of Germination under Water Stress with Field Emergence of Naturally Aged Seed Lots

Comparisons of germination percentages at water potentials of 0 and -0.6 MPa with field emergence of 24 naturally aged seed lots are shown in Table 4.

The 1980 seeds were lowest in viability and this was reflected in lower germination percentages by all three

test procedures. Germination at -0.6 MPa generally showed a good relationship to field emergence for the 1980, 1982 and 1983 seed lots. For an as yet unexplained reason, germination of the 1984 lots of Paha, Luke and Moro were very low at -0.6 MPa, but field emergence was over 90%. However, the correlation coefficients between germination at -0.6 MPa and field emergence were 0.61^{**} and 0.59^{**} for untreated and Arasan-treated seeds, respectively. Because of the poor correlation of field emergence and -0.6 MPa results for 1984 seed of three of the cultivars, the correlation with germination in water was actually higher at 0.95^{**} and 0.96^{**} for untreated and treated seed, respectively. In general, germination percentages in water overestimated field emergence, while germination under water stress underestimated field performance.

Germination under Oxygen Stress

The effects of reduced oxygen levels on the germination percentages of high, medium and low vigor seed lots are shown in Figure 3. The germination percentage at each oxygen level is the percentage of that obtained in air.

Germination of low vigor seed generally declined with each reduction of oxygen level while that of high vigor seed remained nearly constant. The germination differential between high and low vigor seed widened to as

much as 30% in 2% oxygen, at 30°C, while medium vigor seed was intermediate.

Germination rate also declined at each lower oxygen level, with the GRI ratio of low vigor seed falling at the fastest rate (Table 5). At 2% oxygen at 30°C, the GRI ratio for low vigor seed had fallen to 48%, while that of high vigor seed was 76%.

The reduction in germination percentage and germination rate did not appear to be as temperature-dependent as the reductions from reduced water potential. There appeared to be a trend for oxygen stress to inhibit germination more at the higher temperatures. This trend was not pronounced enough to draw firm conclusions, however. This work will be continued to determine if greater differential germination would occur at oxygen levels below 2%.

It is clear from these studies that environmental stresses reduce the germination of low vigor seeds more than that of high vigor seeds. Of the stresses applied, low vigor seeds are least affected by temperature. Temperature has an influence, however, when acting in conjunction with water or oxygen stress. Since germinating seeds are often exposed to unfavorable conditions of temperature, moisture, and oxygen in the field, a laboratory test based on one or more of these

stresses should be a realistic method of evaluating seed lots for ability to resist these stresses in the soil.

A practical seed vigor test must be easily manipulated, not labor-intensive, reproducible, and not be cultivar-specific. Vigor tests based on temperature, water or oxygen stress appear to meet these requirements. Methods of controlling these factors are not overly complicated and the results are readily interpreted in terms of germination percentages. Investigation of the potential for adapting these techniques as practical vigor tests should be continued and extended to additional crops. It is expected that appropriate test conditions will vary and must be determined for each species.

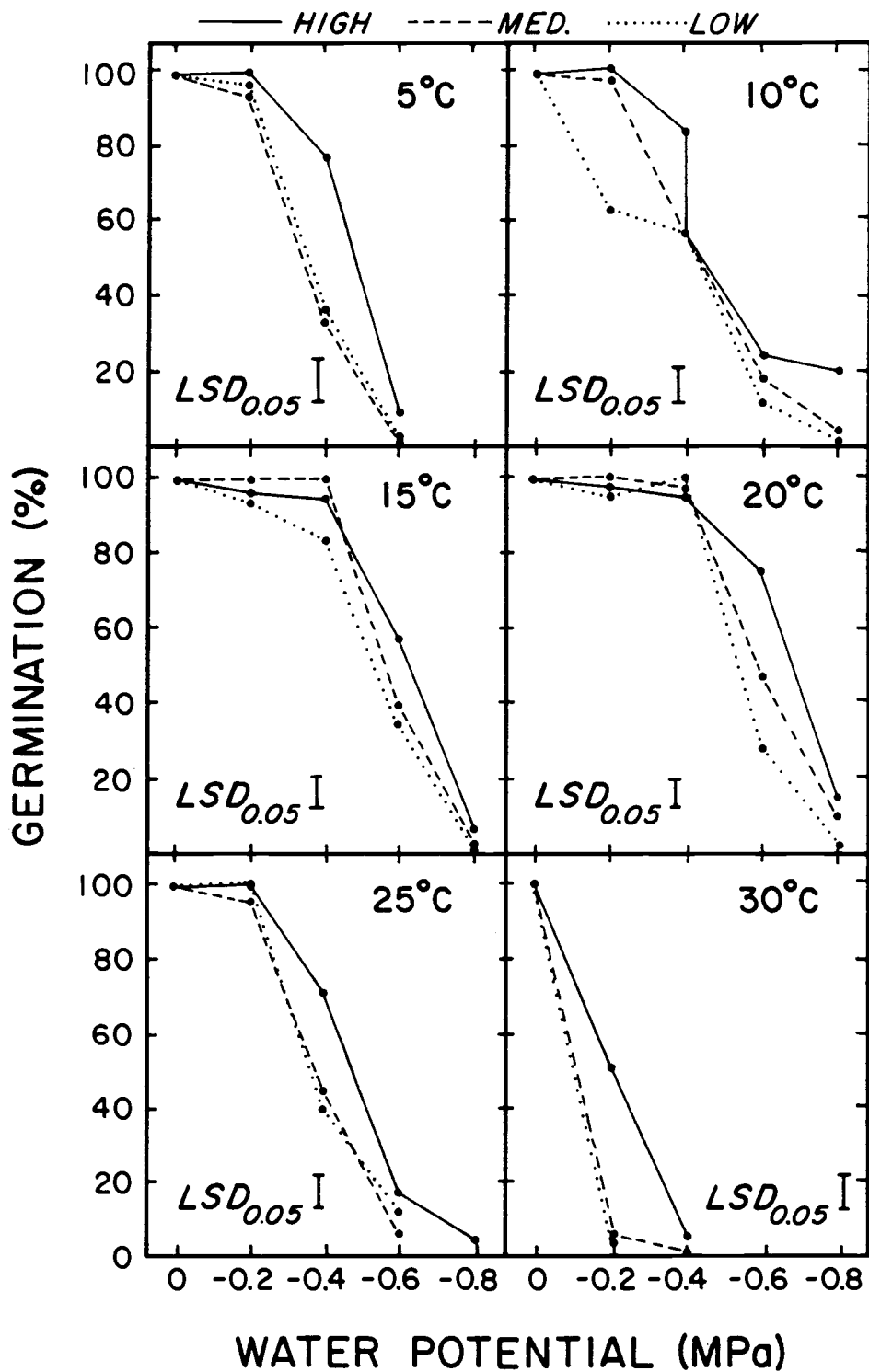


Figure 1. Effects of reduced water potential on the germination percentages of high, medium, and low vigor wheat seed lots at six temperatures.

Table 1. Germination Rate Indices (GRI) and GRI ratios of high, medium and low vigor seed lots under reduced water potentials at six temperatures.

Temp	Seed Lot Vigor	Water potential (MPa)					Water potential (MPa)				
		0	-0.2	-0.4	-0.6	-0.8	0	-0.2	-0.4	-0.6	-0.8
°C		GRI †					GRI ratios ‡				
5	High	9.97	7.58	4.10	0.45	-	100	76	41	5	-
	Medium	8.95	6.62	1.85	0.09	-	100	74	20	1	-
	Low	7.33	5.42	1.58	0.13	-	100	74	21	2	-
10	High	9.36	5.63	4.50	1.40	1.13	100	60	48	15	12
	Medium	8.20	4.84	2.70	0.93	0.20	100	52	29	10	2
	Low	7.15	2.73	2.46	0.60	0.07	100	38	34	8	1
15	High	17.53	11.85	7.97	3.50	-	100	68	45	20	-
	Medium	15.60	10.34	7.00	2.16	-	100	66	45	14	-
	Low	14.33	8.72	5.16	1.75	-	100	60	36	12	-
20	High	18.42	13.39	9.26	4.86	0.93	100	73	50	26	5
	Medium	16.66	12.40	7.46	2.58	0.56	100	74	45	16	3
	Low	14.58	9.90	6.66	1.50	0.15	100	59	40	9	1
25	High	26.37	14.26	5.60	1.26	0.32	100	54	21	5	1
	Medium	23.50	8.39	3.20	0.30	-	100	36	14	1	-
	Low	19.57	8.08	2.30	0.62	-	100	41	12	3	-
30	High	26.40	5.50	0.51	-	-	100	21	2	-	-
	Medium	18.44	0.36	0.10	-	-	100	2	1	-	-
	Low	17.90	0.30	-	-	-	100	1	-	-	-

† Germination rate index.

‡ GRI ratios calculated as a percentage of the GRI in water.

Table 2. Germination of high, medium and low vigor wheat seed lots in water at six temperatures.

Seed Lot Vigor	Temperature (°C)					
	5	10	15	20	25	30
	% germination [†]					
High	96	96	98	95	93	93
Medium	98	89	93	93	94	82
Low	85	83	91	87	85	80

[†] Actual number of total germination.

Table 3. Germination Rate Indices (GRI) and GRI ratios of high, medium and low vigor seed lots at six temperatures.

Seed Lot Vigor	Temperature (°C)											
	5	10	15	20	25	30	5	10	15	20	25	30
	GRI †						GRI ratios ‡					
High	9.97	9.36	17.53	18.42	26.37	26.40	54	51	95	100	143	143
Medium	8.95	8.20	15.60	16.66	22.50	18.44	54	49	94	100	141	111
Low	7.33	7.15	14.33	14.58	19.57	17.90	50	49	98	100	134	123

† Germination rate index.

‡ GRI ratios calculated as a percentage of the GRI at 20°C.

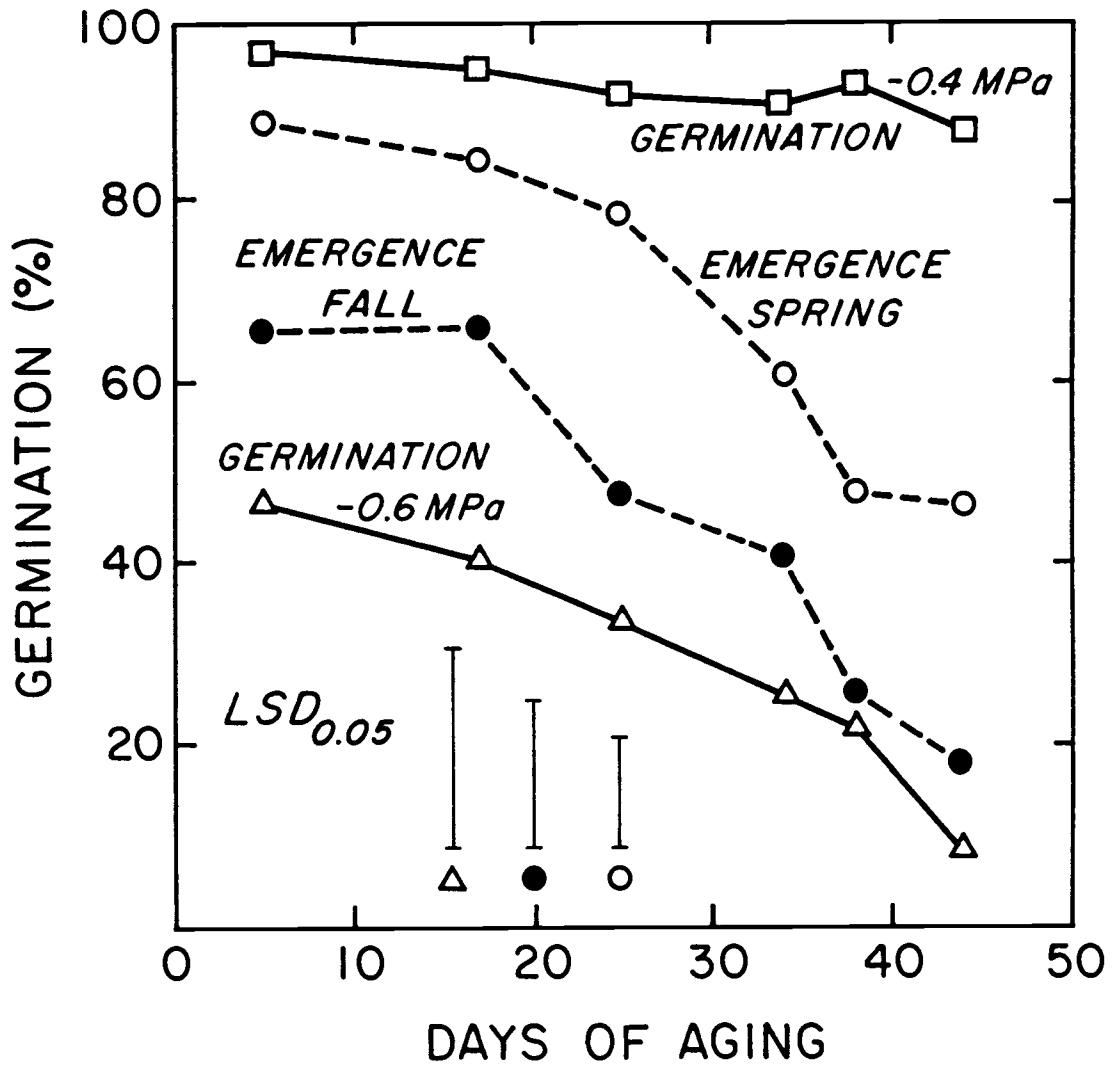


Figure 2. Comparison of germination percentage under water stress and field emergence percentage of six artificially aged (5, 17, 25, 34, 38 and 44 days) wheat seed lots.

Table 4. Germination of 24 naturally aged wheat seed lots under water stress and field conditions.

Cultivar	Year seed produced	Untreated			Arasan-treated		
		Water	-0.6MPa	Field	Water	-0.6MPa	Field
		% germination					
Kharkov	1980	84	51	77	79	77	79
	1982	96	76	87	92	88	80
	1983	96	78	91	97	91	92
	1984	98	44	89	97	92	92
Paha	1980	83	14	74	91	55	81
	1982	95	63	80	87	76	82
	1983	99	89	92	97	93	92
	1984	98	57	94	97	41	92
Nugaines	1980	94	49	88	96	80	89
	1982	90	54	83	86	85	82
	1983	96	78	91	99	90	89
	1984	97	68	92	97	91	98
Wanser	1980	80	35	72	85	84	82
	1982	92	66	83	93	85	83
	1983	96	71	85	96	96	88
	1984	98	57	87	95	89	92
Luke	1980	71	33	38	57	45	44
	1982	91	51	83	88	67	86
	1983	96	77	87	97	90	91
	1984	99	54	92	98	73	94
Moro	1980	52	12	34	62	20	50
	1982	95	46	89	93	58	86
	1983	98	55	90	94	57	91
	1984	99	34	95	97	56	92
Avg for method		91	55	82	90	74	84
LSD 0.05		5.77	11.77	6.66	4.68	14.67	7.18
Avg for year							
	1980	77	32	64	78	60	71
	1982	93	59	84	90	77	83
	1983	97	75	89	97	86	91
	1984	98	52	92	97	74	93
LSD 0.05		2.35	4.80	2.72	1.91	6.00	2.93
Avg for variety							
	Kharkov	94	62	86	91	87	86
	Paha	94	56	85	93	66	87
	Nugaines	94	62	89	95	87	90
	Wanser	92	57	82	92	89	86
	Luke	89	54	75	85	69	79
	Moro	86	37	77	87	48	80
LSD 0.05		2.88	5.88	3.33	2.34	7.34	6.45

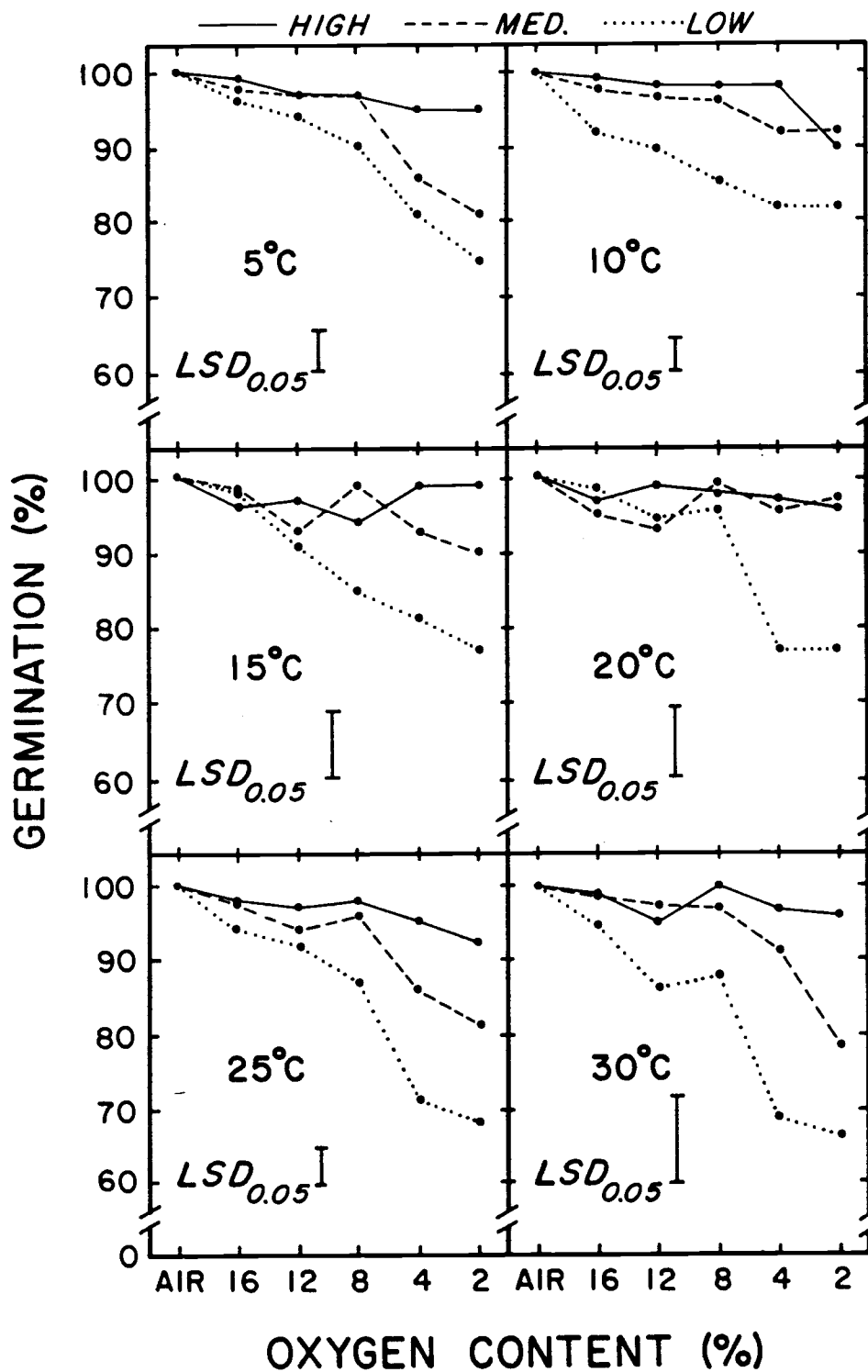


Figure 3. Effects of reduced oxygen levels on the germination percentages of high, medium and low vigor wheat seed lots at six temperatures.

Table 5. Germination Rate Indices (GRI) and GRI ratios of high, medium and low vigor seed lots under reduced oxygen levels at six temperatures.

Temp	Seed Lot Vigor	Oxygen content (%)						Oxygen content (%)					
		Air	16	12	8	4	2	Air	16	12	8	4	2
°C		GRI †						GRI ratios ‡					
5	High	10.20	9.24	8.87	8.34	8.09	7.94	100	91	87	82	79	78
	Medium	8.78	8.54	8.26	8.05	6.71	6.79	100	97	94	91	76	77
	Low	7.04	6.56	6.22	5.97	5.35	4.77	100	93	88	85	76	67
10	High	9.73	9.16	8.90	8.60	8.41	7.70	100	94	91	88	86	79
	Medium	9.11	8.22	7.96	7.80	7.10	6.90	100	90	87	86	78	76
	Low	7.14	6.33	6.20	5.60	5.40	5.40	100	88	87	78	75	75
15	High	23.07	22.50	23.00	22.00	23.60	21.80	100	97	99	95	99	94
	Medium	20.70	20.70	20.20	20.52	18.00	15.93	100	101	97	99	89	77
	Low	16.90	16.20	14.50	14.32	14.66	10.45	100	96	86	85	86	62
20	High	26.16	24.86	26.40	24.50	27.18	21.76	100	95	100	93	103	83
	Medium	24.40	24.40	21.95	22.80	21.20	18.93	100	100	90	93	87	77
	Low	19.76	18.95	18.42	17.88	14.60	11.50	100	96	93	90	74	58
25	High	31.76	31.76	28.60	29.16	26.41	21.66	100	100	90	92	83	68
	Medium	28.30	28.16	25.0	23.96	16.68	13.96	100	99	88	85	59	49
	Low	21.50	22.28	20.46	18.57	14.38	12.35	100	100	95	86	66	57
30	High	34.91	32.53	31.75	32.23	31.76	26.63	100	93	91	92	91	76
	Medium	27.68	26.75	26.23	22.52	19.65	17.23	100	96	95	81	71	62
	Low	24.75	24.75	20.40	20.30	14.46	11.88	100	100	82	82	58	48

† Germination rate index.

‡ GRI ratios calculated as a percentage of the GRI in air.

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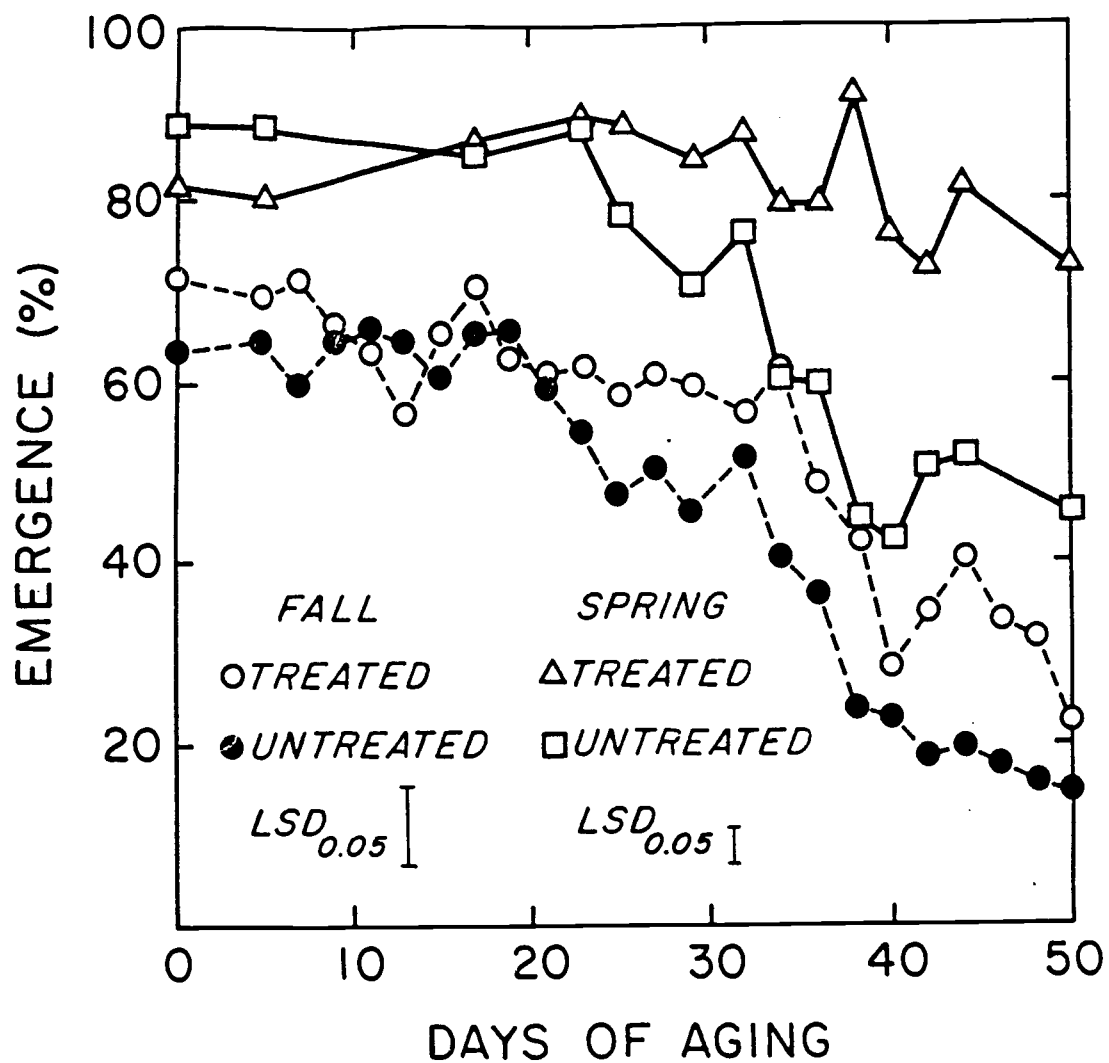
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APPENDIX



Appendix Figure 1. Field emergence percentages of artificially aged wheat seed lots from fall and spring planting. Comparisons were made between Arasan-treated and untreated seed.