



Evaluation of seed vigor tests for safflower
by Diane Luth

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Agronomy
Montana State University
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Abstract:

Safflower is an oilseed crop which is well adapted for production in parts of central and eastern Montana. The current practice of pre-plant incorporation of herbicides often results in a dry seedbed, unfavorable for field emergence. Producers need high quality seed for satisfactory emergence under these conditions. The standard germination test is currently the principle criterion for evaluating seed quality. However, this test, which measures percentage viability under optimal conditions, has not always been successful in predicting field emergence. Seed vigor tests have been developed for many crops to predict field emergence under a variety of stress conditions; their use for safflower has not been reported. Nine seed lots were evaluated for emergence under field and greenhouse conditions. The emergence data were correlated with results of seven laboratory tests, including, standard germination, seedling growth rate, accelerated aging, electrical conductivity, respiration, ATP content, and glutamic acid decarboxylase activity. Greenhouse total emergence suggested that while the standard germination test remains a useful criterion for seed quality, seed vigor tests can be important when evaluating seed lots which meet acceptable germination levels. For those seven seed lots with germination values $> 80\%$, accelerated aging had the highest correlation with total emergence ($r = .80$). In a stepwise multiple regression analysis accelerated aging and respiration provide the most useful model for predicting total emergence ($R = .93$).

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MONTANA STATE UNIVERSITY
Bozeman, Montana

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of a thesis submitted by

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ABSTRACT

Safflower is an oilseed crop which is well adapted for production in parts of central and eastern Montana. The current practice of pre-plant incorporation of herbicides often results in a dry seedbed, unfavorable for field emergence. Producers need high quality seed for satisfactory emergence under these conditions. The standard germination test is currently the principle criterion for evaluating seed quality. However, this test, which measures percentage viability under optimal conditions, has not always been successful in predicting field emergence. Seed vigor tests have been developed for many crops to predict field emergence under a variety of stress conditions; their use for safflower has not been reported. Nine seed lots were evaluated for emergence under field and greenhouse conditions. The emergence data were correlated with results of seven laboratory tests, including, standard germination, seedling growth rate, accelerated aging, electrical conductivity, respiration, ATP content, and glutamic acid decarboxylase activity. Greenhouse total emergence suggested that while the standard germination test remains a useful criterion for seed quality, seed vigor tests can be important when evaluating seed lots which meet acceptable germination levels. For those seven seed lots with germination values $> 80\%$, accelerated aging had the highest correlation with total emergence ($r = .80$). In a stepwise multiple regression analysis accelerated aging and respiration provide the most useful model for predicting total emergence ($R = .93$).

INTRODUCTION

Safflower (Carthamus tinctorius L.) is an oilseed gaining popularity as an alternative crop in eastern and central Montana. When included in a crop rotation and grown in conjunction with a chemical weed control program, the number of grassy weeds infesting small grain fields can be decreased and subsequent grain yields enhanced (Bergman et al., 1979).

Stand establishment and weed control are two major problems facing safflower producers (Wichman, 1983). Pre-plant incorporation of herbicides decreases weed problems, but can result in a dry, crusty seedbed unfavorable for germination and emergence. Producers need high quality seed capable of emerging under these stressful conditions.

Seed quality has traditionally been evaluated by the standard germination test (McDonald 1980). This test is conducted in controlled environments under optimum conditions. Percentage germination correlate well with stand establishment under favorable field conditions (Tekrony and Egli, 1977; Johnson and Wax, 1978; Egli and Tekrony, 1979), but is not indicative of performance under stress conditions.

An additional component of seed quality, known as seed vigor, has been recognized. Seed vigor is evaluated through laboratory tests measuring growth rate, stress response, or biochemical parameters of the seeds. These tests have been successful in predicting field performance of several crops (Association of Official Seed Analysts, 1983), but their use for safflower has not been reported. The objective of this study was to evaluate six vigor tests and determine which tests could be useful for predicting field performance of safflower.

LITERATURE

Seed Vigor

Fredrich Nobbe, in 1876, first discussed the concept of seed vigor. In addition to germination, he believed speed and uniformity were important parameters of seed quality (Copeland and McDonald, 1985). The concept of seed vigor has been reviewed extensively by Heydecker (1972), Woodstock (1973), and McDonald (1975; 1980). The Vigor Test Committee of the Association of Official Seed Analysts (AOSA) adopted the following definition of seed vigor: ".... those 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 International Seed Testing Association.

Seed vigor testing has become an increasingly important component of seed quality evaluation. A practical vigor test should be reproducible, rapid, economical, objective, uncomplicated, and provide a good indication of field performance potential (AOSA, 1983).

Seedling Growth Rate

The seedling growth rate test is conducted similarly to the standard germination test, but a defined amount of moisture is applied to the media. At the end of the germination period, seedling growth is determined either by measuring linear growth or dry weight of the seedling (AOSA, 1983). Seedling dry weight is more commonly used in the United States. The principle of this test is that vigorous seeds have a greater ability to synthesize and transport new materials to the growing embryonic axis, resulting in greater total seedling growth (Copeland and McDonald, 1985).

Perry (1977) found that under stressful field conditions, plumule growth of barley (Hordeum vulgare L.) was better correlated with field emergence than the standard germination test. This test was also successful for corn (Zea mays L.) (Woodstock, 1969b). However, Yaklich and Kulick (1979) concluded that total shoot length did not correlate with field emergence of soybean (Glycine max L. Merr.).

Pinthus and Kimel (1979) found a close relationship between dry weight matter accumulation and subsequent development of soybean plants throughout all growth stages including seed production. Mock and McNeil (1979) observed seedling dry weight was significantly correlated to

seedling emergence, emergence index, and grain yield of corn.

These tests are inexpensive and relatively rapid, but have limitations. Seedling elongation can be inherently different among cultivars, and moisture and temperature must be closely monitored (AOSA, 1983).

Accelerated Aging

The accelerated aging test functions by exposing seeds to high temperatures (40-45 C) and high relative humidities for short periods of time. Seeds deteriorate rapidly under these stressful conditions. Halmer et al., (1962) first observed that the response of crimson clover (Trifolium incarnatum L.) following exposure to high temperature and humidity was correlated with seed vigor and field emergence. Delouche and Baskin (1973) have proposed that the decline in viability following accelerated aging is proportional to the initial physiological condition of the seed.

Extensive reviews on the mechanisms of accelerated aging have been written (Priestly, 1986; McDonald and Nelson, 1986). It is generally believed that viability decreases mainly as a result of membrane deterioration.

In a study on biochemical changes in safflower following accelerated aging, Kole and Gupta (1982)

concluded that membrane permeability remained unaffected. However, their aging regime increased seed moisture levels to only 15%, far below the recommended 30% levels (Tekrony, 1985). With soybean, Stewart and Bewley (1980) noted a loss of unsaturated fatty acids presumably caused by lipid peroxidation. Priestly and Leopold (1979) found no changes in unsaturated fatty acids following accelerated aging, and in a later study (Priestly et al. 1980) analyzed soybeans for the levels of tocopherol, an antioxidant. They again concluded that lipid peroxidation does not play a significant role in causing membrane deterioration following accelerated or natural aging.

Problems with reproducibility and standardization hampered the use of accelerated aging as a vigor test. McDonald (1977) showed that initial seed moisture levels influenced the degree of deterioration from accelerated aging of barley and soybean seeds. The technique was further refined by McDonald and Phaneedranath (1978) who recommended that a single layer of seeds be placed on wire mesh trays in plastic boxes. This replaced the method of placing seeds in a wire basket over a measured amount of water in a sealed jar (Baskin, 1977).

Tao (1979) confirmed McDonald's conclusions regarding initial seed moisture of soybeans, and suggested that using 40 ml of water in the boxes decreased variation. In 1984,

the AOSA vigor subcommittee recommended McDonald and Phaneedranth's (1978) method be adopted as standard procedure.

Accelerated aging has also been used to predict field performance of peanut (Arachis hypogaea) (Baskin, 1970), bean (Phaseolus vulgaris L.) (Roos and Manalo, 1971), soybean (Tekrony and Egli, 1977; Kulik and Yaklich, 1982) and cotton (Gossypium hirsutum L.) (Bishnoi and Delouche, 1975; 1980).

Electrical Conductivity

Membranes, during seed maturation, are transformed from a hydrated to a nearly dessicated state. Dessicated membranes are disorganized, and no longer form an intact barrier around the cytoplasm of each cell (Simon and Mills, 1983). There is a short period at the start of imbibition, while membrane constituents are reorganizing, that solutes, including sugars, amino acids, and proteins, leak out of the seed (Simon and Raja Harun, 1972).

It has been hypothesized that membrane deterioration is the first stage in loss of seed vigor (Powell and Matthews, 1977). The formation of free radicals by peroxidation, and hydrolysis of membrane phospholipids could result in membrane deterioration. These topics have been reviewed by Powell (1986) and Priestly (1986).

Vigorous seeds may re-establish membranes at a faster rate and with less leakage than seeds with poor vigor (AOSA, 1983). Measuring the conductivity of the seed leachate is the principle behind the electrical conductivity test.

A secondary consequence of increased leakage of organic metabolites during germination is that they encourage growth of microorganisms. There is evidence that solutes leached from seeds can stimulate the growth of fungal pathogens (Flentje and Saskena, 1964; Schroth and Cook, 1964). A direct correlation has also been reported between seed rot and the quantity of carbohydrates exuded from seeds of soybean, pea and garden bean (AOSA, 1983).

Parrish and Leopold (1978) observed increased leakage rates before a decline in germinability of soybean seeds. Electrical conductivity of seed leachate has been shown to correlate with vigor in seeds of barley, rice (Oryza sativa L.), corn, pea (Pisum sativa L.), and soybean (AOSA, 1983; McDonald, 1975). However, Halder and Gupta (1980; 1981) reported a positive correlation between leachate conductivity and viability in sunflower (Helianthus annuus L.), while no correlations were found for muskmelon (Cucumis melo L.) (Pesis and Ng, 1983), and sorghum (Sorghum bicolor L.) (Perl et al., 1978).

Tao (1978) reported several factors could cause variation in conductivity measurements of soybean seeds.

Initial seed moisture content and seed injury, as well as technique could affect conductivity readings. Simon and Wiebe (1975) also observed that the extent of leakage from imbibing pea embryos depended on initial moisture content.

Perl et al. (1978) believed the release of solutes was primarily controlled by the number of dead seeds in a sample, and that no studies have clearly demonstrated that low vigor seeds are themselves leaky.

Respiration Test

Respiration is a fundamental cellular activity whereby food reserves are oxidized resulting in the liberation of energy for seed germination and seedling growth. Three respiratory pathways are assumed to be active in the imbibed seed; glycolysis, the pentose phosphate pathway, and the citric acid cycle (Bewley and Black, 1985).

The respiration test for seed vigor is based on the concept that vigorous seeds, which germinate and grow rapidly, use energy rapidly and thus require high respiratory activity. Woodstock and Grabe (1967) found significant correlations between oxygen consumption during imbibition, and rates of germination and seedling growth in corn.

Both embryonic and nonembryonic parts may exhibit depressed oxygen consumption in less vigorous seeds (Wahab

and Burris, 1971; Parrish and Leopold, 1977). However, in wheat (Triticum aestivum L.), Anderson and Abdul-Baki (1971) found embryonic oxygen consumption decreased as a result of seed deterioration, but endosperm was unaffected. They suggested biochemical and physiological studies of seed deterioration should focus on the embryonic axis rather than intact seeds, as a decrease in the metabolic activities of the embryonic axis will be directly associated with seed vigor (Abdul-Baki and Anderson, 1973b).

Depressed rates of oxygen consumption have usually been ascribed to mitochondrial deterioration (Priestly, 1986). In vigorous seeds, considerable mitochondrial differentiation occurs during imbibition. This occurs by either modification of existing organelles, or synthesis of new ones (Morahashi et al., 1981). During this period, the ability of the mitochondria to oxidize exogenous substrates is greatly enhanced, and phosphorylation coupling becomes evident (Pradet, 1982; Priestly, 1986).

Oxygen consumption associated with less vigorous seeds may be depressed by direct lesions in the mitochondrial structure or inferior mitochondrial development (Priestly, 1986). Evidence of lesions was found by Leopold and Musgrave (1980). While studying the embryonic axes of accelerated aged soybeans, they proposed the aged axes suffered a deterioration of the cyanide sensitive electron

transport chain through loss of cytochrome c oxidase activity. An alternative electron transport pathway which is cyanide insensitive and less efficient becomes engaged. In vigorous seeds, this alternative pathway is apparently inactive. Woodstock et al. (1984) noted this lower oxygen consumption associated with artificially aged embryonic axes was apparent within the first minutes of imbibition.

Evidence of inferior mitochondrial development has also been reported. Abu-Shakra and Ching (1967) investigated mitochondria from pea embryonic axes at 4 days post-imbibition. They concluded the organelles were fewer and less efficient in low vigor seedlings, presumably due to improper development. Using succinate as a substrate, Woodstock et al. (1984) found mitochondria with depressed rates of oxidation and a decreased amount of inorganic phosphate esterified into ATP per unit oxygen consumption.

The respiration test is quantitative, rapid, easy to standardize and perform, well suited to routine testing of large numbers of seed samples, and, with suitable precautions, reliable (Woodstock, 1966). Several factors, including temperature (Woodstock and Pollack, 1965) and the presence of microflora on the seed (Oxley and Jones, 1944), can influence respiration rates. However, Ragai and Loomis (1954) concluded that surface microorganisms did not affect respiration rates significantly. Mechanical injury can

damage seeds, yet increase respiration rates (Woodstock, 1969a).

Adenosine Triphosphate (ATP) Content

Seed germination and seedling growth are energy requiring processes. ATP is the main source of energy for most biological activities (Perl, 1986). ATP content has received increased attention as a seed vigor test in recent years (Ching, 1982; Priestly, 1986).

ATP levels are low in dry seeds and increase rapidly upon imbibition (Obendorf, 1974). Moreland et al. (1974) found this increase to follow a triphasic time course in radish (Raphnus sativus L.) seeds. Oxidative phosphorylation may develop too slowly to account for the sudden surge of ATP levels (Mayer, 1977). Perl (1980; 1981) suggested that ATP accumulates rapidly in imbibing seeds through a specialized enzyme system utilizing adenosine monophosphate (AMP), phosphoenol pyruvate, and inorganic orthophosphate.

Investigators who believe oxidative phosphorylation is responsible for ATP levels have reported slower rates of increase in less vigorous seeds. Ching (1973) found the ATP content of crimson clover (Trifolium incarnatum cv. 'Dixie'), annual ryegrass (Lolium multiflorum L.), and rape (Brassica napus L.) seeds to be significantly correlated

with seedling weight and indicative of vigor. Negative correlations were found between ATP content and lack of vigor in lettuce (Lactuca sativa L.) seeds subjected to accelerated aging (Ching and Danielson, 1972).

Van Onckelen et al. (1974) observed depressed ATP levels in both embryos and endosperms of accelerated aged barley at 6 hours post-imbibition. A significant correlation between ATP levels at 7 hours post-imbibition and 30 day dry matter growth for cauliflower (Brassica oleracea L.) was noted by Lunn and Madsen (1981).

Diminished ATP accumulation in imbibing axes of low vigor soybean was also reported (Anderson, 1977). There is also evidence that the accumulation of other nucleoside triphosphates is hindered during imbibition of low vigor seeds (Standard et al., 1983).

Other studies question the utility of ATP measurements for determining seed vigor. Harman et al. (1976) reported the ATP content of the pea oscillated during the first 24 hours of imbibition, and the time that measurements are made can have a profound effect on results. They found differences between aged and unaged axes were most apparent at 9 hours, less so at 3 hours and absent at 15 hours post-imbibition. Styer et al. (1980) studied maize, radish, cucumber (Cucumis sativus L.) and onion (Allium cepa L.) seeds. The reported ATP content at 4 hours of imbibition

did not correlate with changes in germination, germination rate, or radicle length for most seeds.

Mazor et al. (1984) concluded ATP is subject to very rapid turnover and there is no reason to assume that steady state levels reflect rates of synthesis or utilization. Perl (1986) states that most data supporting ATP content and seed vigor was published based on the assumption that ATP production is the result of oxidative phosphorylation. Since the ATP synthesizing system described by Perl (1980; 1981) becomes active at the same time as ATP requiring systems, the accumulated ATP is a result of the balance between synthesis and utilization. Thus, large amounts of accumulated ATP could result from either active ATP synthesis, reflecting high vigor seeds, or from impaired ATP utilization, indicating low vigor seeds.

Glutamic Acid Decarboxylase Activity (GADA)

Proteins are hydrolyzed during seed germination, releasing amino acids which are further catabolized to provide energy. Glutamic acid is particularly important in seed germination because it comprises a high percentage of the total amino acids in seeds (AOSA, 1983). In a series of reactions, glutamic acid is catalyzed to form gamma-amino butyric acid and CO_2 . The measurement of CO_2 is the basis for the GADA test.

Glutamic acid decarboxylase has been shown to be highly active in vigorous seeds and less active in seeds of lower vigor (McDonald, 1975). Linko and Sogn (1960) discovered that GADA was significantly correlated to germination and percentage germ-damaged wheat seeds. Linko (1961) then developed a rapid method to determine GADA for use as a quality index for wheat. Grabe (1964) found GADA to be a good indicator of seed deterioration and seedling vigor for corn and oats (Avena sativa L.). Bautisa (1964) and Azizul-Islam et al. (1973) used the test successfully to predict viability and vigor of rice.

Other reports, such as Burris et al. (1969) and Abdul-Baki and Anderson (1973a) showed no relationship between GADA and seedling vigor in soybean. In bean, GADA remained high among low vigor seeds (James, 1968). Abdul-Baki and Anderson (1973a) have criticized this test because of the high respiration rate of germinating seeds or seed parts, only a small portion of the CO₂ evolved came directly from glutamic acid decarboxylation.

MATERIALS AND METHODS

Seed lots were obtained from samples of safflower seed submitted to the Montana State University Seed Testing Laboratory. Nine lots were selected based on varying levels of percentage germination. Seed lots 1, 2 and 3 were the cultivar "Oker", lots 4, 5 and 6 were "Hartman", and lots 7, 8 and 9 were "S-541". All nine lots were used in the laboratory, greenhouse, and field experiments. The seed lots were stored at room temperature (22 C) in the laboratory during the course of these experiments. All experiments were conducted in a randomized complete block design with four replications.

Laboratory Studies

Standard Germination

One-hundred seeds per replication were placed in 13 x 13.5 cm plastic germination boxes containing two moistened blotters, treated with 1500 ppm Thiram fungicide. The seeds were germinated for 14 days at 20 C in a dark germinator. Normal seedlings were counted according to "Rules for Testing Seed" (AOSA, 1981), and expressed as percentage germination.

Seedling Growth Rate

Seedling growth rate test was conducted as suggested by AOSA (1983), with minor procedural modifications. Fifty seeds were used per replication. Twenty five seeds per row were oriented radical downward on a #76 paper towel, 25.4 x 38.1 cm, which had been moistened with 30 ml 1500 ppm Thiram fungicide. The top row was approximately 6.25 cm from the top of the towel, and the second row was 12.5 cm from the top. The seeds were covered with another paper towel treated as above. The two towels were loosely rolled in a 30.4 x 45.7 cm wax paper sheet and placed upright in a 15.5 x 17 cm container. Each container was covered with a plastic bag and placed in a dark 20 C germinator for 11 days. The seed hulls were removed from normal seedlings which were then placed in coin envelopes and dried for 24 hours at 80 C. Weights were recorded to the nearest mg and total dry weight was divided by number of normal seedlings to determine mean seedling growth rate (mg/seedling).

Accelerated Aging

The accelerated aging test was conducted as recommended by AOSA (Tekrony, 1985). Thirteen grams of seed per replication were surface sterilized in 100 ml 1.5% sodium hypochlorite for 15 minutes, rinsed 5 times with sterile water, oven-dried at 32 C for 24 hours, and placed in a dessicator 24 hours prior to initiating the test.

Twelve grams of seed per replication were dry treated with Thiram , and were uniformly distributed on 10.4 cm² wire mesh trays inside 11.4 cm² covered plastic boxes containing 40 ml sterile water. The boxes were placed inside the accelerated aging chamber (Stults Engineering Corp.) at 38 C, and 100% relative humidity. After 72 hours, the seeds were removed and standard germination tests conducted. An additional 50 seeds per lot were removed, weighed, oven-dried at 105 C for 24 hours and re-weighed to determine seed moisture content.

Electrical Conductivity

The electrical conductivity test was conducted as recommended by AOSA (1981) with minor procedural modifications. Twenty five intact seeds from each seed lot were weighed and placed in a 75 ml test tube with 50 ml double deionized water. The tubes were swirled, covered with parafilm and placed in a dark 20 C germinator for 24 hours. The seeds were gently stirred, the electrical conductivity of the solution measured, and reported in micromhos per gram seed.

Respiration

Oxygen uptake was measured manometrically using a Gilson Differential Respirometer. Twenty seeds were weighed, and placed in a reaction flask containing 2 ml

sterile water with 0.2 ml 10% KOH and a paper wick in the center well. The reaction flasks were placed on the respirometer in 20 C water bath for four hours of imbibition. The system was equilibrated for 15 minutes and flasks shaken at 78 oscillations per minute. Four readings were taken at 15 minute intervals. Respiration rate was reported as microliters of oxygen consumed per gram seed per minute at standard temperature and pressure.

ATP Content

Twenty seeds per lot were weighed, placed in a plastic germination box between two layers of filter paper moistened with 2.5 ml distilled water, and imbibed for four hours at 20 C in a dark germinator.

The seeds were then homogenized in 9 ml dimethyl sulfoxide. A 50 microliter aliquot of the liquid was diluted with 200 microliters Hepes buffer (pH 7.5) and placed on ice. One hundred fifty microliters was pipetted into an 8 x 50 mm plastic test tube which was then placed in a Turner TD-20e luminometer. One hundred microliters of luciferin-luciferase enzyme preparation (Turner Designs) was injected into the sample and the luminescence, in photo units, recorded. Luminescence is proportional to ATP content which was calculated for each sample using a standard curve, and reported in nanograms ATP per gram seed.

GADA

Glutamic acid decarboxylase activity was measured as described by Linko (1961) and Grabe (1964) with minor procedural modifications. Carbon dioxide production was measured manometrically using a Gilson Differential Respirometer. Twenty grams of each seed lot were finely ground, and a 1 gram sample was placed in the reaction flask with 2.5 ml reaction mixture and mixed gently with a glass rod. The reaction mixture consisted of a 0.1 M glutamic acid solution in 0.067 M phosphate buffer at pH 5.8 (Ram, 1983). The flasks were attached to the respirometer, placed in a 30 C water bath and shaken at 78 oscillations per minute. Following a 10 minute equilibration period, CO₂ evolution was measured at 15 minute intervals for 45 minutes. The enzyme activity was measured as microliters of CO₂ per gram seed per minute at standard temperature and pressure.

Oil Content

Total percentage oil was measured using nuclear magnetic resonance spectroscopy at the Eastern Montana Experiment Station, Sidney, Montana. Each sample was comprised of twenty five grams of seed.

Field Studies

Field plantings were made in May 1986 at the Arthur H. Post Agricultural Research Center, Bozeman, Montana, and at the Central Agricultural Research Center, Moccasin, Montana. Soils at the Bozeman location are Amsterdam silt loam variants classified in the fine-silty, mixed family of Typic Haploboralls. Soils at the Moccasin location are described as Danvers Judith clay loam.

Plot size was 6.1 m by 1.2 m. Each plot contained four rows spaced 0.3 m apart. Seeding rate was 7 seeds per 0.3 m row length and planting depth was 5 cm. A 1 m section of row was marked prior to emergence in the center two rows of each plot. Emergence rate and total emergence counts were taken in the marked section and the average count per meter obtained. Emergence rate was measured at the Bozeman location only and total emergence was measured at both locations.

Speed of emergence index was calculated as described by Carlton et al. (1968). Emergence index was calculated as follows:

$$EI = \frac{\text{no. emerged seedlings}}{\text{days to first count}} + \dots + \frac{\text{newly emerged seedlings}}{\text{days to final count}}$$

Greenhouse Studies

In the greenhouse, 56 x 41 x 11 cm flats were filled within 3 cm of the top with soil. Each flat represented a

replication and contained 60 seeds planted 5 cm deep. The flats were watered once per week to create stress conditions, and the temperature was maintained at 25 C with natural light supplemented 4 hours per day with artificial light.

Total emergence and emergence index were calculated. Mean seedling dry weight was determined for each flat by harvesting above ground portions of the seedlings, oven drying at 105 C for 24 hours, and dividing total weight by the number of established seedlings.

RESULTS

Significant differences ($p = 0.5$) were found among seed lots for all laboratory tests (Table 1). The results of the standard germination tests (Table 1) indicate that seven of the seed lots were of generally acceptable quality ($> 80\%$). The mean of all lots tested was 87.7% . Cultivar effects were observed in seedling growth rate, accelerated aging, electrical conductivity, ATP, and GADA tests (Table 2).

Oil content of the seed lots ranged from $39.7-48.8\%$ (Table 3). Moisture content following accelerated aging ranged from $26-34\%$ (Table 4).

No significant location $\times$ treatment effects were observed between the Bozeman and Moccasin locations. The total emergence values obtained from both locations were averaged and a mean value for combined locations used. Total field emergence ranged from 12 to 22 plants/m (Table 5). Lot 6 had significantly lower total field emergence, than lots 1, 2, 5 and 9. No other significant differences in field emergence were observed among seed lots. Field emergence index was highly correlated with total field emergence ($r = .97$). No cultivar effect was observed in the field for total emergence and emergence index.

Table 1. Mean comparisons among standard germination and vigor tests for nine seed lots of safflower

Seed Lot	Laboratory parameters						
	Std. germ.	1/ SGR	Accel. aging	Elec. Conduct.	Respiration	ATP	GADA
	%	mg/plant	%	umhos/g	ulO ₂ /g/min	ng/g	ulCO ₂ /g/min
1	93d ^{2/}	14.3b	61c	240c	.82de	144cde	7.6c
2	83c	14.7b	64c	190b	1.24de	138b-e	7.4c
3	97d	13.0a	87ef	169b	1.11cde	76a	7.8c
4	66a	19.3cd	4a	284d	.46a	100abc	4.9a
5	91d	19.1cd	48b	297d	.95cd	106a-d	5.9b
6	93d	17.9c	61c	175b	1.30e	152de	5.3a
7	98d	20.3e	93f	122a	1.26de	172e	6.3b
8	94d	21.7d	82e	95a	.65ab	60a	5.1a
9	75b	20.2d	71d	91a	1.1cde	91ab	4.8a
C.V.% ^{3/}	6	4	11	7	5	29	7

1/ Seedling growth rate

2/ Means within a column followed by the same letter do not differ significantly at the 5% level according to Newman-Keuls mean separation test

3/ Coefficient of variability

Table 2. Mean comparisons among cultivars for six vigor tests averaged over three seed lots

Cultivar	Vigor Tests					
	2/ SGR	Accel. aging	3/ ECT	4/ Resp.	ATP	GADA
	mg/plant	%	umhos/g	ulO ₂ /g/min	ng/g	ulCO ₂ /g/min
Oker	14.02a ^{1/}	70.67b	199.5b	1.06a	81a	7.6b
Hartman	18.77b	37.67a	252.2c	0.91a	132c	5.4a
S-541	20.75c	82.25b	102.8a	0.99a	98b	5.4a
Overall mean	17.85	63.53	184.8	0.98	94	6.2

1/ Means within a column followed by the same letter do not differ significantly at the 5% level according to Newman-Keuls mean separation test

2/ Seedling growth rate

3/ Electrical conductivity test

4/ Respiration rate

Table 3. Mean comparisons among oil contents for nine seed lots of safflower

Seed Lot	Percentage Oil
	1/
1	45.79 e
2	44.92 d
3	41.70 b
4	39.74 a
5	41.44 b
6	42.36 c
7	48.69 g
8	46.48 f
9	45.75 e
2/	
C.V.%	4

1/ Means followed by the same letter do not differ significantly at the 5% level according to Newman-Keuls mean separation test.

2/ Coefficient of variability

Table 4. Mean comparisons among moisture contents of nine seed lots of safflower following 72 hours of accelerated aging

Seed Lot	Percentage Moisture
	1/
1	28.44 ab
2	28.55 ab
3	28.44 ab
4	34.43 c
5	30.20 b
6	29.55 ab
7	25.51 a
8	27.15 ab
9	28.26 ab
2/	
C.V.%	5

1/ Means followed by the same letter do not differ at the 5% significance level by Newman-Keuls mean separation.

2/ Coefficient of variability.

Table 5. Mean comparisons among field total emergence and emergence index for nine lots of safflower

Seed Lot	Total Emergence 2/ plants/m 1/	Emergence Index 3/
1	21 b	31 b
2	20 b	29 b
3	18 ab	25 ab
4	19 ab	24 ab
5	20 b	29 b
6	12 a	12 a
7	18 ab	22 ab
8	15 ab	18 ab
9	22 b	28 b
C.V.% ^{4/}	19	26

- 1/ Means followed by the same letter do not differ significantly at the 5 % level according to Newman-Keuls mean separation test
 2/ Means from combined Bozeman and Moccasin locations
 3/ Means from Bozeman location only
 4/ Coefficient of variability

Greenhouse total emergence ranged from 42-92 % (Table 6). However, six of the seed lots did not differ significantly ($p = .05$). The correlation between greenhouse emergence index and total emergence in the greenhouse was high ($r = .87$). No cultivar effects were observed.

None of the simple correlation coefficients between field performance and laboratory tests were significant (Table 7). When correlations were made between greenhouse performance and laboratory tests (Table 8), standard

germination, accelerated aging, and respiration all showed significant correlations with greenhouse total emergence. Greenhouse emergence index was significantly correlated with standard germination and respiration.

Table 6. Mean comparisons among greenhouse total emergence and emergence index for nine lots of safflower

Seed Lot	Total Emergence %	Emergence Index
	1/	
1	68 bc	7.6 bcd
2	77 bcd	8.4 bcd
3	86 cd	9.3 bcd
4	42 a	3.1 a
5	71 bc	7.7 bcd
6	82 bcd	10.4 d
7	92 d	9.8 cd
8	77 bcd	5.6 ab
9	64 b	5.9 abc
C.V.% ^{2/}	12	24

1/ Means followed by the same letter do not differ significantly at the 5 % level according to NewmanKeuls mean separation test.

2/ Coefficient of variability

These data were reanalyzed using only the seven seed lots with standard germinations above 80% (eliminating lots 4 and 9). Glutamic acid decarboxylase activity showed a significant positive correlation (Table 9) with field total emergence and emergence index. Reanalyzing the greenhouse data (Table 10), revealed significant correlations for greenhouse total emergence with accelerated aging, and greenhouse emergence index with respiration.

Table 7. Simple Correlations among standard germination, vigor tests and field performance variables for nine seed lots of safflower

	<u>Laboratory Parameters</u>						
	Std. germ.	1/ SGR	2/ AA	3/ ECT	Resp	ATP	GADA
Field Parameters							
Total emer. (plants/m)	- .40	- .24	- .15	.25	- .10	- .03	.31
Emerg. index	- .27	- .42	- .13	.34	- .12	- .06	.48
1/ Seedling growth rate							
2/ Accelerated aging							
3/ Electrical conductivity							

Table 8. Simple Correlations among standard germination, vigor tests and greenhouse performance variables for nine seed lots of safflower

	<u>Laboratory Parameters</u>						
Greenhouse Parameters	Std. germ.	1/ SGR	2/ AA	3/ ECT	Resp	ATP	GADA
Total emer. (%)	.88**	-.17	.88**	-.51	.74*	-.06	.43
Emerg. index	.77*	-.39	.64	-.20	.88**	.57	.53
* r(.05) = 0.63							
** r(.01) = 0.76							
1/ Seedling growth rate							
2/ Accelerated aging							
3/ Electrical conductivity							

Table 9. Simple Correlations among standard germination, vigor tests and field performance parameters for seed lots with germination > 80 %

Field Parameters	Laboratory Parameters						
	Std. germ.	1/ SGR	2/ AA	3/ ECT	Resp	ATP	GADA
Total emer. plants/m	-.33	-.47	-.21	.56	-.13	.10	.73*
Emerg. index	-.38	-.54	-.28	.61	-.20	.01	.75*

* $r(.05) = 0.70$

1/ Seedling growth rate

2/ Accelerated aging

3/ Electrical conductivity

Table 10. Simple correlations among standard germination, vigor tests and greenhouse performance parameters for seed lots with germination > 80 %

Greenhouse Parameters	Laboratory Parameters						
	Std. germ.	1/ SGR	2/ AA	3/ ECT	Resp	ATP	GADA
Total emer. (%)	.56	-.17	.80*	-.68	.59	.18	-.09
Emerg. index	.23	-.29	.12	.01	.93**	.64	.19

* $r(.05) = 0.70$

** $r(.01) = 0.83$

1/ Seedling growth rate

2/ Accelerated aging

3/ Electrical conductivity

A stepwise multiple regression analysis was conducted on the seven acceptable seed lots to determine if any combination of laboratory tests could more accurately predict performance. No combination of laboratory tests provided a significant multiple correlation coefficient for field performance. However, the best model for predicting greenhouse total emergence (Table 11) included accelerated aging, respiration, and GADA. A combination of respiration and standard germination tests provided the best model for predicting the greenhouse emergence index (Table 12).

Table 11. Summary of multiple stepwise regression comparing greenhouse total emergence with laboratory tests for seven lots of safflower with germination > 80%

Step	Variable	² R	Regression equation
1	Accelerated aging (AA)	.64	$y = 50.29 + 0.41(AA)$
2	Respiration (R)	.93	$y = 33.16 + .38(AA) + .30(R)$
3	GADA (G)	.97	$y = 42.48 + .39(AA) + .32(R) + -1.68(G)$

Table 12. Summary of multiple stepwise regression comparing greenhouse emergence index with laboratory tests for seven lots of safflower with germination > 80%

Step	Variable	² R	Regression equation
1	Respiration (R)	.86	$y = 2.10 + .10(R)$
2	Std. Germ (SG)	.94	$y = -6.83 + .09(R) + .10 (SG)$

DISCUSSION

Although there were significant differences in the laboratory tests, no significant correlations could be made with field performance due to the lack of differences among seed lots in the field. Seven of the seed lots had total field emergence values between 18 and 22 seedlings per meter. The general lack of variation in field performance may have been caused by favorable conditions at planting time. Uneven seed placement and small sample size are other possible explanations for lack of differences among seed lots for field parameters.

The greenhouse, with low moisture being the only environmental stress imposed, showed more variation among seed lots than the field. The germination test was important in predicting greenhouse performance and continues to be the primary test for seed quality. Seed lots 4 and 9 had standard germinations below 80%, generally the minimum acceptable level. In a seed testing laboratory, vigor testing would not be conducted on seed lots which had low standard germination. Using only the seven seed lots of acceptable viability, accelerated aging and respiration provided the best model for correlating vigor tests to greenhouse performance. Seeds with high

respiration may have increased ability to germinate and establish when moisture is available. When moisture becomes limiting, as in the greenhouse experiment, these seedlings could better tolerate the stress. Seeds which do well in accelerated aging may have more resilient membranes and be able to hydrate more efficiently and partition cellular functions more effectively. Such seeds would have more efficient metabolic processes and be better able to withstand stress. The combination of high respiration and structurally intact membranes would create a high rate and efficiency of energy production in germinating seeds. This could explain why the combination of respiration and accelerated aging tests correlated well with greenhouse performance under moisture stress.

Cultivar effects were apparent for all vigor tests, with the exception of respiration rate. This requires further study with a larger number of samples of each cultivar tested. If cultivar effects prove important, the range of values relative to each cultivar must be established for each test, and results must be interpreted within genotypes.

Seed lot 7, with the highest oil content, performed best in the accelerated aging test and had the highest greenhouse emergence. The high oil content and hydrophobic nature explain why it had the lowest moisture content.

following accelerated aging. More research is needed to investigate the effects of varying oil contents on safflower cultivars subjected to accelerated aging. Inherent properties of the seed which protect it from damage following accelerated aging may also be functional in preserving seed vigor under natural aging conditions.

Seed lots 1 and 5 performed poorly under accelerated aging and electrical conductivity tests, both of which relate to membrane integrity (Priestly, 1986). Studies in other crops have shown that seed lots performing poorly in both these tests will not emerge well under cold, wet soil conditions (McDonald, 1975). As early field plantings are necessary in cold climates to insure maturation before killing frosts, these tests may be predictive of stress performance in certain environments.

Further research is needed to evaluate the influence of genotype on vigor test performance and to determine whether the model developed for greenhouse emergence is predictive of field performance under stressful conditions. The type of stress encountered may influence the best combination of tests included in the model. Continued vigor testing with field plantings conducted under a variety of environments would be most useful in developing the emergence prediction models.

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