Relationship between seed vigor testing and field performance of regar meadow bromegrass (bromus biebersteinii Roem and Schult) by Reginald Denny Hall, II

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:
The standard germination test continues to be the primary method for determining seed quality. However, germination results often overestimate actual field emergence. Seed quality in grasses has been shown to affect field emergence and subsequent stand establishment. Several physiological and biochemical tests have been developed for evaluating seed vigor in various crops, but the relationship between vigor testing and field performance has not been studied extensively in grasses.

This study evaluated several laboratory vigor tests to determine their relationship to field performance of 'Regar' meadow bromegrass. Standard germination and accelerated aging tests were used to evaluate four seed lots during preliminary studies in 1985. Field performance of these lots were evaluated by determining speed of emergence, total emergence, and forage yield. Total emergence and forage yield were significantly correlated with accelerated aging. The following year, standard germination, seedling growth rate, accelerated aging, electrical conductivity, respiration rate, and ATP content were used to evaluate ten seed lots planted in the field. Accelerated aging, respiration rate, and ATP content were significantly correlated with forage yield. Multiple stepwise regression selected accelerated aging and respiration rate as the best model for predicting first year forage yield.
RELATIONSHIP BETWEEN SEED VIGOR TESTING AND FIELD PERFORMANCE OF 'REGAR' MEADOW BROMEGRASS
(Bromus biebersteinii Roem and Schult)

by
Reginald Denny Hall, II

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy

MONTANA STATE UNIVERSITY
Bozeman, Montana
May 1987
APPROVAL

of a thesis submitted by

Reginald Denny Hall, II

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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The standard germination test continues to be the primary method for determining seed quality. However, germination results often overestimate actual field emergence. Seed quality in grasses has been shown to affect field emergence and subsequent stand establishment. Several physiological and biochemical tests have been developed for evaluating seed vigor in various crops, but the relationship between vigor testing and field performance has not been studied extensively in grasses.

This study evaluated several laboratory vigor tests to determine their relationship to field performance of 'Regar' meadow bromegrass. Standard germination and accelerated aging tests were used to evaluate four seed lots during preliminary studies in 1985. Field performance of these lots were evaluated by determining speed of emergence, total emergence, and forage yield. Total emergence and forage yield were significantly correlated with accelerated aging. The following year, standard germination, seedling growth rate, accelerated aging, electrical conductivity, respiration rate, and ATP content were used to evaluate ten seed lots planted in the field. Accelerated aging, respiration rate, and ATP content were significantly correlated with forage yield. Multiple stepwise regression selected accelerated aging and respiration rate as the best model for predicting first year forage yield.
CHAPTER I

INTRODUCTION

'Regar' meadow bromegrass (*Bromus biebersteinii* Roem and Schult), an improved cool season grass cultivar, was selected for its rapid regrowth characteristics. Regar is winterhardy, moderately drought tolerant, and its bunchgrass habit is compatible when planted with other perennial grasses and legumes. Regar is a popular cultivar for forage and seed production in the northern United States and Canada.

Stand establishment problems of small seeded forage species, including Regar meadow bromegrass, is often encountered. Adverse environmental conditions or improper cultural practices can result in erratic stand establishment. These factors impose stress on germinating seeds and growing seedlings, depleting their energy supply, and subsequently preventing their emergence. Therefore, it is important to plant high quality seed which possess the potential to germinate and produce rapid growing seedlings under a wide range of field conditions.

The standard germination test is the primary method for determining seed quality. However, germination results often overestimate actual field emergence. Germination tests are
performed under optimum conditions seldom encountered in the field.

The seed vigor concept was developed to provide a more accurate appraisal of seed quality as it relates to field emergence. Laboratory seed vigor tests utilize both physiological and biochemical methods to evaluate seed vigor in various crops.

Grass seed quality affects field emergence and subsequent stand establishment. However, the relationship of vigor tests with field performance has not been studied extensively in grasses. The objective of this study was to evaluate several laboratory vigor tests and determine their relationship to field performance of Regar meadow bromegrass.
CHAPTER II
LITERATURE REVIEW

Significance of Vigor Testing

The Association of Official Seed Analysts (AOSA), "Rules for Testing Seeds" (1981) defines germination in the laboratory as, "the emergence and development from the seed embryo of those essential structures which, for the kinds of seed in question, are indicative of the ability to produce a normal plant, under favorable conditions." Whenever field conditions, at planting, are near optimum, standard germination tests correlate well with field emergence (Abdalla and Roberts, 1969; Perry, 1977; Egli and Tekrony, 1979). However, actual field conditions are often far from optimum. Under suboptimum field conditions, standard germination tests differ significantly from field emergence for a given seed lot (Sherf, 1953; Delouche and Caldwell, 1960; Tekrony and Egli, 1977; Johnson and Wax, 1978; Yaklich and Kulik, 1979; Naylor, 1981).

The seed vigor concept was developed to provide a more accurate appraisal of seed quality in relationship to field emergence. The definition of seed vigor has been reviewed extensively by Heydecker (1972), Woodstock (1973), and McDonald (1975, 1980a). The International Seed Testing
Association (ISTA), defined seed vigor as the "sum of those properties which determine the potential level of activity and performance of the seed lot during germination and seedling emergence" (Perry, 1978). Seed vigor was defined by AOSA as "those properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (McDonald, 1980b).

Seed vigor testing has become an increasingly important component of seed analysis (McDonald, 1975). Successful vigor tests are reproducible, correlate with emergence under field conditions, rapid, objective, simple, and economically practical (AOSA, 1983). Vigor tests are designed to differentiate between seed lots of high and low performance under field conditions (Heydecker, 1972). Marshall and Naylor (1984) reported that improved grass emergence could be achieved by planting high vigor seed; that is, seed that establishes well under poor conditions.

**Seedling Growth Rate**

Seedling growth rate tests are conducted similarly to standard germination tests, but require specific moisture content of media. Seedling growth is measured in terms of dry weight or linear growth after a given period of growth.

Woodstock (1969a) reported that measuring linear growth
of corn (*Zea mays* L.) seedlings was a sensitive indicator of seed quality. Even under optimum conditions, seedling growth rate tests revealed vigor differences not detected by percentage germination (Woodstock and Grabe, 1967).

Perry (1977) evaluated plumule growth as a possible vigor test for barley (*Hordeum vulgare* L.). Field emergence and grain yield were more closely correlated with plumule length than the standard germination test when soil conditions were unfavorable. However, no significant correlations were found between shoot length and field emergence for soybeans (*Glycine max* L. Merr.) (Yaklich and Kulik, 1979).

Seedling dry weight has been suggested by the AOSA Vigor Testing Committee (Woodstock, 1976). Total dry weight of soybean seedlings, excluding cotyledons, were closely associated with vigor (Edje and Burris, 1970). Mock and McNeill (1979) evaluated cold tolerance responses in several inbred lines of corn. Seedling dry weight sampled 42 days after planting correlated significantly with grain yield.

**Accelerated Aging**

The accelerated aging test stresses seed prior to germination. Seeds are subjected to high temperature (40-45 C) and high humidity (nearly 100%) for varying times, depending on the kind of seed, after which a germination
test is performed. In theory, high vigor seed should tolerate the high temperature – high humidity conditions better, and retain its ability to germinate. This test was developed to predict relative storability of seed lots (Delouche, 1965; Delouche and Baskin, 1973). Later, AOSA suggested using the accelerated aging test to determine which seed will perform well under field conditions (Woodstock, 1976).

The first method involved placing seed in open plastic boxes and then inserting the boxes inside a large chamber where humidity and temperature were held constant. Baskin (1977) modified the procedure by placing seeds in wire baskets over a measured amount of water in a sealed jar, and placing the jars in a chamber. McDonald (1978) found that the original seed moisture of soybeans and barley influence the degree of deterioration during the accelerated aging test. Subsequently, McDonald and Phaneedranath (1978) further modified the accelerated aging test by placing seeds in a single layer on wire mesh trays over a measured amount of water inside plastic germination boxes. Tao (1979) reported that the addition of 40 ml water to the tray method results in minimum test variation. This method produced excellent repeatability in a national vigor referee test for soybeans (Tao, 1980). In 1984, the AOSA Vigor Subcommittee suggested that McDonald and Phaneedranath's
(1978) method be adopted as the recommended method for accelerated aging (Tekrony, 1985).

Accelerated aging has been used to predict field performance of bean (*Phaseolus vulgaris* L.) (Roos and Manalo, 1971), soybean (Byrd and Delouche, 1971; Tekrony and Egli, 1977; Kulik and Yaklich, 1982), and cotton (*Gossypium hirsutum* L.) (Bishnoi and Delouche, 1980). Controlled deterioration, a technique similar to accelerated aging, has been significantly correlated with field emergence of Italian ryegrass (*Lolium multiflorum* L.) (Marshall and Naylor, 1985).

**Electrical Conductivity**

The electrical conductivity test measures electrolyte leakage from deteriorating seeds. Ching and Schoolcraft (1968) reported that deteriorated seeds leach more cellular components than nondeteriorated seeds, presumably because of increased membrane permeability. Roberts (1979) associated the loss of seed viability with a loss of membrane integrity resulting in the enhanced efflux of cellular constituents in seed leachates.

Membrane integrity is important for many biochemical reactions that occur in living cells. Changes in membrane ultrastructure and permeability in aged wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) seed have been
detected by electron microscope studies (Anderson et al., 1970; Harman and Granett, 1972).

Abdul-Baki and Anderson (1970) reported that sugars leaching from barley seed appears to be regulated primarily by sugar utilization rate during germination, rather than changes in dry seed membrane permeability. Glucose utilization rate was faster in high quality seeds, and the glucose concentration in the leachate was lower.

Woodstock et al. (1985) suggested that mineral leaching in weathered cotton seeds was a better indicator of seed vigor than the total release of electrolytes. The release of $^+\text{K}$ and $^{++}\text{Ca}$ was significantly correlated with measurements of seed quality.

Electrical conductivity measurements were significantly correlated with field emergence in corn and soybean in an AOSA refereed vigor test (Tao, 1980a; Tao, 1980b). The results were also repeatable among seed laboratories.

The electrical conductivity test correlates well with vigor in seeds of pea (Matthews and Bradnock, 1967; Carver and Matthews, 1975), rice (Oryza sativa L.) (Agrawal, 1977), corn (Gill and Delouche, 1973; Tao, 1980a; Tao, 1980b), soybean (Abdul-Baki and Anderson, 1973; Yaklich et al., 1979; Tao, 1980a; Tao, 1980b), crimson clover (Trifolium incarnatum L. 'Dixie') (Ching and Schoolcraft, 1968) and sal (Shorea robusta Gaertn. f.) (Nautiyal and Purohit, 1985).
However, poor correlations between electrolytic leakage and seed vigor have been reported in sorghum (Sorghum bicolor L. Moench) (Perl and Gelmond, 1978), muskmelon (Cucumis melo L.) (Pesis and Ng, 1983), and Italian ryegrass (Marshall and Naylor, 1985).

Respiration

Seed germination and seedling growth require metabolic energy supplied by respiration. Vigorous seed germinates and grows more rapidly than nonvigor seed, requiring more energy through increased respiratory activity. McDonald (1975) reported that mitochondrial dysfunction decreases respiratory activity and results in little or no embryonic axis elongation.

Vigor evaluation by respiration tests are quantitative, rapid, easy to standardize and perform, well-suited for routinely testing many seed samples and, with suitable precautions, reliable (Woodstock, 1966). However, seed treatments can influence seed respiration. Pretreating corn and lima bean (Phaseolus lunatus L.) with chilling temperatures reduced respiration and subsequent seedling growth (Woodstock and Feeley, 1965; Woodstock and Pollock, 1965), respectively. Exposing corn seed to gamma-radiation treatments markedly inhibited seedling growth and respiration rate (Woodstock and Combs, 1965). Mechanically
injured seed was less vigorous and had increased rather than decreased respiration rates (Woodstock, 1969b). Respiration of pea embryo axes infected with *Aspergillus ruber* Thom and Church was lower than noninfected peas after imbibition (Harman and Drury, 1973).

Bewley and Black (1982) reported respiratory patterns exhibited by deteriorating seeds are variable, and sometimes precede, accompany, or lag behind germination. Halmer and Bewley (1984) suggested that the correlation between oxygen uptake and seedling vigor varies with time after imbibition at which respiration is measured, and the number of days after which seedling vigor is determined.

Respiration correlates well with vigor or field performance in seeds of wheat (Kittock and Law, 1968; DasGupta and Austenson, 1973), soybeans (Burris et al., 1969; Abdul-Baki and Anderson, 1973), peas (Carver and Matthews, 1975), corn (Woodstock and Grabe, 1967; Cantrell et al., 1972), cotton (Bishnoi and Delouchche, 1980; Woodstock et al., 1985), and Italian ryegrass (Marshall and Naylor, 1985).

However, some researchers have reported poor correlations between respiration and seed quality. Abdul-Baki (1969) observed that in deteriorating barley the decrease in oxygen uptake lagged behind changes in germination, shoot growth, and glucose utilization. Anderson
(1970) reported that in barley, oxygen uptake did not correlate with either percentage germination or age, although respiratory quotients were consistently higher in older seeds. Byrd and Delouche (1971) observed that in deteriorating soybean the decrease in oxygen uptake lagged behind germination and seedling growth rate; however, respiratory quotients increased as germination decreased. Bonner (1974) reported that germination rates did not correlate with oxygen uptake in deteriorating cherrybark oak (*Quercus falcata* var. *pagodaefolia*) acorn.

**Adenosine Triphosphate (ATP) Content**

Seed germination is an energy requiring process. The energy needed for biochemical reactions in living cells is stored in high energy compounds such as ATP. Extraction of ATP has been performed in boiling water (Ching, 1973), or by homogenizing seeds in chilled perchloric acid solution (Yaklich et al., 1979). Extracted ATP is then measured by a luminescence photometer (Ching, 1973), or a liquid scintillation counter (Tao et al., 1974) using a luciferin–luciferase system. This method is rapid, but requires specialized equipment and trained personnel to conduct the test (AOSA, 1983).

Adenosine triphosphate is produced rapidly following seed imbibition; however, the initial sources of ATP during
early imbibition is still unknown. Ching (1972) reported that the rapid ATP increase in seeds following imbibition comes not only from oxidative phosphorylation, but also from substrate level phosphorylation such as glycolysis, since glycolytic enzymes are active in dry or imbibed seed. Willson and Bonner (1971) found that peanut seed mitochondria were lacking in cytochrome c until approximately 16 hours after imbibition started. Respiratory activity before that time was "alternate" respiration, and not sensitive to cyanide. Moreland et al. (1974) observed ATP production in imbibed radish (Raphanus sativus L.) seed under anaerobic conditions and in the presence of glycolysis inhibitors. They reported decreases in adenosine monophosphate (AMP) concentrations rather than adenosine diphosphate (ADP) during the early stage of germination, suggesting an unknown system that phosphorylates AMP to ATP. Perl (1980), using onion (Allium cepa L.) seed powder, developed an in vitro system for ATP synthesis requiring AMP, phosphoenolpyruvate (PEP), and orthophosphate.

Mitochondria isolated from seedling axes of new and old soybean seed exhibited differential phosphorylative efficiency when identical cofactors and substrates were added (Abu-Shakra and Ching, 1967). The amount of inorganic phosphate esterified into ATP per volume of oxygen consumed in aged seeds was less than 50% that of unaged seeds. They
suggested that mitochondria in aged seeds may be partially uncoupled, and their inability to produce ATP efficiently contributes to reduced germination vigor.

Ribonucleic acid (RNA) and protein synthesis in axes excised from dry soybean seeds were assayed after being subjected to varying accelerated aging treatments (Anderson, 1977). Lower rates of RNA and protein synthesis in deteriorated seeds were associated with reduced ATP tissue content.

Adenosine triphosphate content in imbibed seeds was significantly correlated with seedling weight of lettuce (Lactuca sativa L.) (Ching and Danielson, 1972), ryegrass, crimson clover, and common rape (Brassica napus L.) (Ching, 1973). In soybean, ATP content was significantly correlated with field emergence in one trial, but not in another (Yaklich et al., 1979). Pea seeds infected with Aspergillus ruber Thom and Church exhibited decreased seed vigor and ATP content compared to uninfected peas (Tao et al., 1974). However, ATP content was not correlated with reduced germination or vigor in corn, cucumber (Cucumis sativus L.), onion, and radish seeds (Styer et al., 1980).
CHAPTER III

RELATIONSHIP BETWEEN SEED VIGOR TESTING AND FIELD PERFORMANCE OF 'REGAR' MEADOW BROMEGRASS (Bromus biebersteinii Roem and Schult)

Introduction

The standard germination test is the primary method for determining seed quality. When field conditions, at planting, are near optimum, standard germination tests correlate well with field emergence (Abdalla and Roberts, 1969; Perry, 1977; Egli and Tekrony, 1979). However, under suboptimum field conditions, standard germination tests differ significantly from field emergence for a given seed lot (Tekrony and Egli, 1977; Johnson and Wax, 1978; Yaklich and Kulik, 1979; Naylor, 1981).

Seed quality in grasses affects field emergence and subsequent stand establishment (Marshall and Naylor, 1984). Several physiological and biochemical tests have been developed for evaluating seed vigor in various crops. However, these relationships have not been studied extensively in grasses. Controlled deterioration and respiration tests have both been significantly correlated with field emergence of Italian ryegrass (Lolium multiflorum L.) (Marshall and Naylor, 1985). Electrical conductivity correlates well with germination of crimson clover
Trifolium incarnatum L. 'Dixie'), but not perennial ryegrass (Lolium perenne L.) (Ching and Schoolcraft, 1968). Poor correlations were also observed between electrolytic leakage and field emergence of Italian ryegrass (Marshall and Naylor, 1985). Ching, (1973) observed significant correlations between ATP content in imbibed ryegrass seed and seed weight or seedling size and weight.

Burris et al. (1969) questioned whether one test can measure both viability and vigor. Subsequently, various combinations of physiological and biochemical tests have been utilized to predict field performance in several crops (Tekrony and Egli, 1977; Ram, 1983).

The objective of this research was to evaluate several laboratory vigor tests to determine their relationship to field performance of Regar meadow bromegrass.

**Materials and Methods**

Seed lots evaluated were obtained from certified seed samples of Regar meadow bromegrass submitted to the Montana State University Seed Testing Laboratory. Selections were based on varying levels of percentage germination. Four seed lots were evaluated during preliminary studies in 1984-85, and one lot (854) was subjected to accelerated aging to lower quality prior to field planting. Ten seed lots were evaluated during 1985-86. Two of these lots (868 and 8610)
exhibited low viability due to over-heating after harvest, and one lot (869) had been mechanically injured during harvesting and/or cleaning. None of the seed lots evaluated in 1985 were used in the 1986 studies. All seed lots were stored at room temperature (22°C) in the laboratory during the course of these experiments.

**Laboratory Studies**

**Standard Germination Test**

Four groups of 100 seeds from each seed lot were placed in 13 x 13.5 cm plastic germination boxes containing two moistened blotter papers. Seeds were germinated for 14 days at alternating temperatures of 15°C for sixteen hours and 25°C for eight hours (15-25°C). Normal seedlings were evaluated according to "Rules for Testing Seed" (Association of Official Seed Analysts [AOSA], 1981) and expressed as percentage germination.

**Seedling Growth Rate**

Seedling growth rate test was conducted as suggested by AOSA (1983) with minor procedural modification. The basic procedure was a rolled-towel germination test with four replications. Each roll consisted of two heavy weight (#76) paper towels, 25.4 x 38.1 cm, one below the seed and one above the seed. Towels were moistened with 30 ml water per
towel. Fifty seeds per towel were arranged as described by AOSA. The two paper towels were loosely rolled within 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 15-25°C germinator for 14 days. Normal seedlings were cut free from their caryopses and placed in 8 x 9 cm coin envelopes for drying. Seedlings were dried at 105°C for 24 hours, then weighed to the nearest mg. Total dry weight was divided by the number of normal seedlings to determine seedling growth rate (mg/seedling).

**Accelerated Aging**

Preliminary accelerated aging tests during 1984-85 were conducted as recommended by McDonald and Phaneedranath (1978) with minor procedural modifications. Two-hundred seeds were evenly distributed on wire mesh trays inside covered plastic boxes containing 30 ml of water and boxes were placed in a Stults Scientific accelerated aging chamber for eight days. Temperature and humidity were maintained at 41°C and 100% relative humidity, respectively. Following the aging period, seed was removed and standard germination tests conducted using four replications of 50 seeds per seed lot. Heavy infestations of storage fungi on some poor quality seed lots made repeatability difficult.

During 1985-86 studies, the accelerated aging test was
conducted as recommended by AOSA (Tekrony, 1985) with minor procedural modifications. Four grams of seed per lot were surface sterilized in 30 ml of 1.5% sodium hypochlorite (NaOCl) for 15 minutes, rinsed six times with sterile water, oven-dried at 32°C for 24 hours, and placed in a desiccator 24 hours prior to conducting accelerated aging tests. Seed was uniformly distributed on wire mesh trays inside covered plastic boxes containing 40 ml of water. Plastic boxes were placed inside a Stults Scientific accelerated aging chamber for four days. Temperature and humidity were maintained at 41°C and 100% relative humidity, respectively. Following the aging period, seed samples were removed and standard germination tests conducted, using four replications of 50 seeds per seed lot. An additional 50 seeds per seed lot were removed, weighed immediately to the nearest mg, oven-dried at 105°C for 24 hours, and re-weighed to determine seed moisture content (wet-weight basis).

Electrical Conductivity

The electrical conductivity test was conducted as recommended by AOSA (1981) with minor procedural modifications. Four replicates of 400 seeds from each seed lot were weighed and placed in 50 ml test tubes containing 40 ml deionized water. Tests tubes were incubated at 20°C for 24 hours, after which time the contents of each test tube
was gently stirred, and electrical conductivity measured immediately. Electrical conductivity was measured with a Markson 4503 conductivity meter and as umhos per gram seed weight (umhos g\(^{-1}\)).

**Respiration**

Oxygen uptake was measured with a Gilson Differential Respirometer. Four replicates of 25 seeds from each seed lot were imbibed in a reaction flask containing 2 ml of distilled water for 24 hours, after which time 0.2 ml of 10% KOH was added to the center well of each reaction flask. The reaction flasks were placed on the respirometer and submerged in a 25°C water bath and were shaken 78 oscillations min\(^{-1}\). The system was equilibrated for 30 minutes and readings were taken every hour for four hours. Respiration rate was reported as microliters of oxygen absorbed per seed per hour (uL O\(_2\) seed hr\(^{-1}\)).

**ATP Content**

Four replications of 0.5 g of seed from each seed lot were weighed and counted. Each replication was placed between two layers of Whatman No. 1 filter paper (9.0 cm) which was moistened with 2.5 ml distilled water in a plastic germination box for four hours at 20°C. Imbibed seeds were ground in a Wiley laboratory grinder (20 mm mesh) and a 0.3
g sample removed and placed in a test tube for extraction. Nine ml of dimethyl sulfoxide (99%) was added to the ground sample and mixed with a Vortex laboratory mixer for one minute, after which time the particulate matter was allowed to settle for two minutes. A 50 microliter aliquot was transferred from each test tube into small plastic ependorf tubes, and immediately diluted with 200 microliters of Hepes buffer (pH 7.5) and placed on ice. Extracted samples were quickly transported for assaying. Four replications of 150 microliters of the diluted seed extract were delivered into small plastic test tubes (8 x 50 cm), and tubes placed in a Turner TD-20e Luminometer. One-hundred microliters of luciferin-luciferase enzyme preparation was then injected into each test tube and the luminescence reading recorded for each sample. Sample readings were compared to an ATP standard curve and ATP content of each sample calculated as $10^{-12}$ picograms of ATP per seed.

Dimethyl sulfoxide (DMSO) used for ATP extraction was purchased from Sigma Chemical. ATP standard, Hepes buffer, and the luciferin-luciferase preparation was purchased from Turner Designs.

**Field Studies**

All seed lots evaluated in the laboratory were planted at the Arthur H. Post field research laboratory near
Bozeman, Montana. The soil is classified as Amsterdam variant of silt loam (fine-silty, mixed family of Typic Haploborolls).

Four seed lots were planted in 1985 and 10 seed lots in 1986. All field experiments were conducted as a randomized complete block with four replications. Plot size was 6.09 m x 1.83 m and each plot contained six rows spaced 0.305 m apart. Seeding rate was 15 pure live seed per 0.305 m of row, and seed was planted 12.7 mm deep.

After planting and before emergence of seedlings, two 0.5 m of row were marked in the two center rows of each plot. Emergence rate and total emergence counts were obtained from these areas. Speed of emergence index was calculated similarly to speed of germination index as described by Maquire (1962). Emergence rate index was calculated as follows:

\[
x = \frac{\text{number of seedlings emerged}}{\text{increase of seedlings emerged from previous count}} + \ldots + \frac{\text{days to first count}}{\text{days to final count}}
\]

Forage yield was determined by harvesting 6.09 m of the two center rows using a Rem flail harvester. Random moisture samples were taken from each plot; samples were oven-dried, and percentage moisture determined on a wet-weight basis.
Forage yield was corrected for moisture content and reported as kg ha\(^{-1}\) (dry-weight basis).

**Statistical Analysis**

All variables were subjected to analyses of variance. Means were separated using Neuman - Kuels. Simple correlations were computed from the mean values using "MSUSTAT" (Lund, 1983). A multiple stepwise regression model building procedure was used to determine which 1986 seed vigor tests best predict first year forage yield (SAS Institute Inc., 1985).

**Results and Discussion**

Significant differences were found among seed lots for total seedlings emerged and forage yield in 1985 preliminary studies (Table 1). Seed lot 852 had the highest total seedling emergence and first year forage yield and was significantly higher than seed lot 854 for both traits. Speed of emergence ratings did not differ significantly, although seed lots which had high forage yields also had high speed of emergence values.

Similar field results were obtained in 1986 when ten seed lots were evaluated (Table 2). Significant differences were found among seed lots for total seedling emergence and first year forage yield. Seed lot 863 had the highest forage
yield and was significantly different from seven other seed lots. However, seed lot 864 had the highest total seedling emergence and was significantly different than seed lots 869 and 868. Speed of emergence ratings did not differ significantly.

High variability among seed lots for speed of emergence was indicated by high coefficient of variation percentages of 69% (Table 1) and 37% (Table 2) respectively for 1985 and 1986 studies. Small sample size (1 meter of row) and uneven seeding depth and placement may have contributed to high coefficient of variation for speed of emergence.

Table 1. Mean comparisons among standard germination, accelerated aging, and field performance variables for four seed lots of Regar meadow bromegrass - Bozeman, MT. 1985

<table>
<thead>
<tr>
<th>Seed Lot</th>
<th>Speed of emerg.</th>
<th>Total emerg.</th>
<th>Forage yield</th>
<th>Std. germ.</th>
<th>Accel. aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>852</td>
<td>8.24</td>
<td>39b</td>
<td>1669b</td>
<td>88c</td>
<td>33c</td>
</tr>
<tr>
<td>851</td>
<td>5.77</td>
<td>36b</td>
<td>1534ab</td>
<td>94d</td>
<td>38c</td>
</tr>
<tr>
<td>853</td>
<td>3.63</td>
<td>27ab</td>
<td>997a</td>
<td>84b</td>
<td>20b</td>
</tr>
<tr>
<td>854</td>
<td>2.24</td>
<td>17a</td>
<td>918a</td>
<td>60a</td>
<td>0a</td>
</tr>
</tbody>
</table>

2/ Coefficient of variability
Laboratory tests conducted during 1985 were standard germination and accelerated aging. Significant differences were found among seed lots for both of these tests (Table 1). Seed lots 851 and 852, which had the highest first year forage yield and total seedling emergence, also had the highest standard germination percentage and accelerated aging values.

Laboratory tests conducted in 1986 were standard germination, seedling growth rate, accelerated aging, electrical conductivity, respiration, and ATP content. Significant differences were found among seed lots for all laboratory tests (Table 2); however, some laboratory tests were better predictors of field performance than others.

Standard germination percentages for seed lots evaluated during both years (Tables 1 and 2) ranged from commercially acceptable to unacceptable (less than 80% standard germination). Each seed lot was planted with the same number of live seed per meter of row, thus seed viability should not have influenced field performance.

Simple correlations were calculated among laboratory tests and field performance in 1985. Accelerated aging was significantly correlated with total seedling emergence ($r = 0.97^{**}$) and first year forage yield ($r = 0.88^*$), but not with speed of emergence (Table 3). Standard germination was correlated with total seedling emergence ($r = 0.91^*$), but
Table 2. Mean comparisons among standard germination, vigor tests, and field performance variables for ten seed lots of Regar meadow bromegrass – Bozeman, MT, 1986.

<table>
<thead>
<tr>
<th>Seed Lot</th>
<th>Speed of emerg. index</th>
<th>Total emerg. plants/m</th>
<th>Forage yield kg/ha</th>
<th>Std. germ. %</th>
<th>SGR2/ mg/plant</th>
<th>ECT3/ μhos/g</th>
<th>Resp. %</th>
<th>Resp. 4/ μLO₂/seed/hr.</th>
<th>ATP picog./seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>863</td>
<td>21.61</td>
<td>35ab</td>
<td>5582d</td>
<td>94ed</td>
<td>2.16cd</td>
<td>84.47a</td>
<td>76f</td>
<td>.95d</td>
<td>7.11b</td>
</tr>
<tr>
<td>867</td>
<td>22.22</td>
<td>33ab</td>
<td>5243dc</td>
<td>86d</td>
<td>1.96abc</td>
<td>284.09d</td>
<td>43cb</td>
<td>.79cbd</td>
<td>4.44a</td>
</tr>
<tr>
<td>866</td>
<td>34.31</td>
<td>47ab</td>
<td>4986dc</td>
<td>88ed</td>
<td>2.22d</td>
<td>242.35cb</td>
<td>69fe</td>
<td>.90cd</td>
<td>4.77a</td>
</tr>
<tr>
<td>868</td>
<td>20.31</td>
<td>30a</td>
<td>4538bc</td>
<td>75c</td>
<td>2.12bcd</td>
<td>228.19b</td>
<td>51cd</td>
<td>.67cbd</td>
<td>3.34a</td>
</tr>
<tr>
<td>864</td>
<td>38.42</td>
<td>55b</td>
<td>4207ab</td>
<td>92ed</td>
<td>1.90ab</td>
<td>322.16e</td>
<td>64fe</td>
<td>.55b</td>
<td>2.97a</td>
</tr>
<tr>
<td>865</td>
<td>30.31</td>
<td>38ab</td>
<td>4149ab</td>
<td>90ed</td>
<td>1.85a</td>
<td>271.33cd</td>
<td>35b</td>
<td>.52b</td>
<td>3.90a</td>
</tr>
<tr>
<td>861</td>
<td>19.40</td>
<td>32ab</td>
<td>4090ab</td>
<td>96e</td>
<td>2.20d</td>
<td>265.72cbd</td>
<td>33b</td>
<td>.64cb</td>
<td>4.01a</td>
</tr>
<tr>
<td>862</td>
<td>22.50</td>
<td>35ab</td>
<td>3934ab</td>
<td>94ed</td>
<td>1.96abc</td>
<td>249.09cbd</td>
<td>58ed</td>
<td>.97cd</td>
<td>4.45a</td>
</tr>
<tr>
<td>869</td>
<td>21.33</td>
<td>28a</td>
<td>3837ab</td>
<td>60b</td>
<td>1.94abc</td>
<td>343.61e</td>
<td>15a</td>
<td>.79cbd</td>
<td>5.08a</td>
</tr>
<tr>
<td>8610</td>
<td>19.11</td>
<td>34ab</td>
<td>3350a</td>
<td>49a</td>
<td>1.83a</td>
<td>252.65cbd</td>
<td>13a</td>
<td>.26a</td>
<td>3.49a</td>
</tr>
</tbody>
</table>

C.V.%\(^5/\) 37 27 10 5 5 7 16 19 21

1/ Means followed by the same letter are not significantly different (P = 0.05) according to Neuman-Keuls mean separation test
2/ Seedling growth rate
3/ Electrical conductivity test
4/ Respiration rate
5/ Coefficient of variability
not with speed of emergence \((r = 0.74)\) and first year forage yield \((r = 0.76)\). The high \(r\) values are not significant due to few degrees of freedom.

Accelerated aging, respiration rate, and ATP content were significantly correlated with first year forage yield in 1986 (Table 4). None of the laboratory tests were correlated with speed of emergence and total emergence. The inconsistent relationship among speed of emergence or total seedling emergence with laboratory tests during both years (Tables 3 and 4) suggest that this association may be effected by the presence or lack of stress conditions in the field. First year forage yield was the only parameter which consistently reflected vigor differences among seed lots during both years.

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Std. germ.</th>
<th>Accel. aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of emerg. ((\text{index}))</td>
<td>0.74</td>
<td>0.83</td>
</tr>
<tr>
<td>Total emerg. ((\text{plants/m}))</td>
<td>0.91*</td>
<td>0.97**</td>
</tr>
<tr>
<td>Forage yield ((\text{kg/ha}))</td>
<td>0.76</td>
<td>0.88*</td>
</tr>
</tbody>
</table>

* \(r(0.05) = 0.88\)
** \(r(0.01) = 0.96\)
Standard germination percentage did not correlate well with first year forage yield (Table 4) during 1986 studies \((r = 0.53)\), which agrees with the 1985 data (Table 3). Seed lots 861 and 862 had standard germination percentages of 96% and 94% (Table 2), respectively. However, seed lot 863 with 94% germination, had a significantly higher first year forage yield than seed lots 861 and 862 (Table 2). This supports previous research by Naylor (1981) and Marshall and Naylor (1985) who found that Italian ryegrass seed lots with

Table 4. Simple correlations among standard germination, vigor tests and field performance variables for 10 seed lots of Regar meadow bromegrass – Bozeman, MT. 1986

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Std. germ.</th>
<th>1/ SGR</th>
<th>2/ ECT</th>
<th>3/ AA</th>
<th>4/ Resp</th>
<th>5/ ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed of emerg. (index)</td>
<td>0.38</td>
<td>-0.09</td>
<td>0.26</td>
<td>0.47</td>
<td>0.008</td>
<td>-0.25</td>
</tr>
<tr>
<td>Total emerg. (plants/m)</td>
<td>0.37</td>
<td>-0.04</td>
<td>0.14</td>
<td>0.54</td>
<td>-0.06</td>
<td>-0.25</td>
</tr>
<tr>
<td>Forage yield (kg/ha)</td>
<td>0.53</td>
<td>0.58</td>
<td>-0.58</td>
<td>0.71*</td>
<td>0.69*</td>
<td>0.60*</td>
</tr>
</tbody>
</table>

* \(r(.05) = 0.60\)
1/ Seedling growth rate
2/ Electrical conductivity test
3/ Accelerated aging
4/ Respiration
5/ Adenosine triphosphate
similar and acceptable standard germination percentages may not perform similarly in the field. Seed lots 869 and 8610 had standard germination percentages of 60% and 49% (Table 2), respectively. Even though these seed lots had the same number of live seed per meter of row as seed lot 863, their first year forage yields were significantly lower (Table 2). These data suggest that differences in seed vigor affects first year forage yield and that compensating for seed viability does not improve field performance of low viability seed lots.

Neither seedling growth rate nor electrical conductivity were correlated with first year forage yield in 1986 (Table 4). Seedling growth rate was different among seed lots (Table 2), but these differences were not related (Table 4) to standard germination or first year forage yield. Seed lot 863 and 869 had significantly different electrical conductivity readings, while the remainder of the seed lots did not differ greatly (Table 2). Seed lot 869 was mechanically damaged, which resulted in the palea and lemma being stripped from the caryopses. This damage may have contributed to higher solute leakage. Marshall and Naylor (1985) found a poor relationship between the conductivity test and field performance in Italian ryegrass. They suggested that leaching of electrolytes from the lemma and palea may mask differences among seed lots, or the lemma and
palea might slow water passage into caryopses and electrolytes out.

First year forage yield was significantly correlated with accelerated aging ($r = 0.71^*$) and respiration rate ($r = 0.69^*$) (Table 4). Seed lot 863 had the highest accelerated aging value (Table 2) and was significantly higher than seed lots 861 and 862. These two seed lots had standard germination percentages similar to seed lot 863. Seed lots 869 and 8610 performed poorly in the field and had the lowest accelerated aging values (Table 2). Both of these seed lots contained high amounts of storage fungi, which could not be controlled with surface sterilization. Consequently, seed from these lots deteriorated rapidly when exposed to accelerated aging conditions. Seed lot 863 had the highest respiration rate among seed lots and was significantly higher than seed lots 861, 864, 865, and 8610 (Table 2). However, seed lot 869, which had been mechanically injured, exhibited respiration rates statistically equal to seed lot 863 (Table 2). Woodstock (1969b) reported that mechanically injured seed was less vigorous, but may have increased rather than decreased respiration rates.

There was a significant correlation between ATP content of imbibed seeds and first year forage yield ($r = 0.60^*$) (Table 4). Seed lot 863 had the highest ATP content per seed
and was significantly different than all other seed lots (Table 2). Seed lot 869, which had one of the highest respiration rates, also had a high ATP value (Table 2). High respiration rates may contribute to the high ATP production of seed lot 869, which is supported by the significant correlation between respiration rate and ATP \((r = 0.71^*)\) (Appendix–Table 7). Ching (1973) observed significant correlations between ATP content of imbibed ryegrass seed and seedling size and weight. Even though present techniques for measuring ATP content in seeds may be impractical as a seed vigor test, its requirement for seed germination and early seedling growth makes it an important biochemical tool for evaluating seed vigor and providing an explanation for the phenomenon of seed vigor.

A multiple stepwise regression model building procedure was used to determine which 1986 seed vigor tests were associated with first year forage yield. Accelerated aging was selected first and was most closely related to first year forage yield \((R = 0.71^*)\) (Table 5). Accelerated aging also had the highest correlation with first year forage yield during 1985 preliminary studies (Table 4). Respiration rate strengthened the above relationship and was the only other variable added to the model \((R = 0.78^{**})\) (Table 5).

These data suggest that one vigor test is not enough to measure overall seed quality. A combination of tests, which
measure both physiological and biochemical aspects of seed vigor should be utilized. For Regar meadow bromegrass, first

Table 5. Summary of multiple stepwise regression comparing first year forage yield with vigor tests for 10 seed lots of Regar meadow bromegrass—Bozeman, MT. 1986

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>R</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Accelerated aging (A.A.)</td>
<td>0.51</td>
<td>( \hat{y} = 3355 + 22(A.A.) )</td>
</tr>
<tr>
<td>2</td>
<td>Respiration (Resp.)</td>
<td>0.61</td>
<td>( \hat{y} = 2794 + 15(A.A.) + 1332(Resp.) )</td>
</tr>
</tbody>
</table>

year forage yield was closely related to accelerated aging and respiration rate. Adenosine triphosphate content of imbibed seeds may be a useful biochemical index of seed vigor.
LITERATURE CITED
LITERATURE CITED


APPENDIX

Table 6. Simple correlations among standard germination, accelerated aging and field performance variables for four seed lots of Regar meadow bromegrass - Bozeman, MT. 1985

<table>
<thead>
<tr>
<th>Field parameters</th>
<th>Laboratory parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of emerg.</td>
<td>Total emerg.</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1 1.00</td>
<td>2 0.94*</td>
</tr>
<tr>
<td>2 0.94*</td>
<td>1.00</td>
</tr>
<tr>
<td>3 0.96**</td>
<td>0.94*</td>
</tr>
<tr>
<td>4 0.73</td>
<td>0.91*</td>
</tr>
<tr>
<td>5 0.83</td>
<td>0.96**</td>
</tr>
</tbody>
</table>

* $r(0.05) = 0.88$
** $r(0.01) = 0.96$
Table 7. Simple correlations among standard germination, vigor tests, and field performance variables for ten seed lots of Regar meadow bromegrass - Bozeman, MT, 1986.

<table>
<thead>
<tr>
<th>Field parameters</th>
<th>Laboratory parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of emerg.</td>
<td>Std. germ.</td>
</tr>
<tr>
<td>Total emerg.</td>
<td>1</td>
</tr>
<tr>
<td>Forage yield</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* \( r(0.05) = 0.60 \)
** \( r(0.01) = 0.73 \)
1/ seedling growth rate
2/ electrical conductivity test
3/ respiration rate