



Physical and physiological attributes affected by size and density separations in thickspike wheatgrass, *Elymus lanceolatus*
by Harold Ray Armstrong

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

Thickspike wheatgrass is a native cool season rhizomatous grass adapted to large areas of the Northern Great Plains. High seed costs and stand establishment problems may preclude its use to reclaim dryland range. High quality, vigorous seeds will increase ease of establishment. Seed conditioners can make seed separations based on size or density, which may impact seedling vigor and year of establishment forage yields. Three seed lots were separated into thirteen seed size-density separations for evaluation of 100 seed weight, total germination and speed of germination. A greenhouse study of the size-density separations was evaluated to determine total seedling emergence, speed of emergence, seedling mortality, 45 day dry seedling forage weight, 45 day dry seedling root weight, 45 day total dry seedling weight and 30 day dry seedling forage regrowth. A field study was evaluated for total seedling emergence, speed of emergence, forage production and seed head production during the year of establishment. Rate of imbibition and respiration rates were determined for whole seeds, naked caryopsis, endosperm and embryos of large heavy and small light seed separations. Seed vigor was increased with maximum gain for minimal expenditure by density separation. Light seed and bulk clean seed showed no difference in vigor for most traits measured. Large seed separations increased seed vigor although not to the degree found in heavy density separations. Combinations of heavy large or large heavy seed separations were superior in most traits measured although it is doubtful the extra expenditure of dual separation would be justified by minimal gains over density separation alone. Density separations may increase stand establishment success especially during period of environmental stress. Light seed can be planted under favorable environmental conditions and results will be similar to planting nonseparated seed.

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AFFECTED BY SIZE AND DENSITY SEPARATIONS
IN THICKSPIKE WHEATGRASS, Elymus lanceolatus

by

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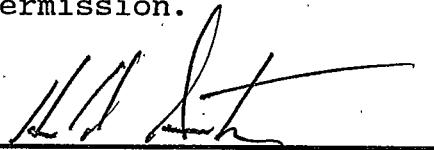
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ABSTRACT

Thickspike wheatgrass is a native cool season rhizomatous grass adapted to large areas of the Northern Great Plains. High seed costs and stand establishment problems may preclude its use to reclaim dryland range. High quality, vigorous seeds will increase ease of establishment. Seed conditioners can make seed separations based on size or density, which may impact seedling vigor and year of establishment forage yields. Three seed lots were separated into thirteen seed size-density separations for evaluation of 100 seed weight, total germination and speed of germination. A greenhouse study of the size-density separations was evaluated to determine total seedling emergence, speed of emergence, seedling mortality, 45 day dry seedling forage weight, 45 day dry seedling root weight, 45 day total dry seedling weight and 30 day dry seedling forage regrowth. A field study was evaluated for total seedling emergence, speed of emergence, forage production and seed head production during the year of establishment. Rate of imbibition and respiration rates were determined for whole seeds, naked caryopsis, endosperm and embryos of large heavy and small light seed separations. Seed vigor was increased with maximum gain for minimal expenditure by density separation. Light seed and bulk clean seed showed no difference in vigor for most traits measured. Large seed separations increased seed vigor although not to the degree found in heavy density separations. Combinations of heavy large or large heavy seed separations were superior in most traits measured although it is doubtful the extra expenditure of dual separation would be justified by minimal gains over density separation alone. Density separations may increase stand establishment success especially during period of environmental stress. Light seed can be planted under favorable environmental conditions and results will be similar to planting nonseparated seed.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Thickspike wheatgrass, Elymus lanceolatus (Scribn. and Smith) Gould (Dewey, 1983) or more commonly Agropyron dasystachyum (Hook.) Scribn., is a cool season, drought tolerant, perennial grass native to the northern great plains. Physical appearance is similar to western wheatgrass, Agropyron smithii (Rybd.) Love, although the leaves are finer, light green in color and it has smaller glumes. It is a rhizomatous sod former.

Thickspike wheatgrass is widely distributed and is adapted to a range of soils from sand to clay. It grows well in a 15-50 cm precipitation zone and at altitudes up to 3500 meters. It is more drought tolerant than western wheatgrass. Dryland forage yields range from 250 kg · ha⁻¹ to 1600 kg · ha⁻¹ in pure stands. Seed yields at Bridger, Montana have averaged 300 to 350 kg · ha⁻¹ under irrigation (Anonymous, 1977).

Thickspike wheatgrass is commonly used to revegetate roadsides, mechanically disturbed sites, gas or oil drill sites and mine spoils. The variety 'Critana' was developed

by the Soil Conservation Service Plant Materials Center at Bridger, Montana from collections made in the Havre, Montana area.

The recommended seeding rate is $4.5 \text{ kg} \cdot \text{ha}^{-1}$ for forage production or 80 to 100 pure live seeds (PLS) per meter of row with a 60 cm row spacing for dryland seed production. Seedlings are small and compete well with weeds. Stand establishment can be adversely affected by poor seedbed preparation or drought.

Failure to obtain a field stand can be a very expensive investment at current wholesale seed prices of \$7.70 to \$13.20 per kilogram.

In order to increase the likelihood of obtaining a stand this study was developed to determine: 1). if seed separations result in increased stand and seedling vigor; 2). whether seed sizing, seed density separation or a combination of techniques was more effective in increasing seed germination, seedling establishment and seedling vigor; and 3). the mechanism or reason for increased seed vigor due to seed separations.

Seed Size and its Contribution to Seed Vigor

Grass stand establishment under dryland conditions can be risky due to variable moisture supply. Hassanyar, et al. (1979) found that rapid emergence and establishment is dependent on rapid root growth to best utilize available soil moisture. Rapid root growth can be attributed to seed

or seedling vigor, which McDaniel (1973) defines as "superior performance of a genotype after planting, compared to the same genotype or other genotype, under defined experimental conditions".

Plant scientists in the mid 1800's attempted to increase seed vigor by selecting for large seed. Hicks and Dabney (1896) espoused the use of large and heavy seed to increase: leaf numbers, plant height, early maturity, crop yield, seed weight, and seedling vigor. Heavier test weights and greater yield in oats, Avena sativa L., barley, Hordeum vulgare L., and spring wheat, Triticum aestivum L., was obtained by selecting for large seed (Zavitz, 1908). Zavitz (1908) increased yield of rape, Brassica napus L., potatoes, Solanum tuberosum L., and a variety of rootcrops by selecting for large seed. Cummings, in 1914, found that peas, Pisum sativum L., lettuce, Lactuca sativa L., spinach, Spinacia oleracea L., parsley, Petroselinum crispum (Mill.) Nyman ex A. W. Hill, pumpkin, Cucurbita maxima Duchesne, radish, Raphanus sativa L., and bean, Phaseolus vulgaris L., yields were improved by selecting for large seeds. Findley (1919) found that large seed generally produced more vigorous, higher yielding plants. Larger seed of crimson clover, Trifolium incarnatum L., exhibited increased seedling vigor (Moore, 1943). Rogler (1954) published a landmark paper on the relationship of seed size to increased seedling vigor in native and

introduced grasses. Scientists are still trying to define the mechanism of apparent increased seedling vigor (Wichman, 1983; Carlson, 1982).

Seed size separations were first made by hand sorting and visual classification, (Kiesselbach, 1924). Sieving (Beveridge and Wilsie, 1959) and using slotted screens (Carver, 1977) afforded more precise and repeatable dimensional grading of seed classes. Knipe (1970) removed the lemma and palea prior to screening to reduce variability due to external seed covering.

Several groups have made size or weight separations based upon varietal differences (Findley, 1919; Hunt, 1954; Christians et al., 1979). Variation from 4413 seeds per gram to 1847 seeds per gram were observed among Kentucky bluegrass, Poa pratensis L., cultivars (Christians et al., 1979). Hunt (1954) based differences in seed size among cultivars of ryegrass, Lolium spp. L., timothy, Phleum pratense L., tall fescue, Festuca arundinacea Schreb., meadow fescue, Festuca pratensis Huds., and orchardgrass, Dactylis glomerata L., on 1000 seed weight basis. Padilla et al. (1978) found that cultivars of western wheatgrass, varied in 100 seed weight from 377 to 951 mg.

Early work with density separations was conducted by Clark (1904) using salt solution density gradients for seed separation in several crops: eggplant, Solanum Melongana L.; peppers, Piper nigrum L.; grapes, Vitus spp. L.;

timothy; clover, Trifolium spp. L.; peas, Pisum sativum L.; cauliflower, Brassica oleracea Botrys group L.; and cabbage, Brassica oleracea Capitata group L. An urea phosphate slurry diluted with water was used by Maranville and Clegg (1977) for density separation in grain sorghum, Sorghum bicolor (L.) Moench. Weight separation was made by pouring safflower, Carthamus tinctorius L., seed in front of a fan and splitting the lot on the basis of distance blown (Wichman, 1983). Kittock and Patterson (1962) utilized a screening operation followed by aspiration to obtain differences in size and weight. Commercial separation into density classes can be obtained by aspiration or by using a gravity table.

Size is an ambiguous term which does not adequately define the physical size and weight of the seed. The term may indicate differences in length or width which may not necessarily result in differences in mass. One may use solutions varying in density, aspiration or gravity tables to obtain differences in mass per seed. Seed weight within a lot can vary considerably. Differences may occur between cultivars (Christians et al., 1979), among plants within the same cultivar over years (Lawrence, 1963), or within a single spike (Hatcher, 1940; McAllister, 1943).

Dimensional sizing using slotted screens results in a homogeneous appearance. The mass per seed within a screened lot of peas or beans is consistent, but that is

not true for grasses since the lemma and palea are loosely adhering (Kneebone and Cremer, 1955). This factor makes it difficult to obtain repeatable homogeneous lot separations of grasses.

The ratio of embryo length to width may vary considerably during seed development. The resultant seed may reflect resultant size due to differences in seed development. The embryo rapidly differentiates to a critical mass. As the caryopsis continues to fill, the embryo expands concurrently with the endosperm (Bewley and Black, 1978). Since the embryo stores the enzymes necessary for germination, its size may be very critical. The problem is how to select seed with the largest embryos. Larger embryo's store more of the hydrolytic enzymes that mediate germination than smaller embryos. Size separations in grasses are based on similar length lemmas and paleas which may conceal a small caryopsis. Density separations may merely segregate dense endosperm.

Embryo size may be modified by mode of pollination, environmental factors, interplant competition, floret position on the flowering shoot and genetic makeup (Hatcher, 1940; Wood et.al., 1977). Brown (1976) found that embryo size increased with seed weight in 'Westerwolds' ryegrass, Lolium multiflorum ssp. gaudinii Lam. Hatcher (1940) found a high correlation between seed size and embryo size in tomatoes, Lycopersicon lycopersium

(L.) Karst. ex Farw., but little advantage for large embryos. Bremer et al. (1963) stated that if the same amount of reserve material was available to what were originally large and small embryos, there would be no difference in their growth rates or in the size of plants produced. Thus, the dominant factor in the seed size (plant size⁻¹) relationship in wheat is the extent of the energy source available to the developing seed. This was substantiated by Paluska et al. (1979) finding that twice as much endosperm was exhausted in large barley seeds.

Cultivars characterized by different seed sizes have been compared against each other in a number of species (Davies, 1960; Murphy and Arny, 1939). Ahring and Todd (1978) found that seed size was inherent to a particular cultivar and among cultivars in Bermudagrass, Cynodon dactylon (L.) Pers. A lack of interaction between size and cultivars was found in barley (Baniaameur and Caddel, 1976) and spring wheat (Lafond and Baker, 1986). Carlson (1982) however found an interaction between seed size and two versus six row barley. Findley (1919) cautioned against comparisons of cultivars based on seed size as a small seeded cultivar may exhibit more inherent vigor than a large seeded cultivar.

Brown (1977) estimated that seed weight heritability was approximately 10 % in ryegrass. Righter (1945) suggests that the portion of size variation in pine seeds,

Pinus L., due to heredity leads to inherently greater seed vigor whereas that portion of size variation not due to heredity is of questionable value. Size variation not due to heredity, however may lead to valuable incites into causes of seed size variation due to environmental and cultural practices. Kittock and Patterson (1962) concluded that the closer the genetic background, the higher the correlation between seed weight and seedling vigor in a number of wheatgrasses and brome grass.

Ashby (1930) found that greater initial embryo size in corn led to an advantage in the plant which was maintained throughout the growing season. The question arises whether vigor differences are due to increased number of cells, greater cell size, greater food reserves or to more enzymatic protein. Burriss et al. (1971) attributed increased respiration rates in soybeans, Glycine max (L.) Merr. to increased number of cells per seed. McDaniel (1969) attributed increased respiration rates to increased quantities of mitochondrial protein in large barley seed.

Large seeds have higher protein content in spring wheat (Evans and Bhatt, 1977) and barley (Pinthus and Osher, 1966). At constant seed size, 86 % of the variation in seed weight in wheat was due to seed protein content (Lowe et al., 1972). Seed protein levels in wheat and barley can be increased by nitrogen fertilization, although much of the increased protein is stored in the endosperm (Lopez and

Grabe, 1971). Greater protein content may explain why Arnott (1974) found a greater portion of the endosperm was depleted in large ryegrass seed. Bremner et al. (1963) however found that small wheat embryo's mobilized storage materials faster. They hypothesized that the scutellum is larger in relation to embryo size in small seed.

Size or density separations may lead to higher germination of large or heavy seed than small or light seed of the same seed lot (Clark, 1904). Many investigators have concluded that small seeds within a seedlot may be immature (Kidd and West, 1919; McAllister, 1943; Ahmed and Zuberi, 1973; Lam and Ridout, 1984). Under stress conditions the embryo may not be fully developed in small or light seed. While germination is positively correlated with seed size and seed weight (McDaniel, 1973), speed of germination is commonly negatively correlated with seed size or weight in barley (Carlson, 1982). Water imbibition is slower in large corn seed due to the greater volume to be wetted (Muchena and Grogan, 1977) or lower surface area to volume ratio (Bewley and Black, 1985). The total water volume imbibed by large soybean seed was greater (Burris et al., 1971).

Burris et al. (1971) found that large seed exhibited less respiration per gram of dry weight due to lesser percentage loss of cotyledon weight. They found large soybean seed had greater overall respiration on an embryo

basis. They concluded that differences in embryo physiology must be responsible for differential growth of sized seed. Carlson (1982) also confirmed that respiration of large barley seed was greater on a per embryo basis but not on a per gram seed weight basis. McDaniel (1967) found that increased mitochondrial protein was linked to greater respiration rate and a higher adenosinetriphosphate (ATP) level in barley. He developed a technique to measure the adenosinediphosphate \cdot oxygen⁻¹ (ADP/O) consumption ratio which indicates mitochondrial respiration efficiency (McDaniel, 1973).

Heavier or larger seed produce larger seedlings than light or small seed in: crested wheatgrass, Agropyron desertorum (Fisch ex Link) Schult. and A. cristatum (L.) Beauv., species and cultivars (Rogler, 1954); subterranean clover, Trifolium subterreneum L., (Black, 1956); wheat (Evans and Bhatt, 1977); and carrot, Daucus carota L., seed (Auston and Longden, 1967). Boyd et al. (1971) found that barley seed size and germination resistance, as indicated by speed of emergence index, accounted for 95 % of variability in seedling weight. Seed size was positively related to coleoptile length in intermediate wheatgrass, Elytrigia intermedia (Host.) Nevski, (Hunt and Miller, 1963). Soybean seed weight, however, was negatively related to hypocotyl length (Payne and Koszykowski, 1979). Bremner et al. (1963) indicated that

relative growth of the wheat seedling was due to the extent of the energy source available to the developing seedling. This may be the reason several researchers have observed that large seeds emerge from greater depths than small seeds.

Stand density may not be affected by seed size in alfalfa, Medicago sativa L., (Beveridge and Wilsie, 1959) and in grain sorghum (Maranville and Clegg, 1977). However, sand bluestem, Andropogon hallii Hack., stand establishment was positively correlated with seed weight (Kneebone, 1956). Kneebone and Cremer (1955) found that grass seedlings from large seed emerged faster and exhibited greater seedling vigor. Spring wheat seedlings from large seed emerged faster than those from small seed (LaFond and Baker, 1986).

Faster seedling emergence with larger seeds may be dependent on depth of planting. Rogler (1954) observed that percentage emergence of crested wheatgrass seedlings was reduced by increased planting depth of both large and small seeds. Significant size by depth of planting interactions were found for seedling emergence of clovers (Multamaki, 1962). Kalton et al. (1959). reported that smooth brome grass, Bromus inermis Leyss., depth of planting was negatively correlated with speed of emergence. Speed of emergence at greater depths of planting was positively associated with seed size. Russian wildrye,

Psathyrostachys juncea (Fisch.) Neverski, seedling emergence from greater than 3.7 cm was positively related to seed weight (Lawrence, 1963).

Arnott (1969) observed that ryegrass seedlings from heavy seeds developed faster with greater mean number of leaves and tillers. The proportion of shoot emergence declined with depth of seeding as seed size diminished. Harkness (1965) found that larger seeds within a cultivar of Italian ryegrass, Lolium multiflorum Lam., produced more tillers by 30 days after emergence. Perennial ryegrass, Lolium perenne L., tiller numbers at 47 and 62 days after planting were positively correlated with seed weight (Thomas, 1966). Increased tiller numbers from seeds with greater seed weight were apparent throughout the establishment year in Westerwolds ryegrass (Brown, 1977). Naylor (1980) found that ryegrass seed size did not correlate with subsequent tillering and production. He found that speed of emergence was a good predictor of speed of tillering and tiller number until the onset of maturity.

Grass emergence and establishment under dryland conditions is dependent on rapid root growth (Hassanyar and Wilson, 1979). Kittock and Patterson (1962) observed greater seven week root penetration with heavy grass seed. Evans (1973) observed faster root growth of large wheat seed. Total root tissue has been found to be positively correlated with seed size (Paluska, et al., 1979).

Harkness (1965) found that use of large seeds within a variety resulted in heavier root weights. Seed weight is highly correlated with plant performance in sainfoin (Fransen, 1975). Use of heavy seed resulted in greater plant height and leaf length in grasses (Kittock and Patterson, 1959). Winter barley coleoptile length was positively associated with seed weight (Ceccarelli and Pegiati, 1980). Native grass seedlings (Kneebone and Cremer, 1959) and ryegrass seedlings (Arnott, 1969; Harkness, 1965) from heavy seeds developed faster with greater mean number of leaves and tillers. Brown (1977) also found that ryegrass tiller numbers were positively associated with seed weight.

Total plant weight has often been associated with seed weight and size. This increase in vegetative yield might be attributed to increased plant spread. Padilla et al. (1978) did not find a correlation between seed weight and rhizome numbers in western wheatgrass. Increased seed size in birdsfoot trefoil, Lotus cornicalatus L., resulted in increased regrowth, although the second cutting showed less pronounced differences than the first cutting (Henson and Tayman, 1961). Harkness (1965) attributes the positive attributes of various vegetative characteristics to control by genetic factors rather than seed size. Seed separations based on size or weight can be used as a screening test to increase various vegetative components (Tossel, 1960).

Planting large seeds increased seed yield in Russian wildrye (Lawrence, 1963), barley (Demirlicakmak et al., 1963) and wheat (Hampton, 1981). These increases in seed yield have been attributed to increased culm numbers, more heads per plant (Demirlicakmak et al., 1963; Kiesselbach, 1924; Kaufmann and McFaddon, 1960) or to increased number of kernels per head (Pinthus and Osher, 1966). Use of large seeds increased spikes \cdot plant⁻¹ by 10 % and kernels \cdot spike⁻¹ by 11 % resulting in yield increases in wheat and barley (Pinthus and Osher, 1966). Kaufmann and McFaddon (1960) found that large seeded barley outyielded small seeded barley 1:.54 under close spacing. Maranville and Clegg (1977) found no difference in seed yield attributable to seed size in grain sorghum. Wood et al. (1977) state that seed size may be important for increasing seed yields of crops especially if the initial yield is great, growing season is short or with low plant density.

A good stand can be reduced in vigor and seedling survival by interplant competition. The optimal seeding rate is based on seed size, plant density, fertilizer applied and cutting or harvesting technique (Rogers, 1966). Planting large seeded alfalfa at the same planting density as small seeded alfalfa resulted in more plants per square meter of soil (Ericson, 1946). Black (1957) observed that under optimal spacing subterranean clover plants maintained differences among plant weight proportional to initial seed

weights throughout the season. In mixed swards (stands) plants from large seed progressively suppressed the other plants, and eventually contributed 90 % of the dry weight and leaf area in the sward (Black, 1958). Eliminating of the effects of seed size under normal seeding rates was found to be improbable (Demirlicakmak et al., 1963). Wide row spacing and limited competition resulted in highest yield from large snapbean seed, Phaseolus vulgaris L., (Clark and Peck, 1968). When snapbeans were planted on an equal seed number basis large seed was superior. Approximately 30 % more small seeds per meter were planted to result in equal yield. Orchardgrass has shown a definite forage yield response to both seed weight and sowing rate (Carpenter, 1960). In light stands seed weight negatively affected plant density while positively affecting forage yield. Under heavy sowing rates seed weight negatively affected plant density and forage yield.

Many models have been proposed that elucidate the relationships which lead to seed size and those relationships which are affected by subsequent seed size. Kidd and West (1919) proposed a general model for many crops in which seed size was dependent on: climate and edaphic features; seed positioning on the parent plant; genetics; maturity and storage. An alternative model found that seed weight was a product of: number of fertile tillers per plant; number of grains per ear and the mean

weight per grain (Harper, 1977). Blackman's (1919) compound interest law defined seedling vigor as the rate of change in seedling weight which was proportional to the quantity itself (initial seed weight). Ultimately dry weight is dependent on: weight of seed; rate at which material present is employed to produce new material and the time during which the plant is increasing in weight. Mathematically this can be expressed as:

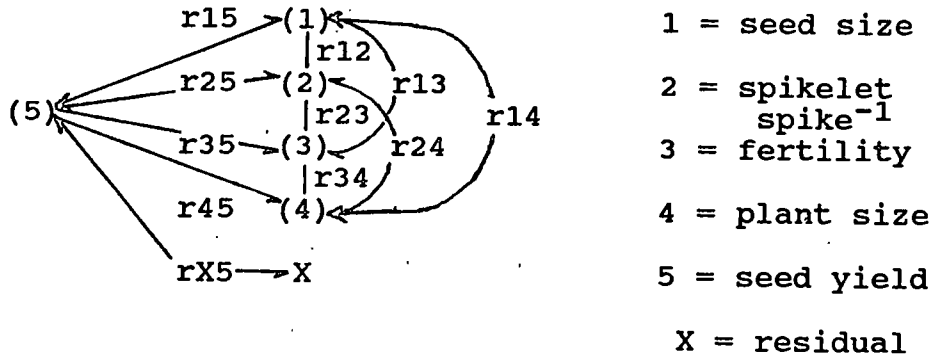
$$W_1 = W_0 * e^{rt}$$

where W_1 = final weight, W_0 = initial weight, r = rate of interest (rate of growth and endosperm depletion), t = time and e = base of the natural log (Blackman, 1919). If the rate of enzymatic protein synthesis is constant per unit mass the most important variable is the initial seed weight. The weight of the mature barley seed can be expressed as:

$$W_g = W_g(i) + (R_g * D_g)$$

where W_g = mean weight grain-1 at harvest, $W_g(i)$ = mean weight grain-1 at initial time of the linear phase of growth, R_g = mean growth rate grain-1 during the linear phase and D_g = duration of linear phase of grain growth (Gallagher et al., 1976). This model reflects back to the variables leading to seed weight proposed by Kidd and West (1919).

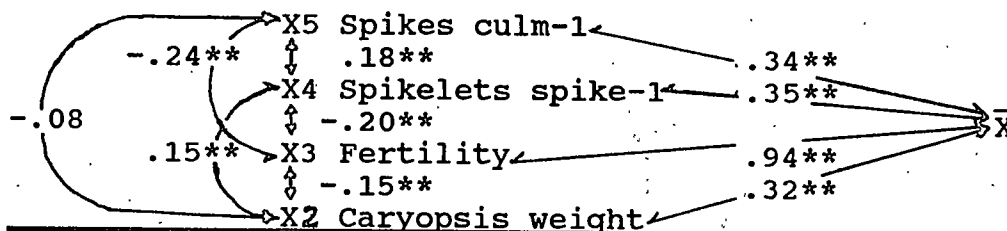
Table 1. A correlation and path-coefficient analysis of components of crested wheatgrass production. Dewey and Lu, 1959.



	seed size	spklt.s. spike-1	fert.	seed wt. spike-1	plant size	seed yield
seed size	--	.260*	-.640**	-.280*	.495**	.007ns
spikelet spike-1		--	-.279*	.208ns	.443**	.432**
fertility			--	.823**	-.568**	.257*
seed wt. spike ⁻¹				--	-.273*	.579**
plant size					--	.504**
seed yield						--

Dewey and Lu (1959) used path coefficient analysis to predict crested wheatgrass production (Table 1.). Genetic characteristics are an important component of seed weight in blue grama. (Wilson et al., 1981).

Table 2. Relationships among components of seed yield in blue grama (Wilson et al., 1981).



They found that the contribution of caryopsis weight to resulting seed yield was significant yet fertility was the most significant component of yield (Table 2).

Many seed or seedling attributes are related to seed weight. Seed weight of barley was positively ($r = 0.18$) correlated with germination (McDaniel, 1973). Seedling weight correlations to seed weight ranged from $r = 0.58$ to $r = 0.80$ in alfalfa and trefoil (Carleton and Cooper, 1972) to $r = 0.97$ to $r = 0.99$ in barley (McDaniel, 1973). Emergence was positively correlated to seed weight ($r = 0.98$) in grasses (Kittock and Patterson, 1962) while speed of emergence was negatively correlated to seed weight ($r = -0.83$) in ryegrass (Brown, 1977) and a variety of forages, ($r = -0.49$), (Kaulton et al., 1959).

Initial stands had high correlations to seed weight of $r = 0.88$ and $r = 0.99$ in bluegrass (Kneebone, 1956) and a variety of grasses (Kittock and Patterson, 1962) respectively. Carleton and Cooper (1972) found a negative $r = -0.10$ correlation between seed weight and seed raceme⁻¹ in three legumes. Yield was positively correlated with seed weight ($r = 0.40$) in bluegrass (Kneebone, 1956). Mitochondrial protein correlation to seed weight ranged from $r = 0.86$ to $r = 0.98$ in wheat (Evans and Bhatt, 1977) and barley (McDaniel, 1973). The correlation of emergence from depth to seed weight ranged from $r = 0.58$ to $r = 0.92$ in crested wheatgrass (Rogler, 1954). Barley root and

shoot weights were also positively correlated with seed weight at correlation coefficients of $r = 0.95$ and $r = 0.91$ to $r = 0.97$ (McDaniel, 1973).

CHAPTER 2

LABORATORY GERMINATION AND VIGOR TESTING

Materials and Methods1984 Study

A preliminary study was conducted to determine if Critana thickspike wheatgrass responded to seed separation. A 1982 seed lot of known purity and germination from the Bridger SCS Plant Materials Center was selected and was used as a control. The remainder of the seed lot was hand screened. The portion remaining over a 0.13 x 1.27 cm screen was termed large seed. That portion which passed through the 0.13 x 1.27 cm screen was classified as small seed. Both large and small seed lots were divided equally for density using a South Dakota blower¹ set at a guide number of 42 for 1 minute. The 100 seed weight of each seed treatment combination was determined. Total germination and tetrazolium viability were obtained using the methods outlined in the AOSA Rules For Testing Seeds

¹ Mention of a trademark, proprietary product, or vendor is included for the benefit of the reader, and does not imply endorsement by Montana State University or the Montana Agricultural Experiment Station to the exclusion of other suitable products.

(1983). Speed of germination index was calculated using Maguire's (1962) formula with counts made weekly:

$$\text{SGI} = \frac{\# \text{ seeds germinated}}{\text{count \# (1)}} + \frac{\text{add. germ.}}{\text{count \# (2)}} + \dots + \frac{\text{add. germ.}}{\text{count \# (n)}}$$

Speed of germination indices were calculated from four 100 seed samples. These data were evaluated utilizing the MSUStat¹ analysis of variance, multifactor program. Mean comparisons were made using Newman-Keuls multiple comparison at the P = 0.05 level.

1985 Study

In the second year, three Critana thickspike wheatgrass lots were utilized. Seed lots were selected from environments with an average 25 cm · year⁻¹ precipitation zone over a period of three consecutive years; 1982, 1983 and 1984, each from a different field. A representative control sample was taken from each seed lot. The remaining seed was then evenly split, half to be sized first and half to be density separated first.

Seed lots were sized using precision hand screens. Seed remaining above a 0.13 x 1.27 cm screen was classified as large and that which passed through a 0.12 x 0.79 cm screen was classified as small. Half of each size classification was then volumetrically split into heavy and light seed separations using an Oregon continuous blower¹.

The portion of the initial lot which was to be density separated was evenly split by an Oregon blower into heavy and light density separations. Half of each of the density separations was then sized using hand screens as previously described (Table 3).

Table 3. Composition of seed size and density treatments used in the three seed lots to determine vigor of Critana thickspike wheatgrass.

Treatment	Size Separation			Density Separation		
	None	Small	Large	None	Light	Heavy
Control	x			x		
Large			x	x		
Small		x		x		
Large Heavy			x			x
Large Light			x		x	
Small Heavy		x				x
Small Light		x			x	
Heavy	x					x
Light	x				x	
Heavy Large			x			x
Heavy Small		x				x
Light Large			x		x	
Light Small		x			x	

Four replications of 100 seeds were weighed and germinated according to AOSA standards. Speed of germination indices were calculated using McGuire's formula.

Results and Discussion

1984 Study

Small light, large light, small heavy and large heavy size classes were 86%, 89%, 130% and 136% respectively as heavy as the control seed treatment (Table 4). Density

treatments did result in seed separations while size separations reflected a trend toward expected seed weight. The control was comprised of 45% small seed and 55% large seed.

Table 4. 1984 Critana thickspike mean 100 seed weight, germination, and speed of germination index (SGI) for seed treatments.

Seed Separation	Seed weight	Germination	SGI
	mg	%	
Control	274b [#]	43a	26a
Small light	235a	45a	27a
Large light	245a	59b	40b
Small heavy	355c	47a	25a
Large heavy	373d	59b	41b

[#] Means in the same column followed by a common letter are not significantly different (P=.05).

Mean germination of the treatment combinations when compared to the control were: small light 105%, large light 137%, small heavy 109% and large heavy 137%. Low germination of the control was influenced by multiple florets which were screened out in the other seed treatment combinations. Multiple florets failed to germinate or were often abnormal due to mold contamination on sterile florets if they did germinate. Large seed treatments resulted in greater percent germination and faster germination. Greater total germination and increased SGI of large seed separations reflect the need to properly condition seed to insure highest quality seed possible for planting.

These results indicated that seed size separation affect seed weight, germination, and speed of germination. Large seeds significantly outperformed the control or small

seed. Planting of separated seeds on a weight basis may result in either too thick or too thin a stand. If seed separations are used seeding rates need to be adjusted to a pure live seed basis for optimal seeding efficiency.

1985 Study

In 1985, the experiment was expanded to further define the observed increase in seed weight, percentage germination and speed of germination indices due to seed separations. One hundred seed weight was significantly effected by seed lots, treatment combination and the interaction between seed source and treatment combination (Appendix Table 15 and Table 5.). Controls were withdrawn from the data set to examine the main effects; method of separation, density separations and size separations.

Examination of the 100 seed weight interaction between seed source and treatment combination indicated that the 1982 small and 1982 light seed treatments were exceptionally light (Figure 1). This was probably due to environmental conditions. There were no significant differences within the combination pairs: light-small, small-light; light- large, large-light and small-heavy, heavy-small (Table 5.). The heavy large seed treatment was lighter than the large heavy seed treatment. This indicates that whether seed was sized first or density separated first, the resulting treatment combination

Table 5. Critana thickspike wheatgrass means for 100 seed weight, percent germination and speed of germination for seed separated by size and density, 1985.

Main effects	Treatments	100 seed weight	Germ.	SGI
		mg	%	
Seed lots	1982	312a [#]	96b	53c
	1983	352b	94a	39b
	1984	393c	96b	24a
Treatment combinations	Control	349de	95bcd	67ab
	Light no size	@	95bcd	62ab
	Light small	288a	94abc	64ab
	Light large	356e	94abc	57a
	Heavy no size	391g	97de	62ab
	Heavy small	339c	97de	66ab
	Heavy large	417h	98e	65ab
	Small no den.	@	94abc	62ab
	Small light	290a	93ab	61ab
	Small heavy	347cd	98de	68b
	Large no den.	382f	95bcd	61ab
	Large light	351e	92a	60ab
	Large heavy	429i	96cde	58a
Method of separation	Density 1st	350a	97a	40a
	Size 1st	354b	96a	38a
Density separations	Light	321a	93a	37a
	Heavy	383b	98b	41b
Size separations	Small	316a	96a	43b
	Large	383b	95a	35a

[#] Means in the same column followed by a common letter are not significantly different (P=.05).

@ Means withdrawn because of significant interaction effect.

behaved similarly except for the exception noted above. Sizing seed first may result in a slightly heavier seed weight.

Interactions for: year by method, method by density, year by method by density, year by size and density by size were significant (Appendix Table 15.). These interactions are of relatively small magnitude and appear to be the

result of immaturity of small or light seed in the 1982 seed lot. Using size or density separation results in heavy seed, especially during years of environmental stress. The main effect of large seed and heavy seed separations was to increase 100 seed weight.

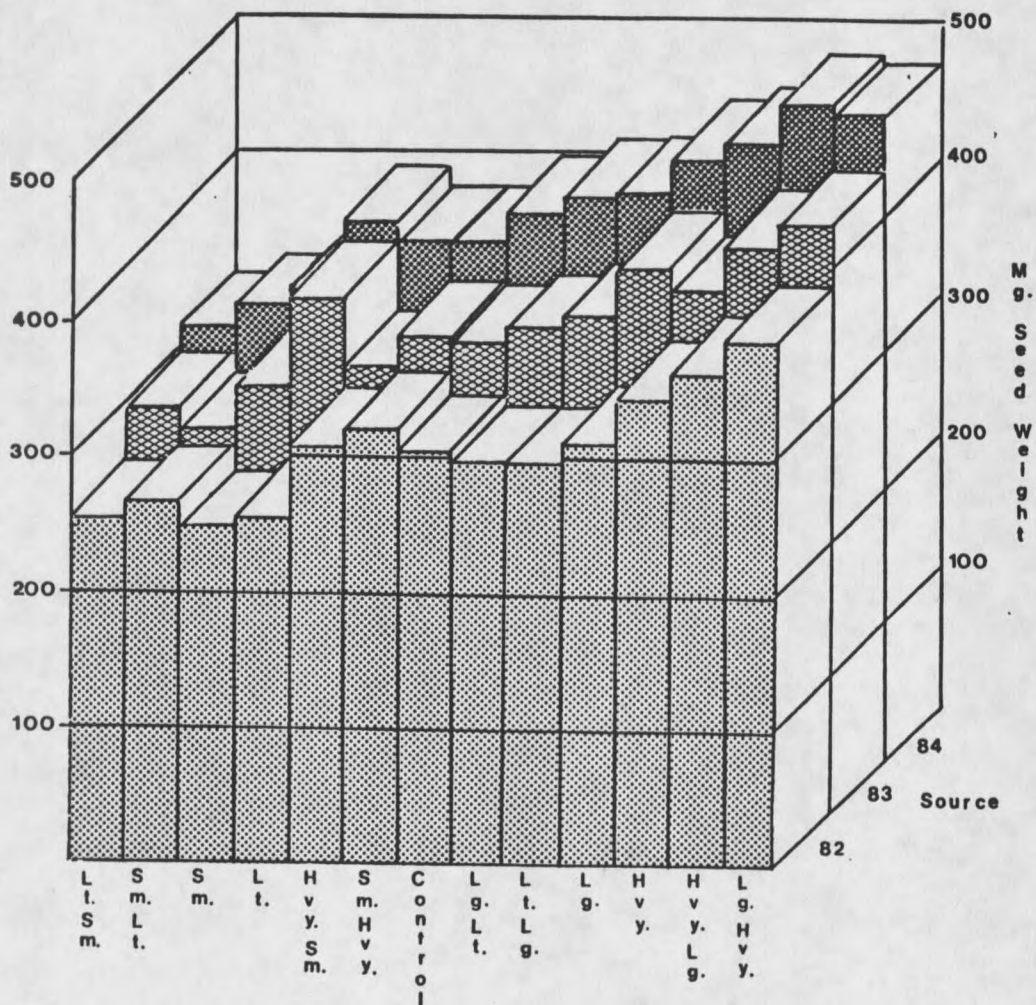


Figure 1. Critana thickspike wheatgrass mean seed weight of thirteen treatments for each of three seed lots as separated by size and density (mg).

Significant differences in percent germination were obtained among the treatment combinations and seed sources (Table 5 and Appendix Table 15). The 1983 seed lot exhibited lower germination than the 1982 or 1984 seed lots. Individual treatments containing the light separation had a significantly lower germination than treatments containing heavy separations. A small method by size interaction was found (Appendix Table 15). The interaction may be due to high germination of the 1983 small heavy seed treatment. There were no significant differences in germination between sizes.

Significant differences were found among seed source and seed treatment combinations for speed of germination indices (SGI) (Table 5). The 1982 seed source was the fastest germinating and the equally viable 1984 seed lot was the slowest germinating (Table 5). The light large and large heavy seed treatment combinations were significantly slower germinating than the small heavy seed. This would agree with the slow rate of imbibition by large seed observed by Muchena and Grogan (1977).

The interaction, method by density by size, was significant. This is attributed to the slow SGI of the 1982 heavy large treatment combination in comparison to the 1982 large heavy treatment combination. The year by density interaction was also significant and the previous explanation applies here also. Small seeds were

significantly faster germinating than large seed which is in agreement with Carlson's (1982) data, however heavy and light seed SGI were not significantly different.

Germination percentage was affected by seed source as was expected from review of the literature (Lawrence and Baker, 1986). Density separation increased total germination within a seed treatment which agrees with previous reports (Clark, 1904; McDaniel, 1973). Investigators reporting increased germination due to sizing (Maranville and Clegg, 1977) may have actually reported the effects of density separations on germination. Proper conditioning of Critana thickspike wheatgrass should include use of density separation to insure the highest germination possible. Speed of germination was negatively related to seed size.

CHAPTER 3

GREENHOUSE STUDY AND SEEDLING VIGOR

Materials and Methods

Each of three seed sources of Critana thickspike wheatgrass with thirteen treatment combinations per seed source (Table 3.) were planted 1.2 cm. deep in 2.5 x 15 cm "Conetainers"¹ (Ray Leach Co., Camby, Or.). Each conetainer was filled with "Sunshine Mix"¹. Seed sources and treatment combinations were the same as in Chapter 2. Two seeds were placed in each of the conetainers to ensure emergence of at least one seedling. Five replications with seven plants each were planted for sampling in a RCB design with a sixth replication held back for replacement. Seedlings were thinned to one per conetainer upon emergence of a second seedling. Emergence counts started one week after planting and seedling counts were made daily for five days and every other day for the next week. Final emergence was counted 40 days after initial emergence.

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Total emergence, speed of emergence index and seedling mortality were determined. Speed of emergence index was calculated by the following formula;

$$\text{SEI} = (\text{Emergence at count } 1/1) + (\text{Emergence at count } 2/2) + \dots + (\text{Emergence at count } n/\text{days from initial emergence}).$$

Four plants were randomly selected per treatment combination and cut at the soil surface 45 days after planting. Fresh top weight and root weight were determined and the samples dried and reweighed after 48 hour in an 105C oven.

The remaining plants were cut at the soil surface and replacement plants were added from the replacement replication. These plants were then evaluated for regrowth. Two seedlings from each treatment combination were cut at the soil surface thirty days after initial harvest and dried in a 105C oven to determine weight of regrowth.

The study was initially analyzed as a two factor RCB with three seed sources and thirteen treatment combinations. Means were compared using Newman-Keuls mean separation test at the $P=0.05$ level. These data were reanalyzed as a completely randomized design (CRD) with unequal cell counts to determine individual effects of density and size separations.

Results and Discussion

Total seedling emergence of the 1984 seed lot was significantly superior to the 1983 seed lot (Table 6.). There were no observed differences among treatment combinations. Density separations were significant when the data were reanalyzed as a three factor CRD. Seedlings from the heavy density separations emerged better than the control. This may be a reflection of the lower germination of the control treatments as discussed in Chapter 2 or the effects of seedling vigor. Differences in total emergence occurred even when external factors were controlled. No differences in total emergence were found among the size separations or the treatment combinations. Field emergence may also be positively affected by density separation.

Total seedling emergence of heavy seeded seedlings was superior to control and light seeded seedlings while the light seeded seedlings emerged as fast as the control. Use of seed separated on the basis of density resulted in seedling emergence as good as or better than control seedling emergence. Therefore, no seed would be wasted by seed separation.

Seedling emergence was faster for the 1982 seed lot than the 1983 and 1984 seed lots (Table 6.). No statistical differences were observed among the treatment combinations. Seedlings from the control and heavy seed density separations emerged faster than the light density

separation. There were no differences in speed of emergence among the size separations. Under conditions of no water stress seedlings from heavier seed emerged faster

Table 6. Critana thickspike wheatgrass comparisons of 1985 treatments for greenhouse percent emergence, speed of emergence (SEI) and seedling loss.

Main Effect	Emergence %	SEI	Seedling Loss %
Seed Source			
1982	89ab [#]	4.15a	28b
1983	84a	3.76b	19a
1984	90b	3.53b	17a
Density Separations			
Light	86ab	3.75a	19a
Control	85a	3.90b	26a
Heavy	91b	3.85b	21a
Size Separations			
Small	87a	3.74a	20a
Control	89a	3.94a	25a
Large	88a	3.82a	21a
Treatment Combinations			
Small Light	84a	3.27a	18a
Light Small	91a	4.07a	20a
Small No Den.	83a	3.80a	18a
Light No Size	87a	3.84a	18a
Large Light	85a	3.79a	14a
Light Large	82a	3.68a	25a
Control	87a	4.15a	35a
Small Heavy	85a	3.60a	26a
Heavy Small	92a	3.87a	20a
Large No Den.	86a	3.75a	26a
Heavy No Size	92a	3.83a	23a
Large Heavy	93a	4.03a	18a
Heavy Large	92a	3.93a	16a

[#] Means in the same column followed by a common letter are not significantly different (P=.05 N.K. test).

than light seed in the greenhouse. Density separation may be a management technique to influence the speed of emergence of Critana thickspike wheatgrass.

The 1982 seed lot exhibited significantly greater

seedling mortality (28%), than the 1983 (19%) and the 1984 (17%) seed lots (Table 6.). Under moisture stress conditions maturity may have been hastened (Battle and Whittington, 1969; Auston, 1963), lowering the seedling vigor of the 1982 seed lot. Seedling mortality does not appear to be affected by size or density separations as no significant differences were found among main effects. Variability due to small sample size appears to mask differences in mortality of the various treatments and treatment combinations as a wide range of seedling loss was observed.

Forage dry weight for the 1983 and 1984 seed lot averaged 2 mg seedling⁻¹ more than for the 1982 seed lot (Table 7). Newman-Keuls mean separation did not separate treatment combinations, however a significant F test for treatment combinations suggests that the significant main effect of density separation impacted the treatment combinations (Appendix Table 16). Seedlings from density separations, whether light or heavy, produced 6-8 mg more forage · seedling⁻¹ than the control. Large seedlings may have competed for sunlight against small seedlings in the rows among the control. This emphasizes the validity of using seed separation to reduce the interrow competition when planting on an equal seed basis. Several studies (Black, 1957; Demirlicakmak et al., 1963; and Clark and Peck, 1968) have indicated interrow competition resulted in

decreased vegetative growth and increased mortality when large or heavy seeds competed against small or light seeds.

Table 7. Critana thickspike wheatgrass seedling weight comparisons of 1985 treatments for greenhouse 45 day dry forage weight, 45 day root dry weight, 45 day total dry plant weight and 30 day dry seedling forage regrowth.

Main Effect	Forage mg	Root mg	Plant mg	Regrowth mg
Seed Source				
1982	43a [#]	55a	98a	20a
1983	45b	50a	94a	19a
1984	45b	58a	102a	20a
Density Separation				
Light	47b	47a	94a	19ab
Control	42a	52a	94a	16a
Heavy	49b	58a	107b	22b
Size Separations				
Small	43a	53a	96a	19a
Control	45a	49a	94a	19a
Heavy	47a	52a	100a	20a
Treatment Combinations				
Small Light	44a	47a	91a	21a
Light Small	47a	49a	96a	18a
Small No Den.	41a	54a	95a	16a
Light No Size	44a	38a	83a	18a
Large Light	53a	56a	109a	21a
Light Large	47a	57a	104a	20a
Control	43a	46a	89a	17a
Small Heavy	42a	58a	99a	25a
Heavy Small	44a	57a	102a	18a
Large No Den.	39a	44a	83a	15a
Heavy No Size	48a	62a	110a	19a
Large Heavy	55a	61a	116a	23a
Heavy Large	51a	50a	101a	24a

[#] Means in the same column followed by a common letter are not significantly different (P=.05 N.K. test).

Root dry weight · seedling⁻¹ was not effected by seed lot, density, size or treatment combinations (Table 7). This indicates that root growth was similar regardless of treatment or seed lot. Under conditions of optimal watering and unlimited space for root growth, during the

period of the study, no differences in root growth would be expected. Inter-root competition could become significant over time in field or greenhouse plantings where roots were not restricted to the same rooting volume. There were no significant differences due to seed source or treatment combinations for total seedling weight (Table 7). Seed density was a significant factor affecting total seedling weight. Seedlings from heavy seed separation yielded more total plant mass than the control or light seed separation.

After a 30 day regrowth period, no differences in seedling weight were observed among seed lots or treatment combinations (Table 7). This agrees with results of regrowth studies in grasses by Lawrence (1963). Seedlings from heavy seed separations recovered significantly faster than control seedlings while seed size had no effect on recovery. Density separations may be an effective means of minimizing the effects of environmental stress after emergence.

Implications of the greenhouse study are threefold:

- 1) seed lot quality can vary due to environmental conditions during seed set and/or seed storage. Total emergence, speed of emergence, and seedling forage production varied among seed lots.
- 2) heavy density separations improved seedling emergence, forage production, total plant weight and regrowth.
- 3) treatment combinations did not affect seedling vigor. Seed sizing can

be important commercially to remove weed seeds but does little to improve thickspike wheatgrass seedling vigor. Density separation, whether light or heavy, reduced interrow competition for sunlight in the containers.

CHAPTER 4

FIELD TESTS

Materials and Methods1984 Study

Critana thickspike wheatgrass was planted May 23, 1984 at the Post farm, MSU, Bozeman, Mt., 2.5 cm deep using a cone seeder with depth bands. Treatments were as described in Chapter 2. The seed treatments planted were; light small, light large, control, heavy small, and heavy large. The seeding rate was 50 seeds · meter row⁻¹. Plots were four rows wide (30 cm centers) and 6 meters long bordered by two rows of tall wheatgrass, Elytrigia pontica (Podp.) Holub. Four replications were planted in a RCB design.

First emergence counts were made on June 5. Emergence counts were made in 0.5 m of the two center rows of each plot. Counts were made at four day intervals for the first three counts and at weekly intervals for the first month of emergence.

Plant height was measured on the longest tiller of six randomly selected plants in each plot in August. A forage harvest was hand clipped at the soil surface of a 0.5 m² area in the center of the plot. The forage was dried for

48 hours at 105C in a large oven drier and forage production was recorded on a dry weight basis.

Considerable fall growth occurred by the end of September and sample number was increased to two 0.5 m² samples to reduce sampling error. These plots were hand clipped at the soil surface. The forage was oven dried for 48 hours at 105C and forage production was recorded on a dry weight basis. All seed heads in the two central rows of each plot were counted.

In 1985, the central two rows of the 1984 planting were harvested for forage at a 2.5 cm height in September using a flail harvester. Harvest area was 0.6 m by 6 m. Field weights were recorded and plant samples were oven dried for 48 hours at 105C to determine field moisture. Dry forage weight was recorded. Seed yield was determined by hand clipping a 0.5 M by 1 M area. Seed yield was determined after seed conditioning using a cone thresher and then screening to remove inert matter.

1985 Study

Three seed lots with 13 size - density combinations per seed lot (Table 3.) were planted at the Post farm in 1985 in a RCB design with 4 replications. Two seed lots were from the Bridger, Mt. Soil Conservation Service Plant Materials Center (SCS PMC). One lot was from a field taken out of production after harvest in 1982 and the other from a field planted in 1981 and harvested in 1983. The third

seed lot was from Manhattan, Mt. grown in 1984. The methods of obtaining the treatment combinations were described in Chapter 2. Seeding rate was 50 PLS · meter⁻¹ row. Plots were four rows (30 cm center) and 6 m long. The plots were bordered by two rows of tall wheatgrass. Seeding depth was 2.5 cm. Planting date was May 15 and initial emergence occurred May 23. Emergence counts were made 2, 5, 12, and 16 days after emergence. Hail occurred on May 28 and it was cold and rainy through July 1.

Forage was harvested in the plots Oct. 24. Harvested area was 60 cm by 6 m with the central two rows harvested at 5 cm height with a flail harvester. Samples were weighed in grams after being oven dried for 48 hours at 105C. Dry forage weight was determined. Seed heads were counted and recorded from the two remaining rows (area = 2*30 cm x 6m).

Results and Discussion

1984 Study

Percentage emergence of the heavy large seed in the 1984 study was significantly greater than the control or light large seed (Table 8). Speed of emergence index (SEI) for the heavy large seed was significantly greater than for all other treatments.

Significant differences were not found for plant height. Visual evaluation of the field indicated that

differences might be expected. Low sample numbers and sampling variability may have lead to the results observed.

Table 8. Critana thickspike wheatgrass field performance traits for the 1984 planting taken in 1984 and 1985. (Post Farm, MSU, Bozeman, Mt.).

Treatment	Seed Treatment				
	Light Small	Light Large	Control	Heavy Small	Heavy Large
1984					
% Emergence	62ab [#]	46a	45a	74ab	96b
SEI	45a	36a	32a	52a	77b
Plant Height (cm)	17.2a	21.2a	19.8a	20.8a	22.7a
Aug. Forage Yield (Kg Ha ⁻¹)	204.5a	184.2a	137.0a	291.8a	567.2b
Sept. Forage Yield (Kg Ha ⁻¹)	1173a	1093a	940.9a	1261a	1897b
1985					
1985 Forage Yield (Kg Ha ⁻¹)	4370a	4653a	3827a	4213a	4842a
Seed Yield	301.3a	279.6a	291.7a	258.6a	327.6a

[#] Means in the same row followed by a common letter are not significantly different (P=.05 N.K. test).

* Speed of Emergence Index

There was a two to three fold August, 1984 forage yield increase of the heavy large seed treatment over all other treatments (Table 8.). The low August yield was due to a dry summer followed by a moist fall. This resulted in large forage yield increases due to fall green-up. By September the significant difference in yield was reduced to a two fold forage increase of the heavy large seed over all other treatments.

In 1985, the 1984 field planting had overcome the effects of initial seedling vigor and there were no significant differences among treatments for forage or seed yield (Table 8.). Although effects of seed separations were not apparent in the second year, this does not imply that seed separation is not a valid tool to increase plant establishment. The critical period of stand establishment is during the first year. Thereafter Lawrence (1963) has shown that there is a tendency for the surviving plants to compensate for differences during the year of establishment as long as an adequate stand has been established. The high forage yields were a reflection of irrigation and high soil fertility.

1985 Trial

Total emergence (plants \cdot meter⁻¹ \cdot row⁻¹) of the 1985 planting was not effected by treatments or treatment combinations (Table 9.). The 1982 and 1983 seed lots exhibited a significantly faster SEI than the 1984 seed lot. No differences were found among treatment combinations. Heavy density separations emerged faster than other density separations. No differences in SEI were observed among size separations.

If vigorous seedlings are more aggressive and are better able to complete their life cycle, increasing number of seed heads \cdot row⁻¹ may reflect increased vigor. The 1983 and 1984 seed lots produced significantly more seed

heads \cdot row⁻¹ than the 1982 seed lot (Table 9.). While the 1984 seed lot showed a low SEI, the seed lot produced a high number of seed heads. No differences were observed

Table 9. Critana thickspike wheatgrass 1985 field performance of thirteen treatment combinations with three seed lots for field emergence, speed of emergence and seed heads \cdot two rows⁻¹ (Post Farm, MSU, Bozeman, Mt.).

<u>Main Effect</u>	<u>Emergence</u> Plant \cdot M ⁻¹	<u>SEI</u>	<u>Seed Heads</u> Heads \cdot 2 Rows ⁻¹
Seed Source			
1982	36a [#]	14.22b	1.2a
1983	36a	12.45b	3.5b
1984	33a	8.07a	2.7b
Density Separation			
Light	34a	11.17a	1.8a
Control	32a	10.50a	2.8b
Heavy	36a	13.07b	3.0b
Size Separation			
Small	36a	11.97a	1.8a
Control	36a	12.06a	2.9b
Large	33a	13.71a	3.0b
Treatment Combinations			
Small Light	36a	10.33a	0.9a
Light Small	36a	11.70a	1.7a
Small No Den.	32a	11.55a	2.3a
Light No Size	37a	13.39a	2.7a
Large Light	29a	9.68a	1.8a
Light Large	32a	10.75a	2.7a
Control	36a	10.90a	3.8a
Small Heavy	40a	14.26a	2.2a
Heavy Small	34a	12.02a	2.2a
Large No Den.	29a	9.05a	1.9a
Heavy No Size	35a	11.88a	2.7a
Large Heavy	34a	14.03a	4.4a
Heavy Large	38a	13.15a	3.0a

[#] Means in the same column followed by a common letter are not significantly different (P=0.05 N.K. test).

among treatment combinations although a wide range in the number of seed heads existed. The heavy seed separation and control had more seed heads \cdot row⁻¹ than the light seed

separation. The small seed separation had significantly fewer seed heads than other size separations. The small seed lot might be expected to have a lower seed yield if this trend was to continue in the future.

Table 10. Critana thickspike wheatgrass field performance of the 1985 field planting of thirteen treatment combinations with three seed lots for forage yield (Post Farm, MSU, Bozeman, Mt.).

Treatment	Forage Yield
	Kg · Ha ⁻¹
Seed Source	
1982	1017a [#]
1983	1159b
1984	1077ab
Density Separation	
Light	1043a
Control	1027a
Heavy	1183b
Size Separation	
Small	1006a
Control	1136a
Large	1110a
Treatment Combinations	
Small Light	825a
Light Small	919ab
Small No Den.	1003sb
Light No Size	1140ab
Large Light	1140ab
Light Large	1092ab
Control	1033ab
Small Heavy	1089ab
Heavy Small	1196b
Large No Den.	1044ab
Heavy No Size	1235b
Large Heavy	1178b
Heavy Large	1165ab

[#] Means in the same column followed by a common letter are not significantly different (P=.05 N.K. test).

Seeding year forage yield (kg · ha⁻¹) for the 1985 planting (Table 10.) was similar to seeding year September

forage yields from the 1984 planting when averaged over density treatments (Table 8). Significantly more forage was produced by the 1983 seed lot than the 1982 seed lot. The heavy small, heavy no size and large heavy treatments significantly outyielded the small light treatment. Forage yield of heavy seed separations was $140 \text{ kg} \cdot \text{ha}^{-1}$ greater than for other treatments. No differences in forage yield existed among size separations.

Density separation for heavy seed appears to be a means of increasing most field performance traits during the year of establishment (Table 9). While stand emergence was not significantly improved by seed density separation, an increase of one to two seed heads $\cdot \text{row}^{-1}$ was observed in the field. A combination of seed size and density separations did not improve most field performance traits except for the large heavy seed forage yield.

If a seed lot is weed free the minimal expense of density separation is likely to result in improved field performance in thickspike wheatgrass. The minor yield increase may not pay for the cost of seed separation but this procedure may insure a stand under adverse field conditions. While light seeds do not perform as well as heavy seeds, they do perform as well as unseparated seed. The light seed left over after density separation could be utilized under more optimal field conditions and no expensive seed would be discarded.

CHAPTER 5

PHYSICAL AND PHYSIOLOGICAL EXAMINATION OF THE CARYOPSIS

Materials and Method

Lemma and palea were removed from seeds to determine physical differences in seed length and width among four seed treatment combinations. The treatment combinations were the extremes of size and density and consisted of four replications of 100 seeds \cdot treatment⁻¹ \cdot replication⁻¹ of the following; small-light, light-small, large-heavy, heavy-large. The method of obtaining the treatment combinations was discussed in Chapter 2. The number of infertile florets \cdot 100 seeds⁻¹ was recorded.

Physical attributes measured were the caryopsis length and width and the embryo length and width. Measurements were determined using an American Optical Corp.¹ dissecting scope fitted with a 10X Baush and Lomb¹ ocular. The magnification scale was five grids = 1.76 mm with the basal objective lens at a height of 17.3 cm above the platform.

¹ Mention of a trademark, proprietary product, or vendor is included for the benefit of the reader, and does not imply endorsement by Montana State University or the Montana Agricultural Experiment Station to the exclusion of other suitable products.

The weight of each subsample of 100 seeds was recorded in ug and mean weight of the treatment combination reported in Table 11.

Imbibition curves were established for each treatment to determine baseline imbibition for respiration rate determination. Three replications of 100 seeds treatment⁻¹ for each of the following treatments were used; whole seed, naked caryopsis, endosperm and embryo. Naked caryopsis had the lemma and palea removed. The endosperm treatment consisted of embryo removal from the naked seed. Naked caryopsis were conditioned on moistened blotter paper and the embryo was excised with a scalpel using a dissecting scope. Seed of the treatments were placed on 12.5 by 15.5 cm blotter paper, Anchor Paper Co., St. Paul, Mn¹ which had been moistened with water for imbibition studies. The seed parts were gently blotted and weighed every 6 hours for 54 hours. Visual sign of germination, swelling and protrudence of the radicle, was observed and the time of germination recorded. Upon completion of imbibition, the treatments were placed in a 105C forced air oven for 24 hours to determine dry weight. Fresh weight moisture percentage and percentage moisture uptake was calculated for each of the weighing periods.

Fifty seeds from each of the four seed separations and three treatments; (whole seed, naked caryopsis and excised embryo's) were premoistened on blotter paper to 30%

moisture. Fresh weight and imbibed weight were measured until 30% water uptake had occurred. The treatments were then placed in a 15 ml reaction vessel with 2 ml water and 200 ul of 10% Potassium hydroxide (KOH) in the center well along with a paper wick. Reaction vessels were attached to a precalibrated 14 station volumometer Gilson Differential Single Valve Style Respirometer (Gilson Medical Electronics, Inc., Middleton, Wisc.)¹ equipped with a 25C continuous flowing water bath. Oxygen uptake was recorded at 30 minute intervals for a 2 hour period with concurrent barometric pressure readings recorded after a 1 hour equilibration period. The study was replicated five times.

The standard volume of Oxygen uptake was determined by the following formula:

$$X = -Vg \cdot \frac{PT'}{P'T}$$

where: X = volume in microliters at STP
 P = barometric pressure (mm Hg)*
 P' = standard pressure (760 mm Hg)
 T = temperature at the micrometer (⁰K)
 T' = standard temperature (273K)
 -Vg = observed change in volume(ul)

Analysis of variance was conducted to determine if differences in physical dimensions of the caryopsis and in respiration rate occurred among size-density separations and seed treatments. Newman-Keuls mean separation at the P=0.05 level was utilized to compare treatment means. Multiple regression analysis was utilized to determine appropriate models to describe caryopsis dimensions.

Results and Discussion

Small light and large heavy treatments represent the extreme differences in separations. In species with adhering lemma and palea, sizing or density separation treatments may not represent the physical dimensions of the seed (Knipe, 1970). Differences in physical dimensions of the caryopsis due to seed fill may occur, yet embryo size and width may not vary.

The large heavy caryopsis was 0.65mm longer and 0.2 mm wider than small light caryopsis (Table 11.). The large heavy embryos were 1.5 times as long and 0.06 mm wider than the small light embryos. These physical differences result in a 0.9 mg weight increase in the large heavy naked caryopsis. Size-density separations did reflect physical differences in the naked caryopsis.

Table 11. Critana thickspike 100 seed mean weight, length and width of the caryopsis and embryo for treatment combinations small light and large heavy.

Treatment	Caryopsis		Embryo		Seed
	Length	Width	Length	Width	Weight
	mm	mm	mm	mm	ug
Small Light	4.132a [#]	1.131a	0.988a	0.517a	1669a
Large Heavy	4.781b	1.308b	1.441b	0.579b	2571b

[#] Means in the same column followed by a common letter are not significantly different (P=.05 N.K. test).

Regression analysis indicated that the naked caryopsis and embryo dimensions can be described by naked caryopsis weight (Table 12.).

Table 12. Critana thickspike wheatgrass regression equation to describe mean length and width of naked caryopsis and by seed weight.

Attribute	Intercept	B(seed weight)	R ²	P-value
mm		mg		
Caryopsis length	= 2.921 +	0.7243E ⁻³ ·B	.9665	.0000
Caryopsis width	= 0.810 +	0.1933E ⁻³ ·B	.9635	.0000
Embryo length	= 0.610 +	0.2068E ⁻³ ·B	.8777	.0007
Embryo width	= 0.401 +	0.6930E ⁻⁴ ·B	.6554	.0017

IMBIBITION

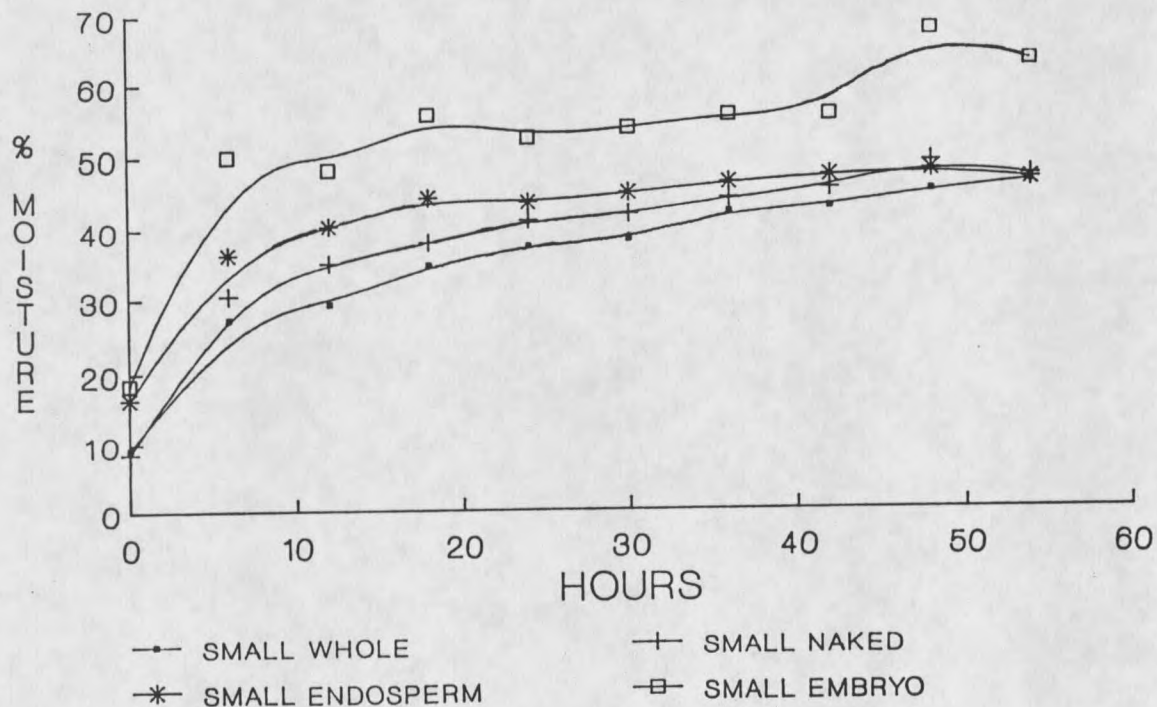


Figure 2. Critana thickspike wheatgrass rate of water uptake of small whole seed, small naked caryopsis, small endosperm and small embryos measured over a 54 hour period.

Rate of imbibition curves were drawn to determine the phases of imbibition (Bewley and Black, 1985): Phase 1.)

rapid water uptake, Phase 2.) lag phase and Phase 3.) germination phase. Imbibition curves appeared to be similar regardless to the seed separation utilized for that component. Since the purpose of the study was to pinpoint the rapid imbibition phase (Figures 2 and 3.) no statistical analysis was made of the data.

IMBIBITION

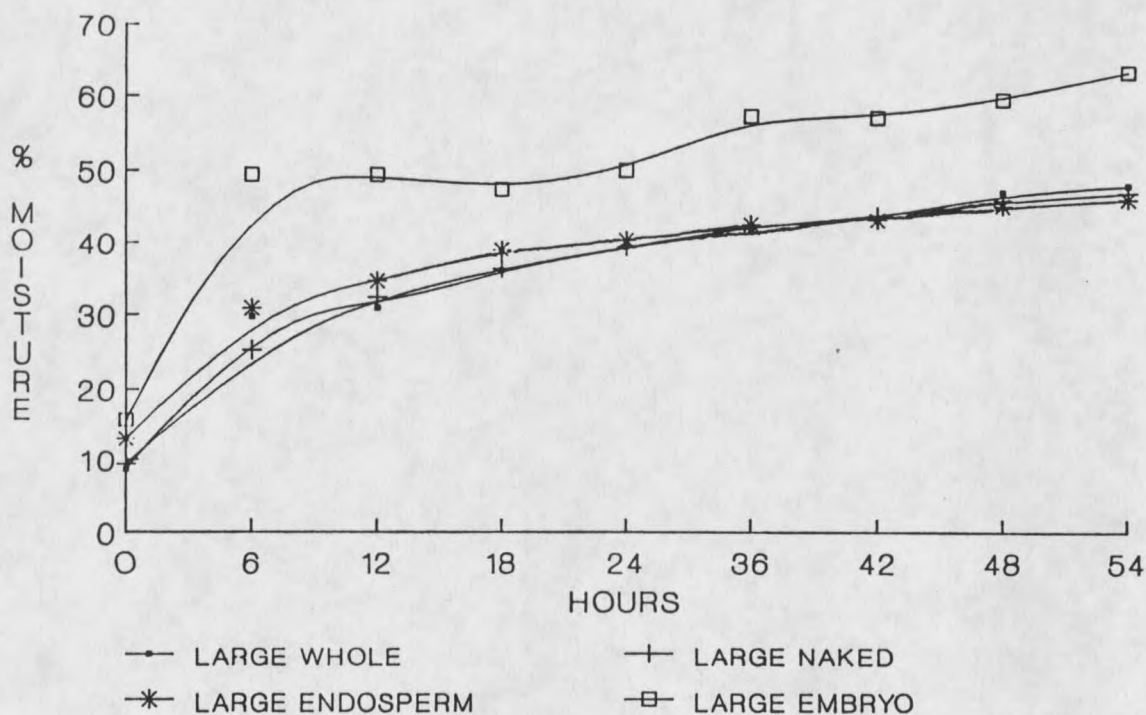


Figure 3. Critana thickspike wheatgrass rate of water uptake of large whole seed, large naked caryopsis, large endosperm and large embryo measured over a 54 hour period.

Naked caryopsis were premoistened prior to embryo excision to facilitate removal of undamaged embryos. The embryos air dried to 15.6 and 18.4% moisture, for large heavy and small light treatments, respectively (Table 13). Endosperms air dried to 12.9 and 16.4% moisture, respectively. Proteins adsorb water readily and starch granules adsorb water slowly until enzyme activation. This may indicate that large heavy caryopsis contain proportionally a higher percentage of carbohydrates on a weight basis in the endosperm and embryo.

Embryos rapidly adsorb water for about 6 hours during phase 1 (Figures 2 and 3). The lag phase (phase 2), continues in the embryo until approximately 42 hours of imbibition. Phase 3 begins when the embryo tissues visually began to swell and as germination is initiated.

Table 13. Critana thickspike wheatgrass moisture content (%) over a 54 hour period for small light and large heavy treatments of whole seed, naked caryopsis, endosperm and embryos.

Hours:	0	6	12	18	24	30	36	42	48	54
Large Whole	8.6	29.7	30.9	36.0	39.4	40.2	41.6	43.3	47.1	48.0
Large Naked	9.3	25.3	32.3	36.2	39.3	39.7	42.4	43.8	46.0	47.0
Large Endosperms	12.9	30.8	34.5	39.9	40.3	41.5	42.6	43.2	45.2	46.1
Large Embryos	15.6	49.4	49.4	49.3	50.0	53.0	57.2	57.0	59.5	63.3
Small Whole	9.2	27.1	29.2	34.7	37.4	38.3	42.2	42.8	45.1	46.4
Small Naked	8.5	30.4	35.0	38.0	41.1	41.9	44.1	45.3	48.2	47.1
Small Endosperms	16.4	36.3	40.3	43.2	43.7	44.9	46.3	47.2	47.9	46.6
Small Embryos	18.4	50.0	48.2	55.6	52.4	55.7	55.4	58.4	67.7	63.1

The endosperm rapidly imbibes water for approximately 18 hours. The moisture uptake during Phase 1 is due to filling of inter-cellular space. A gradual increase in moisture absorption occurred after 18 hours due to water uptake into the starch granules and the granules expanded.

The naked caryopsis mirrors the imbibition pattern of the endosperm during Phase 1. The slow water uptake was apparently due to restricted water uptake of the unbroken seed coat. Phase 2 continues in the naked caryopsis until approximately 42 hours of imbibition. Phase 3 or germination was initiated at this time when swelling of the radicle was noted.

Whole seeds closely followed the imbibition pattern of the naked caryopsis. Moisture content of the embryo during Phase 1 was 48% while the moisture content of the caryopsis was 36 to 38% of the whole seed or naked caryopsis weight (Table 13.). Germination started when moisture content was approximately 57-59% of the embryos weight or about 43% of the whole seed or naked caryopsis weight. Radicle swelling was visible after 46% water uptake by the whole seed or naked caryopsis or at 63% water uptake by the embryo.

Respiration rates were measured during Phase 1. A moisture content of 30% was chosen as the point during Phase 1 that all treatments could be measured and that the treatments would remain in Phase 1 during respiration

testing. Significant differences in $\text{ul oxygen consumption} \cdot \text{seed}^{-1} \cdot \text{hour}^{-1}$ among treatment combinations were found on a whole seed basis (Table 14.). Large heavy embryos consumed 30% more oxygen than small light embryos. Oxygen consumption for the naked large heavy caryopsis was 25% greater than small light caryopsis and 30% greater in large heavy whole seed. No significant differences were found when oxygen consumption was placed on a gram basis.

Table 14. Oxygen consumption rate for 50 Critana thickspike wheatgrass embryos, naked caryopsis and whole seeds.

Basis:	Whole Seed		Naked Seed		Embryo	
	Seed	Gram	Seed	Gram	Seed	Gram
Small Light	49.10a [#]	184.3a	36.32a	242.0a	9.27a	1225a
Large Heavy	63.95b	187.8a	45.69b	220.4a	12.08b	1542a

[#] Means in the same column followed by a common letter are not significantly different (P=.05 N.K. test).

Seed separations based on size and weight can result in differences in weight and physical dimensions of the caryopsis and embryo. A linear regression equation described caryopsis length and width and embryo length and width in relation to seed weight to a high degree of accuracy.

Embryos rapidly imbibe water during the first 6 hours of imbibition while caryopsis's reach the end of Phase 1 at 18 hours. Germination or Phase 3 occurred in Critana thickspike wheatgrass at approximately 42 hours. There are no differences in rates of water uptake between large and small seeds or embryos. Oxygen consumption is

significantly greater per seed for large heavy seeds and large heavy embryos than small light seeds or embryos. Since moisture content is similar, these differences might be attributed to greater quantities of mitochondrial protein.

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APPENDIX

Table 15. Analysis of variance of 100 mean seed weight for main effects (mg).

Source	DF	Sum Square	Mean Square	F-Value	P-Value
Blocks	3	.3065e-3	.1022e-3		
Seed Lots	2	.2025	.1012	1286	0.000***
Separations	12	.2070	.2252e-1	286.2	0.000***
Lot *					
Separation	24	.3160	.1317E-2	16.73	0.000***
Residual	114	.8972e-2	.7870e-4		

*** significant at the 0.005 level

Table 16. Analysis of variance of 100 mean seed weight for subeffects averaged over seed separations chronologically reversed (mg).

Source	DF	Sum Square	Mean Square	F-Value	P-Value
Block	3	.6646e-4	.2215e-4		
Seed Lot	2	.1039	.5194e-1	649.6	0.000***
Method	1	.3619e-3	.3619e-3	4.526	0.034*
Lot * Method	2	.5294e-3	.2647e-3	3.310	0.041*
Density	1	.9181e-1	.9181e-1	1148	0.000***
Lot * Density	2	.3908e-3	.1954e-3	2.444	0.924
Method *					
Density	1	.8773e-3	.8773e-3	10.97	0.002**
Lot * Method					
* Density	2	.1254e-2	.6271e-3	7.84	0.012*
Size	1	.1262	.1262	1579	0.000***
Lot * Size	2	.8993e-2	.4497e-2	56.23	0.000***
Method * Size	1	.2340e-4	.2340e-4	0.29	0.597
Lot * Method					
* Size	2	.1465e-3	.1048e-3	1.31	0.275
Density * Size	1	.1465e-2	.1465e-2	18.32	0.000***
Lot * Method					
* Size	2	.3062e-3	.1531e-3	1.92	0.153
Method * Density					
* Size	1	.1848e-3	.1848e-3	2.311	0.129
Lot * Method *					
Density*Size	2	.1593e-3	.7965e-4	0.996	0.376
Residual	69	.5518e-2	.7997e-4		

*** significant at the 0.005 level

** significant at the 0.01 level

* significant at the 0.05 level

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