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Breaking Seed Dormancy in Three Poaceae Species

By

Heidi Jo Larson

A thesis submitted in partial fulfillment

of the requirements for the

Master of Science

Major in Agronomy

South Dakota State University

2002

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Breaking Seed Dormancy in Three Poaceae Species

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Abstract

Breaking Seed Dormancy in Three Species of the Poaceae Family

Heidi Jo Larson

April 2002

Seed dormancy is a major impediment to germination testing in seed testing laboratories. Dormant seeds are alive but do not germinate under conditions favorable for germination of non-dormant seeds of the same species. Dormancy in many plant species can be broken using a single treatment or a combination of treatments. Treatments commonly used are light or dark, various temperature regimes, prolonged exposure to cold treatments (pre-chilling), and the use of growth hormones or other chemicals.

Three Poaceae species, winter wheat (*Triticum aestivum*), prairie dropseed (*Sporobolus heterolepis*), and prairie cordgrass (*Spartina pectinata*), were the subject of this research. Winter wheat seed dormancy can be a problem because of the short turn around from harvest to planting in the upper Midwest. New crop wheat seeds need to be planted and exposed to cold temperatures (5°C) for five days to often break dormancy. The other two species, prairie dropseed and prairie cordgrass, do not have any standardized or published testing methods.

To break dormancy in winter wheat, different exposure times (3-day and 5-day) to cold temperatures and growth hormones or chemicals were evaluated for seed crops produced during 2000 and 2001. Chemical treatments used were 1000 ppm and 5000

ppm gibberellic acid, potassium nitrate (first year only), and ethephon (first year only), and de-ionized water as a control. The 3-day pre-chill (95.8% mean germination) worked as well as the 5-day pre-chill (95.8% mean germination). Gibberellic acid at 5000 ppm had the highest mean percent germination (94.2%) of the chemical treatments.

With prairie cordgrass, light and dark, use of growth hormones or chemicals, and various temperature regimes were evaluated to start developing optimum methods for germination testing or reducing dormancy. Results indicated that there appears to be no difference between light and dark treatments. The most promising temperature is an alternating 20-30°C. The chemical of choice would be 0.2% potassium nitrate. Gibberellic acid at 5000 ppm caused abnormalities resulting in stunted root growth.

Prairie dropseed was exposed to a pre-chill treatment, varying germination temperature regimes, and growth hormones or chemicals to break dormancy. The seeds were exposed to a pre-chill (5°C) for two weeks and then planted at various temperature regimes with different growth hormones or chemicals. A set of each sample for each treatment and temperature was placed straight into the germinators to examine the effect of the pre-chill. The chemicals used were potassium nitrate, 500 ppm, 1000 ppm, and 5000 ppm gibberellic acid, 1.0 mM ethephon, and de-ionized water control. The temperature that appears to break dormancy and maintain viability is either 20-30°C or 25°C. The chemical treatment that yielded the lowest dormancy and maintained viability was potassium nitrate or de-ionized water.

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List of Abbreviations

AOSA: Association of Official Seed Analysts

GA_3: Gibberellic acid

H_2O: de-ionized water

KNO_3: Potassium nitrate

PPM: parts per million

TZ: tetrazolium

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Introduction

Seed dormancy is a major impediment in seed testing laboratories, because it prolongs the time from when the sample arrives in the lab to when the results are sent to the client. One of the other problems that dormancy creates in laboratories is that seeds do not germinate, and labs need to rely on additional testing to determine if they are dormant.

Treatments commonly used to break seed dormancy in labs are light or dark, chemicals or growth hormones, various temperature regimes (constant or alternating), and exposure to cold treatments (pre-chilling at 5-10°C). The combination of treatments most commonly used is the exposure to cold and the use of chemicals or growth hormones. Another common combination is various temperature regimes and the use of chemicals or growth hormones.

In the seed testing industry, testing methods have been researched, evaluated, and standardized for several hundred species (crops, flowers, vegetables, trees, etc.), but little is known about many species now being collected. There are hundreds of species being used for re-vegetation purposes or some new use that has been developed. The market for these species is growing fast, making it important for seed testing laboratories to test and accurately report germination or total viability of these species. Some species are easily tested (low dormancy) and others have deep-seated dormancy. Usual testing practices do not yield any germination, thus making it necessary for new research to be conducted.

Another problem seed testing labs face is testing time. Seedsmen, seed companies, and farmers want faster results than in the past. Anything that can speed up the germination test period is sought after by the seed industry. This may mean increased usage of growth hormones/chemicals or the re-evaluation of current testing protocols.

The objective of this experiment was to identify a method to shorten the germination test period of new crop winter wheat. Prairie cordgrass and prairie dropseed dormancy is not understood. This is noticeable by the fact that there are no standardized testing methods. The purpose of this research is to begin the process of determining a standardized testing method for these two species.

Literature Review

Dormancy Defined

The Association of Official Seed Analysts (AOSA) (1999) defines seed dormancy as "viable seeds, other than hard seeds, which fail to germinate when provided the specified germination conditions for the kind of seed in question." Seed dormancy has many advantages and disadvantages. Some advantages of dormancy are that it distributes germination over time and enhances the survival of the species. Dormancy also prevents or reduces the rate of seed deterioration under adverse field conditions. To survive in the soil, dormancy must prevent germination while viability is maintained (Wareing 1982). Some disadvantages of dormancy are the long periods of time needed to overcome it, and the contribution of dormancy to weed seed longevity. Seed dormancy also causes problems or complications in seed testing laboratories.

Another definition of dormancy is the blocking of the germination process within an imbibed seed. The easiest definition of dormancy is "the failure of normally viable seeds to germinate given moisture, suitable temperature, and air necessary for seedling growth" (Murdoch and Ellis 2000). The measure of dormancy can be determined by subtracting the portion of seeds germinated from the seed lot viability, usually determined by a tetrazolium test.

Types of Dormancy

There are two main types of dormancy: primary and secondary. Primary dormancy is dormancy that develops on the mother plant during seed maturation. This type of dormancy may occur because the embryo is immature, and advantageous since it

prevents germination while on the mother plant (Murdoch and Ellis 2000). Primary dormancy can be subdivided into two forms: endogenous and exogenous.

Endogenous dormancy involves some characteristic within the embryo preventing germination. Endogenous dormancy can be further broken into physiological, physiological inhibiting mechanisms of germination, and morphologically underdeveloped embryos (Baskin and Baskin 1998). Endogenous or embryo dormancy is believed to be caused by underdeveloped and undifferentiated embryos, blocking of nucleic acid and protein synthesis, failure to mobilize food reserves of embryo, deficiency in plant growth substances, and the presence of inhibitors such as abscisic acid (Bradbeer 1988).

Exogenous dormancy involves some characteristic within the outer structures, including the endosperm seed coat or fruit walls that prevent the embryo from germinating. Exogenous dormancy can be further classified as physical, chemical, and mechanical. Physical exogenous dormancy may involve the seed coat being impermeable to water or the seed coat being selectively permeable to gases. Chemical dormancy is caused by an inhibitor(s) within the seed that prevents germination. Mechanical dormancy involves woody structures or suspected seed covering structures present that restrict germination or protrusion of organs (Baskin and Baskin 1998).

Secondary dormancy is dormancy that is induced after shedding. Secondary dormancy typically lasts for longer periods than primary dormancy. Secondary dormancy is an induced dormancy that may be caused by a different mechanism than primary dormancy, and is also poorly understood.

According to Baskin and Baskin (1998), there are 12 types of seeds based on the embryo morphology, relative amount of the endosperm, and the position of the embryo in relation to the endosperm. The twelve types are broad, capitate, lateral, peripheral, rudimentary, dwarf, micro, linear, spatulate, investing, bent, and folded. The Poaceae family possesses the lateral seed type, which means the seed is large, contains a large amount of endosperm, and the embryo is small. The lateral seed type occurs only in monocotyledons. Baskin and Baskin (1998) believe that the type of dormancy is based on the type of seed.

Breaking Dormancy

Dormancy can often be broken by a single factor such as chilling, dry storage, elevated temperatures, light, leaching, scarification, and exposure to chemicals, or exposure to fluctuating conditions or two or more of these factors (Bradbeer 1988).

Chilling involves holding imbibed seeds at low temperatures, optimally at 5°C. Chilling (an artificial winter) is a natural mechanism, which ensures germination occurs in the spring under field conditions (Probert 2000). In an experiment involving hazel seeds, the primary effect of chilling was to promote a block of gibberellic acid synthesis that occurs in dormant seeds. An increase in the level of gibberellic acid after chilling was an indicator that dormancy had been released (Wareing 1982). When trying to break dormancy, applying a pre-chill treatment increases the endogenous gibberellic acid activity and therefore stimulates enzyme activity (Gasper, et al. 1975). Zarnstorff et al. (1994) showed that through the use of a wet pre-chill, the short-term dormancy of switchgrass seeds was broken.

Dormancy can also be released through dry storage or under natural conditions that occur during a dry season. Dry storage is the storing of seeds at an average temperature of 15-20°C for one to two months. Working with 10 wheat varieties Ching and Foote (1961) found that dormancy was eliminated after four weeks of dry storage at 38°C with a relative humidity of 15%, while dormancy remained or was re-acquired when the seed was stored at 3°C.

Elevated temperatures involve raising the seed temperature to a level that causes dormancy to be broken. This can also be accomplished by using alternating temperatures. The most common alternating temperatures used in seed testing laboratories are 20-30°C, 15-25°C, and 15-30°C. At an alternating temperature the lower temperature is held for 16 hours and then the higher temperature for 8 hours. This simulates natural daytime-nighttime temperature fluctuations.

There are three physiological processes in the seed that are affected by temperature. First temperature, along with the moisture content, determines the rate of deterioration of the seed. Second temperature affects the rate of dormancy loss in dry seeds, and in moist seeds, temperature changes the pattern of dormancy. Third, in non-dormant seed, temperature determines the rate of germination (Probert 2000).

Light can also play a factor in dormancy and germination. Western wheatgrass (*Pascopyron smithii*) germinates better without light than with light. Many other species germinate better with light. In the case of *Sporobolus cryptandrus*, germination without light and using water as a wetting agent was 10%. Germination with light and water as a wetting agent was 31%. With potassium nitrate, a chemical commonly used in seed

testing laboratories, germination without light versus with light was 15% and 67% respectively (Toole 1941).

Leaching is a method of removing chemicals or hormones from the seed by the addition of water, chemicals, or hormones. Leaching is commonly used when an excess of a hormone or chemical is the cause of dormancy. Sometimes the seed covering can prevent leaching of an inhibitor from the embryo, and the seed coat may need to be made penetrable prior to leaching methods.

Seed scarification usually involves abrasion of the seed coat by rubbing the seeds over a rough surface such as sandpaper. In the case of grasses, seed scarification involves the removal of the glumes or lemma and palea. Switchgrass (*Panicum virgatum*) is the most commonly studied species for scarification. Its outer covering (lemma and palea) is the main cause of dormancy. Jensen and Boe (1991) conducted a study on switchgrass seeds using three treatments of scarification (non-scarified, 15 seconds of scarification, and 30 seconds of scarification) prior to germination testing. Three replications of one hundred seeds were germinated at 15-30°C and 20°C. The germination was higher in the 15-30°C than at 20°C. The germination for no scarification was 5% in the 15-30°C germinator. For 15-second scarification, the germination rate increased to 36%. Compared to the 15-second scarification there was little to no additional germination for the 30-second scarification. The wetting agent used for this experiment was de-ionized water, and the planted seeds were exposed to a two-week pre-chill prior to germination.

Exposure to chemicals is one of the most commonly used methods of breaking dormancy in seed testing laboratories. The most commonly used chemical is potassium nitrate (KNO_3). Most often this is the recommended chemical by the AOSA. No one knows for sure the precise role of potassium nitrate in stimulating seed germination. Some of the thoughts are that it substitutes for light, interacts with temperature, affects the respiratory system directly, acts to stimulate oxygen uptake, or serves as a cofactor of phytochrome (Copeland and McDonald 1995).

Toole (1941) conducted an experiment on the dropseeds, *Sporobolus cryptandrus*, *S. flexuosus, S. contractus, S. giganteus, S. airoides, S. wrightii,* and *S. asper*. The samples were germinated in petri dishes using de-ionized water or 0.2% potassium nitrate as wetting agents. With *S. cryptandrus*, the high constant temperatures gave better germination than the lower temperatures. *S. flexuosus* and *S. giganteus* had poor germination at all temperatures. Potassium nitrate and light also had no effect. Neither the potassium nitrate wetting agent nor the two-week pre-chill provided any increase in germination in *S. airoides*. *S. contractus* had poor germination at all temperatures and potassium nitrate was only of some benefit.

Another hormone used to aid in breaking dormancy is gibberellic acid (GA_3). Gibberellins regulate protein synthesis, stem elongation, and plant growth. High concentrations of gibberellic acid cause a rapid increase in germination of seeds because it overcomes dormancy. For seed germination, 2000 parts per million (ppm) gibberellic acid is sufficient (Riley 1987).

The breakdown of starch is assumed to be the essential process of germination. The gibberellic acid enhances the synthesis of hydrolases in the endosperm of cereal grains. This implies that gibberellic acid via amylase synthesis stimulates seed germination (Chen and Park 1973). During seed maturation there is a drop in the levels of free gibberellic acid in *Hordeum vulgare* and *Triticum aestivum*, suggesting that gibberellic acid is necessary for germination (Khan 1982).

Wareing (1982) discussed an experiment involving hazel and beechnut seeds, where the levels of gibberellic acid were measured. At the end of a two-week pre-chilling period, the seeds had low levels of gibberellic acid, while seeds that were not pre-chilled had no detectable level of gibberellic acid. Pre-chilled seeds were transferred to a warm condition of 20°C the level of gibberellins increased even more.

Gaspar et al. (1975) conducted an experiment using spring and winter wheat by soaking seeds in solutions containing 0, 10, 50, 100, 200, 400, and 800 ppm GA_3 for 16 and 20 hours to try and break seed dormancy. Initially, the higher concentrations were needed to break dormancy. Lower concentrations of gibberellic acid are needed following after-ripening of the grain. For barley (*Hordeum vulgare*) seed dormancy was broken at 1000-ppm gibberellic acid concentration. The germination test period of freshly harvested barley could be shortened by the use of a gibberellic acid soak or wetting agent, as opposed to pre-chilling, which takes longer.

Gibberellic acid also is effective in breaking dormancy in weed seeds. Holm and Miller (1972) studied eleven species of weed seed germination using gibberellic acid. Nine out of eleven species had a consistent increase in germination when treated with

10^{-6} and 10^{-5} M gibberellic acid.

An experiment conducted on bladderpod (*Lesquerella fendler*) determined what effect gibberellic acid had on seeds and seedlings. The germination rate increased 26% when using GA_3. The use of gibberellic acid caused an increase in the seedling height but at the expense of the leaves, which were lesser in number and narrower. The effects appear to be long lasting considering the short amount of exposure time. The difference in traits that were present at 45 days were still present at 115 days (Evans, et al. 1996).

Ethylene is another growth hormone/chemical used. Ethylene is synthesized in seeds and is involved in radicle elongation and increases the germination rate of after-ripened embryos (Feghahati and Reese 1994). Ethylene is also effective in breaking seed coat imposed dormancy. Ethylene has also been proven to overcome secondary dormancy in lettuce and celery (Wareing 1982).

In purple coneflower (*Echinacea angustifolia*) 1.0 mM ethephon was used as a wetting agent, and the seeds were pre-chilled for two weeks at 5°C. They were then transferred to a 25°C chamber for an additional two weeks. The germination after the two additional weeks was 80%. The control using de-ionized water only produced a germination rate of only 15% (Feghahati and Reese 1994).

Ethylene already exists in peanuts at a low rate, and as dormancy is released, the rate of ethylene production increases. The natural release of dormancy is dependent upon the ability of the peanut seeds to increase the production of ethylene. The raising of the internal level of the ethylene concentration to a threshold value necessary for germination aids in the natural release of dormancy (Wareing 1982).

Another treatment that has been used to aid in increasing germination rates and decreasing dormancy is fusicoccin, a fungal toxin. Fusicoccin acts as a "super auxin". It causes coleoptile sections to grow and excrete hydrogen ions at a faster rate. This is important in cell elongation (Moore 1989).

The Grass Family

The Poaceae or grass family contributes the most to the prairie plant biomass. The humus from the decay of the root system is responsible for the rich, black color of the grassland soil.

Grasses are either sod forming or bunch-forming species. The sod forming species reproduce sexually by seed and asexually from rhizomes. Rhizomes extend a few inches to a few feet from the parent plant, resulting in a dense stand. Prairie cordgrass (*Spartina pectinata*) and switchgrass (*Panicum virgatum*) are two examples of sod forming grasses. Bunch forming species have an erect growth of tillers or shoots at the base and reproduce both sexually and asexually. Little bluestem (*Schizachyrium scoparium*) and prairie dropseed (*Sporobolus heterolepis*) are two examples of bunch forming grasses.

Beyond the taxonomic classification, the grasses are also grouped according to their climatic origin. They are classified as either cool season or warm season grasses. Cool season grasses renew their growth in the early spring and mature from April to early July. They become semi-dormant in the hot summer months and renew growth in the autumn. Examples of cool season grasses are needlegrass (*Hesperostipa spartea*), blue joint grass (*Calamagrostis canadensis*), and winter wheat (*Triticum aestivum*).

Examples of warm season grasses are big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), prairie cordgrass, and prairie dropseed. Warm season grasses grow through late spring and early fall and mature during the mid fall (Kirt 1995).

Species Utilized in This Study

Three grasses (Poaceae) were used in this research. Winter wheat is a winter annual cereal that is used to make flour for breads. Wheat was first planted in the United States in 1777 as a hobby crop. Wheat is one of the most widely grown small grain crops in the United States. It is grown in 42 states in the United States. About 50% of the wheat produced in the U.S. is exported each year, making us the largest exporter (Wheat Facts 2001). Winter wheat dormancy can be a major problem at harvest but it is typically lost before the seed is re-sown. The seed dormancy of winter wheat is greatly modified by environmental conditions during ripening of the seed on the parent plant (Simpson 1990). *Triticum* dormancy is a non-deep dormancy, since it usually can be broken by a 5-day period of cold stratification (Baskin and Baskin 2000).

Prairie cordgrass, also known as slough grass or ripgut, is found in wet prairies, prairie marshes, and shores and streams in upland prairies (Ladd 1995). It can be found in 41 of the 51 states in the United States. It is found from Washington and Oregon and south to North Carolina, Arkansas, Texas, and New Mexico. Prairie cordgrass is an indicator of land with a high water content and poor soil aeration. At the same time, cordgrass protects the soil against erosion and rushing floodwaters (Weaver 1968). Prairie cordgrass is a warm season grass that has branched rhizomes. The plant grows 1-

2.5 meters tall and has long, thick coarse leaves. The plant flowers from mid July to August (Kirt 1995). Prairie cordgrass has been used as thatching for roofs and as a fuel source. Prairie cordgrass makes suitable hay if it is cut before the stems are mature.

Prairie dropseed is found on hills in prairies and rocky prairie slopes. It can be found from Wyoming and Colorado east to Connecticut and Massachusetts and south to Texas. Dropseed is a bunch grass that grows in fountain looking tufts, 10-20 cm in diameter. Prairie dropseed is a warm season grass that indicated virgin prairie conditions. The height of the plant is 0.6-1.2 m. The plant flowers in August and has a fragrant aroma while flowering (Kirt 1995). The seeds mature in September. Dropseed can dominate local areas by forming 80% or more of the vegetation (Weaver 1968). Dropseed makes excellent grazing forage during the spring and summer months. Native Americans used the seeds as flour for food (Ladd 1995).

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Materials and Methods

Winter Wheat

Fifty samples of winter wheat seed were obtained from South Dakota certified seed growers from the 2000 and 2001 harvests. Samples from certified seed growers were used to be sure of varietal purity. The samples were stored at -12°C from the time received in the lab until planted to maintain dormancy.

Pure seed units, as defined by AOSA Rules for Testing Seeds (1999), were obtained from each sample. The pure seed unit consisted of the caryopsis. Each sample was then planted on moistened germination paper towels free of any dyes or chemicals that may interfere with germination. Each sample was planted in four replications of 100 seeds. Seeds were planted on two bottom towels and covered with one towel on top. The towels were rolled up, placed in germination containers (dye and chemical free plastic bags), and germinated at a temperature of 20°C. The samples that were pre-chilled for 3 or 5-days were exposed to a temperature of 5°C. Germination counts were made following 7 days at 20°C. Evaluation of normal seedlings was conducted according to the AOSA Rules for Testing Seeds (1999). A normal seedling is one that has all the essential structures (roots, coleoptile, etc.) necessary for germination. A tetrazolium (TZ) test was conducted on the ungerminated seeds to determine viability. The methods described in the AOSA Tetrazolium Handbook (2000) were followed.

The control samples were planted using de-ionized water as the wetting agent and germinated for 7 days at 20°C. The standard testing method described in the AOSA

Rules for Testing Seeds, for freshly harvested wheat seeds, is a 5-day pre-chill at 5°C followed by 7-day germination at 20°C with de-ionized water as a wetting agent.

Other treatments used were 1) 3-day pre-chill (5°C) and 7 day germination (20°C); 2) in place of the pre-chill treatments, chemicals used as wetting agents were 1000 parts per million (ppm) gibberellic acid, 5000 ppm gibberellic acid, 0.2% potassium nitrate (KNO_3), and 1.0 mM ethephon. Samples subjected to chemicals were germinated for 7 days at 20°C.

For the 2001 harvest the same procedures were followed except the 0.2% potassium nitrate and 1.0 mM ethephon wetting agents were dropped, as in 2000 they did not prove to be promising for breaking dormancy.

Prairie Cordgrass

There are no published or recommended procedures for the germination of prairie cordgrass. Samples of prairie cordgrass were obtained from USDA-NRCS Plant Materials Center in North Dakota for 1994 and 1996 harvests. The samples were stored at -12°C since they were received. The samples were cleaned and pure seeds were obtained according to the AOSA Rules for Testing Seeds (1999). The pure seed unit includes the caryopsis, lemma, and palea. Seeds were counted out in sets of 50. The treatments used were four wetting agents x ten temperature regime combinations on a thermogradient plate.

One replication of 50 seeds for each treatment and temperature were placed in a 12 cm x 12 cm plastic germination box. Two blotters soaked with various wetting agents were placed on the lid as the planting medium. The wetting agents used were 0.2%

potassium nitrate, 1000-ppm gibberellic acid, and 5000-ppm gibberellic acid. Seeds were placed on top of the wet blotters and the box bottom was placed on the lid. The samples were pre-chilled at 5°C for 14-days then transferred to a thermogradient plate for a 21-day germination period.

The thermogradient plate (Figures 1 and 2) is a square special aluminum alloy lined box that measures 76 cm x 76 cm. A plastic lid closes over the top of the box. The plate has a range of alternating temperatures when connected to circulating water baths. The coolest area was the top left corner and warmest area was the bottom right corner. Temperatures across the rest of the plate varied from left to right and top to bottom. The temperature was controlled through the use of a warm and cold water bath. The cold water bath was a 1:1 mixture of ethylene based antifreeze and water, with the temperature set at 4°C. The warm water bath consisted of de-ionized water, with the temperature set at 40°C. A 24-hour cycle timer system was used to create alternating temperature regimes by changing the flow direction of the baths. The timer system was set on an eight and sixteen hour cycle to simulate day and night temperature fluctuations.

Ten temperatures used routinely in seed testing labs and that had proven to be successful in a previous undergraduate experiment were selected on the thermogradient plate. They were 24-17°C, 26-18°C, 31-19°C, 33-22°C, 31-21°C, 23-21°C, 19-24°C, 26-26°C, 21-29°C, and 33-30°C.

The thermogradient plate was covered with aluminum foil to seal out the light. This was done to determine if light was an influence in germination and breaking dormancy.

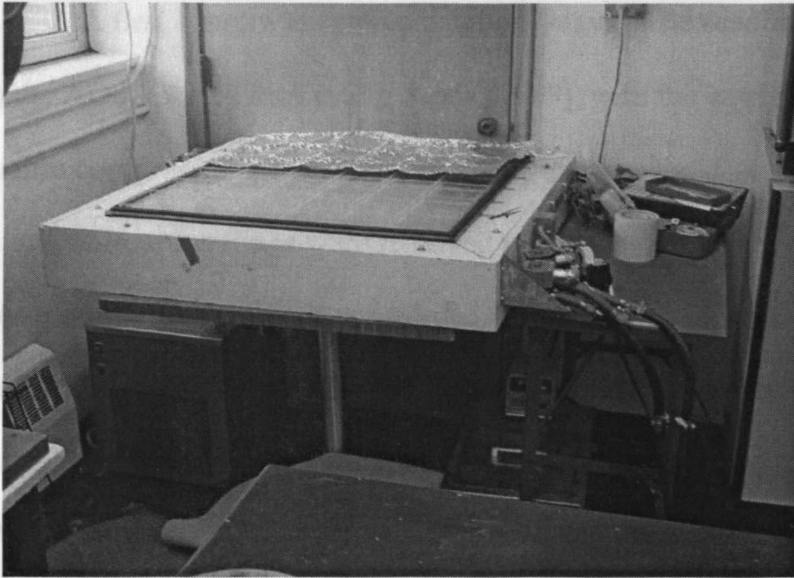

Figure 1. Exterior of the thermogradient plate.

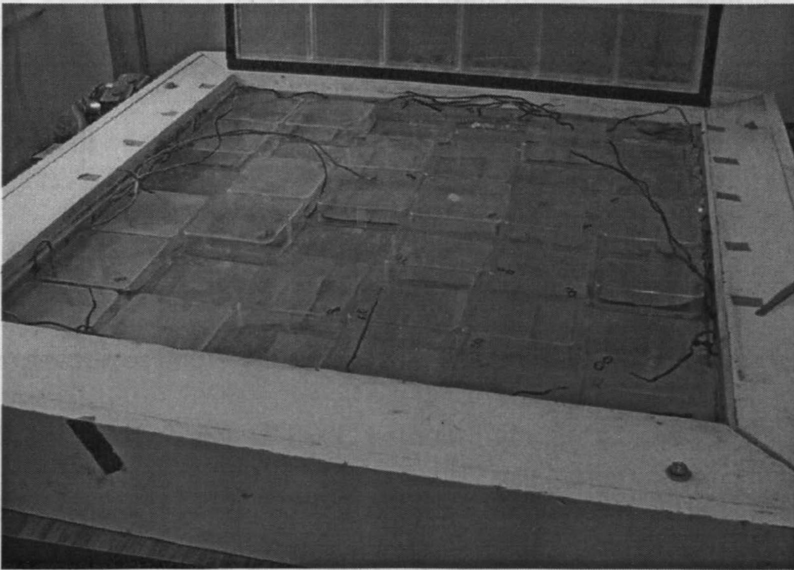

Figure 2. Interior of the thermogradient plate.

A first count was conducted after 7-days. The seedlings were evaluated according to the AOSA Rules for Testing Seeds (1999), with the exception of the roots. Roots of prairie cordgrass do not grow well on artificial media. Every other day, after the first count, normal seedlings were removed and the blotters were remoistened with de-ionized water until a final count was recorded at 21-days. A TZ test was conducted on the ungerminated seeds to determine if they were dead or dormant. The evaluation of the seeds was conducted according to the AOSA Tetrazolium Testing Handbook (2000).

Prairie Dropseed

Prairie dropseed has no published or recommended testing procedures. Samples of prairie dropseed were obtained from SDSU Seed Lab customers out of Minnesota. The samples were stored at -12°C until needed. The samples were cleaned and pure seed units were obtained according to the AOSA Rules for Testing Seeds (1999). The pure seed unit consists of the caryopsis, lemma, palea, and sometimes the glumes.

Two replications of 100 seeds were planted on top of two blotter papers inside a 12 cm x 12 cm plastic germination box. The samples were then exposed to various temperatures and wetting agents. The temperature regimes used were 15-25°C, 20-30°C, 15-30°C with light, 15-30°C without light, 20°C, and 25°C. Two replications of 100 seeds were placed directly into each germinator at its specific temperature for 14-days. Another two replications of 100 seeds were pre-chilled at 5°C for 14 days before being placed in the various germination chambers for an additional 14-days. The wetting agents used were 0.2% potassium nitrate, 500, 1000, and 5000-ppm gibberellic acid, and 1.0 mM ethephon, with de-ionized water as a control.

After seven days a first count was made. After the fourteenth day of the germination period, a final count was conducted. The seedlings were evaluated according to the AOSA Rules for Testing Seeds (1999), with the exception of the roots. Roots of prairie dropseed do not grow well on artificial media. A TZ test was conducted on the ungerminated seeds to determine viability, according to the AOSA Tetrazolium Handbook (2000).

The statistical analysis for the winter wheat and prairie dropseed was conducted using SAS and the Proc ANOVA command. For prairie cordgrass, the analysis was conducted using Excel spreadsheet and the ANOVA two way analysis command. For all analysis the p value = 0.05.

Results and Discussion

Winter Wheat

Data for 2000 and 2001 harvest were analyzed separately. For 2000, mean germination percentages for each replication using different chemical treatments are presented in Table 1. Mean germination percentages ranged from 54% (#3439 no pre-chill) to 99% (#3382 no pre-chill). Mean dormancy percentage ranged from 0% to 39% (#3439 no pre-chill).

Viability is determined by adding the percentage of dormancy to the percentage of seeds that germinated. Viability was maintained throughout the experiment for 2000. The highest viability was 96.6% (ethephon). The lowest viability was 95.2% (1000 ppm GA_3). The difference of 1.4% was not significant to the seed testing industry.

Of the fifty samples, twenty-four were used for statistical analysis. The others had a mean germination percentage higher than 90% for the no pre-chill, 7-day germination control. Dormancy, at 10% or less, was an acceptable level for planting purposes and determining seeding rates.

Table 2 summarizes the information for all treatments. The no pre-chill control, 7-day germination at 20°C followed by a TZ test on ungerminated seeds, was used to determine if dormancy existed in the sample. The mean dormancy across all samples was 17.2% and mean germination was 78.7%. There was a significant ($P<0.05$) difference between the no pre-chill versus the current standard of a 5-day pre-chill. Mean percentage of dormancy for the 5-day pre-chill treatment was 0.4%. Mean percentage of germination for this treatment was 95.8%. The mean viability was 96.3%.

Table 1. 2000 Winter wheat mean percentage of germination and dormancy of four replications for each treatment

Sample Number	No	Prechill	3 Day	Pre-chill	5 Day	Pre-chill	KNO		1000 GA		5000GA		Ethephon	
	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm
3338	84	13	95	0	96	0	92	7	88	1	94	1	86	11
3339	77	19	97	0	97	0	84	10	95	0	96	0	84	14
3340	83	16	95	0	96	0	89	9	90	5	95	0	82	15
3341	77	18	96	0	95	0	88	5	95	0	95	0	91	6
3342	82	13	98	0	96	0	87	8	92	3	94	0	84	12
3343	81	15	95	1	98	0	90	7	91	5	95	0	87	10
3344	97	0	97	0	97	0	99	0	97	0	95	0	98	0
3345	95	1	96	0	97	0	97	1	96	0	95	0	96	1
3346	96	1	98	0	95	0	96	0	95	0	95	0	98	0
3347	93	2	90	0	93	0	95	1	89	2	89	0	89	4
3348	93	2	95	0	96	0	93	1	94	0	94	0	95	1
3349	93	2	92	1	95	0	95	1	90	3	96	0	90	7
3350	64	31	96	0	93	3	80	15	84	11	87	7	73	22
3351	62	31	91	4	87	2	78	14	83	11	87	6	69	26
3352	76	11	92	0	94	0	88	3	87	2	88	0	85	8
3354	79	16	95	0	95	0	89	6	93	1	92	1	86	7
3357	91	5	95	0	94	0	92	3	93	1	96	0	91	2
3358	95	4	98	0	98	0	97	1	98	0	98	0	96	2
3359	81	16	97	0	97	0	90	7	94	2	97	0	91	6
3360	83	13	96	0	93	2	92	3	95	2	95	0	85	11
3361	83	14	97	0	97	0	91	3	94	0	96	0	87	8
3362	86	8	96	1	97	0	94	1	94	1	94	0	92	4
3363	97	1	98	0	98	0	97	0	98	0	98	0	97	0
3364	97	1	98	0	99	0	98	0	99	0	98	0	94	3
3365	93	5	98	0	98	0	98	0	97	0	97	0	94	3
3366	75	20	96	0	97	0	93	2	92	1	93	1	91	5
3377	78	19	99	0	98	0	93	5	97	1	96	0	89	10
3378	73	24	97	0	97	0	89	9	95	2	97	0	85	14
3379	80	18	98	0	98	0	93	5	95	2	97	0	85	14
3380	90	9	98	0	99	0	96	2	95	1	97	0	84	15
3381	92	6	99	0	99	0	96	2	98	0	99	0	92	6
3382	99	0	98	0	98	0	99	0	98	0	99	0	98	0
3383	91	7	99	0	98	0	98	0	98	0	98	0	98	1
3384	84	12	99	0	99	0	95	3	97	0	98	0	93	6
3385	85	9	88	0	90	0	85	4	89	0	90	0	81	10
3438	79	16	97	0	97	0	96	2	94	3	95	0	92	6
3439	54	39	91	0	92	0	74	15	82	10	90	3	74	20
3440	86	10	96	0	96	0	95	0	91	1	93	1	91	6
3441	97	2	98	0	98	0	98	0	98	0	98	0	98	0
3442	94	1	94	0	95	0	91	0	93	0	94	0	94	0
3444	92	5	95	0	97	0	93	0	94	1	95	0	91	2
3445	89	2	93	0	93	0	93	0	93	0	94	0	92	2
3446	97	0	96	0	96	0	97	0	96	0	98	0	96	1
3447	98	0	99	0	99	0	98	0	98	0	99	0	98	0
3458	97	0	98	0	98	0	98	0	97	0	98	0	97	0
3462	97	1	98	0	99	0	99	0	98	0	98	0	98	0
3463	97	0	98	0	98	0	97	0	97	0	96	0	97	0
3464	95	1	97	0	97	0	96	0	95	1	95	0	94	2
3465	97	0	98	0	98	0	98	0	96	0	96	0	96	0
3466	98	0	98	0	99	0	97	0	98	0	98	0	97	0

Table 2. Summary Analysis using Duncan's Test (alpha=0.05) for winter wheat 2000 crop.

Treatment	Mean Germination[I]	Mean Dormancy[I]	Mean Viability[I]
No Pre-chill	78.7 e	17.2 a	95.9 a, b, c
3 day Pre-chill	95.8 a	0.4 e	96.2 a, b
5 day Pre-chill	95.8 a	0.4 e	96.3 a, b
1000 ppm GA_3	92.2 b	3.0 d	95.2 c
5000 ppm GA_3	94.2 a	1.1 e	95.3 c
KNO_3	89.5 c	6.2 c	95.7 b, c
Ethephon	85.9 d	10.9 b	96.6 a

[I] Treatments with the same letter are not significantly different at the alpha 0.05 level

The mean germination for the 3-day pre-chill was 95.8%. Less than a 0.1% difference was found between the 3-day and 5-day pre-chill treatments. This was not significantly different at the 0.05 level. The mean dormancy was 0.4%. This was not significant from the 5 day pre-chill (0.4%).

Gibberellic acid at 5000 ppm was not significantly different at the 0.05 level from the 5 or 3-day pre-chill. Mean germination for this treatment was 94.2% and mean dormancy was 1.1%. At a rate of 1000 ppm, germination was significantly different ($p<0.05$) from the 5-day pre-chill, 3-day pre-chill, and the rate of 5000 ppm gibberellic acid. The mean percentage germination was 92.2%. The mean percentage dormancy was 3.0%.

Potassium nitrate was significantly different from all other treatments, as demonstrated by lower germination rates and higher rates of dormancy. The mean germination for the KNO_3 was 89.5%, while mean dormancy was 6.2%. The ethephon

Table 3. 2001 Winter Wheat Mean Percentage Germination and Dormancy of the four replications for each treatment.

Sample Number	No Pre-chill		3 Day Pre-chill		5 Day Pre-chill		1000 GA		5000 GA	
	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm	Germ	Dorm
4815	88	7	98	0	96	0	97	0	98	0
4816	72	24	96	0	96	0	92	4	92	2
4823	74	23	96	0	98	0	96	0	95	0
4824	50	44	94	0	94	0	81	7	88	1
4825	73	20	95	0	93	0	89	3	93	0
4826	65	29	90	0	92	0	89	5	87	0
4828	57	37	90	1	90	0	86	4	91	1
4829	55	40	93	0	93	0	87	9	92	3
4833	78	13	89	0	88	0	92	1	92	0
4834	89	7	95	0	94	0	94	2	95	0
4839	67	24	94	0	95	0	90	4	86	5
4840	48	47	96	1	94	0	81	14	80	9
4841	56	33	93	1	89	0	86	5	82	5
4842	61	32	95	0	93	0	90	5	92	3
4843	72	15	87	0	84	0	86	4	86	0
4848	94	2	96	0	96	0	96	0	96	0
4849	64	29	91	0	90	0	85	2	91	0
4850	51	41	90	0	91	0	84	7	87	0
4851	60	24	87	0	85	0	79	2	82	0
4852	60	24	86	0	83	0	85	1	80	6
4854	81	14	96	0	94	0	93	1	93	0
4855	75	20	93	0	95	0	91	1	95	0
4856	63	15	86	0	85	0	82	2	85	0
4857	70	16	89	0	88	0	78	3	85	0
4859	63	31	93	0	93	0	87	3	95	0
4860	63	27	91	0	90	0	85	4	91	0
4861	63	27	91	0	89	0	88	3	91	0
4862	80	10	94	0	95	0	93	0	92	0
4865	78	16	97	0	95	0	89	4	96	0
4866	83	16	99	0	98	0	97	0	96	1
4867	89	9	98	0	98	0	97	0	98	0
4869	62	20	83	0	79	0	79	2	80	0
4870	95	2	96	0	98	0	98	0	98	0
4872	93	0	95	0	92	0	94	0	93	0
4876	97	1	98	0	98	0	98	0	99	0
4880	94	4	98	0	99	0	97	1	99	0
4881	93	5	98	0	99	0	98	0	97	0
4882	96	2	98	0	98	0	97	0	98	0
4884	94	3	98	0	98	0	98	0	97	0
4885	72	11	87	0	82	0	83	0	81	0
4898	97	0	99	0	98	0	98	0	98	0
4903	95	3	98	0	98	0	97	0	97	0
4904	67	24	90	0	95	0	92	0	92	0
4908	77	19	95	0	94	0	91	0	97	0
4912	89	0	90	0	86	0	87	0	86	0
4914	81	4	92	0	86	0	89	0	88	0
4915	85	5	89	0	90	0	86	0	88	0
4916	96	0	96	0	96	0	97	0	94	0
4917	98	1	97	0	97	0	95	0	96	0
4918	94	1	96	0	94	0	94	0	93	0

treatment was also significantly different at the 0.05 level from all other treatments. It gave the lowest mean germination of 85.9% and mean dormancy of 10.9%.

Due to the fact that potassium nitrate and ethephon treatments in 2000 were significantly different (lower germination and dormancy), they were not used as chemical treatments with the 2001 study. The 2001 mean germination and dormancy percentages for each sample at each treatment is presented in Table 3. The mean percentage germination ranged from 48% (#4840 no pre-chill) to 99%. The mean percentage dormancy ranged from 0% to 47% (#4840 no pre-chill).

The highest mean viability across all samples was 92.6% (3 day pre-chill). The lowest mean percentage of viability was 91.2% (5000 ppm GA_3). This is a difference of 1.4%, which is not significant in the seed testing industry. The goal of maintaining viability for the year 2001 was achieved.

Of the fifty samples, thirty-seven were used for statistical analysis in 2001. The other thirteen had germinations above 90% with the no pre-chill control and were dropped from the study. The no pre-chill control across all samples had a mean germination of 70.2% and mean dormancy of 22.0%. The mean viability was 92.2%.

The standard testing procedure of a 5-day pre-chill had a mean germination of 91.4% and 0% dormancy. In comparison, the 3-day pre-chill had a mean germination of 92.4% and mean dormancy of 0.2%. At the $p = 0.05$ level, there is no significant difference between the 3 and 5-day pre-chill (Table 4).

Table 4. 2001 Winter wheat Summary Analysis Using Duncan's Test

Treatment	Mean Germination[1]	Mean Dormancy[1]	Mean Viability[1]
No Pre-chill	70.2 d	22.0 a	92.2 a, b
3 day Pre-chill	92.4 a	0.2 c	92.6 a
5 day Pre-chill	91.4 a, b	0.0 c	91.4 a, b
1000 ppm GA_3	88.2 c	3.3 b	91.5 a, b
5000 ppm GA_3	90.2 b	1.1 c	91.2 b

[1] Treatments with the same letter are not significantly different at the 0.05 level.

The mean germination for the 5000-ppm gibberellic acid treatment was 90.2%. It had a mean dormancy of 1.1%. This treatment was not significantly different at the 0.05 level from the 3 and 5-day pre-chills. For the 1000-ppm gibberellic acid treatment mean germination was 88.2% and mean dormancy was 3.3%. At the 0.05 level, 1000-ppm gibberellic acid was significantly different from all the other treatments.

A combined statistical analysis was conducted for the two years and summarized in Table 5. This showed that there was no significant difference between the 3 and 5-day pre-chill. There is a significant difference between the 5000 ppm GA_3 and the 3 and 5-day pre-chill. For 5000 ppm GA_3, the mean germination was 91.7%. This was compared to 93.7% (3-day) and 93.2% (5-day).

The use of gibberellic acid as a substitute for the 5-day pre-chill was effective. Unfortunately, it is not economically feasible as twenty-five grams of gibberellic acid costs around $100. Twenty-five grams would be enough to test approximately 40 samples, adding $2.50 to the test fee just for the chemical alone.

Table 5. Combined years analysis of winter wheat using Duncan's Test (0.05 level)

Treatment	Mean Germination[1]	Mean Dormancy[1]	Mean Viability[1]
No Pre-chill	73.5 d	20.1 a	93.7 a, b
3-day Pre-chill	93.7 a	0.3 c	94.0 a
5-day Pre-chill	93.2 a	0.2 c	93.3 a, b
1000 ppm GA_3	89.7 c	3.2 b	93.0 b
5000 ppm GA_3	91.7 b	1.1 c	92.8 b

[1] Treatments with the same letter are not significantly different at the 0.05 level.

Something else to consider in the future is what affects wheat seed dormancy. Records showed the varieties used and where they were grown for each sample. This could allow someone to go back and evaluate if the samples that were high in dormancy were specific to a variety or a specific area. This will help determine whether dormancy was due to environmental factors, varietal factors, or some combination of factors.

Another area that could be examined closer is if the number of pre-chill days could be reduced even more in further tests. It has been shown that there is no significant difference between the 3 and 5-day pre-chill. Is there a significant difference between 3-day pre-chill and a 1 or 2-day pre-chill?

With the chemical treatments, the use of fusicoccin as a wetting agent should have been looked at also. Maybe this chemical would be even more successful at breaking dormancy and increasing germination than gibberellic acid at a rate of 5000 ppm. This chemical may also be more economically feasible.

Prairie Cordgrass

There are no standardized or published testing methods for prairie cordgrass. Due to dormancy, prairie cordgrass does not usually have a high germination rate. The mean germination and dormancy for prairie cordgrass with light and without light for each chemical and treatment is presented in Tables 6 and 7. The lowest mean germination for all treatments was 0% and the highest mean germination was 61%.

The temperature that exhibited the highest germination rate was 21-29°C at a mean of 61%. This temperature most closely resembled widely used laboratory germination temperatures of 20-30°C. Three of the six different treatments exhibited their highest germination at this alternating temperature.

This can be reinforced by a prairie cordgrass referee that was conducted by the South Dakota State University Seed Lab (1999). Eight different laboratories, that are members of the Seed Analysts of the Midwest (SAM), were sent three different lots of prairie cordgrass to germinate at 20°C and 20-30°C. The three lots had mean germinations of 4%, 17%, and 5% at 20°C. At 20-30°C, the three lots had mean germinations of 9%, 37%, and 20%. The percentage of dormant seeds was also lower at 20-30°C than at 20°C. The mean percentage of dormancy at 20°C, for the three lots, was 5%, 13%, and 13%. For 20-30°C, the mean percentage of dormancy was 1%, 2%, and 2%.

Table 6. Mean germination and dormancy percentages of prairie cordgrass in the presence of light

Temperature	Chemical	Mean Germ	Mean Dormancy
24-17°C	KNO_3	52	0
	1000 ppm GA_3	46	0
	5000 ppm GA_3	20	2
26-17°C	KNO_3	50	0
	1000 ppm GA_3	50	0
	5000 ppm GA_3	18	0
31-19°C	KNO_3	38	0
	1000 ppm GA_3	34	2
	5000 ppm GA_3	2	2
33-22°C	KNO_3	34	0
	1000 ppm GA_3	38	0
	5000 ppm GA_3	0	0
31-21°C	KNO_3	50	0
	1000 ppm GA_3	60	0
	5000 ppm GA_3	2	0
23-21°C	KNO_3	20	20
	1000 ppm GA_3	14	42
	5000 ppm GA_3	0	42
19-24°C	KNO_3	46	0
	1000 ppm GA_3	48	0
	5000 ppm GA_3	28	0
26-26°C	KNO_3	38	2
	1000 ppm GA_3	36	6
	5000 ppm GA_3	12	12
21-29°C	KNO_3	60	0
	1000 ppm GA_3	50	0
	5000 ppm GA_3	16	0
33-30°C	KNO_3	22	0
	1000 ppm GA_3	24	4
	5000 ppm GA_3	0	0

Table 7. Mean germination and dormancy percentages of prairie cordgrass without light.

Temperature	Chemical	Mean Germ	Mean Dormancy
24-17°C	KNO_3	46	2
	1000 ppm GA_3	42	2
	5000 ppm GA_3	18	0
26-17°C	KNO_3	54	0
	1000 ppm GA_3	50	0
	5000 ppm GA_3	14	0
31-19°C	KNO_3	32	0
	1000 ppm GA_3	34	0
	5000 ppm GA_3	0	0
33-22°C	KNO_3	26	0
	1000 ppm GA_3	22	0
	5000 ppm GA_3	0	0
31-21°C	KNO_3	48	0
	1000 ppm GA_3	28	0
	5000 ppm GA_3	4	0
23-21°C	KNO_3	6	48
	1000 ppm GA_3	14	38
	5000 ppm GA_3	0	30
19-24°C	KNO_3	52	0
	1000 ppm GA_3	36	0
	5000 ppm GA_3	16	0
26-26°C	KNO_3	48	4
	1000 ppm GA_3	40	6
	5000 ppm GA_3	6	4
21-29°C	KNO_3	58	0
	1000 ppm GA_3	60	0
	5000 ppm GA_3	14	0
33-30°C	KNO_3	18	2
	1000 ppm GA_3	10	4
	5000 ppm GA_3	0	0

In this study, the chemical treatment that appeared to most stimulate or promote germination and decrease dormancy was potassium nitrate (KNO_3). Of the ten different temperature regimes, seven had the highest percentage of germination with potassium nitrate. The other three had the highest percentage of germination with 1000-ppm gibberellic acid. The mean germination for all ten temperature regimes was at 40% with KNO_3. With 1000 ppm GA_3, the mean germination percentage for all ten temperature regimes was 40%. The mean dormancy, for all ten temperature regimes, with KNO_3 was 4%. With 1000-ppm gibberellic acid, the mean dormancy was 5%.

Out of the ten temperature regimes, six had higher percentage germination with light versus three that had higher percentage germination without light. The temperature of 21-29°C with light and without light had the highest germination at 61%. It appears that temperatures are a more important and deciding factor than light in the germination of prairie cordgrass when using KNO_3 as a wetting agent.

The rate of 5000-ppm gibberellic acid proved to be too strong for the prairie cordgrass, as it caused seedling abnormalities exhibited by stunted root growth. The shoots were of sufficient growth to be counted as normal seedlings but the root was absent or less than 1/8" long.

The root growth, for prairie cordgrass, is slower than the shoot growth. The roots for most of the samples were only about 1/8" long and some roots appeared to be bound up in the seed coat occasionally. This needs to be taken into consideration when determining normal seedlings. The 5000-ppm treatment resulting in even shorter root growth than normal.

Another problem that occurred with the prairie cordgrass is that mold became a problem. Mold growth took over the samples. This inhibited the growth of the seedlings. This may have caused some of the abnormal seedlings. The mold makes the examination of normal seedlings harder.

This experiment needs to be examined closer. Only two different seed lots were obtained from the same source. To be more accurate additional samples from different areas need to be obtained. This would particularly hold true if the environment influences the dormancy. Secondly, more than one replication of each sample, with each treatment and temperature, needs to be conducted. Due to time limitations only one replication of each treatment was completed. To treat more lots and treatments a two-year study period would be minimal. This would then allow a more complete statistical analysis to be conducted. An initial conclusion, with what has been completed, would be to use potassium nitrate as a wetting agent, and to germinate the seeds at the alternating temperature of 20-30°C with light. At present there are no standardized or published methods for obtaining optimum germination of prairie cordgrass.

Another treatment that could have been tried is using fuscicoccin. Fuscicoccin can aid in breaking dormancy. An experiment with *Spartina alterniflora* (Plyer and Carrick 1993) was conducted to determine the effect of fuscicoccin on dormancy. Fuscicoccin at the rate of 10 mg/Liter was applied to a petri dish containing the seeds. In the control, the germination of the *S. alterniflora* had dormancy and no germination during the incubation period. The germination rate using fuscicoccin as a wetting agent

was 88%. In seeds that had the scutellum punctured and used fuscicoccin the germination rate was 100%.

Prairie Dropseed

There are no standardized or published testing methods for prairie dropseed. The first step was to determine whether a two-week pre-chill would be beneficial in breaking dormancy. The F value is 101.41 and the p value is 0.0001, indicating that there is a significant difference between a two-week pre-chill and a no pre-chill for breaking dormancy.

The mean germination for no pre-chill was 10.6%, and the mean dormancy was 6.7%. For the two week pre-chill, the mean germination was 11.9%, and the mean dormancy was 3.4%. At the 0.05 level, there was a significant difference between no pre-chill and the two-week pre-chill. The mean germination and dormancy for with pre-chill and without pre-chill are presented in Tables 8 and 9.

The next step was to determine the optimum germination temperature. The mean germination and dormancy based on temperature is presented in Tables 10 and 11. For no pre-chill, the highest mean germination was 12.0% (15-25°C). The lowest mean germination percentage was 8.6% (15-30°C without light). The highest mean dormancy was 8.5% (20°C), and the lowest mean dormancy was 5% (25°C). The highest mean germination for pre-chill was 14.0% (15-25°C), and the lowest mean germination was 10.2% (15-30°C without light). The highest mean dormancy was 4.3% (15-30°C without light), and the lowest mean dormancy was 2.0% (25°C).

Table 8. Mean germination and dormancy of prairie dropseed with no pre-chill.

Temperature	Treatment	Sample No.	Mean Germ	Mean Dormancy	Sample No.	Mean Germ	Mean Dormancy
20	KNO_3	6123	10	10	6124	10	8
20	Water	6123	7	7	6124	14	10
20	500 ppm GA_3	6123	8	8	6124	10	8
20	1000 ppm GA_3	6123	10	8	6124	17	7
20	5000 ppm GA_3	6123	8	10	6124	8	2
20	Ethephon	6123	16	12	6124	16	9
20-30	KNO_3	6123	7	11	6124	14	8
20-30	Water	6123	12	6	6124	14	6
20-30	500 ppm GA_3	6123	10	5	6124	14	11
20-30	1000 ppm GA_3	6123	10	4	6124	12	3
20-30	5000 ppm GA_3	6123	2	6	6124	4	1
20-30	Ethephon	6123	11	10	6124	15	3
15-25	KNO_3	6123	11	5	6124	18	6
15-25	Water	6123	11	6	6124	19	6
15-25	500 ppm GA_3	6123	12	5	6124	19	10
15-25	1000 ppm GA_3	6123	7	6	6124	14	4
15-25	5000 ppm GA_3	6123	2	10	6124	8	3
15-25	Ethephon	6123	7	9	6124	13	5
25	KNO_3	6123	7	6	6124	19	4
25	Water	6123	7	6	6124	18	1
25	500 ppm GA_3	6123	5	7	6124	15	7
25	1000 ppm GA_3	6123	5	4	6124	20	3
25	5000 ppm GA_3	6123	1	6	6124	3	0
25	Ethephon	6123	4	8	6124	17	5
15-30 light	KNO_3	6123	12	5	6124	13	8
15-30 light	Water	6123	10	5	6124	19	9
15-30 light	500 ppm GA_3	6123	7	6	6124	14	9
15-30 light	1000 ppm GA_3	6123	9	9	6124	10	8
15-30 light	5000 ppm GA_3	6123	3	8	6124	5	1
15-30 light	Ethephon	6123	10	6	6124	11	8
15-30 dark	KNO_3	6123	5	10	6124	20	10
15-30 dark	Water	6123	8	11	6124	15	8
15-30 dark	500 ppm GA_3	6123	3	2	6124	11	11
15-30 dark	1000 ppm GA_3	6123	0	3	6124	10	5
15-30 dark	5000 ppm GA_3	6123	1	8	6124	3	3
15-30 dark	Ethephon	6123	10	6	6124	14	7

Table 9. Mean germination and dormancy of prairie dropseed with two-week pre-chill

Temperature	Treatment	Sample No.	Mean Germ	Mean Dormancy	Sample No.	Mean Germ	Mean Dormancy
20	KNO_3	6123	7	5	6124	19	3
20	Water	6123	10	2	6124	18	5
20	500 ppm GA_3	6123	9	3	6124	17	4
20	1000 ppm GA_3	6123	12	4	6124	16	3
20	5000 ppm GA_3	6123	1	5	6124	6	3
20	Ethephon	6123	16	3	6124	20	8
20-30	KNO_3	6123	9	2	6124	17	1
20-30	Water	6123	7	2	6124	20	2
20-30	500 ppm GA_3	6123	4	1	6124	15	2
20-30	1000 ppm GA_3	6123	8	1	6124	13	4
20-30	5000 ppm GA_3	6123	1	2	6124	7	4
20-30	Ethephon	6123	11	3	6124	20	2
15-25	KNO_3	6123	12	3	6124	15	1
15-25	Water	6123	14	2	6124	19	4
15-25	500 ppm GA_3	6123	12	3	6124	19	4
15-25	1000 ppm GA_3	6123	11	2	6124	16	3
15-25	5000 ppm GA_3	6123	4	5	6124	6	7
15-25	Ethephon	6123	12	2	6124	25	5
25	KNO_3	6123	12	2	6124	16	2
25	Water	6123	9	2	6124	18	4
25	500 ppm GA_3	6123	6	2	6124	13	0
25	1000 ppm GA_3	6123	5	1	6124	10	2
25	5000 ppm GA_3	6123	5	2	6124	3	2
25	Ethephon	6123	14	1	6124	21	3
15-30 light	KNO_3	6123	16	3	6124	12	2
15-30 light	Water	6123	9	2	6124	14	4
15-30 light	500 ppm GA_3	6123	7	3	6124	18	4
15-30 light	1000 ppm GA_3	6123	7	4	6124	12	1
15-30 light	5000 ppm GA_3	6123	3	4	6124	5	7
15-30 light	Ethephon	6123	10	1	6124	22	8
15-30 dark	KNO_3	6123	5	1	6124	12	4
15-30 dark	Water	6123	5	2	6124	18	5
15-30 dark	500 ppm GA_3	6123	11	3	6124	14	6
15-30 dark	1000 ppm GA_3	6123	4	1	6124	13	3
15-30 dark	5000 ppm GA_3	6123	2	4	6124	6	4
15-30 dark	Ethephon	6123	14	4	6124	15	7

Table 10. Mean germination and dormancy of prairie dropseed based on temperature (pre-chill).

Temperature	Pre-chill	Mean Germination[1]	Mean Dormancy[1]	Mean Viability[1]
15-25°C	Yes	14.0 a	3.7 a, b	17.7 a
15-30°C dark	Yes	10.2 b	4.3 a	14.5 a, b
15-30°C light	Yes	12.0 a, b	3.8 a	15.8 a, b
20°C	Yes	12.9 a, b	4.2 a	17.1 a, b
20-30°C	Yes	11.0 a, b	2.5 b, c	13.5 b
25°C	Yes	11.2 a, b	2.0 c	13.2 b

[1] Treatments with the same letter are not significantly different at the 0.05 level.

Table 11. Mean germination and dormancy of prairie dropseed based on temperature (no pre-chill).

Temperature	Pre-chill	Mean Germination[1]	Mean Dormancy[1]	Mean Viability[1]
15-25°C	No	12.0 a	6.4 b, c	18.4 a, b
15-30°C dark	No	8.6 a	7.3 a, b	15.9 a, b
15-30°C light	No	10.4 a	7.1 a, b	17.5 a, b
20°C	No	11.4 a	8.5 a	19.9 a
20-30°C	No	10.7 a	6.1 b, c	16.8 a, b
25°C	No	10.3 a	5.0 c	15.3 b

[1] Treatments with the same letter are not significantly different at the 0.05 level.

At the 0.05 level, there was no significant germination difference between 15-25°C, 20°C, 25°C, 20-30°C, and 15-30°C with light for a two week pre-chill. For the no pre-chill there was no significant germination difference between any of the temperature regimes. For the two-week pre-chill dormancy, there was no significant difference between the temperature regimes of 25°C and 20-30°C. For the two-week pre-chill, there was no significant difference at the 0.05 level between the temperature regimes of 25°C, 20-30°C, and 15-25°C.

There was no significant difference between temperatures at the 0.05 alpha level. The F value is 1.10 and $p=0.3639$ for germination without pre-chill. For the viability with pre-chill, the F value was 1.99 and the $p=0.0825$. The F value is 1.70 and $p=0.1388$ for viability without pre-chill. The F value is 1.36 and $p=0.2444$ for germination with pre-chill.

The mean germination and dormancy based on treatment are presented in Table 12 and 13. For the pre-chill tests, the highest mean germination was 16.9% using 1.0 mM ethephon, while the lowest germination was 4.7% using 5000 ppm GA_3. The highest mean dormancy was 4.6% using 5000 ppm GA_3, while the lowest mean dormancy was 2.8% using 0.2% KNO_3. Without pre-chill, the highest mean germination was 13.0% using de-ionized water, while the lowest mean germination was 4.2% using 5000 ppm GA_3. The highest mean dormancy was 7.8% using 0.2% KNO_3, while the lowest mean dormancy was 5.1% using 5000 ppm GA_3.

Table 12. Mean germination and dormancy of prairie dropseed based on treatment (pre-chill).

Treatment	Pre-chill	Mean Germination[1]	Mean Dormancy[1]	Mean Viability
500 ppm GA_3	Yes	12.4 b, c	3.1 b, c	15.5 b
1000 ppm GA_3	Yes	10.6 c	2.8 c	13.4 b
5000 ppm GA_3	Yes	4.7 d	4.6 a	9.3 c
Ethephon	Yes	16.9 a	4.3 a, b	21.2 a
KNO_3	Yes	13.0 b, c	2.8 c	15.8 b
Water	Yes	13.7 b	2.9 c	16.6 b

[1] Treatments with the same letter are not significantly different at the 0.05 level

Table 13. Mean germination and dormancy of prairie dropseed based on treatment (no pre-chill).

Treatment	Pre-chill	Mean Germination[1]	Mean Dormancy[1]	Mean Viability[1]
500 ppm GA_3	No	10.8 a	7.7 a	18.5 a, b
1000 ppm GA_3	No	10.6 a	5.5 b, c	16.1 b
5000 ppm GA_3	No	4.2 b	5.1 c	9.3 c
Ethephon	No	12.2 a	7.2 a, b	19.4 a, b
KNO_3	No	12.5 a	7.8 a	20.3 a
Water	No	13.0 a	7.0 a, b	20.0 a

[1] Treatments with the same letter are not significantly different at the 0.05 level.

The three levels of gibberellic acid (500 ppm, 1000 ppm, and 5000 ppm) were not very effective in promoting germination for the two week pre-chill. The germination rates were 12.4%, 10.6%, and 4.7%, respectively. These three levels are all significantly

different from one another at the 0.05 level. The 500-ppm gibberellic acid was not significantly different from the potassium nitrate or de-ionized water control.

For the two week pre-chill, the ethephon treatment had the highest percentage of germination and the highest percentage of viability. It was significantly different from all other treatments. Still, it was not very successful in breaking dormancy, as its mean dormancy was 4.3%. This was not significantly different from 5000 ppm GA_3, which has the highest mean percentage of dormancy.

The de-ionized water control, for the two-week pre-chill, had a mean germination of 13.7%, which was not significantly different from ethephon. The mean dormancy was 3.0%, which was not significantly different from KNO_3, 1000 ppm GA_3, and 500 ppm GA_3.

Potassium nitrate, for the two week pre-chill, had a mean germination of 13.0%, which was not significantly different at the 0.05 level from 500 ppm GA_3 and the de-ionized water control. The mean dormancy was 3.0%, which is not significantly different from the KNO_3, 500 ppm, and 1000 ppm gibberellic acid treatments.

Without pre-chill, the three levels of gibberellic acid had little to slight effects on the germination but decreased the level of dormancy from the de-ionized water control. The germination for 1000 ppm was 10.6%, while the germination for 500-ppm gibberellic acid was 10.8%. There was no significant difference between these two. The rate of 5000 ppm had a lower mean germination of 4.2%. This was significantly different from the 500 ppm and 1000 ppm GA_3 treatments. The percentage of dormancy for 1000 ppm GA_3 was 5.5%, while the percentage of dormancy for 5000 ppm was 5.1%. These

two treatments were not significantly different from one another. The percentage of dormancy for 500 ppm was 7.7%, which was significantly different from the 1000-ppm and 5000 ppm gibberellic acid treatments.

The mean germination for no pre-chill and for the de-ionized water control was 13.0%. This was not significantly different from the potassium nitrate and ethephon treatments. The mean germination for potassium nitrate was 12.5%, and the mean germination for ethephon was 12.2%. The mean dormancy for the de-ionized water control was 7.0%. This was not significantly different from the ethephon, potassium nitrate, and 500 ppm gibberellic acid treatments. The mean dormancy for ethephon was 7.2%, while the mean dormancy for potassium nitrate was 7.8%.

There was no significant difference between the treatment x temperature interaction. The F value was 0.85 and $p=0.6747$ for germination without pre-chill. The F value was 0.45 and $p=0.9878$ for germination with a two week pre-chill. For dormancy without pre-chill, the F value was 0.97 and $p=0.5130$. The F value was 0.60 and $p=0.9280$ for dormancy with a two week pre-chill.

Through the study conducted by Toole (1941) and in this experiment, it appears that finding a standard optimum germination test protocol for *Sporobolus* species is going to be difficult. Short or bound up roots were even more problematic in prairie dropseed than in prairie cordgrass. *Sporobolus* is a genus that has great variability among its species, which makes it difficult to determine an optimum germination protocol (temperature, wetting agents, etc.). What works with one species does not work with others.

When TZ tests were conducted on the prairie dropseed seeds, the staining of the embryos was also a problem. The embryo is dark green, indicating it has chlorophyll, which interferes with staining. There is a great amount of dormancy in prairie dropseed. A properly stained embryo should be pink to red in color. Some of the embryos were still a dark green color. This is a sign of an embryo that has a high amount of dormancy. An embryo that is dead will be unstained or have significant areas (radicle, cotyledons, etc.) unstained and the tissue may be flaccid. The embryos that were dark green in color were counted as dormant embryos when the tissue was still turgid, along with the seeds exhibiting normal red stained embryos.

The ANOVA tables for all three experiments are presented in the appendix.

Conclusion

Seed dormancy is important to seed testing laboratories. It has been a problem in viability testing for many years. It is important to find methods to break dormancy, so a more accurate viability assessment can be provided to clients. Through this experiment, three specific species of the Poaceae family were examined.

Winter wheat

Winter wheat was studied because a twelve-day testing period is too long with such short turn around time between harvest and planting. Farmers typically only have about a month from harvest to planting. Twelve of these days are spent testing the seed, not including the time it takes to clean the seed, get the sample to the laboratory, the report to the customer, bag the seed, and deliver it. Through this experiment, the three-day pre-chill was shown to not be significantly different from the five day pre-chill. This would allow seed testing laboratories to decrease the pre-chill testing period from five to three days. This would assist farmers and seedsmen.

Another way to reduce the testing period would be to use 5000-ppm gibberellic acid. This was not significantly different from the standard test of a 5-day pre-chill or from the 3 day pre-chill. Although gibberellic acid is an expensive chemical, it would result in decreasing testing time by 5-days. This would be beneficial to producers that could not wait the extra days needed for pre-chilling. This could possibly be offered as a quick test. The cost for a GA_3 test would be higher, but the customer would know about it up front and it would be their choice.

Prairie Cordgrass

More work needs to be conducted on this species before a complete recommendation can be made. Using the current data, the current recommendation would be to use potassium nitrate as the wetting agent. The use of 1000-ppm gibberellic acid appears to be just as effective. Most laboratories have potassium nitrate on hand, and it is less expensive than gibberellic acid. From the results found in this preliminary study, it would be best to plant using 0.2% KNO_3 as a wetting agent, a pre-chill (5°C) for 14 days, followed by 21-days at 20-30°C with 8 hours of light.

Prairie Dropseed

It is hard to make recommendations or conclusions with prairie dropseed. However, there is definitely the need for a two-week pre-chill to assist in breaking dormancy. The only temperature treatment that was significantly different from the others for germination was 15-30°C without light. The lowest levels of dormancy existed at 20-30°C and 25°C. The temperatures that should be used appear to be 20-30°C or 25°C. This species may have more than one optimum temperature.

The chemical or growth hormone that appears to be of most use in breaking dormancy was potassium nitrate. The de-ionized water control was just as effective. These two treatments have a higher rate of germination and a low percentage of dormancy. Potassium nitrate would be the first choice because it had the lowest percentage of dormancy.

More samples (both in number and diversity) should be obtained and studied. Two obstacles one will have to overcome that it is inherently a short-lived seed, and it is

expensive to purchase when it can be found. Hand harvesting of native stands should be used in future studies, as they may act differently than mechanically harvested lots.

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Appendix

Table A-1. ANOVA for 2000 Winter Wheat Germination.

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	6	22471.34226190	3745.22371032	116.76	0.0001
Error	665	21331.07291667	32.07680138		
Corrected Total	671	43802.41517857			
R-Square		**C.V.**	**Root MSE**	**Germ Mean**	
0.513016		6.272707	5.66363853	90.29017857	

Table A-2. ANOVA for 2000 Winter Wheat Dormancy.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	6	23364.59226190	3894.09871032	163.31	0.0001
Error	665	15856.35416667	23.84414160		
Corrected Total	671	39220.94642857			
R-Square		**C.V.**	**Root MSE**	**Dorm Mean**	
0.595717		86.94773	4.88304634	5.61607143	

Table A-3. ANOVA for 2000 Winter Wheat Viability.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	6	162.63988095	27.10664683	3.09	0.0054
Error	665	5825.64583333	8.76036967		
Corrected Total	671	5988.28571429			
R-Square		**C.V.**	**Root MSE**	**Viab Mean**	
0.027160		3.086562	2.95979217	95.89285714	

Table A-4. ANOVA for 2001 Winter Wheat Germination.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	50410.42432432	12602.60608108	233.54	0.0001
Error	735	39663.43918919	53.96386284		
Corrected Total	739	90073.86351351			
R-Square		**C.V.**	**Root MSE**	**Germ Mean**	
0.559657		8.496346	7.34600999	86.46081081	

Table A-5. ANOVA for 2001 Winter Wheat Dormancy.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	52688.26216216	13172.06554054	402.63	0.0001
Error	735	24045.54729730	32.71503034		
Corrected Total	739	76733.80945946			
R-Square		**C.V.**	**Root MSE**	**Dorm Mean**	
0.686637		107.4532	5.71970544	5.32297297	

Table A-6. ANOVA for 2001 Winter Wheat Viability.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	212.04054054	53.01013514	1.96	0.0988
Error	735	19878.90540541	27.04612980		
Corrected Total	739	20090.94594595			
R-Square		**C.V.**	**Root MSE**	**Viab Mean**	
0.010554		5.666965	5.20058937	91.77027027	

Table A-7. ANOVA Combined Analysis of Two Years for Winter Wheat Germination.

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	6944.08688525	17361.02172131	347.60	0.0001
Error	1215	60682.95901639	49.94482224		
Corrected Total	1219	67627.04590164			
R-Square		**C.V.**	**Root MSE**	**Germ Mean**	
0.533664		7.996458	7.0671650	88.37868852	

Table A-8. ANOVA Combined Analysis of Two Years for Winter Wheat Dormancy.

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	71569.13606557	17892.28401639	619.94	0.0001
Error	1215	25066.22131148	28.86108750		
Corrected Total	1219	106635.35737705			
R-Square		**C.V.**	**Root MSE**	**Dorm Mean**	
0.671158		107.9405	5.3722516	4.97704918	

Table A-9. ANOVA Combined Analysis for Winter Wheat Viability.

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr>F
Model	4	249.97049180	62.49262295	2.64	0.0324
Error	1215	28739.27868852	23.65372732		
Corrected Total	1219	28989.24918033			
R-Square		**CV**	**Root MSE**	**Viab Mean**	
0.008623		5.210019	4.8635097	93.34918033	

Table A-10. ANOVA Two Replication Analysis of Prairie Cordgrass with Light

SUMMARY	Temp1	Temp2	Temp3	Temp4	Temp5	Temp6	Temp7	Temp8	Temp9	Temp10	Total
Chem1-KNO											
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	104	102	78	70	100	42	96	78	122	44	836
Average	52	51	39	35	50	21	48	39	61	22	41.8
Variance	8	50	98	18	128	242	72	450	50	8	222.273
Chem2-1000 GA											
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	92	100	68	78	120	30	98	74	100	50	810
Average	46	50	34	39	60	15	49	37	50	25	40.5
Variance	32	200	128	338	72	98	242	2	8	2	228.578
Chem3-5000 GA											
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	42	36	6	2	4	0	56	26	32	0	204
Average	21	18	3	1	2	0	28	13	16	0	10.2
Variance	242	0	18	2	8	0	648	18	128	0	155.747
Total											
Count	6	6	6	6	6	6	6	6	6	6	
Sum	238	238	152	150	224	72	250	178	254	94	
Average	39.7	39.7	25.3	25	37.3	12	41.7	29.7	42.3	15.7	
Variance	272.7	331.9	353.1	420.4	810.7	161.6	304.7	261.5	477.5	151.1	

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12788.9	2	6394.46	57.955	4.96E-11	3.31583
Columns	6589.7	9	732.185	6.6361	3.58E-05	2.21069
Interaction	1625.7	18	90.3185	0.8185	0.666	1.96011
Within	3310	30	110.3			

Table A-11. ANOVA Two Replication Analysis of Prairie Cordgrass Without Light.

SUMMARY *Chem1-KNO*	Temp1	Temp2	Temp3	Temp4	Temp5	Temp6	Temp7	Temp8	Temp9	Temp10	Total
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	94	110	66	52	96	12	106	98	116	36	786
Average	47	55	33	26	48	6	53	49	58	18	39.3
Variance	50	162	18	8	8	8	18	98	200	200	334.4315
Chem2-1000 GA											
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	86	102	68	46	112	30	72	82	122	20	740
Average	43	51	34	23	56	15	36	41	61	10	37
Variance	98	50	32	338	72	2	72	50	50	32	316
Chem3-5000 GA											
Count	2	2	2	2	2	2	2	2	2	2	20
Sum	36	30	0	0	10	2	34	14	28	0	154
Average	18	15	0	0	5	1	17	7	14	0	7.7
Variance	0	2	0	0	18	2	18	18	8	0	57.8
Total											
Count	6	6	6	6	6	6	6	6	6	6	
Sum	216	242	134	98	218	44	212	194	266	56	
Average	36	40.3	22.3	16.3	36.3	7.3	35.3	32.3	44.3	9.3	
Variance	227.2	431.1	309.5	231.1	621.5	42.7	281.1	431.1	605.5	111.5	
ANOVA											
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*					
Sample	12415.6	2	6207.8	114.1139	9.48E-15	3.315832					
Columns	9412	9	1045.777	19.22385	3.79E-10	2.210697					
Interaction	2412.4	18	134.0222	2.463643	0.014028	1.960117					
Within	1632	30	54.4								

Table A-12. ANOVA for Prairie Dropseed Germination with Pre-chill for Treatments.

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	2002.78472222	400.55694444	18.59	0.0001
Error	138	2973.70833333	21.54861111		
Corrected Total	143	4976.49305556			
R-Square		**C.V.**	**Root MSE**	**Germ Mean**	
0.402449		39.11381	4.64204816	11.86805556	

Table A-13. ANOVA for Prairie Dropseed Dormancy with Pre-chill for Treatments.

Source	D.F.	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	77.38888889	15.47777778	3.25	0.0083
Error	138	657.25000000	4.76268116		
Corrected Total	143	734.63888889			
R-Square		**C.V.**	**Root MSE**	**Dorm Mean**	
0.105343		64.13457	2.18235679	3.40277778	

Table A-14. ANOVA for Prairie Dropseed Viability with Pre-chill for Treatments.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	1827.89583333	365.57916667	11.45	0.0001
Error	138	4404.54166667	31.91696860		
Corrected Total	143	6232.43750000			
R-Square		**C.V.**	**Root MSE**	**Viab Mean**	
0.293287		36.99543	5.64951047	15.27083333	

Table A-15. ANOVA for Prairie Dropseed Germination with Pre-chill for Temperatures.

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	5	233.20138889	46.64027778	1.36	0.2444
Error	138	4743.29166667	34.37167874		
Corrected Total	143	4976.49305556			
R-Square		**C.V.**	**Root MSE**	**Germ Mean**	
0.046861		49.39930	5.86273646	11.86805556	

Table A-16. ANOVA for Prairie Dropseed Dormancy with Pre-chill for Temperatures.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	5	104.13888889	20.82777778	4.56	0.0007
Error	138	630.50000000	4.56884058		
Corrected Total	143	734.63888889			
R-Square		**C.V.**	**Root MSE**	**Dorm Mean**	
0.141755		62.81588	2.13748464	2.40277778	

Table A-17. ANOVA for Prairie Dropseed Viability with Pre-chill for Temperatures.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr>F**
Model	5	419.64583333	83.92916667	1.99	0.0835
Error	138	5812.79166667	42.12167874		
Corrected Total	143	6232.43750000			
R-Square		**C.V.**	**Root MSE**	**Viab Mean**	
0.067333		42.50011	6.49012163	15.27083333	