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Dormancy in Green Needlegrass Seed :Its Nature, Mode of Action, and Methods of Reproduction

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DORMANCY IN GREEN NEEDLEGRASS SEED: ITS NATURE, MODE OF ACTION, AND METHODS OF REDUCTION

BY

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DORMANCY IN GREEN NEEDLEGRASS SEED: ITS NATURE, MODE OF ACTION, AND METHODS OF REDUCTION

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Green needlegrass (Stipa viridula Trin.) in the past few years has received wide acceptance for use in range renovation throughout the Northern Great Plains. It is a cool season native bunchgrass which has a good seedling vigor and a high forage palatability. The large demand for seed and high price per pound has greatly increased the amount of green needlegrass seed harvested and handled by commercial seed companies.

Maximum germination of newly harvested green needlegrass seed cannot be obtained due to a high incidence of seed dormancy.

Germination procedures set forth in a report by the Subcommittee on Germination of Range Grasses of the Association of Official Seed Analysts (9) prescribe six weeks prechill of dormant green needlegrass seeds. Germination readings obtained, when using the above method, varied from 8 to 18 percent. The low germinating samples can be checked with a triphenyl tetrazolium chloride solution to determine actual viability and potential germination. Tetrazolium tests indicated the seeds were capable of much higher germination and that the green needlegrass seeds are in a temporary state of dormancy.

This study was undertaken (a) to determine the nature of dormancy present in green needlegrass seeds, (b) to study the mode of expression of factors that causes dormancy in the seeds, and (c) to seek a method which will reduce the dormancy in order to obtain maximum germination readings in the laboratory and in field plantings.

REVIEW OF LITERATURE

Seed Dormancy

Many times the seed of freshly harvested grains and grasses show delayed germination or dormancy. Dormancy, as defined in the Yearbook of Agriculture Seeds (14), "is an internal condition of the chemistry or stage of development of a viable seed that prevents its germination although good growing temperatures and moisture are provided."

Stone (17) tested rye, wheat, and timothy at 5-day intervals up to 20 days. Germination percentages were considerably higher after 20 days than at 5 days.

Rogler (15) and McWilliams (11) studied the effects of age on dormancy of green needlegrass seed. They found a decrease in dormancy with increasing age of seed. Peak germination was reached at 7 years of age after which time there was a gradual decrease in germination for 21 years.

As early as 1906, Crocker (2) used wild oats seed to show that the seed coats of grasses affect germination. Samples which had their seed coats entire germinated 8 percent, and those with the seed coat broken germinated 96 percent.

Coukos (1) found that hammer mill processing, a type of mechanical scarification, tended to induce germination in dormant caryopses of several native grasses. Even the slight scarification obtained by combining, as compared to hand harvest, was effective in breaking dormancy in a large percentage of the caryopses of big and

little bluestem. Acid scarification, both concentrated and diluted treatments, induced germination, but it was also observed that such treatments were detrimental to seeds which were nondormant. From the above results, Coukos suggested that seed coats of grasses affect dormancy. Seed coat restriction of germination in grasses is not similar to dormancy of "hard seeds" as found in many legumes, for the seed coats of dormant grass permit entrance of water even in unscarified seeds. In conclusion, Coukos suggested the answer to dormancy in native grasses may lie in the theory of "gas-exchange" restrictions in seed coats or membranes.

Coukos (1) also studied the effect of certain temperature-humidity relationships on seed viability. Low temperature and low humidity or high temperature and low humidity were influential in prolonging high viability in nondormant seeds or seeds which had after-ripened.

The use of various temperature-humidity relationships to induce germination was also tested by Coukos (1). Although low temperature and high humidity had a tendency to induce germination more rapidly and produce higher germination percentages than any other temperature-humidity combination, the difference was not sufficiently significant to be considered in overcoming prolonged seed dormancy.

Dawson and Heinrichs (3) found by removing the seed coats of green stipagrass, without prechill, induced more seeds to germinate than prechill treatment alone. A combination of removal of seed coat and prechill for 3 weeks was equal to germination obtained with acid

scarification. Immersion in 95 percent sulphuric acid for 10 minutes produced the highest germination, but immersion for 15 minutes caused injury to the seeds. They found seeds could be soaked in 72 percent sulphuric acid up to 80 minutes without causing any damage to the seeds, however, maximum germination was not obtained. Dawson and Heinrichs concluded that the easiest and most practical procedure to induce germination was to soak seeds in 95 percent sulphuric acid for two and one-half minutes, then prechill for 3 weeks. This technique produced a germination of 76 percent compared to an 80 percent tetrazolium reading. Removal of seed coats and 3 weeks prechill produced a germination of 78 percent.

Villiers (18) promoted germination of dormant unleached unchilled embryos of Fraxinus excelsior with an application of extract obtained from chilled germinating embryos. Based on these results, he suggested the role of prechill was not to remove an inhibitor but to cause the promotion of a germination promoting substance which counteracted the inhibitor within the tissues of the embryo. Photoperiodic treatment of Fraxinus excelsior seeds was found to replace the chilling treatment. Villiers also found that an application of gibberellic acid replaced both the light and prechilling requirements necessary for germination of dormant seeds.

Delouche (4), in his work with Kentucky bluegrass, concluded the more immature the seed, the greater the degree of dormancy. Schaaf and Rogler (16) demonstrated that immature seeds of green needlegrass are dormant as well as mature seed. McAlister (10) observed

an extended dormancy in the seed of green needlegrass. Mature seeds overcame this dormancy more quickly than the dough or pre-milk stage seed. Mature samples of green needlegrass seed produced 24 percent germination 4 months after harvest and a 98 percent germination when tested 51 and 58 months after harvest. Pre-milk stage seeds germinated 5 percent at 4 months and 53 percent 51 months after harvest.

Schaaf and Rogler (16) selected strains of green needlegrass for low seed dormancy. Low seed dormancy was found to be transmitted from parent to progeny. One strain which they have selected has maintained consistent low dormancy in 6 years of testing.

Laboratory Methods

Most native grasses present a problem in obtaining maximum germination in the laboratory. Green needlegrass has an extreme dormancy which gives germination percentages from 8 to 18 percent in the laboratory under normal germination procedures used for most grasses. This low percentage is not the maximum germination possible for most samples and does not give a fair and accurate estimate of the germination capability of the seed. Another reason for finding a germination procedure is that the Association of Official Seed Analysts does not have an accepted standard procedure for germinating green needlegrass.

Griswold (8) studied the effects of alternate moistening and drying conditions on several range grasses. The alternate moistening and drying conditions were divided into two groups: (a) seeds which were dried rapidly, and (b) seeds which were dried slowly. Several

species were inconsistent in their reaction to alternate moistening and drying. Some species had increased germination with rapid drying and some had decreased germination with rapid drying. Similar results were obtained with slow drying. Two species of Stipa were tested and each reacted differently to the treatment. Stipa lettermani gave an increase in germination with both types of drying, whereas, Stipa columbiana gave negative results with both types of drying. Griswold gave these possible effects of drying on germination:

1. Lack of moisture may stop the growth of the embryo.
2. Drying of the embryo at a critical stage of growth may injure the embryo and cause decrease in germination.
3. Drying may bring about changes in seed coat, which could either increase or decrease germination.
4. Some species will withstand long periods of moistening and drying and some cannot withstand this stress.

Rogler (15) stratified green needlegrass seed in moist sand for 60 days at 2° to 4°C. After stratification the seed was rapidly dried and germinated. This method gave high germination of new seed.

Stand Establishment

The last objective of this study is to obtain a seed treatment that can be used to facilitate stand establishment. The Soil Conservation Service (12) recommends planting of green needlegrass in late fall in order to establish a stand.

Frischknecht (6) suggested using vernalized seed in spring planting of some range grasses. Vernalization of seed may help obtain

a field stand when fall planting has not been feasible. Vernalization may also produce a crop of seed from spring planting a year earlier and may increase the yield the following year, but would have no increase over fall planting. The grasses used by Frischknecht in this study were intermediate and crested wheatgrass, great basin wild rye, and indian ricegrass.

White and Horner (20) found best survival of seedlings was obtained from those plants which had emerged from the soil and reached the two or three leaf stage of growth before a killing frost. Seedlings not emerging from the soil also had a high mortality rate.

Musil (13) studied pretreatment of buffalograss for field planting. The pretreatments which increased germination of buffalograss were: (a) prechilling in petri-soil at 5°C for 6 weeks, or (b) soaking seed in tap water 48 hours, drying thoroughly at room temperature, then prechilling at 5°C for 6 weeks. These two treatments gave high germination of buffalograss in 7 out of 8 samples tested.

Wenger (19) also treated buffalograss to improve germination. He recommended soaking dormant seeds for 24 hours in 0.5 percent potassium nitrate, prechilling at 41°F for 6 weeks. After prechilling seeds were dried at a temperature under 120°F. This treatment was found to increase the germination of dormant buffalograss seed at least 75 percent. Wenger also stated that temperatures near or below freezing were not as effective in breaking dormancy as were warmer temperatures. Temperatures of -10°F for as long as 15 days did not injure seed viability, but failed to increase germination.

MATERIALS AND METHODS

Green needlegrass seed lots were collected in the Spring and Summer of 1960 for maturity studies. Dates of collection were June 27th, July 1st, 5th, 8th, and 21st, and all collections were taken from the same location south of Lake Campbell. Each collection date was designated to correspond to a maturity stage. The first three collections were designated as follows: milk stage, medium dough stage, and hard dough stage; the last two collections were designated as mature stage. The samples of seed were obtained by hand stripping of the seed heads. Each seed lot of a maturity class was air dried and hand threshed. Germination tests were made to determine the amount of dormant seed present. Germination procedures used were those set forth in a report published by the Subcommittee on Range Grasses of the Association of Official Seed Analysts. The rules prescribed for germinating most grasses consisted of planting seeds on blotters moistened with 0.2 percent potassium nitrate and germinated at an alternating[1] temperature of 20-30°C. Pretreatment recommended for dormant seeds consisted of prechill at 2-4°C for 6 weeks. Other green needlegrass samples used in this study were commercial samples received for testing by the South Dakota Seed Laboratory.

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[1]Hereafter alternating temperature will refer to 8 hours at the higher temperature and 16 hours at the lower temperature unless otherwise specified.

Preliminary germination and viability studies undertaken were: removing glumes, chipping seed coats, excision of embryos, clipping tip of caryopsis, and mechanical scarification. Chipping of seeds was completed with a dissecting needle and razor blade. Excision of embryo was accomplished by removing the embryo according to the techniques described by Flemion (5). Seed scarification was accomplished with an electrical seed scarifier which was allowed to run for periods of 5, 10, and 20 seconds. After pretreatment, the seeds were planted on blotters moistened with 0.2 percent potassium nitrate solution and germinated in an alternating 15-30°C germinator. Each test consisted of germinating two 100-seed lots, except the excised embryo test which was conducted on only 50 embryos. Seeds which received the pretreatments were germinated for 1 month. Excised embryos were germinated for 1 week.

Late in the Fall of 1960, two 100-seed lots of five selected green needlegrass samples were planted in flats which were placed outside during the Winter. Early in the Spring these flats were placed in a sunny location, watered, and seedling emergence was observed.

Tests were conducted to determine the germination response of green needlegrass to treatment of seeds with a 0.2 percent potassium nitrate solution. Six selected green needlegrass samples were used in this study and two 100-seed lots of each sample were planted. Samples were germinated in an alternating 15-30°C germinator for 3 months. The treatment consisted of moistening germination blotters

with a 0.2 percent solution of potassium nitrate; water was used as the moistening agent for the control.

An intensive study was conducted to determine the effects of prechill and the duration of prechill treatment necessary to stimulate germination of green needlegrass. All samples were placed in plastic boxes on blotters moistened with a 0.2 percent potassium nitrate solution and prechilled at 2-4°C. Three lengths of prechill were used; 2 weeks, 12 weeks, and 20 weeks. After the prescribed length of prechill each sample was germinated in an alternating 15-30°C germinator for 1 month.

A modified form of the tetrazolium test described by Grabe and Delouche (7) was used to determine seed viability. Two 100-seed lots of each sample were soaked in water for 12 hours at 30°C. Each seed was then cut longitudinally in order to slice the germ in half. Half of each seed was placed in a petri dish containing 0.1 percent of 2, 3, 5-triphenyl-2H-tetrazolium chloride salt in an aqueous solution for 4 hours and examined for a color change with a binocular microscope.

Maturity studies conducted in the Summer of 1961 were somewhat changed from those conducted in 1960. Seed maturity classes were separated within each panicle instead of making a judgment as to stage of maturity of the entire panicle as was done the previous year. A panicle of green needlegrass can be divided into various maturity classes due to the determinate type of inflorescence. The top of the panicle will have mature seed and the bottom of the panicle immature seed. Sections of each head were classified as milk stage, medium

dough stage, hard dough stage, and mature. Collections were made on the 28th and 29th of June; all seed was collected from the same location south of Lake Campbell. Each maturity separation was dried at room temperature and threshed by hand. Seed viability was check with 2, 3, 5-triphenyl-2H-tetrazolium chloride salt in an aqueous solution, and the degree of dormancy was determined by a standard germination test.

Twelve week prechill showed excellent possibilities of stimulating germination in the 1960 tests, therefore, this treatment was repeated in the Summer of 1961 with freshly harvested seed collected for the maturity studies. Each 100-seed sample was germinated in a plastic germination box on blotters moistened with 0.2 percent potassium nitrate solution and placed in a refrigerator at a temperature of 2-4°C for 12 weeks. At the conclusion of the prechill treatment all samples were removed from the prechill and placed in an alternating 15-30°C germinator for 1 month. Results obtained from the 1960 studies of 2 and 20 week prechill did not warrant continuation of these two studies.

Many other possibilities of breaking the dormancy and getting green needlegrass seeds to germinate were explored. The extreme alternation of temperature procedure consisted of planting the seeds in plastic germination boxes on blotters moistened with 0.2 percent potassium nitrate solution, prechilling at a -18°C temperature for 6 days, removing from prechill chamber, allowing the seeds to dry at a temperature of 28°C for 5 hours. Then the samples were again moistened, put back into the prechill for 3 days, removed and dried, and

once again placed into prechill for 3 days. At the conclusion of the prechill treatment all samples were placed in an alternating 15-30°C germinator for 1 month.

A second temperature alternation test with less extreme temperature changes was conducted for 16 weeks. During this test, samples were alternated at 1 week intervals from a prechill of 2-4°C to an alternating 15-30°C germinator. Germination counts were made at 1 week intervals.

A series of tests were conducted in an attempt to determine some of the physical and chemical properties associated with dormancy in green needlegrass. A sample of the 1961 seed was selected which had a high degree of dormancy. The seed was ground with a Wiley Mill and placed in a plastic container for storage. Individual extractions of the ground seed were made with water, absolute alcohol, acetone, and ether.

Five grams of seed material were placed in a beaker with 50 cc of water. The extraction was allowed to continue for 15 minutes; the diffusate was then decanted and filtered through a Butner funnel. After the diffusate had been filtered, a biological test was made to determine if an inhibitor had been extracted. The procedure was modified slightly when extractions were made with the following volatile liquids: alcohol, acetone, and ether. The diffusate was filtered and an equal volume of water added to the filtrate. The volatile extractor was allowed to evaporate off. All the volatile extracting liquid was removed by this procedure, and the extracted

material remained in the water solution.

The biological test consisted of germinating seeds which were very sensitive to inhibiting compounds on blotters moistened with the water solution containing the extracted material.

The sensitive seeds consisted of flax, radish, and lettuce. Test crop inhibition was determined by the percent germination of each seed replication as compared to the control.

Several tests were conducted to determine some of the physical properties of the inhibitor present in green needlegrass seeds. One test used was extraction of the inhibitor from ground seeds with water for 48 hours, filtering the diffusate, and adding an equal volume of acetone to coagulate the proteins thus allowing them to precipitate out of solution. The acetone was allowed to evaporate out of the solution and the precipitated proteins removed. At this point a biological test was made to determine if the inhibitor had been removed with the protein fraction. Ether was then added to the diffusate to remove the ether soluble material. The ether extraction continued for 15 minutes and then was removed from the diffusate with a separatory funnel. The diffusate was again tested to determine if the inhibitor had been removed with the ether soluble material.

Heat stability of the inhibitor was tested by boiling ground green needlegrass seeds in water for 10 minutes and testing to determine if the inhibitor had been destroyed.

The location of the inhibitor in the seed was determined by hand removal of the glumes and by removing the embryo from the

endosperm. An alcohol extract was made on each of the three separations and a biological test made to determine the location of the inhibitor.

Using the results obtained from the study of chemical and physical properties of the inhibitor, a series of tests were made using absolute alcohol to stimulate germination. Green needlegrass seeds were soaked in absolute alcohol for various lengths of time, washed in tap water, and germinated in plastic boxes on blotters moistened with a 0.2 percent potassium nitrate solution. Four lengths of treatment were used; one treatment consisted of soaking seeds in absolute alcohol for 15 minutes, the other treatments were similar except the seeds were soaked for 10, 8, and 5 minutes. A control was planted for comparison. All seeds were germinated in an alternating 15-30°C germinator for 1 month.

Various concentrations of thiourea were used in an effort to stimulate germination. The concentrations used were 0.1, 1, and 4 percent thiourea. Seeds were planted as previously described except that the blotters were moistened with the various thiourea concentrations. Seeds were germinated for 1 month in an alternating 15-30°C germinator.

Seed treatment similar to that used by Musil (13) and Wenger (19) on buffalograss was used to treat green needlegrass to reduce dormancy. The treatment used was to soak two 100-seed lots for 25 hours in a 0.2 percent solution of potassium nitrate, with each sample in a separate cloth bag. After soaking the samples were placed

in moist sand and prechilled in a refrigerator at 2-4°C for 6 weeks. Upon conclusion of the prechill, samples were removed from the moist sand and dried in an oven at 38°C for 3 hours. The seed lots were placed in a germinator for 2 months at a temperature of 15-30°C.

Acid scarification was used in this study to determine its effect on germination of green needlegrass. Seeds were soaked in 20 cc of 95 percent sulphuric acid for 2½, 5, 10, and 15 minutes. After soaking, seeds were removed from the acid and washed in tap water for 4 hours, planted in plastic boxes on blotters moistened with a 0.2 percent solution of potassium nitrate and germinated in an alternating 15-30°C germinator for 2 months.

Studies on stand establishment of green needlegrass were started the Spring of 1962. Three plantings were made; the first planting on May 3, the second June 28, and the third November 5, 1962. Three replications were planted at each planting date and samples selected at random within each replication. A total of 39 samples were planted in each replication.

Pretreatments consisted of soaking 9 lots of 1961 seed in 0.2 percent potassium nitrate for 24 hours, draining, and prechilling moist for 6 weeks or 3 months. At the conclusion of the prechill each sample was immediately dried at a temperature of 38°C and stored until planting. After each lot of seed had received a pretreatment, 100 seed subsamples were counted to be used in the field plantings. An untreated lot of each seed sample was planted as a control. Field plantings were made by hand and 100 seeds planted per row.

EXPERIMENTAL RESULTS

Preliminary germination studies conducted in the Summer of 1960 revealed dormant conditions existed in green needlegrass seeds. Germination tests conducted in the Winter and Spring of 1961 also exhibited the effects of dormancy. Statistical analysis of these data demonstrated that continued studies were warranted.

Dormancy Studies

Germination percentages of freshly harvested green needlegrass seed, using the germinating procedure prescribed by the Association of Official Seed Analysts, indicate that a high degree of dormancy is present in freshly harvested seed. Germination of freshly harvested samples of green needlegrass seed is shown in Table 1. Tetrazolium[1] readings indicate the percentage of viable seed in each sample. Attempts were made to classify seed samples collected on a particular date into one maturity group, such as all seed samples collected on the 27th of June were classified as milk stage of maturity. Likewise all seed samples collected on the 21st of July were classified as mature seeds. Average germination of freshly harvested maturity groups was 5.6 percent, compared to an average tetrazolium reading for the same samples of 85.5 percent. In 1961 maturity studies were conducted in a somewhat different manner. An attempt was made to

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[1]Hereafter tetrazolium readings will refer to testing with 0.1 percent 2, 3, 5-triphenyl-2H-tetrazolium chloride salt solution.

find a stage of maturity in which seed dormancy was not present. Dormancy was found to be present in seed of all the immature seed groups as well as mature seed groups. Germination percentages of maturity studies are presented in Table 1.

Table 1. Germination Percentages of 15 Hand Harvested Samples of Green Needlegrass Seed Collected in 1960 and 1961, Showing Date of Harvest, Date Tested, Maturity Classification, and Tetrazolium Readings

Sample no.	Date harvested	Date tested	Maturity class	Percent germination	Percent TZ*
			1960 Harvest		
1	6/27/60	7/6/60	Milk	9	77
3	7/1/60	7/6/60	Med. dough	10	86
5	7/5/60	7/8/60	Hard dough	3	88
6	7/8/60	7/15/60	Hard dough	2	93
8	7/13/60	7/23/60	Hard dough	2	94
12	7/21/60	7/26/60	Mature	18	87
			1961 Harvest		
A_1	6/29/61	8/30/61	Milk	10	40
A_2	6/29/61	8/30/61	Med. dough	21	73
A_3	6/29/61	8/30/61	Hard dough	16	87
A_4	6/29/61	8/30/61	Mature	9	97
B_1	6/28/61	8/30/61	Milk	32	44
B_2	6/28/61	8/30/61	Med. dough	35	83
B_3	6/28/61	8/30/61	Hard dough	31	97
B_4	6/28/61	8/30/61	Mature	23	95
C	7/18/61	8/30/61	Mature	11	90

*Indicates tetrazolium readings.

Several tests were conducted to determine the cause of dormancy within the seed and if dormancy could be broken. Hand removal of glumes, mechanical scarification, punctured seed coats, and clipping tip of caryopsis gave increased germination over standard germination test in almost every instance, but germination readings did not

compare with tetrazolium readings. Excision of embryos gave embryo viability readings comparable to tetrazolium readings. Table 2 shows results of germination tests conducted using the following treatments: removal of glumes, punctured seed coats, clipping the tip of caryopsis, mechanical scarification, excision of embryos, tetrazolium readings, and a control or standard germination test.

A 0.2 percent potassium nitrate solution increased germination of green needlegrass seeds when compared to water as a moistening agent. Germination percentages obtained by use of potassium nitrate solution were still substantially below the tetrazolium readings for each sample. According to the readings presented in Table 3, a potassium nitrate solution aids in breaking the dormancy of green needlegrass; therefore, it was used as the moistening agent throughout the remainder of this study. Table 3 presents the results of potassium nitrate test along with several other tests conducted in an effort to increase germination of green needlegrass. Two tests using alternating temperatures were conducted. Extreme alternation of temperature was very detrimental to green needlegrass seeds, whereas, mild alternation did increase germination to a limited extent, although it did not compare with the tetrazolium readings. The buffalograss and 0.1 thiourea treatments of seeds showed increases over the controls in most cases but again did not equal tetrazolium readings. In all tests presented in Table 3, tetrazolium readings were significantly higher than the treatment readings.

Table 2. Germination Percentages of Three Green Needlegrass Seed Samples Receiving the Following Treatments: Glumes Removed, Punctured Seed Coats, Clipped Caryopsis Tip, Excised Embryos, and Scarified Mechanically. Germination Percentages of the Control and Tetrazolium Percentages are Shown for Comparison

Sample no.	Removed glumes	Punctured seed coats	Clipping caryopsis tip	Excised embryos	Scarif. mech.	TZ	Control
6	-	77	-	-	40	93	36
12	-	82	-	-	40	87	18
24	43	78	91	98	40	96	45

Table 3. Germination Percentages of Green Needlegrass Seed Samples Which were Harvested in 1960 and Given Pretreatments of Extreme and Mild Alternation of Temperatures, the Buffalograss Treatment, and Soaked in Solutions of Thiourea, Potassium Nitrate, and Water. Tetrazolium Readings Show the Percent Viable Seed in Each Sample

	Treatments							
Sample no.	Extreme alternation	Mild alternation	Buffalograss treatment	Thiourea	KNO_3	H_2O	Control	TZ
1	0	-	6	32	49	25	25	77*
3	-	59	-	-	53	30	10	86*
6	1	-	73	70	60	29	58	93*
9	-	22	-	-	49	17	2	94*
18	0	-	18	30	34	24	26	51*
24	3	46	74	62	69	27	71	96*

*Significant at the 5 percent level.

Removal of seed coats, acid scarification, chipping of seed coats, and clipping of caryopsis tip all increased germination over the standard germination procedures but did not equal tetrazolium readings. According to these results dormancy in green needlegrass is more than a physical type of dormancy, since it must also involve some type of chemical block within the seeds. Several methods of extraction were used in an attempt to extract an inhibitor from green needlegrass seeds. The most successful extraction was obtained by use of absolute alcohol. Water also removed an inhibitor from green needlegrass seeds, but the quantity was very small and much time was necessary for extraction. The inhibitor extracted with absolute alcohol completely prevented germination of all test crops, whereas, water extraction for the same length of time as alcohol extraction showed germination percentages of test crops as follows: radish, 66 percent; lettuce, 92 percent; and flax, 64 percent. Table 4 shows germination percentage of test crops germinated on blotters moistened with extracts obtained from green needlegrass seeds with water, alcohol, ether, and acetone extraction. Extractions made with ether and acetone had very little effect on germination of test crops.

Figure I illustrates the germination obtained when radish, lettuce, and flax were germinated on blotters moistened with a solution containing an inhibitor which was extracted with alcohol.

Table 4. Germination Percentages of Test Crops Germinated on Blotters Moistened with a Solution Containing Extracts Obtained with Water, Alcohol, Ether, and Acetone Extraction of Ground Green Needlegrass Seed

Test crops	Control	H_2O ext.	Alcohol ext.	Ether ext.	Acetone ext.
Radish	98	66	0	98	98
Lettuce	98	92	0	98	98
Flax	92	64	0	86	88

Some of the chemical properties of the inhibitor were examined in an attempt to study the mode of expression of factors that cause dormancy in green needlegrass seeds. The chemical properties studied were (a) is the inhibitor proteineceous or lipid, and (b) is it heat stable? Test on the diffusate after the protein had been removed with acetone showed that the inhibitor was still present and had not been removed with the proteins. Ether was then added to this same diffusate to remove the lipids present. The diffusate was separated from the ether and tested for presence of the inhibitor. Germination percentages of test crops showed that some of the inhibitor had been removed with the lipids, although a large portion of the inhibitor still remained in the diffusate. Germination percentages of test crops after proteins and lipids were removed from a water solution containing the inhibitor are presented in Table 5.

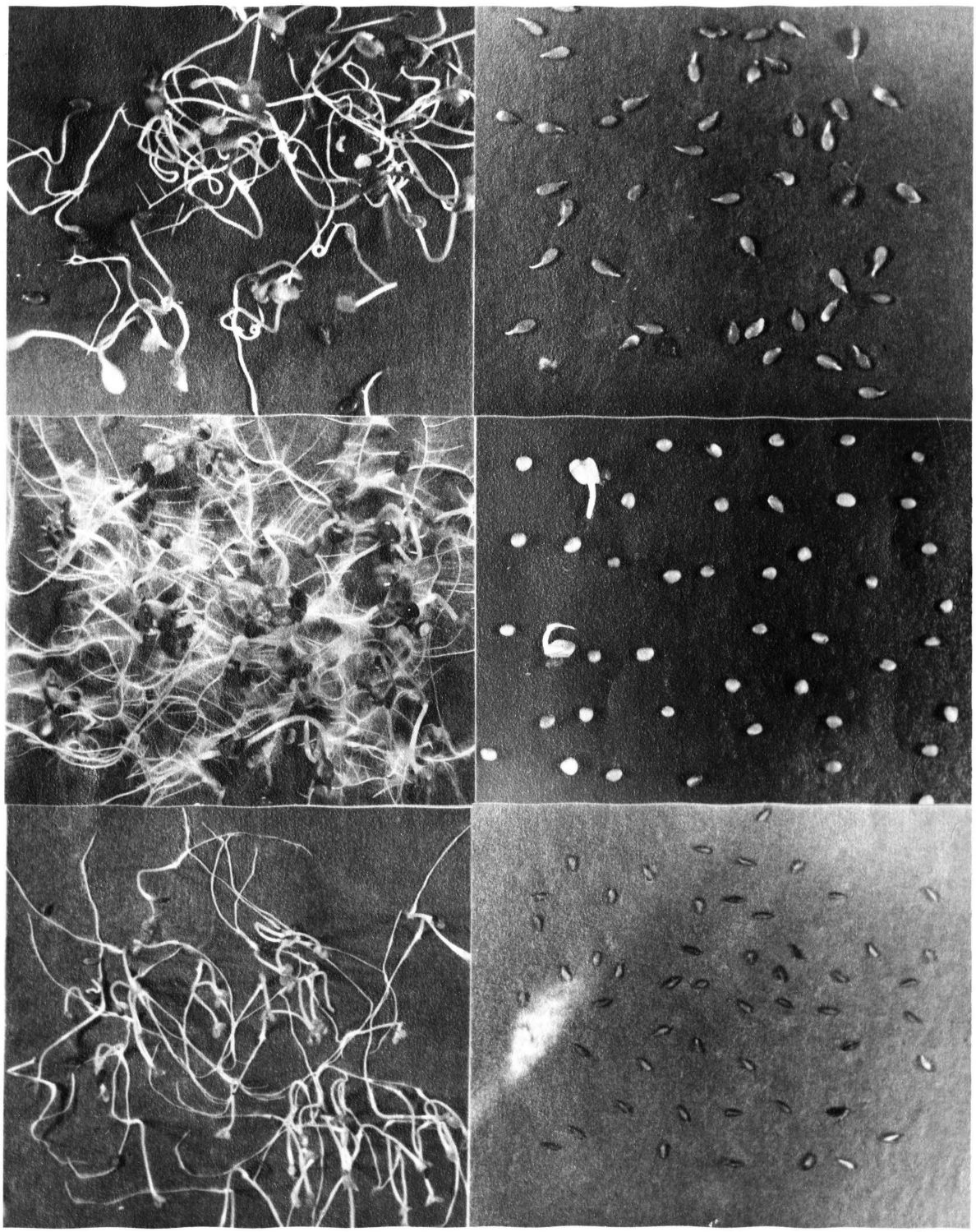

Figure I. Germination of test crops on blotters moistened with water (left column) and with inhibitor solution (right column)

Table 5. Germination Percentages of Test Crops Germinated on Blotters Moistened With a Water Extract Containing the Inhibitor Which had the Proteins and Lipids Removed

Test crops	Control	H_2O ext.	Proteins removed	Lipids removed
Radish	98	66	66	90
Lettuce	98	92	80	76
Flax	92	64	64	80

Heating a water solution containing ground green needlegrass seed during extraction freed more inhibitor than a normal water extraction. Heating did not destroy the inhibitor. Table 6 contains germination percentages of normal water extracts and heated water extracts.

Table 6. Germination Percentages of Test Crops When Comparing Water Extraction of Inhibitor from Ground Green Needlegrass Seed at Room Temperature and Extraction at 100°C. Test Crops were Germinated on Blotters Moistened with Water Solutions Containing Material Extracted from Green Needlegrass Seed

	Type of extraction		
Test crops	Normal H_2O ext.	Heated H_2O ext.	Control
Lettuce	92	24	98
Flax	64	66	86

The inhibitor is located predominently in the seed coats and endosperm of green needlegrass seeds. The embryo was shown to contain some inhibitor. Germination percentages obtained from alcohol extractions of the seed coats, endosperm, and embryos of green needlegrass seeds are presented in Table 7.

Table 7. Germination Percentages of Test Crops Germinated on Blotters Moistened With a Water Solution Containing Material Which was Extracted with Alcohol from Ground Seed Coats, Endosperm, and Embryos of Green Needlegrass Seeds

Test crops	Control	Structures of seed		
		Seed coats	Endosperm	Embryos
Radish	98	0	0	16
Lettuce	98	0	0	38
Flax	92	0	0	36

Study of Laboratory Germination

This study was undertaken to find a method which would reduce the dormancy in order to obtain maximum germination of green needlegrass in the laboratory. Preliminary tests showed that the seed coat has some effect on dormancy; therefore, acid scarification was used in an attempt to reduce the percent of dormant seeds within a sample. The highest germination was obtained by soaking green needlegrass seed for 15 minutes in 95 percent sulphuric acid. Results of acid scarification are shown in Table 8. A germination of 36 percent was significant when compared with the control but not significant when compared with an 86 percent tetrazolium reading.

Green needlegrass seeds when soaked in absolute alcohol for 5 minutes germinated 68 percent. The percent of germination obtained when using the standard germinating procedure was 88 percent, and the viability, as shown by a tetrazolium test, was 96 percent. Lower germination percentages were obtained when seeds were soaked in

alcohol for longer than 5 minutes. Table 8 presents results of alcohol treatments of green needlegrass seeds.

Table 8. Germination Percentages of Green Needlegrass Seed Soaked in Absolute Alcohol and 95 Percent Sulphuric Acid. Seeds were Soaked in Absolute Alcohol for 5, 8, 10, and 15 Minutes

Sample no.	Minutes of Soaking						
	2½	5	8	10	15	Control	TZ
			Alcohol treatment				
A_1	-	12	15	19	18	28	40
B_3	-	25	38	37	26	64	97
C	-	27	29	16	12	53	90
24	-	68	54	29	25	88	96
			Acid treatment				
I	28	30	--	33	36	17	86

Several investigators (6, 13, 15, 18, 19) have reported increased germinations when using a prechill treatment prior to germination. None of these germination increases were compared to tetrazolium readings, therefore, it is not known if these responses were comparable to the maximum germination capabilities of these samples.

The use of prechill along with a potassium nitrate solution gave favorable responses in this study. Potassium nitrate solution plus 2 weeks prechill increased germination to a limited extent, although it did not equal tetrazolium readings. Twelve weeks prechill plus 0.2 percent potassium nitrate gave the greatest germination increase. Germination readings obtained with 12 weeks prechill closely equaled tetrazolium readings. A statistical analysis of these data

confirmed the results. A 0.2 percent potassium nitrate solution plus 20 weeks prechill also produced a germination increase and, in many cases, equaled tetrazolium readings. From a practical point of view 12 weeks prechill is superior to 20 weeks prechill. Results of 2, 12, and 20 weeks prechill are presented in Table 9.

Table 9. Germination Percentages of 29 Commercial and Hand Harvested Green Needlegrass Seed Samples Germinated in an Alternating 15-30°C Germinator After Receiving Pretreatment of 2, 12, and 20 Weeks Prechill at 2-4°C. Green Needlegrass Samples Tested Represent Seed from Both 1960 and 1961 Harvests

Sample no.	Length of Prechill 2 wk.	12 wk.	20 wk.	TZ	Sample no.	12 wk.	TZ
		1960				1961	
1	-	69	54	77	A_1	62	40
3	-	84	84	86	A_2	74	73
5	-	94	90	88	A_3	81	87
6	59	92	92	93	A_4	95	97
8	-	92	95	94	B_1	58	44
9	-	84	87	86	B_2	79	83
12	-	64	77	87	B_3	88	97
13	15	19	10	21	B_4	80	95
14	-	32	30	43	C	83	90
15	-	36	29	39			
17	-	54	46	56			
18	-	63	46	51			
19	6	13	12	17			
20	-	61	50	59			
22	-	42	40	42			
23	-	55	48	57			
24	60	96	80	96			
25	-	31	42	64			
Mean	35	60	56	64		77	78

The following year, 1961, the 12 weeks prechill treatment was again tested to confirm findings of the previous year. Germination

percentages using 12 weeks prechill obtained in 1961 showed results similar to those obtained in 1960. The samples used in the 1961 test were freshly harvested samples collected for maturity studies and ranged in maturity from milk stage to fully mature. Tetrazolium readings for the immature samples were very hard to determine because of the small size of the caryopsis present. Results of the 1961 twelve weeks prechill experiment are shown in Table 9.

The value of using potassium nitrate in conjunction with 12 weeks prechill is quite evident when observing data presented in Table 10.

Table 10. Germination Percentages of Green Needlegrass Seed Showing the Value of Using 0.2 Percent Potassium Nitrate in Conjunction with 12 Weeks Prechill

Sample no.	Treatments			
	0.2% KNO_3 12 wk. PC*	12 wk. PC	No KNO_3 No PC	TZ
24	96	87	50	96

*Indicates prechill.

The germination percentage when potassium nitrate and 12 weeks prechill were used together was 96 percent, equal to the tetrazolium reading. The standard germinating test or control with prechill was considerably lower when potassium nitrate solution was not used to moisten the blotters. When both the prechill and potassium nitrate solution were eliminated, the control was even lower in germination.

Stand Establishment Studies

Presently the recommendation for establishing a field stand of green needlegrass consists of planting in late Fall. The validity of this recommendation was tested in the Fall of 1960. Four samples of seed harvested in the Summer of 1960 were planted, and the maximum germination obtained for any one sample was 45 percent. The sample which germinated 45 percent had a tetrazolium reading of 96 percent. Germination percentages of green needlegrass samples planted in the Fall of 1960 are presented in Table 11.

Table 11. Percent Emergence of Dormant Green Needlegrass Seed Samples Planted in Flats Which were Placed Outside During the Winter

	Sample Numbers				
	1	6	12	18	24
Germ	17	26	17	30	45
TZ	77	93	87	51	96

Field plantings of treated green needlegrass seeds made in the Spring and Summer of 1962 were not successful, due to several environmental conditions which could not be controlled in the field. Time of planting was the only substantial data obtained from field plantings. Average germination of the planting made June 28th was less than 1 percent, and the average germination of the earlier planting was 5 percent. The May 3rd planting showed evidence of germination considerably higher than 5 percent, but a one-half inch crust on top of the soil did not allow the seedlings to emerge. A soil

crust did not form when planting was done on June 28th. Table 12 presents results of field plantings.

Table 12. Percent Emergence of Green Needlegrass Field Plantings of May 3rd and June 28th, 1962, Showing the Type of Pretreatment Each Sample Received. Emergence Readings Represent the Average of Three Replications. Seedling Emergence Counts were Taken One Month After Planting

	May 3rd planting			June 28th planting		
Sample no.	6 wk. PC	12 wk. PC	Control	6 wk. PC	12 wk. PC	Control
A_1	1.00	1.60	.66	.75	.33	0
A_2	3.00	3.00	1.00	0	.33	0
A_3	2.30	2.30	3.00	0	1.00	2.30
A_4	6.30	6.50	11.30	-	-	-
B_1	1.00	3.60	.33	.33	.33	0
B_2	4.00	5.60	1.00	0	0	.33
B_3	6.00	10.60	4.60	.33	.33	0
B_4	7.30	23.60	12.00	.33	1.00	1.00
C	6.00	2.30	8.60	2.00	0	.33
Means	4.10	6.50	4.70	.47	.42	.49

DISCUSSION

This study on dormancy in green needlegrass was undertaken with three objectives. The first objective was to investigate the nature of dormancy in green needlegrass seeds. The second and most important objective of this study was to obtain satisfactory laboratory germinations. The third objective was to obtain a seed treatment which would facilitate stand establishment in the field.

Data in the foregoing sections indicate that dormancy is present in the seed of green needlegrass and is caused both by physical and chemical blocks. The presence of a physical block was indicated by germination increases when the following treatments were performed: removal of glumes, punctured seed coats, clipping caryopsis tip, excised embryos, and mechanical scarification. The increased germinations due to the breaking of seed coats and underlying membranes reveal that these structures of the seed must account for some of the dormancy problem in green needlegrass.

The increased germination of green needlegrass seed to such chemicals as thiourea and potassium nitrate also indicates that dormancy is more than just a physical restriction of the seed coat and underlying membrane. Dormancy in green needlegrass seeds, which still persisted after several of the physical restrictions were removed, indicated that chemical inhibitors are also present.

Absolute alcohol and water extracts from green needlegrass seeds were found to contain chemical inhibitors. Extraction of an inhibitor with alcohol was far superior to extraction with water in

both amount of inhibition of test crops and quantity of inhibitor extracted. The inhibitor was not soluble in either ether or acetone.

Studies on some of the properties of the extracted inhibitor indicate the inhibitor is heat stable, and large quantities of it can be extracted by heating a water solution during extraction. The inhibitor became considerably more soluble in water when heated. Tests conducted on a solution containing the inhibitor indicate that it is not protein in composition. The inhibitor was still present after proteins were removed with acetone. Tests made on the above solution after ether was added to remove the lipids indicate the inhibitor may have something to do with the lipids, because an increase in germination, of the test crops, occurred when the lipids were removed from the solution. Some inhibitor remained in the solution after the proteins and lipids were removed indicating the inhibitor is of more than one chemical composition.

Separation of the seed coats, endosperms, and embryos of green needlegrass seeds and soaking each portion in absolute alcohol helped to determine the location of the inhibitor within the seed. The inhibitor was predominently present in the seed coats and endosperm. A trace of inhibitor was present in the embryos, but it may be that the inhibition which occurred originated from the endosperm material attached to the embryo due to faulty embryo excision technique.

Maturity studies conducted in 1960 and 1961 indicate that seeds of green needlegrass are dormant in the immature stage as well as in the mature stage. Dormancy was exhibited in all tests conducted with

seeds harvested in the milk, medium dough, hard dough, and mature stages of maturity.

Laboratory germination procedures for green needlegrass, set forth by the Subcommittee on Range Grasses of the Association of Official Seed Analysts, were used and found to produce low germinations. Several laboratory methods were tested and found to increase germination, but only one method, 12 weeks moist prechill, equaled tetrazolium viability readings.

Moistening the germination blotters with a solution of 0.2 percent potassium nitrate gave germination responses which were significantly greater than the controls, although these readings were not equal to tetrazolium readings. A solution of 0.2 percent potassium nitrate was shown to produce the greatest germination response of any of the moistening agents used in this study.

Soaking green needlegrass seeds in absolute alcohol for various lengths of time seemed to depress germination of green needlegrass seed. The highest germination was obtained with five minutes of soaking in absolute alcohol, and this reading was substantially lower than the tetrazolium reading for that sample.

Acid scarification of green needlegrass seeds for $2\frac{1}{2}$, 5, 10, and 15 minutes gave limited germination increases. The highest germination was obtained with 15 minute scarification in 95 percent sulphuric acid. All germination percentages obtained with acid scarification were significantly higher than the controls but were not comparable to tetrazolium readings. Acid scarification alone is not

enough to give maximum germination of green needlegrass; some other treatment is necessary to overcome the chemical inhibitor within the seeds. The use of sulphuric acid as a treatment to aid in reducing dormancy may have a practical application if care is taken to see that all seed receives the exact length of scarification. A short period of immersion will not accomplish complete scarification and too long a period of immersion will injure the seeds.

Highest and most consistent germination readings were obtained with 12 weeks moist prechill at 2-4°C and germination in an alternating 15-30°C germinator for four weeks. All samples remained in the germinator four weeks to be reasonably sure germination was completed. The first two weeks in the germinator was sufficient time for most of the seeds to germinate. A statistical analysis of data showed that 12 weeks prechill readings were equal to tetrazolium readings. Tests conducted in 1960 on 20 green needlegrass samples and tests in 1961 on 9 samples indicated that 12 weeks moist prechill was a practical and accurate method of laboratory germination. Some germination of green needlegrass seeds would occur before samples were removed from the prechill chamber if the prechill temperature was not kept between 2-4°C.

Field plantings made the Spring of 1962 were not successful due to various environmental conditions which could not be controlled in the field. Data obtained from these plantings may possibly indicate that early plantings would be advantageous over late plantings made in June. Plantings made in June did not show 1 percent emergence,

whereas, plantings made in May had 5 percent emergence and showed capabilities of much higher germination if it had not been for a formation of a soil crust approximately one-half inch thick. June planting did not have this crust. It was noted that the only plants producing seed that summer were from seed samples which had received some type of treatment. None of the plants from the control (untreated) seed lots produced seed the first year after planting.

SUMMARY

Seed dormancy was found to be the main cause of low germination of green needlegrass, both in the laboratory and in the field. Dormancy was present in seeds of green needlegrass each year this study was conducted. Maturity studies conducted indicate dormancy is present in green needlegrass seeds in the immature stage of maturity as well as in the fully mature stage.

Two types of dormancy were found to be present in green needlegrass--an external restriction and a chemical block within the seed.

The special treatment for germination of green needlegrass seed prescribed by the Subcommittee on Range Grasses of the Association of Official Seed Analysts did not entirely break this dormancy.

Limited germination increases were obtained by removing the glumes, puncturing seed coats, and clipping the tip of the caryopsis, and also by mechanical and acid scarification. Solutions of thiourea and potassium nitrate also gave limited germination increases.

Viability readings of excised embryos were comparable to tetrazolium readings and also maximum germination percentages.

The use of potassium nitrate solution as the moistening agent for blotters and 12 weeks prechill produced germination readings which indicate that dormancy was completely overcome.

A chemical inhibitor was extracted from ground green needlegrass seed with absolute alcohol. The inhibitor was found to be heat stable and only slightly soluble in water, although it is highly soluble in alcohol.

The inhibitor was found to be located in the seed coat and also in the endosperm of the seed. It also may be present in the embryo, but more work is necessary before this can be substantiated.

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