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Dormancy in the Seed of Western Wheatgrass (Agropyron smithii, Rydb.)

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DORMANCY IN THE SEED OF WESTERN WHEATGRASS (*AGROPYRON SMITHII*, RYDB.)

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, 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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TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
Seed Dormancy	3
Dormancy in Native Grasses	8
Seed Response to Light	11
Hormones and Seed Dormancy	14
METHODS AND MATERIALS	16
Dormancy and Maturity	16
Dormancy and Location	17
Breaking Seed Dormancy	17
Quantified Light Effects	20
Hormones and Red Light	20
RESULTS AND DISCUSSION	22
Dormancy and Maturity	22
Dormancy and Location	27
Breaking Seed Dormancy	29
Quantified Light Effects	31
Hormones and Red Light	34
SUMMARY AND CONCLUSIONS	37
LITERATURE CITED	39
APPENDIX	46

LIST OF TABLES

		Page
TABLE 1.	Yearly number of samples received by South Dakota Seed Testing Laboratory	1
TABLE 2.	Effect of successive red and far red light treatments on lettuce seed germination	12
TABLE 3.	The counties in which western wheatgrass samples were collected arranged in their respective regions . . .	18

LIST OF FIGURES

		Page
Figure 1.	Phytochrome response system	12
Figure 2.	The percent dormancy occurring in the seed lots with reference to collection dates in the maturity study .	23
Figure 3.	The percent dormancy occurring in the seed lots with reference to collection locations in the maturity study	24
Figure 4.	The percent germination occurring in the seed lots with reference to collection dates in the maturity study	25
Figure 5.	The percent germination occurring in the seed lots with reference to collection locations in the maturity study	26
Figure 6.	The average dormancy of western wheatgrass seed according to regions within South Dakota	28
Figure 7.	The effects of various light and temperature treatments on the incidence of dormancy in western wheatgrass seed	30
Figure 8.	The effects of various exposure times to red and far red light on the incidence of dormancy in western wheatgrass seed	33
Figure 9.	The effects of various wetting agents and red light on the incidence of dormancy in western wheatgrass seed	35

LIST OF APPENDIX TABLES

Page

TABLE 1. The percent dormancy in the western wheatgrass seed collections from the Samuel H. Ordway Memorial Prairie maturity study 46

TABLE 2. Analysis of variance for the dormancy results of the maturity study 47

TABLE 3. The percent germination of the western wheatgrass seed collections from the Samuel H. Ordway Memorial Prairie maturity study 48

TABLE 4. Analysis of variance for the germination results of the maturity study 49

TABLE 5. The percent dormancy in the August Collections in 33 counties of South Dakota 50

TABLE 6. Analysis of variance of regional effects on dormancy of western wheatgrass 51

TABLE 7. The percent dormancy in the August and October western wheatgrass seed collections in various counties of South Dakota 51

TABLE 8. Analysis of variance for the difference in dormancy between the August and October collections of western wheatgrass seed in various counties of South Dakota 52

TABLE 9. The percent dormancy in seed lots tested under various light and temperature treatments 52

TABLE 10. Analysis of variance for the effects of various light and temperature treatments on dormancy in western wheatgrass seed 53

TABLE 11. The percent dormant seeds in seed lots tested with various exposure times to red and far red light . . . 53

TABLE 12. Analysis of variance of the results of the effects of various exposure times to red and far red light on dormancy in western wheatgrass 54

TABLE 13. The percent dormancy in seed lots tested with various wetting agents and red light treatment . . . 55

Page

TABLE 14. Analysis of variance of data testing the effects of various wetting agents and red light on the germination of western wheatgrass 56

INTRODUCTION

Western wheatgrass (Agropyron smithii, Rydb.) is a native, cool season, sod-forming grass of the northern Great Plains region. With the advent of range renovation through interseeding methods, western wheatgrass became one of the most important native grasses in the seed industry. Today it still is sold in far larger quantities than the seed of other native grasses. An indication of this can be seen in the number of samples of western wheatgrass received by the South Dakota State Seed Testing Laboratory in comparison to other grasses. The number of western wheatgrass samples received in the 5-year period from 1971-1975 was greater than the total number of samples of all other native grasses received. These included Blue grama (Bouteloua gracilis, (HBK) Lag. ex Steud), Side-oats grama (Bouteloua curtipendula, (Michx.) Torr.), Big bluestem (Andropogon gerardi, Vitman), Little bluestem (Andropogon scoparius, Michx.), Green needlegrass (Stipa viridula, Trin.), and Switchgrass (Panicum virgatum, L.).

TABLE 1. Yearly number of samples received by South Dakota State Seed Testing Laboratory.

Year	Western Wheatgrass	Other Native Grasses
1971	78	65
1972	88	65
1973	136	88
1974	55	72
1975	97	45
Total	454	335

As is the case with many of the native grass species, western wheatgrass can at times possess a high amount of seed dormancy. This dormancy makes the determination of pure live seed difficult. Consequently, laboratory methods have been sought to completely break this dormancy in order to obtain a true determination of seed viability. Such methods as embryo excision, lemma and palea removal, caryopsis clipping, alternating temperatures, and others have been used with varying success. The method now employed by the South Dakota State Seed Laboratory to determine the viability of ungerminated grass seeds is the tetrazolium test (7). After the 28-day germination period, the ungerminated seeds are bisected longitudinally and placed in 1.0% tetrazolium solution for four hours. At the end of that period the seeds which have red or pink embryos are considered dormant seeds. The rest are considered dead.

The purpose of this study was to attempt to determine the possible cause of the induction of dormancy in western wheatgrass seed and assess the effects of alternate seed treatment methods on the breaking of this dormancy.

LITERATURE REVIEW

Seed Dormancy

Seeds are the basic means of survival for most plant species. They are responsible for the dispersion as well as the preservation of the species. They are a way by which embryonic life can be almost suspended and then revived to new development. Built into these little capsules of life is the ability to moniter the environment; to determine when the conditions are right for embryonic growth and development. Until these conditions are met, the seed remains in a low metabolic state. This inactive state is called dormancy.

Dormancy is the means by which seeds are able to remain viable for long periods of time. Various experiments have been conducted to determine the maximum longevity of seeds. One such experiment was conducted by J. W. T. Duvel, of the Department of Agriculture (50). Duvel placed the seeds of 107 species in various flower pots and buried the pots. At predetermined intervals he recovered the pots and tested the germination of the seed. After 20 years, 51 of the 107 species tested still germinated. After 39 years 36 or one third of the species still germinated. Many other examples demonstrating the longevity of seed viability can be sited. Among them are the seeds of mullein and evening primrose which have remained viable for over 70 years. The seeds of Mimosa and Cassia have germinated after being kept in a herbarium for over 200 years. Lotus seeds also have germinated which were estimated to be between 800 and 1,200 years old (5). The oldest

known seeds to still show viability were Arctic lupine (Lupinus arcticus, Wats.) found in the Yukon Territory with an estimated age of at least 10,000 years (40).

What then is dormancy and how does it insure the viability of seeds for long periods of time? This is the question that has puzzled scientists for years. The term dormancy is a vague concept. Here it shall be defined as the inhibition of seed germination; whether it is because of external or environmental factors, or due to inherent qualities of the seed.

Three main environmental conditions are monitored by the seed. These conditions must be met before the seed will germinate. These include a suitable temperature, an adequate water supply, and a normal atmospheric composition. In some species presence or absence of light is also a determining factor in germination. If any of the required conditions is not met, the seed remains dormant.

Many times, however, the conditions for germination are met and still the seeds do not germinate. This is due to the inherent qualities of the seed itself, safety devices which help insure survival of the seed. These inherent qualities have been delineated into two major categories; Exogenous and Endogenous dormancy (38). Exogenous dormancy is dormancy caused by properties of the outer covers of the seed, the seed coat and the pericarp. Endogenous dormancy is dormancy caused by properties of the embryo or inner covers directly surrounding it, the endosperm or seed coat.

Exogenous dormancy entails three main types of dormancy; physical dormancy, chemical dormancy, and mechanical dormancy. Physical dormancy usually is caused by the inability of seeds to imbibe water. This can be due to the orientation of the cells of the seed coat, or to a waxy cutin layer covering the seed. This type of dormancy is very common in the family Leguminosae. Some seeds of this type have what is called a strophiolar cleft. This is a hole in the seed coat through which water can pass when the strophiolar plug has been decomposed or removed by abrasion. Mechanical or chemical abrasion is many times needed to break this type of dormancy. It occurs naturally due to alternate freezing and thawing, mechanical abrasion against soil particles, chemical abrasion due to passage through the digestive tract of higher animals or microbial activity on the seed coat. Scarification or acid treatment is used to break this type of dormancy in seeds of economical importance.

Chemical inhibition is due to the presence of certain chemicals either in the seed or in the pericarp, which impede the germination process. Seeds imbedded in juicy fruits such as orange, lemon, and grapefruit, do not germinate. Inhibition may be due to a chemical in the fruit or juice, may be due solely to the osmotic pressure caused by the high concentration of sugar, or the adverse pH of the juice. Many researchers, however, have shown that when the pH has been neutralized in the case of lemons and the osmotic pressure due to the sugar content has been reduced in the case of grapes, the juices of these two fruits still inhibit germination (38). This would seem

to suggest that a specific chemical in the juice inhibits germination. Removal of the fleshy pericarp and/or washing of the seeds normally restores the germination potential of those seeds. Dormancy of this nature is generally not very deep.

In the case of many desert plants, chemical inhibition is due to chemicals found within the seed itself. Graves et al. (22), doing studies on six desert species, found the inhibiting chemicals to be highly water soluble and easily leached from the seed. When leaching had reduced the inhibiting chemicals sufficiently, the seeds would germinate. This is a means of assuring an adequate supply of moisture for seedling development after the germination process begins.

Mechanical dormancy is a physical inhibition of the embryo growth. Most generally this is due to the inner most layer of the pericarp, the endocarp, which in many species is highly lignified, and many times highly cutinized. Examples of such seeds include plum, peach, walnut, hazelnut, and Russian olive. The degree by which germination is inhibited in this manner varies greatly amongst different species. Researchers have shown that water is indeed imbibed by these seeds and that at least in the case of Russian olive, the sole deterrent for germination is the suppression of embryonic growth due to the hard endocarp (38).

Endogenous dormancy entails two main types of dormancy; Morphological dormancy and Physiological dormancy. Morphological dormancy is due to an underdeveloped embryo. After the seeds are seemingly ripe and fall from the plant, the embryo continues to develop

using the endosperm as its food source. These seeds can not germinate until the embryo has reached a certain stage of development. It is for this reason that the seeds of many plants of the temperate regions will not germinate until spring even though the fall temperature, moisture, and light regimes are very similar to spring conditions. Some species in which morphological dormancy occurs are snowberry, carrot, holly, many types of palm trees, and even to some extent in wheat (38). The period required for the embryo to overcome morphological dormancy is called the afterripening period. Seeds can vary to a great extent in their requirements for afterripening. Some require dry storage while others must be imbibed and/or submitted to varying temperatures.

Physiological dormancy is caused by the impermeability of either the seed coat or the endosperm to gases. An example of this is cocklebur in which Thornton (48) found that the presence of deep dormancy in the second seed was due to the impermeability of the seed coat of that seed to oxygen uptake. In this case all other factors were right for germination except for one, the amount of oxygen needed for the respiration rate to reach the critical point for germination to begin. Roberts (41) and Toole (49) also found the same effect in various grass species.

In recent years speculation has also arisen as to whether or not an oxygen arrestor chemical is also present in the seed coat which is activated or deactivated by certain wavelengths of light. Of the types

of dormancy mentioned above, physiological dormancy is the least understood.

Dormancy in Native Grasses

The native grasses of the Great Plains region generally exhibit a high degree of seed dormancy. Because of their economical importance in range renovation, various seed treatment methods have been devised to break the seed dormancy to more accurately assess the actual seed viability of these grasses. Commonly used treatments developed for overcoming dormancy in grass seed include rupturing the seed coat, prechilling, and alternating temperatures (10) (13).

Wiesner (56), working with Green needlegrass, found that hand removing the glumes, puncturing the seed coat, and clipping the tip of the caryopsis, each increased germination over the standard germination procedures in almost all tests. Byers (7) found that hull removal increased Switchgrass germination by 17%, Indiangrass germination by 19%, and Big bluestem germination by 8%. Clipping the caryopsis tip increased the germination of Indiangrass and Big bluestem by 6% and 8%, respectively.

Rogler (42) and Wiesner (56) found that prechilling Green needlegrass resulted in a higher germination over standard procedures. Clark and Bass (9) and Emal and Conard (14) found that Indiangrass germinated better with a 4-week prechill. Byers (7) found that Indiangrass, Switchgrass and Big bluestem all germinated higher after prechill.

The stimulatory effect of alternating temperatures on seed germination has been known for many years. According to Harrington

(23) the evidence of this effect was first noted in the early 1880's when Cieslar observed that yellow light had more of a stimulatory effect on Poa nemoralis, L. seed germination than white light. Harrington demonstrated this stimulatory effect on the germination of a number of grass species. Morinaga (37) was able to show that Bermudagrass (Cyndon dactylon, L.) and Canada bluegrass (Poa compressa, L.) germinated higher under alternating temperature conditions. In fact, this stimulatory effect is so wide spread throughout the plant kingdom that of the 191 plant species listed under the heading Agricultural Seeds in Rules for Testing Seeds, 1970 (1), the germination of 140 of those species is recommended to be done under alternating temperature conditions.

The stimulatory effect of KNO_3 has also been known for some time. Morinaga (37) states that the stimulatory effect of KNO_3 was known as early as 1911 when Gassner observed that KNO_3 increased germination of Chloris ciliata, Swartz. Morinaga also found that KNO_3 increased the germination of Canada bluegrass and Bermudagrass. In 1938 Toole (52) demonstrated the same effect in a number of grass species including two Bouteloua species. The stimulatory effect of KNO_3 being widespread throughout the plant kingdom, is recommended as the wetting agent for a number of the Agricultural Seeds in Rules for Testing Seed (1) principally amongst the grass species.

Delouche and Bass (13) stated that the impermeability or low permeability of hulls, seed coats and other seed membranes to gas exchange, ie. physiological dormancy, is one of the primary causes of

dormancy in grass seeds. Increases in germination due to hull removal and caryopsis clipping would substantiate that theory. They also suggest that the stimulatory effect of fluctuating temperatures on germination may be due to an increase in the permeability of the seed membranes to gases due to the stress on those membranes caused by the radical temperature changes.

Germination studies with Agropyron smithii would seem to substantiate the theory that the principal cause of dormancy in that species is physiological dormancy. Kinch (29)(30), Delouche and Bass (13), Delouche (11) have shown that hull removal, exposure of the embryos, and caryopsis clipping all increased the germination of western wheatgrass significantly over the control. Plummer (39) and Knipe (31) were able to show that fluctuating temperatures gave a significantly higher germination of western wheatgrass over constant temperature controls. Delouche (11) demonstrated that the germination of A. smithii was increased when partial pressure of oxygen in the atmosphere was increased above normal, and that the germination decreased when the partial pressure of oxygen was decreased below normal atmospheric concentration. Hay (25) found that prechilling was inhibitory on western wheatgrass germination, but Delouche (11) found that rupturing the seed coat removed that inhibition. An increase in O_2 uptake due to the different treatments would seem a likely explanation for the results of those studies.

Light was also found to affect western wheatgrass germination. Hay (25), Delouche and Bass (13), Bass (2), and Delouche (11) all

found that exposure to white light during germination inhibited the germination of western wheatgrass. Delouche (11) found that puncturing the seed coat did not remove the inhibitory effect of light and concluded that inhibition by light was not caused by the alteration of the gas permeability of the seed coat.

Seed Response to Light

That light may affect seed germination has been known since 1860 when Caspary discovered that Bulliardea aquatica, D.C. seeds germinated better in full sunlight than diffuse light (15). Stebler attempted to quantify that finding (15). By working with the seeds of several grasses, he was able to prove that these grasses germinated better in light than in total darkness. Heinrecher (15) was the first to report that light could also inhibit germination, as was the case with Acanthostachys strobilacea.

Flint and McAlister (17) and Flint (16) were the first to study component parts of white light to determine if any one wave length had more effect than another on lettuce seed germination. They found that radiations of 520 nanometers (nm.) to 700 nm. (red light) had a stimulatory effect on germination, the critical wavelength being 660 nm. Furthermore, they found that radiations of 700 nm. to 860 nm. (far red light) had a highly inhibitory effect on seed germination, the critical wavelength being 760 nm.

Borthwick et al. (4) were the first to show the reversibility of the effect of red light and far red light in Grand Rapids lettuce seed. The type of treatments used and their effect can be seen in

Table 2. Borthwick et al. (4) were also able to show a similarity between the stimulatory and inhibitory effects of red and far red light, respectively, in flower initiation as well as seed germination.

TABLE 2. Effect of successive red and far red light treatments on lettuce seed germination.

Light Treatment	Germination
none	8.5%
red	98.0%
red-far red	54.0%
red-far red-red	100.0%
red-far red-red-far red	43.0%
red-far red-red-far red-red	99.0%
red-far red-red-far red-red-far red	54.0%
red-far red-red-far red-red-far red-red	98.0%

The theory was then developed that the photoreceptors responsible for these reactions were in actuality one pigment. This pigment existed in two states; an active and an inactive state. The inactive state (Pr) was responsive to red light radiation which changed the pigment to the active state (Pfr). The active state was responsive to far red radiation which in turn reverted the pigment back to the inactive state. This hypothesized pigment was named phytochrome.

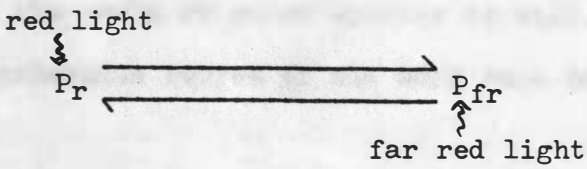


Figure 1. Phytochrome response system

Butler et al. (6) were the first to isolate phytochrome from plant tissue. It was found to be a blue chromoprotein with a molecular weight of about 60,000. Galston (21) using a microspectrophotometric technique demonstrated the presence of phytochrome in the nuclear membrane of etiolated oat and pea seedlings. Later work done by Haupt (24) showed that phytochrome was localized in the plasmalemma of the cell.

Two hypotheses exist as to the action of phytochrome in the cell. One hypothesis offered by Mohr (36) is that the primary action of phytochrome is that of gene activation and gene repression. Other work would indicate, however, that the mode of action of phytochrome is that of changing cell permeability. Results of experiments by Fondeville et al. (18) and Fondeville et al. (19) with Mimosa pudica, L., and Jaffe and Galston (27) with Albizzia julibrissin, Durazz, indicated that the action of phytochrome was too rapid to be that of gene activation. They concluded that phytochrome altered the permeability of the cell membranes and that all other reactions were secondary reactions. Tanada (45) observing the effects of red and far red light on the root tips of barley came to the same conclusion.

The effect of phytochrome action on seed germination and dormancy has been studied extensively with lettuce seed by many researchers and to some extent with the seeds of other species as well. Toole (51) has compiled a comprehensive review of the work done in this area.

Hormones and Seed Dormancy

Various plant hormones have also been shown to be instrumental in the breaking of seed dormancy. Two of these hormones are Gibberellic acid (GA.) and Kinetin. The effects of GA. on plant growth, bud dormancy, and flowering are well known. The effect of GA. in seed germination was first thought to be that of growth stimulation of the seedlings after germination had begun and not that of breaking seed dormancy. However, many experimenters have shown that GA. does in fact break dormancy in many types of seeds. Helgeson and Green (26) were able to show the stimulatory effect of GA. on wild oats seed germination. Franklin (20) and Clark and Bass (9) were also able to demonstrate that effect in hazel, beech and rowan seeds and Indian ricegrass seeds, respectively. Chen and Varner (8) and Vidaver and Hsiao (54) were able to show that GA. removed the light requirement in light sensitive lettuce seeds. Delouche (12) found the same response in Bracted plantain. On the other hand Speer et al. (44) gave evidence to the fact that GA. interacted with red light exposure to stimulate germination. Working with lettuce seed, he was able to show that the application of GA. in darkness had no significant stimulatory effect over the control, while in conjunction with red light, GA. had a decisive stimulatory effect.

Cytokinins have also been shown to have a stimulatory effect on shoot growth and development. Various and conflicting results have been obtained concerning the effect of cytokinins on seed dormancy and germination. Tao et al. (46) were able to demonstrate the stimulatory

effect of kinetin on cocklebur and Indian ricegrass seed. Franklin (20) and McCoy and Harrington (35) were able to demonstrate this also with lettuce seed and hazel, beech and rowan seed, respectively. Both Chen and Varner (8) and Waring et al. (55) claim that kinetin can remove the chilling and light requirements of lettuce seed. Leff (32) was able to demonstrate, however, that kinetin had no stimulatory effect on lettuce seed germination in the dark. She was able to show an increase in germination of the lettuce seed when red light and kinetin treatments were both given. This was also found by Bewley et al. (3) in lettuce seeds and Khan (28) in cocklebur seeds. They concluded that kinetin acted by making the seeds more sensitive to red light radiation.

METHODS AND MATERIALS

Dormancy and Maturity

This study was designed to determine at what stage of maturity dormancy becomes expressed in western wheatgrass seed. For this study five locations were chosen at the Samuel H. Ordway Memorial Prairie, eight miles west of Leola, South Dakota. The sites were chosen so that each represented a different topographic area. The locations included an east facing slope, a west facing slope, a south facing slope, a knoll, and a bottom flat. Beginning July 22, 1975, seed collections were randomly made within each site at 3-day intervals until August 9, 1975. One final collection was made October 7, 1975. The seeds from these collections were subdivided according to their development. The state of development was determined by the relative length of the caryopsis to the palea. The two classifications were: (1) the caryopsis being less than one half the length of the palea, and (2) the caryopsis being greater than one half the length of the palea. The standard method for testing western wheatgrass germination as outlined later in this paper was used to determine the germination percentage of the seed lots. At the end of the 28-day germination period, the ungerminated seeds were tested for the presence of dormancy by the tetrazolium viability test (47). The ungerminated seeds were bisected longitudinally, exposing the embryo. One half of each seed was placed in 1% tetrazolium solution for four hours. At the end of that period the seeds which contained red or pink embryos were

considered to be dormant seeds. All other seeds were considered dead. This procedure was used to determine the dormant seed percentage in all of the germination tests.

Dormancy and Location

An attempt was also made to determine if the dormancy in western wheatgrass seed was a function of the regional location in which the seed was produced. For this study a site was randomly chosen in each of 33 counties in South Dakota, both east and west of the Missouri River. One seed collection was made at each site in August and in October another collection was taken in many of those counties. The counties were grouped into four regions: east of the Missouri River; the Black Hills; west of the Missouri River, north of 44° 22' North Latitude; and west of the Missouri River, south of 44° 22' North Latitude. Germination tests were made on the collections from each site and the tetrazolium test was used to determine the percent of dormant seeds.

Breaking Seed Dormancy

In this study various alternative seed treatment procedures were tested in comparison to the standard method for determining western wheatgrass germination to determine their relative efficiency in breaking seed dormancy. The control was the standard procedure for testing the germination of western wheatgrass seed as employed by South Dakota State Seed Testing Laboratory. The seeds were planted on special seed germination blotting paper in 12 x 12 x 2.8 cm. covered

TABLE 3. The counties in which western wheatgrass samples were collected arranged in their respective regions.

East River	Black Hills	West River, North 44° 22' N. Lat.	West River, South 44° 22' N. Lat.
Buffalo	Custer	Butte	Bennett
Campbell	Fall River	Corson	Gregory
Edmunds	Lawrence	Dewey	Haakon
Faulk		Harding	Jackson
Hand		Meade	Jones
Hughes		Perkins	Lyman
Hyde		Stanley	Mellette
McPherson		Ziebach	Pennington
Potter			Shannon
Sully			Tripp
Walworth			Washabaugh

plastic boxes. The seeds were treated with the fungicide Polyram* before being placed on the blotters. A 0.2% KNO_3 solution was used as the wetting agent. The boxes were wrapped in aluminum foil and placed in an alternating temperature germinator. The temperature was 15° C for 16 hours followed by an eight-hour period at 30° C. After 14 days the first count was made and the boxes were rewrapped and replaced in the germinator. The final count was made after 28 days and the dormant

*Polyram is Niagara Chemical Division's trade name for a powder which contains a mixture of /ethylenebis /dithiocarbamato/ /zinc and /dithiobis/ (thiocarbonyl) iminoethylene/ / bis / dithiocarbamato/ / zinc.

seed percentage was determined by subjecting the ungerminated seeds to a tetrazolium test. Samples of 100 seeds were used per treatment per repetition.

Four alternative treatments were compared to the standard procedure. Treatment one differed from the control only in that the boxes were not wrapped before being put in the germinator. Consequently, they were exposed to fluorescent white light for nine hours each day. Treatment two differed from the control in that the boxes were not placed in the germinator, but left at room temperature, a constant 24° C, throughout the testing period. Treatments three and four were subjected to exposures of red and far red light, respectively.

The method employed in giving the red and far red light treatments was according to procedures outlined by Machlis and Torrey (33). The treatments were given in a cardboard box lined with aluminum foil to exclude any outside light. The box was constructed in such a way that the seeds would be 18 inches from the light source. The red light source was a 25 watt, 115-125 volt, Sylvania BAS photographic safety light with emissions in the red light region of the spectrum. The far red light source was a 100 watt, 120 volt, General Electric incandescent bulb. This light was passed through a CBS Far Red 750 filter made by the Carolina Biological Supply Company. It filtered out wavelengths less than 750 nm. in length. Before the light treatments were given, the seeds were allowed an 18-hour imbibition period in the 15°-30° C germinator. The light treatments were given during the 30° C part of the germination cycle and in a dark room to insure that the seeds were not exposed to any other light except the specific

treatment until the first count was made after 14 days. Exposure times were four minutes for the red light and eight minutes for the far red light.

Quantified Light Effects

This study was undertaken to quantitatively determine the effect of red and far red light over a range of exposure times. The range of exposure times for red light was 4, 8, and 16 minutes in length. The range of exposure times for far red light was 8, 16, and 24 minutes in length. Two controls were used in this study. One control was the standard procedure for testing western wheatgrass germination as already outlined. The other control differed from the first only in that the boxes were not wrapped; consequently, the seed was exposed daily to white light. The first and final counts were taken at 14 and 28 days, respectively. The dormant seed percentage was determined by the tetrazolium test.

Hormones and Red Light

This study was undertaken to observe the individual effects of KNO_3, Gibberellic acid, and Kinetin on western wheatgrass germination as well as the additive effect of those chemicals given in conjunction with red light. The seeds were subjected to a 24-hour imbibition period in wetting agents of 100 ppm Gibberellic acid, 5 ppm Kinetin, and 0.2% KNO_3, using pure water as a control. One half of the seeds in each treatment were exposed to red light for 8 minutes. The other half of the seeds remained in total darkness. The first and final

counts were taken at 14 and 28 days, respectively, and the tetrazolium test was used to determine the presence of dormant seeds.

RESULTS AND DISCUSSION

Dormancy and Maturity

This study was conducted to determine at what point dormancy becomes expressed in western wheatgrass seed. Seed collections were made at five locations on eight different dates. The seeds from these collections were divided into two maturity classifications: (1) the caryopsis being more than one half the length of the palea, and (2) the caryopsis being less than one half the length of the palea. These seeds were tested for germination and dormancy was determined by use of a tetrazolium test.

The results of this study did not show a significant difference in dormancy among dates, among locations, or between seed sizes (figures 2 and 3). There was a very low incidence of dormancy in the experimental material, the highest dormancy being only 2.4% among dates and 2.1% among locations. Dormancy was highest in those seeds with the caryopsis less than one half the length of the palea, at 1.6% dormancy, but there was only a 0.15% difference in dormancy between the two classifications. The low incidence of dormancy, an average of only 1.5%, is most probably the reason for the failure to show significant differences in dormancy among dates and between seed sizes.

There was a significant difference in germination among dates and locations and between seed sizes (figures 4 and 5). The analysis of variance showed that after the July 28 collection date, the

Figure 2. The percent dormancy occurring in the seed lots with reference to collection dates in the maturity study.

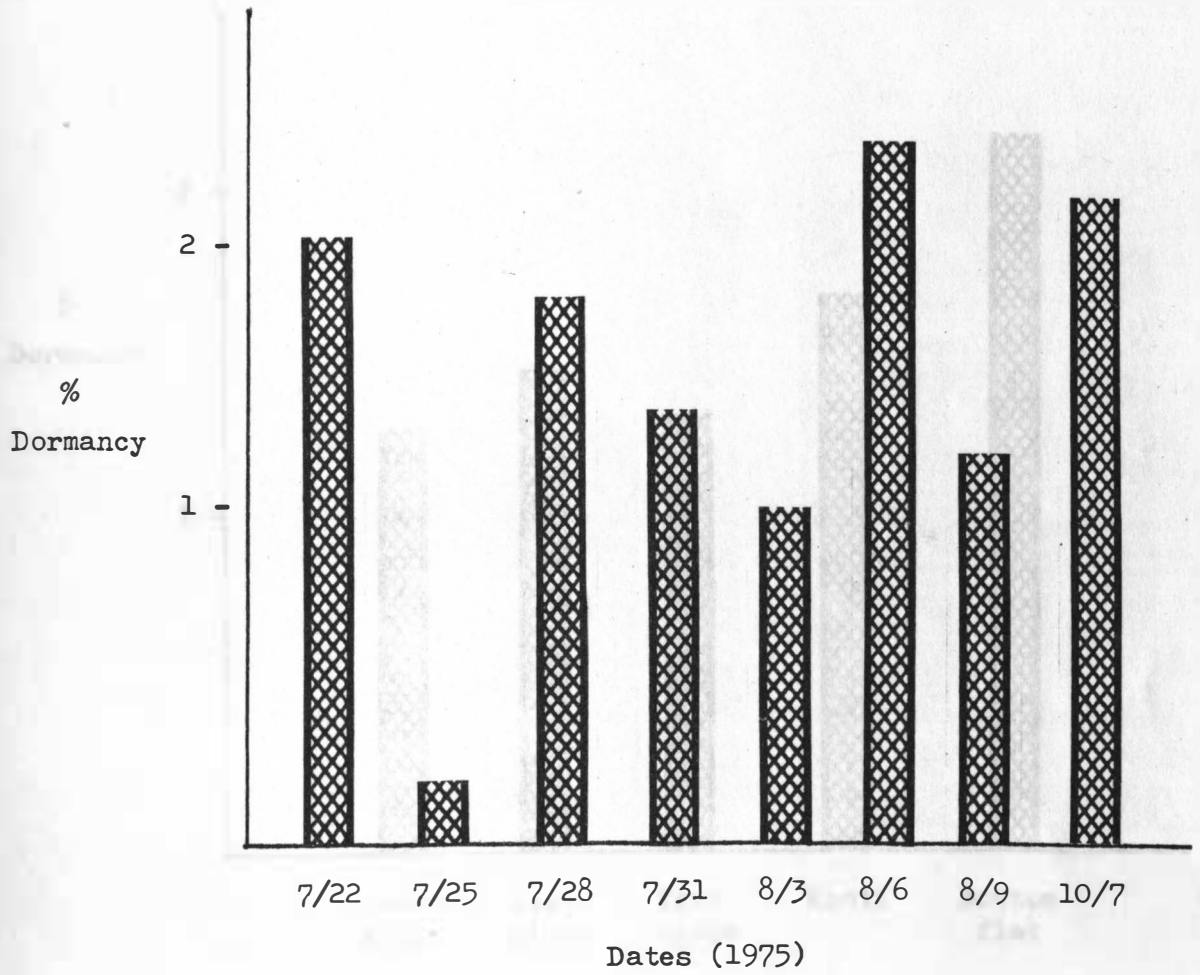


Figure 3. The percent dormancy occurring in the seed lots with reference to collection locations in the maturity study.

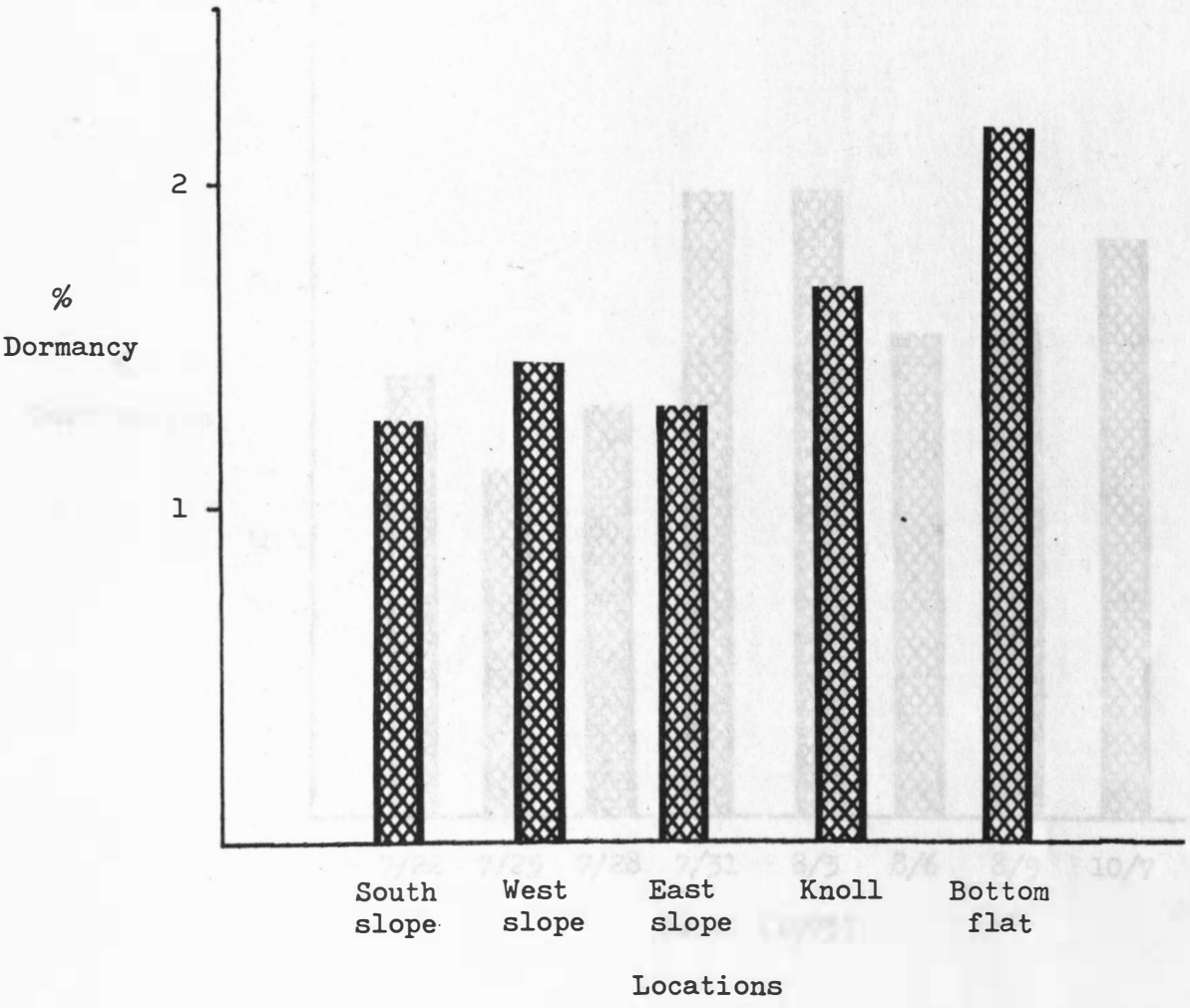


Figure 4. The percent germination occurring in the seed lots with reference to collection dates in the maturity study.

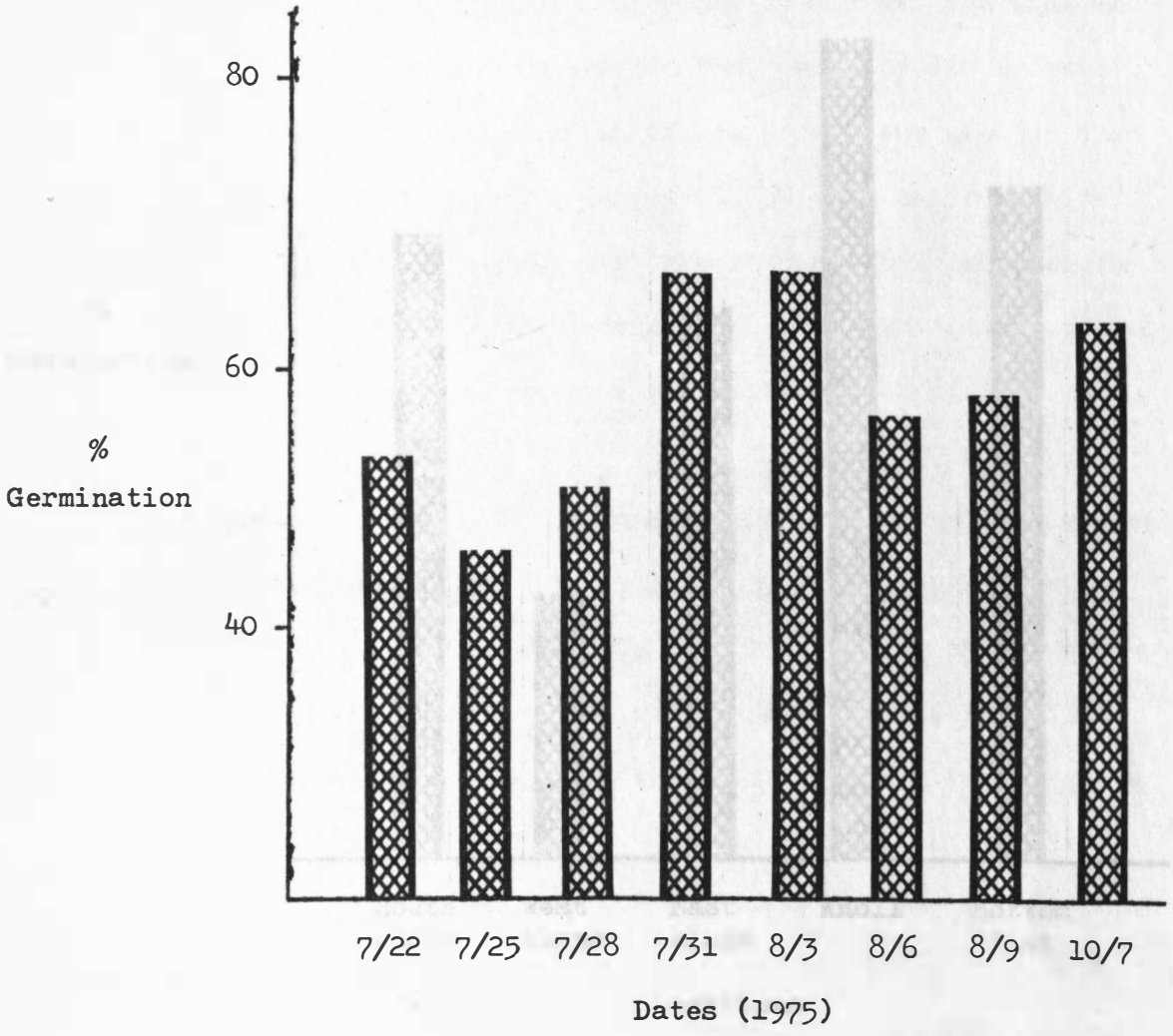
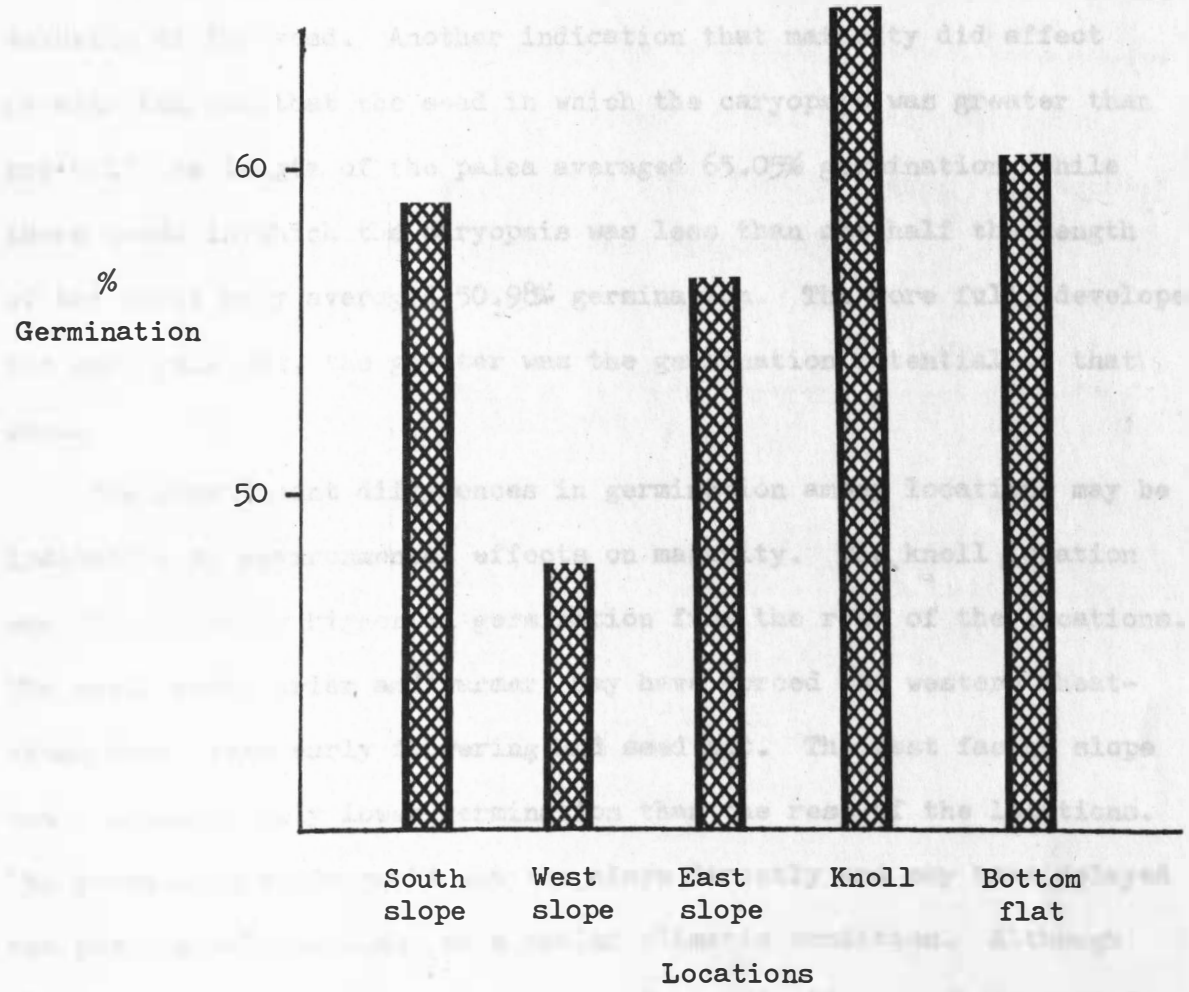


Figure 5. The percent germination occurring in the seed lots with reference to collection locations in the maturity study.



germination of the collections was significantly higher than the collections previous to that time, indicating an overall increase in maturity of the seed. Another indication that maturity did affect germination was that the seed in which the caryopsis was greater than one half the length of the palea averaged 65.05% germination, while those seeds in which the caryopsis was less than one half the length of the palea only averaged 50.98% germination. The more fully developed the caryopsis was, the greater was the germination potential of that seed.

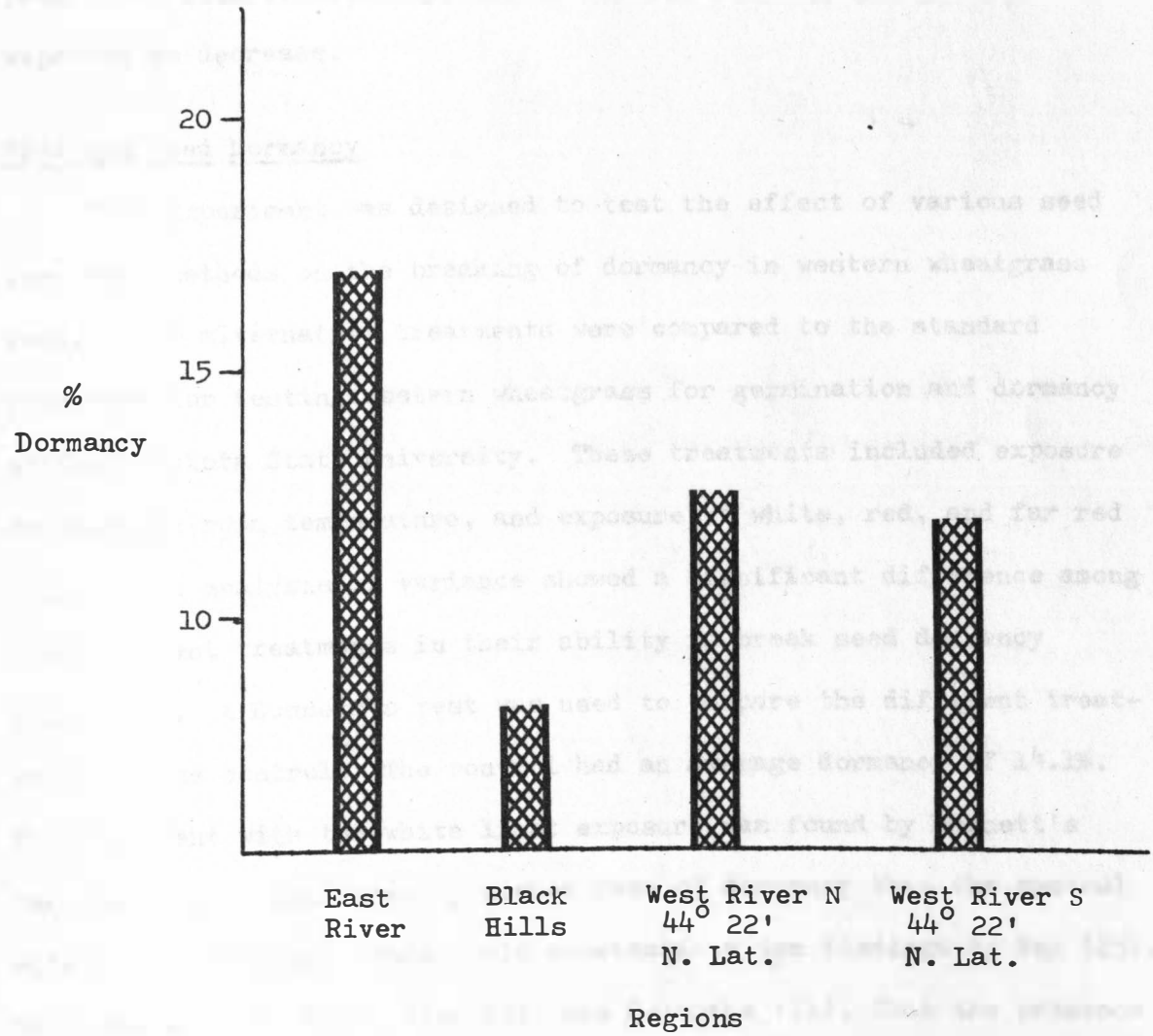
The significant differences in germination among locations may be indicative of environmental effects on maturity. The knoll location was significantly higher in germination from the rest of the locations. The knoll being drier and warmer, may have forced the western wheatgrass seeds into early flowering and seed set. The west facing slope had a significantly lower germination than the rest of the locations. The prevailing winds could hit the slope directly and may have delayed the plant development due to a cooler climatic condition. Although this study indicates that an increase in germination can be expected with an increase in maturity, no indication was found of an increase or decrease in dormancy with an increase in maturity.

Dormancy and Location

The purpose of this study was to determine if the incidence of dormancy in western wheatgrass seed was in any way related to the regional location in which it was produced. The 33 counties in which the collections were made in August were divided into four regions:

east of the Missouri River; the Black Hills; west of the Missouri River, north of 44° 22' North Latitude; and west of the Missouri River, south of 44° 22' North Latitude. An analysis of variance of the data showed no significant differences among regions. There was an indication, however, that the east river counties could have a higher incidence of dormancy in the western wheatgrass seed (figure 6).

Figure 6. The average dormancy of western wheatgrass seed according to regions within South Dakota.

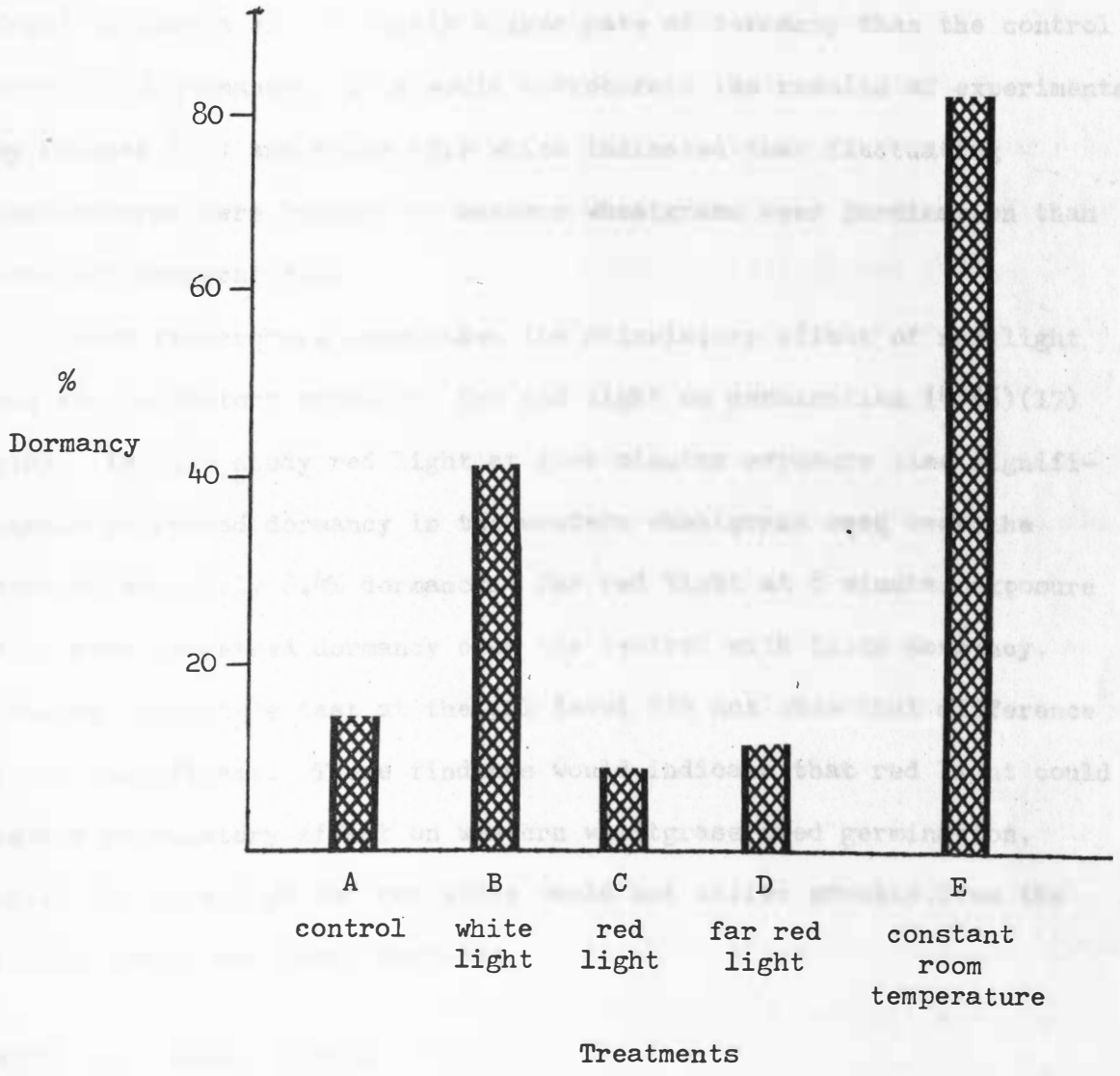


In some of the counties a second seed collection was taken in October. The purpose of this collection was to determine if the change in maturity of the seed had an influence on the incidence of dormancy in the seed. When the analysis of variance was performed on the differences in dormancy between the two collection dates among the different counties, a significant decrease in dormancy was noted in the second collection. There was also a strong interaction between locations and dates. This would indicate that as western wheatgrass seed progresses towards maturity, the incidence of dormancy could be expected to decrease.

Breaking Seed Dormancy

This experiment was designed to test the effect of various seed treatment methods on the breaking of dormancy in western wheatgrass seed. Four alternative treatments were compared to the standard procedure for testing western wheatgrass for germination and dormancy at South Dakota State University. These treatments included exposure to constant room temperature, and exposure to white, red, and far red light. The analysis of variance showed a significant difference among the different treatments in their ability to break seed dormancy (figure 7). A Dunnett's test was used to compare the different treatments to the control. The control had an average dormancy of 14.1%. The treatment with the white light exposure was found by Dunnett's test to have a significantly higher rate of dormancy than the control with 41.5% dormancy. This would substantiate the findings by Hay (25), Delouche and Bass (13), Bass (2), and Delouche (11), that the presence

Figure 7. The effects of various light and temperature treatments on the incidence of dormancy in western wheatgrass seed.



Dunnett's test .01 level

A vs. B Significant
A vs. C Significant
A vs. D Not Significant
A vs. E Significant

of white light during the germination process does inhibit western wheatgrass germination. The constant temperature treatment was also found to have a significantly higher rate of dormancy than the control with 80.4% dormancy. This would corroborate the results of experiments by Plummer (39) and Knipe (31) which indicated that fluctuating temperatures were better for western wheatgrass seed germination than constant temperatures.

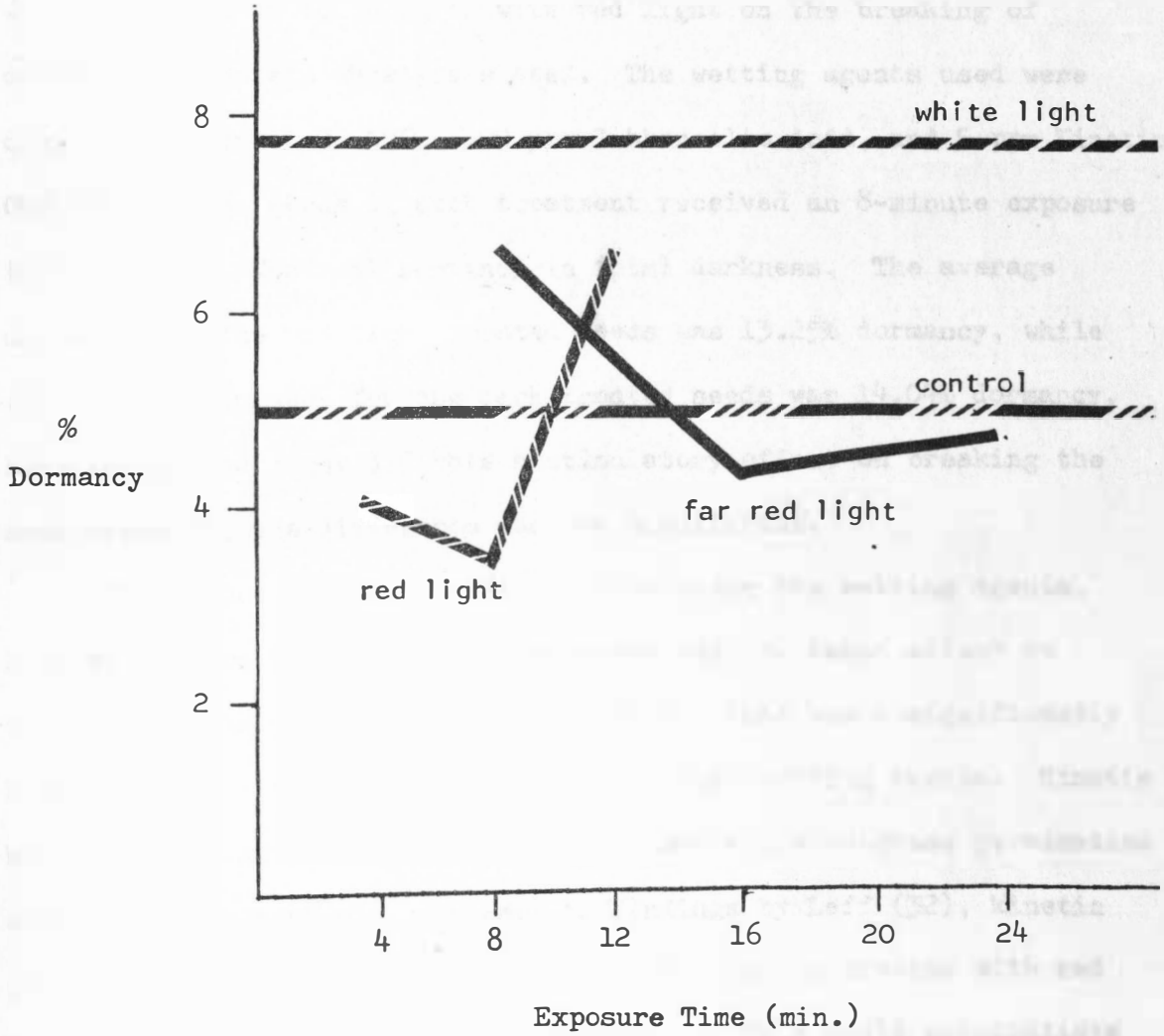
Many researchers have shown the stimulatory effect of red light and the inhibitory effect of far red light on germination (4)(6)(17)(18). In this study red light at four minutes exposure time significantly decreased dormancy in the western wheatgrass seed over the control with only 8.4% dormancy. Far red light at 8 minutes exposure time also decreased dormancy over the control with 11.2% dormancy. However, Dunnett's test at the .01 level did not show that difference to be significant. These findings would indicate that red light could have a stimulatory effect on western wheatgrass seed germination, while the effect of far red light would not differ greatly from the control which was total darkness.

Quantified Light Effects

Since there was an indication that red light stimulated western wheatgrass germination while the effect of far red light seemed to differ little from the total darkness control, an attempt was made to quantify those effects. For this purpose a range of exposure times for both red and far red light was used and compared to a total

darkness control as well as a white light treatment. The red light was given in 4-, 8-, and 12-minute exposure times, while the far red light was given in 8-, 16-, and 24-minute exposure times (figure 8). The control gave an average dormancy of 4.9%. White light again had a significant inhibiting effect on germination and increased dormancy to 7.8%. Red light at the 4- and 8-minute exposures both had stimulatory effects on germination and decreased dormancy to 3.9% and 3.4%, respectively. The 12-minute exposure to red light had an inhibitory effect on germination, however. It increased dormancy to 6.6%, 1.7% higher than the control and almost as high as the white light treatment. This would suggest that prolonged exposure to red light would have the same inhibitory effect on western wheatgrass germination as white light. This might be expected since fluorescent white light contains a high amount of red light. Far red light inhibited germination over the control at the 8-minute exposure time with a dormancy of 6.6%. At the 16- and 24-minute exposure times, the effect of the far red light again seemed to approximate the effect of the total darkness control. Although trends seem to be taking shape, the analysis of variance with the exception of the white light treatment showed no significant difference among treatment means. This again may be due to the low incidence of dormancy in the seed lots tested. The average dormancy for the experiment was only 5.3%.

Figure 8. The effects of various exposure times to red and far red light on the incidence of dormancy in western wheatgrass seed.

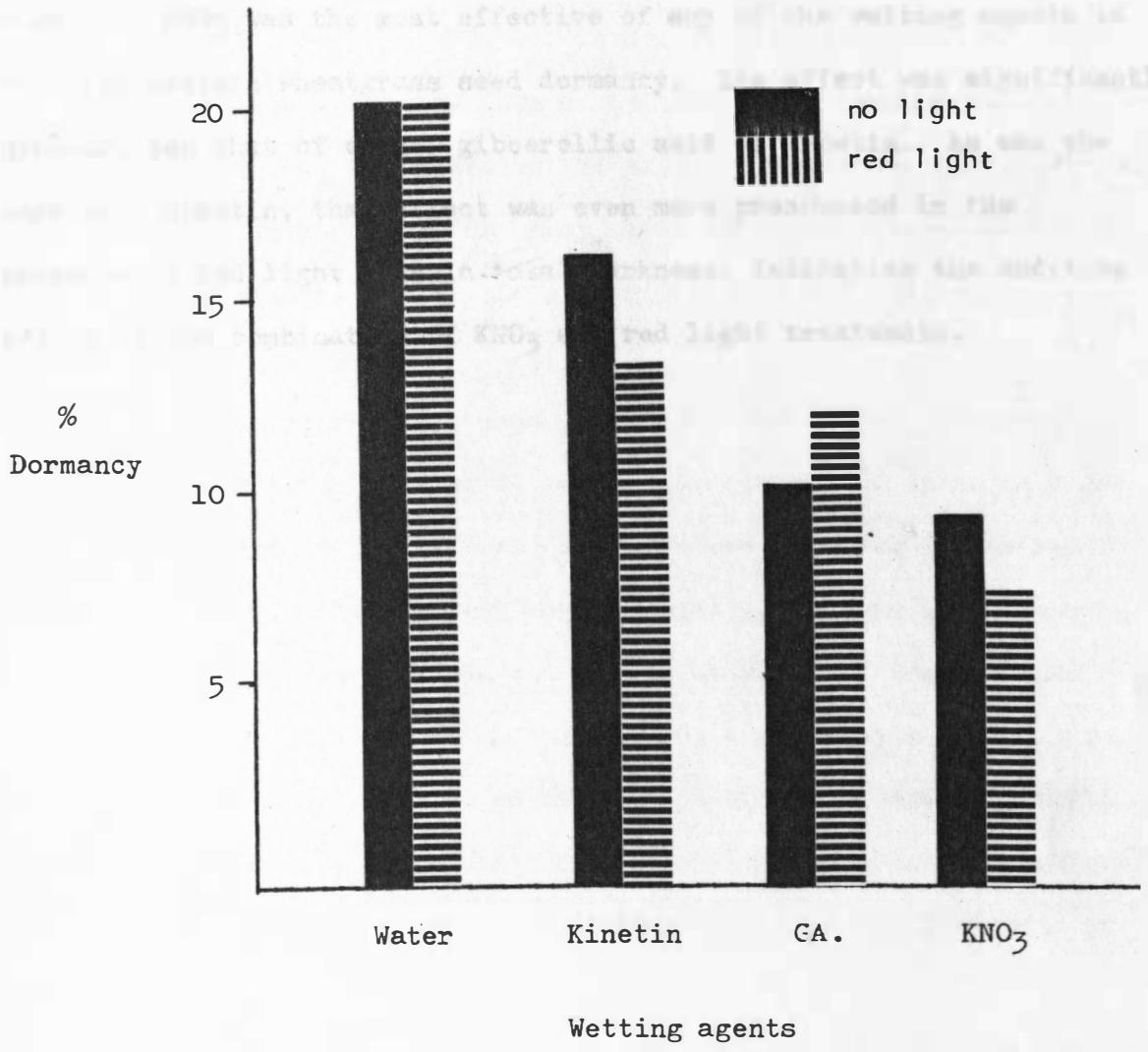


Hormones and Red Light

The purpose of this experiment was to study the effects of various wetting agents in conjunction with red light on the breaking of dormancy in western wheatgrass seed. The wetting agents used were distilled water, 0.2% KNO_3, 100 ppm Gibberellic Acid, and 5 ppm Kinetin. One half of the seeds in each treatment received an 8-minute exposure to red light. The rest remained in total darkness. The average dormancy for the red light treated seeds was 13.25% dormancy, while the average dormancy for the dark treated seeds was 14.04% dormancy. Even though red light did have a stimulatory effect on breaking the seed dormancy, the difference was not significant.

There were significant differences among the wetting agents, however (Figure 9). The distilled water had the least effect on breaking dormancy with a dormancy of 20.8%; that was a significantly higher dormancy than that of any of the other wetting agents. Kinetin had a significant stimulatory effect on western wheatgrass germination over the water control. Contrary to findings by Leff (32), kinetin did have a stimulatory effect in the dark. In conjunction with red light that effect was even more pronounced. This would substantiate the findings by Bewley et al. (3) and Khan (28) that the effects of red light and kinetin on seed germination are additive. Gibberellic acid also had a significant stimulatory effect on western wheatgrass germination over the water control. Contrary to findings by Speer (44), however, gibberellic acid in conjunction with red light in this study had an inhibitory effect, not a stimulatory effect on germination as

Figure 9. The effects of various wetting agents and red light on the incidence of dormancy in western wheatgrass seed.



compared to gibberellic acid alone. There was no significant difference between the stimulatory effects of gibberellic acid and kinetin. KNO_3 was the most effective of any of the wetting agents in breaking western wheatgrass seed dormancy. Its effect was significantly greater than that of either gibberellic acid or kinetin. As was the case with kinetin, that effect was even more pronounced in the presence of red light than in total darkness, indicating the additive effect of the combination of KNO_3 and red light treatments.

SUMMARY AND CONCLUSIONS

A determination of the actual and true germination potential of western wheatgrass seed is very difficult for the seed analyst because of the dormancy almost always present in that seed. The purpose of this study was first, to determine the effect of maturity and regional location on the incidence of dormancy in western wheatgrass seed, and secondly, to determine the effect of various seed treatments on breaking that dormancy.

For the maturity study, seed collections were made throughout the state of South Dakota. The germination and dormancy of those collections were determined by the standard procedure employed by the South Dakota State Seed Testing Laboratory for testing western wheatgrass samples. There was an indication that the maturity of the seed had a bearing on the germination of that seed. On a state-wide basis it was found that with an increase in maturity, a decrease in dormancy could be expected. There was insufficient evidence to indicate that regional locations in South Dakota would have an influence on the incidence of seed dormancy.

In the second part of the study, various alternative seed treatment procedures were tried in comparison to the standard procedures employed to determine the germination and dormancy in western wheatgrass seed. Being exposed to fluorescent white light for a nine-hour period each day had an inhibitory effect on the germination of western wheatgrass seed. Maintaining a constant 24° C during the germination

period was also very inhibitory. There was an indication that red light, up to an eight-minute exposure time, could be effective in breaking some of the seed dormancy of western wheatgrass. Exposure times longer than eight minutes in duration were found to be inhibitory. Exposure to far red light had little effect on the incidence of dormancy in western wheatgrass seed as compared to the dark control.

Gibberellic acid and kinetin used as wetting agents both were significantly effective in breaking seed dormancy over the water control. The difference between the two hormones was not significant however. KNO_3 was the most effective of the wetting agents in breaking the seed dormancy of western wheatgrass, being significantly more effective than either gibberellic acid or kinetin.

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APPENDIX

TABLE 1. The percent dormancy in the western wheatgrass seed collections from the Samuel H. Ordway Memorial Prairie maturity study.

Dates	Caryopsis size	Locations				
		South slope	West slope	East slope	Knoll	Flat
7/22	< 1/2	4	2	0	0	4
	> 1/2	4	2	0	4	0
7/25	< 1/2	0	0	0	0	0
	> 1/2	0	0	0	0	2
7/28	< 1/2	0	0	4	2	2
	> 1/2	4	2	0	4	0
7/31	< 1/2	0	0	4	4	2
	> 1/2	0	2	2	0	0
8/3	< 1/2	0	2	0	2	2
	> 1/2	0	4	0	0	0
8/6	< 1/2	4	2	2	2	0
	> 1/2	2	2	2	2	6
8/9	< 1/2	0	0	2	4	0
	> 1/2	0	4	0	2	0
10/7	< 1/2	0	0	2	0	12
	> 1/2	0	0	2	2	4

TABLE 2. Analysis of variance for the dormancy results of the maturity study.

Source	df	SS	MS	F
Dates	7	36.75	5.250	1.474
Seed Size	1	0.45	0.450	< 1
Locations	4	10.70	2.675	< 1
Dates X Seed Size	7	9.55	1.364	< 1
Dates X Locations	28	140.50	5.018	1.409
Seed Size X Locations	4	16.30	4.075	1.144
Dates X Seed Size X Locations	28	99.70	3.561	
Total	79	313.95		

TABLE 3. The percent germination of the western wheatgrass seed collections from the Samuel H. Ordway Memorial Prairie maturity study.

Dates	Caryopsis Size	Locations				
		South slope 1.	West slope 2.	East slope 3.	Knoll 4.	Flat 5.
1. 7/22	> 1/2	76	52	52	60	52
	< 1/2	72	26	54	44	44
2. 7/25	> 1/2	33	32	62	40	74
	< 1/2	37	26	52	48	60
3. 7/28	> 1/2	44	28	66	70	60
	< 1/2	24	30	52	72	52
4. 7/31	> 1/2	83	50	74	76	74
	< 1/2	48	50	72	72	78
5. 8/3	> 1/2	82	64	68	78	76
	< 1/2	84	68	50	64	34
6. 8/6	> 1/2	88	62	40	66	82
	< 1/2	22	48	38	68	50
7. 8/9	> 1/2	72	56	56	84	90
	< 1/2	42	60	30	70	30
8. 10/7	> 1/2	80	66	78	86	70
	< 1/2	62	48	58	68	32

TABLE 4. Analysis of variance for the germination results of the maturity study.

Source	df	SS	MS	F
Dates	7	4461.69	637.38	5.129**
D_{123} vs. D_{45678}	1	3237.37	3237.37	26.050**
D_1 vs. D_{23}	1	173.37	173.40	1.395
D_2 vs. D_3	1	57.80	57.80	< 1
D_{458} vs. D_{67}	1	915.25	915.25	7.365**
D_6 vs. D_7	1	33.80	33.80	< 1
D_4 vs. D_{58}	1	24.07	24.07	< 1
D_5 vs. D_8	1	20.00	20.00	< 1
Seed size	1	3962.11	3962.11	31.882**
Locations	4	2956.55	739.13	5.948**
L_4 vs. L_{1235}	1	1483.50	1483.50	11.937**
L_2 vs. L_{135}	1	1360.01	1360.01	10.943**
L_3 vs. L_{15}	1	110.51	110.51	< 1
L_1 vs. L_5	1	2.53	2.53	< 1
Dates X Seed size	7	1180.70	168.67	1.357
Dates X Locations	28	7792.25	278.30	2.239*
Seed size X Locations	4	1101.95	275.49	2.217
Seed size X Locations X Dates	28	3479.74	124.28	
Total	79	69595.68		

*Significant at the .05 level
**Significant at the .01 level

TABLE 5. The percent dormancy in the August Collections in 33 counties of South Dakota.

Regions							
East River		Black Hills		West River North 44° 22' N.Lat.		West River South 44° 22' N.Lat.	
Buffalo	1	Custer	10	Butte	4	Bennett	23
	1		9		4		23
Campbell	16	Fall River	1	Corson	9	Gregory	13
	16		1		9		14
Edmunds	49	Lawrence	13	Dewey	5	Haakon	13
	49		13		4		14
Faulk	6			Harding	39	Jackson	6
	6				39		5
Hand	6			Meade	29	Jones	18
	5				15		19
Hughes	57			Perkins	8	Lyman	1
	57				8		0
Hyde	5			Stanley	9	Mellette	33
	4				8		33
McPherson	11			Ziebach	6	Pennington	8
	12				5		8
Potter	15					Shannon	5
	15						2
Sully	10					Tripp	11
	17						10
Walworth	9					Washabaugh	2
	9						2

TABLE 6. Analysis of variance of regional effects on dormancy of western wheatgrass.

Source	df	SS	MS	F
Among Regions	3	535.45	178.48	<1
Among Counties	29	10890.67	375.54	53.65**
Within Counties	33	231.00	7.00	
Total	65	11657.12		

**Significant at the .01 level

TABLE 7. The percent dormancy in the August and October western wheatgrass seed collections in various counties of South Dakota.

County	Aug.	Oct.	County	Aug.	Oct.	County	Aug.	Oct.
Buffalo	1	4	Hand	6	2	McPherson	11	15
	1	1		5	2		12	14
Butte	4	0	Harding	39	0	Pennington	8	5
	4	0		39	0		8	5
Corson	9	5	Hughes	5	4	Potter	15	11
	9	4		4	4		15	11
Edmunds	49	3	Jackson	6	3	Sully	10	10
	48	3		5	4		17	10
Faulk	6	3	Jones	18	8	Walworth	9	8
	6	2		19	7		9	8
Fall River	1	3	Lawrence	13	1	Washbaugh	2	1
	1	2		13	1		2	1
Haakon	13	2						
	14	1						

TABLE 8. Analysis of variance for the difference in dormancy between the August and October collections of western wheatgrass seed in various counties of South Dakota.

Source	df	SS	MS	F
Among Counties	18	3022.63	167.92	< 1
Between Dates	1	1091.37	1091.37	6.490*
Counties X Dates	18	3026.63	168.15	178.883**
Sampling Error	38	35.67	0.94	
Total	75	7176.63		

*Significant at the .05 level
**Significant at the .01 level

TABLE 9. The percent dormancy in seed lots tested under various light and temperature treatments.

Treatments	Lots					
	199	213	216	224	414	861
Control	15	22	11	10	7	22
	17	8	20	10	7	23
	17	7	9	5	6	37
	4	7	16	20	7	31
White Light	33	34	47	44	35	71
	19	34	38	36	37	69
	29	18	30	32	45	68
	36	42	48	46	58	46
Red light	2	4	11	12	3	17
	1	8	2	5	3	22
	7	6	5	5	4	27
	8	5	11	5	7	21
Far red light	3	5	8	13	12	24
	1	2	14	15	16	33
	4	1	12	7	16	15
	4	4	3	19	16	22
Constant 24° C	86	85	84	80	75	86
	76	79	72	79	81	89
	76	78	83	78	78	89
	86	85	73	79	68	84

TABLE 10. Analysis of variance for the effects of various light and temperature treatments on dormancy in western wheatgrass seed.

Source	df	SS	MS	F
Treatments	4	88,687.8	22,171.95	164.25**
Lots	5	4,760.7	952.14	7.05**
Experimental error	20	2,699.8	134.99	3.92**
Sampling error	90	3,098.5	34.43	
Total	119	99,246.8		

**Significant at the .01 level

TABLE 11. The percent dormant seeds in seed lots tested with various exposure times to red and far red light.

Treatments	Lots			
	1243	1255	1429	1469
1. Control	9	0	7	4
	1	2	2	14
2. Red light (4 min.)	6	1	2	4
	10	2	2	4
3. Red light (8 min.)	5	1	3	3
	10	1	3	1
4. Red light (12 min.)	12	1	9	4
	4	3	11	9
5. Far red light (8 min.)	10	4	5	12
	10	5	5	2
6. Far red light (16 min.)	6	1	5	4
	6	3	5	4
7. Far red light (24 min.)	7	5	4	3
	7	2	7	2
8. White light	11	9	10	3
	10	9	7	3

TABLE 12. Analysis of variance of the results of the effects of various exposure times to red and far red light on dormancy in western wheatgrass.

Source	df	SS	MS	F
Lots	3	181.125	60.375	4.92**
Treatments	7	135.750	19.393	1.58
t_8 vs. $t_{1234567}$	1	57.143	57.143	4.64*
t_1 vs. t_{234567}	1	0.024	0.024	< 1
t_{234} vs. t_{567}	1	3.521	3.526	< 1
t_2 vs. t_{34}	1	6.740	6.740	< 1
t_3 vs. t_4	1	42.250	42.250	3.44
t_5 vs. t_{67}	1	25.521	25.521	2.08
t_6 vs. t_7	1	0.551	0.551	< 1
Experimental error	21	258.125	12.292	2.20*
Sampling error	32	179.000	5.594	
Total	63	754.000		

*Significant at the .05 level
**Significant at the .01 level

TABLE 13. The percent dormancy in seed lots tested with various wetting agents and red light treatment.

Wetting agent	Light treatment	199	224	716	1243	1255	1429
KNO_3	Red	11	3	4	15	2	6
	None	4	10	4	19	3	14
GA.	Red	8	10	7	15	11	21
	None	11	3	8	7	5	27
Kinetin	Red	10	16	8	19	9	18
	None	15	23	17	22	2	18
H_2O	Red	10	23	37	24	11	20
	None	18	26	28	25	14	14

TABLE 14. Analysis of variance of data testing the effects of various wetting agents and red light on the germination of western wheatgrass.

Source	df	SS	MS	F
Lots	5	679.86	135.972	3.749**
Treatments	7	1155.75	165.107	4.552**
Wetting agents	3	1107.23	369.077	10.176**
A_4 vs. A_{123}	1	826.56	826.560	22.790**
A_1 vs. A_{23}	1	200.00	200.000	5.515*
A_2 vs. A_3	1	80.67	80.670	2.224
Light treatment	1	7.52	7.520	< 1
Wetting agent X Light treatment	3	41.00	13.667	< 1
Experimental error	35	1269.37	36.268	
Total	47	3104.98		

*Significant at the .05 level
**Significant at the .01 level