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Title: DORMANCY OF KENTUCKY BLUEGRASS (POA PRATENSIS L.) SEED: THE EFFECT OF GERMINATION ENVIRONMENT, HORMONE APPLICATIONS, AND HULLING ON SEED GERMINATION

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Don F. Grabe

The time required for germination of Kentucky bluegrass (Poa pratensis L.) seed is traditionally time consuming, and a handicap to efficient marketing practices. Slow germination characteristics also result in slow establishment of turf and lawns. This study was initiated to find ways to overcome both of these difficulties. Specific objectives of this study were to:

(1) determine if the methods recommended in the current Rules for Testing Seeds are applicable for germination testing of modern varieties,

(2) determine the feasibility of obtaining accurate germination results in a shorter period of time by the application of growth regulators to the germination media, and

(3) determine the feasibility of hulling the seed to enhance the speed of germination.

The first experiment was conducted on seed from nine Kentucky bluegrass varieties and several seed lots of certain varieties that were 18, 6, or 2 months old. Seeds were subjected to four different temperatures (15, 20, 25, and alternating 15-25 C), and light or dark

with either distilled water or a potassium nitrate (KNO_3) solution. Results indicate that the testing conditions currently used by seed labs, i.e., alternating temperature, light, and KNO_3 were most favorable for Kentucky bluegrass seed germination.

The second experiment was conducted on 2-month-old seed from five varieties of Kentucky bluegrass. Several hormones were used at various concentrations, alone and in combination to determine their effectiveness in overcoming Kentucky bluegrass seed dormancy. Gibberellin (GA_3) or ethephon plus a KNO_3 solution reduced the time required for complete germination from 28 to 21 days.

The third experiment was conducted on five varieties of 2-month-old Kentucky bluegrass seed to determine if altering the hulls could change germination responses. Physical treatments included partial and complete hull removal, puncturing the lemma, and removal of the distal seed end. Hull removal resulted in complete germination in 7 days at 15-25 C. Seedling growth was sufficient for evaluation of normal and abnormal seedlings in 14 days.

DORMANCY OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED: THE EFFECT OF GERMINATION ENVIRONMENT, HORMONE APPLICATIONS, AND HULLING ON SEED GERMINATION

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DORMANCY OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED: THE EFFECT OF GERMINATION ENVIRONMENT, HORMONE APPLICATIONS, AND HULLING ON SEED GERMINATION

INTRODUCTION

Kentucky bluegrass is the leading type of turfgrass grown worldwide, as well as an important seed crop in Oregon. Following harvest, the seed exhibits a high degree of dormancy which necessitates extended germination test periods and results in slow establishment of lawn and turfs.

In an effort to standardize the germination testing methods used in all labs, factors were incorporated into the official germination testing process. The Rules for Testing Seeds require that seed germination tests for all varieties of Kentucky bluegrass (*Poa pratensis* L.) be conducted in light at 15-25 C with 0.2% KNO_3 for 28 days following a 5-day prechill period for fresh and dormant seed. These methods were developed for common Kentucky bluegrass, before the advent of the many modern varieties. They were also developed before much of the present knowledge concerning the germination-stimulating effects of growth regulators became known. As a result, concern arises as to the adequacy of the germination methods for present-day seed industry operations. Are the methods applicable to all the varieties that have been released? Can the germination period be shortened by treatment with dormancy-breaking chemicals?

Quicker germination would also be desirable when establishing Kentucky bluegrass for lawns and turf. Previous attempts to hasten germination in the field have frequently involved some form of soaking the seeds in water or chemicals before sowing. Such treatments are

usually not compatible with normal seed conditioning practices and a mechanical treatment, if successful, would be much preferred. Because hulling has been successful in enhancing germination of several other grasses, it is logical to presume that Kentucky bluegrass might also respond to hulling.

These studies were initiated on the premise that application of known principles should result in shorter germination test periods and hasten establishment of Kentucky bluegrass turf. The major emphasis of this thesis dealt with overcoming dormancy in Kentucky bluegrass seed. The specific objectives were:

(1) To determine the consistency of reactions of several varieties, lots, and ages of Kentucky bluegrass seed to the germination environment.

(2) To determine the feasibility of obtaining accurate germination for Kentucky bluegrass seed in a shorter period of time by the application of growth regulators to the germination media.

(3) To determine the feasibility of hulling the seed to enhance speed of germination.

The results are presented in the form of three manuscripts. The first manuscript deals with the response of nine varieties, several lots and ages of Kentucky bluegrass seed to several environments to determine the most appropriate germination environment for the different varieties. The second manuscript deals with the reactions of several Kentucky bluegrass varieties to some common growth regulators in an attempt to increase the speed of and total germination. The third manuscript deals with the effect of hulling on seed germination. Hopefully, this thesis will answer some of these questions and set the stage for further experimentation in this area of study.

LITERATURE REVIEW

Interest in germinating Kentucky bluegrass seed prompted the publishing of several articles on this subject in the early 1900's. E.H. Toole (131, 132) suggested guidelines for testing germination of Kentucky bluegrass seed. He suggested that light was not necessary if mature Kentucky bluegrass seeds were germinated with the proper alternations of temperature and that Kentucky bluegrass seed was easily injured by excess water. By 1923, when Toole wrote these articles, the beneficial effects of KNO_3 were known. Toole suggested the use of potassium nitrate (KNO_3) in a dilute solution for enhancing germination. Since 1923, many new varieties of Kentucky bluegrass have been introduced; however, many of the problems that were encountered when attempting to germinate seed of common Kentucky bluegrass remain a problem today.

The Association of Official Seed Analysts (AOSA) Rules for Testing Kentucky bluegrass (Poa pratensis L.) seeds (6) require that germination tests of all varieties be conducted in the light at 15-25 C with 0.2% KNO_3. Fresh and dormant seed should be prechilled at 10 C for 5 days. The test duration is 28 days, in addition to the prechill period. These general germination requirements have evolved slowly over the years. The 1937 Rules (1) recommended 20-30 C with water and light to be used for fresh seed. In 1949 (2), the temperature was changed to 15-30 C, and 0.1% KNO_3 and light were added to the general requirements. In addition, the provision was added for prechilling dormant seed at 10 C for 5 days. In 1954 (3), the concentration of KNO_3 was increased to 0.2%, and a temperature of 10-30 C was added as an

alternative temperature. in 1970 (5), 15-25 C became the only recommended temperature.

The change in germination methods in the Rules reflect the findings of various researchers who have studied the germination requirements of this species. The proper germination temperature requirement has undergone the greatest change since the original Rules were enacted. Work of several researchers has contributed to bringing about these changes.

Toole (157) reported that the use of alternating temperature was an important factor for germination of Kentucky bluegrass seed. Andersen (16) considered 15-30 C to be the most favorable alternating temperature. Sprague (141) found 10-30 C to be superior to 15-30 C and 20-30 C for germination of freshly harvested seed from greenhouse-grown plants. In addition to alternating temperature, a number of conditions and treatments contribute to Kentucky bluegrass seed germination. All of these various aspects deserve individual attention.

Potassium Nitrate

The effects of KNO_3 on Kentucky bluegrass seed germination are not completely understood. Mayer and Poljakoff-Mayber (104) indicated that KNO_3 affects the general breakdown of stored proteins. Ikuma and Thimann (77) suggested that KNO_3 was taken up during the first phase of germination. They considered this first phase to be the dark imbibition of water. The second phase of germination was light induction when KNO_3 was able to counteract the inhibitory effects of far-red light. Maguire (104) suggested that KNO_3 did not overcome dormancy *per se*,

but acted in conjunction with light and alternating temperature to increase germination of dormant Kentucky bluegrass seed by causing an increase in respiration rate. Maguire also suggested that KNO_3 enhanced or substituted for gibberellin induction of alpha-amylase synthesis during the early phase of germination. The role of KNO_3 may be directly related to its ability to overcome the inhibitor blocking synthesis of alpha-amylase.

Hendricks and Taylorson (65) theorized that nitrate and related compounds promoted germination by inhibiting the decomposition of hydrogen peroxide (H_2O_2) by catalase. This inhibition (66) then started a chain reaction leading to germination. Promotion of germination also depended on the coupling of peroxidase activity to NADPH oxidation. This in turn regulated the pentose phosphate pathway, possibly using pyridine nucleotide quinone reductase as the coupling enzyme. Hendricks and Taylorson came to these conclusions after studying the effect of nitrate, nitrite and hydroxylamine on germination of Amaranthus albus, Lactuca sativa, Phleum pratense, Barbara vulgaris, B. Verna and Setaria glauca seeds. Hendricks and Taylorson (66) used lettuce and pigweed seeds to re-confirm their hypothesis that nitrates were involved in hydrogen peroxide metabolism. This then resulted in the oxidation of NADPH in the pentose phosphate pathway. The theory of catalase inhibition by nitrate compounds was supported by the presence of several enzymes in dry seeds which, according to these authors, could serve to couple with hydrogen peroxide in the oxidation of D-glucose-6-phosphate. The continued functioning of the pentose phosphate pathway then depended on the supply of D-glucose-6-phosphate as well as on the concentration of $NADP^+$ and

on the reoxidation of NADPH.

Prechill

Several authors have reported responses of seeds to a prechill environment. Bostock (31) found that seeds from five perennial weeds (Achilla millefolium, Artemisia vulgaris, Cirsium arvense, Taraxacum officinale, and Tussilago farfara) responded favorably to a chill treatment. Bostock also reported a light and alternating temperature synergism in these seeds which was enhanced by the chill treatment resulting in increased speed of germination. Roberts and Benjamin (134) chilled Chenopodium album, Capsella bursa-pastoris and Poa annua seeds. They reported higher germination results for chill treated seeds than for non-chilled seeds. Roberts and Benjamin also studied the effect of alternating temperature, light and KNO_3 on germination of these seeds and reported a light-alternating temperature synergism as well as a light-KNO_3-alternating temperature interaction. However, after the chill treatment, these interactions became less common. In this study, Roberts and Benjamin concluded that a chill treatment may not necessarily be stimulatory, and that it was important not to chill for extended periods. They also concluded that conditions of light, KNO_3, alternating temperature and chill encouraged germination at or near the soil surface where light, alternating temperatures and high nitrate concentrations were most frequently encountered.

Other researchers have also noted the beneficial effects of a chill treatment on seed germination. Haight and Grabe (62) reported the most beneficial effects on orchardgrass germination were obtained by a chill treatment of 3 to 6 days. Results of ragweed seed

germination studies by Brennan, Wielemsen, Rudd and Frenkel (27) demonstrated that breaking dormancy by stratification was dependent on the availability of oxygen. This led to the production of hydrogen peroxide which in turn oxidized NADPH furnishing the pyridine nucleotide which stimulated the activity of the pentose phosphate respiratory pathway and ultimately resulted in germination. Landgraff and Junttila (100) reported significantly increased germination of reed canarygrass seeds after the seeds were treated with a moist prechill, in light, ethylene, enriched oxygen, or by soaking the seeds in aerated water. Working with reed canarygrass seed, Vose (167) removed dormancy by pricking the seed with a pin and by other scarification methods. He concluded that dormancy was caused by limited oxygen exchange which created an anaerobic condition within the seed. This anaerobic environment led to the formation of inhibitors as a result of fermentation. Vose's results, combined with those of Landgraff and Junttila, led Landgraff and Junttila to speculate that alternating temperature may act as an oxygen pump increasing membrane permeability and oxygen concentrations during the chill period or the low temperature phase of the alternating temperature cycle.

Clark and Bass (41) found that germination of dormant Indian ricegrass was promoted by alternating temperature or a 4-week prechill prior to exposure to high temperature. However, these researchers also found that after scarification, the caryopses no longer required a prechill and that these caryopses generally had a higher germination percentage than whole seeds. Several other people have studied the effects of prechill on hulled seed. Ahring and Todd (13) reported

that a moist prechill treatment tended to reduce the viability of hulled bermudagrass seed while unhulled seeds germinated well after the chill treatment. The beneficial effects of a prechill treatment were apparent only during the first 7 days of the germination period. After 28 days, no differences were observed between prechill and non-prechilled hulled bermudagrass seeds. Hulled bermudagrass seeds germinated better than unhulled seeds regardless of the germination conditions. Canode, Horning and Maguire (35), working with orchardgrass seed, reported significant increases in germination after dehulling. Significant increases in germination were also reported by Canode, et al. (35) for non-dehulled prechilled orchardgrass seeds. In a later study, Fendall and Canode (60) reported that one-third of the inhibitor in Latar orchardgrass seed was present in the lemma and palea. The remaining two-thirds was present in the caryopsis. However, on a per unit weight basis the inhibitor was equally distributed between the hulls (lemma and palea) and the caryopsis. These authors theorized that since both a prechill treatment and dehulling decreased dormancy of orchardgrass, both treatments must reduce the amount of inhibitor associated with the seed. Fendall and Canode reasoned that if this was true, release of orchardgrass seeds from dormancy was dependent on a threshold concentration of inhibitor below which the inhibitor was no longer effective.

Bass (25) reported that mature Kentucky bluegrass seeds did not require a prechill treatment to obtain favorable germination results. He further concluded that the most important consideration for germinating Kentucky bluegrass seed was time of maturity of the seed

at harvest. A later study conducted by Delouche (46) supported Bass' work. Delouche reported that a prechill was required by immature Kentucky bluegrass seed to obtain favorable germination results. In this study, Delouche reported that the Kentucky bluegrass seed had lost most of its dormancy after 8 weeks of storage.

Hulling

Enhanced germination of several grasses by hulling (removal of the lemma and palea) has been demonstrated and is a common commercial practice for bermudagrass and bahiagrass seed. Removal of the outer coverings increased germination of Poa compressa (12), beardless ryegrass (140), bermudagrass (9), Kentucky bluegrass (15), Indian ricegrass (60), orchardgrass (30), and several species of Paspalum (97). Delouche (8) reported increased germination of Kentucky bluegrass, regardless of storage moisture levels and prechill treatment, by removing the lemmas and paleas from the caryopses. In 1932, Andersen (15) found that treatment of Poa annua seeds with a dilute nitric acid solution or removal of the glumes increased germination. Burton (34) increased germination of bahiagrass seed by sanding the hulls. He also reported that germination of dallisgrass and bermudagrass increased when a sulphuric acid pre-treatment was applied to the seed. Ray and Stewart (130) reported that germination of certain species of Paspalum seeds were increased by puncturing the hulls. These researchers concluded that puncturing the hulls allowed moisture to reach the caryopses within. McDonald and Khan (111) reported that the outer seed coat was the cause of dormancy in Indian ricegrass seed and that acid scarification effectively broke the outer coat-

imposed dormancy. These researchers postulated that the seed coat prevented germination by delaying the uptake of water, salts, and hormones. Dormancy in wild buckwheat was also attributed to restricted water uptake. Hsiao (73) reported that once the coats of wild buckwheat seeds were made more permeable by NaOCl, germination increased. Puncturing the hulls of wild rice increased the germination of dormant seeds, according to Cardwell, Oelke and Elliot (37). These researchers reported that dormancy was due to impermeability and mechanical resistance of the hulls.

Water restriction is only one method by which seed coats or hulls prevent germination. Fendall and Carter (59) found that the lemma and palea limited oxygen uptake, carbon dioxide release and water uptake, which resulted in the dormancy associated with green needlegrass seed. Barton (2) reviewed coat-imposed seed dormancy and several possible mechanisms by which this occurred. She proposed that coat-imposed seed dormancy was related to: structural properties resulting in water and gaseous restrictions imposed by the seed coat, environmental conditions that favor development of coat restrictions, germination inhibitors found in the seed coat, and methods of overcoming dormancy caused by this structure. Evenari (57, 58) also reviewed several aspects of dormancy including the effects of seed dehulling. He reported that removing the hull removed several germination requirements, including the requirement for light.

Vose (167) demonstrated that dormancy in reed canarygrass seeds was broken by pricking the palea with a pin. He concluded that dormancy in this case resulted from an interaction between oxygen and an inhibitor. Hay (53) found that leachates from the hull completely

stopped the growth of excised A. fatua embryos. Hay attributed the dormancy of these seeds to oxygen restriction by the hulls, although the actual site controlling dormancy was associated with the embryo. Roberts (133) found that removal of only a small amount of the hull (2 mm) resulted in germination of rice seed. Roberts concluded that the cause of dormancy was not due to an inhibitory substance since such a small amount of the hull was removed. Since rice seeds used in Robert's study imbibed water, he concluded that dormancy was due to restriction of gaseous uptake or release by the seed. Momonoki, Hasegawa, Ota, Kaneki and Suzuki (115) reported the presence of an inhibitor which bound available oxygen within the coats of Bupleurum falcatum seeds. Knapp and Wiesner (97) excised the embryos of beardless wildrye seed which resulted in rapid and complete germination. Knapp and Wiesner concluded that dormancy was imposed by the outer coverings of the beardless wildrye seed. They also speculated, as did Simmonds and Simpson (137), that removal of the hulls allowed more oxygen to reach the embryo which altered the metabolism of the seeds and resulted in germination. This alteration was proposed as a shift from the glycolytic-Krebs cycle to the pentose phosphate pathway which produced precursors for energy production and other substances necessary for germination. Since the pentose phosphate pathway was limited, these researchers theorized that increased oxygen supply was a necessary requirement for this shift to occur. Landgrass and Junttila (100) studied germination and dormancy of reed canarygrass and agreed with Vose's theory that dormancy resulted from limited gas exchange through seed hulls. Hsiao (72) reported that the hull, peri-

carp, and testa were the main barriers of gas exchange and penetration by hormones. Hsiao theorized that water was trapped within spaces in the hull, as well as between the hull and the caryopsis. This barrier to gas exchange also modified the movement of hormones and metabolites essential for germination of the seed. He thought the hulls may also prevent leaching of inhibitors as well as hinder gas exchange. Removal of the hull removed inhibitors and gas barriers and also increased the availability of oxygen. Fendall and Canode (59) reported that one-third of the inhibitor found in orchardgrass seed was located in the lemma and palea while the remaining two-thirds was present in the caryopsis. They also reported that on a per unit weight basis, the inhibitor was equally distributed between the hulls (lemma and palea) and the caryopsis.

Hormones

Various growth regulators enhance germination of many kinds of seeds. Many books and articles have been written on this subject. Several of these include: The Physiology and Biochemistry of Seed Dormancy, by A.A. Khan (86); "Hormones and Dormancy" by P.F. Wareing and P.F. Saunders (162); "Hormones and Seed Dormancy" by S.S. Chen and J.E. Varner (39); "Studies in Seed Dormancy" by P.M. Williams and J.W. Bradbeer (174); "A Model of Seed Dormancy" by R.D. Amen (10); "Physiology of Seed Germination" by E.H. Toole, S.B. Hendricks, H.A. Borthwick and V.K. Toole (160); Seed Germination by P. Koller, A.M. Mayer, A. Poljakoff-Mayber and S. Klein (98); and a number of others.

The gibberellins have received a great deal of attention for their role in stimulating germination. Stokes (143) surveyed the effects

of GA_3 and the history of this growth regulator in seed germination. Stokes cited Khan, Goss and Smith (92) as first reporting gibberellins effective in promoting germination in certain seeds. Naylor and Simpson (118) reported that the onset of dormancy in Avena fatua seeds was prevented by allowing the maturing inflorescences to take up gibberellin. These researchers proposed that dormancy was controlled by a gibberellin-inhibitor antagonism. Later Simpson (138), working with A. fatua, discovered gibberellin was involved in embryo dormancy. He suggested that the function of gibberellin was to promote the synthesis of enzymes, or possibly to activate preformed enzymes necessary for the utilization of the endosperm. Emal and Conrad (52) reported increased germination in the dark of Indiangrass seeds with a gibberellin treatment. Khan, Tao and Roe (94) obtained almost perfect germination of lettuce after treating the seeds with gibberellin. Evans and Fratianne (56) found that gibberellin could substitute for red light in Lepidium virginicum seeds. Gibberellin applications were reported by Hsiao (73) to increase germination of cowcockle seed in the dark. Hendricks and Taylorson (67) working with A. fatua, Lactuca sativa, Barbarea vulgare, and other seeds found that gibberellin reduced the transition temperature for the alternating temperature condition enhancing membrane permeability. According to these authors, this suggested a hysteresis in the period required for membrane reorganization as the temperature was lowered. Reduction of the transition temperature was reported here to be proportional to the concentration of gibberellin.

Ethylene was reported to enhance germination of seeds, tubers and cuttings by Vacha and Harvey (166) in 1927. However, it was not until

much later that ethylene was included on the list of growth regulators. While the effects of ethylene have been reported since 1927, relatively little is actually known about how ethylene works in the seed. Ketring and Morgan (81) reported that low concentrations of ethylene gas effectively stimulated germination of Virginia-type peanut seed. This stimulation appeared directly related to the initiating reaction required for converting the quiescent cells of the seed to an active state of growth. In another study, Ketring and Morgan (81) stated that ethylene reversed the effects of ABA in peanut seeds. They suggested that ethylene interacted with ABA to control seed dormancy. Esashi and Katoh (53) studied the effects of ethylene on germination of cocklebur seeds. In this study, they found that the axis and cotyledons of dormant cocklebur seeds produced little or no ethylene, unlike non-dormant seeds which produced ethylene in these regions. When dormant seeds were exposed to low concentrations of ethylene, dormancy was completely lost. In another study conducted by Katoh and Esashi (78), small seeds were induced to germinate in the presence of ethylene and CO_2. They suggested that after-ripening was characterized by increasing ethylene production. Katoh and Esashi (78) also suggested that ethylene intensified the thrusting power of the embryonic axis and cotyledons which resulted in the germination of these cocklebur seeds. Esashi, Okazaki, Yanai and Hishinuma (54) found that ethylene and an oxygen enriched atmosphere effectively prevented the onset of secondary dormancy in cocklebur seeds. These researchers stated that ethylene in an enriched oxygen environment was most effective in stimulating cocklebur seed germination. Later Esashi, Wakabayashi, Tsukada and Satoh (55) went a step further and

suggested that the stimulation of ethylene may be due to its ability to stimulate alternate respiration in seeds. After studying ragweed seeds, Brennan, Wielemsen, Rudd and Frenkel (32) suggested that ethylene along with oxygen may activate the alternate respiration pathway which in turn led to the formation of hydrogen peroxide through partial reduction of the oxygen. At that point, Taylorson (147) theorized that H_2O_2 served to oxidize NADPH by furnishing the oxidized pyridine nucleotide and stimulated the activity of the respiratory pentose phosphate pathway.

The stimulation of germination with exposure to ethrel (an ethylene releasing liquid) has been recorded for several species by Taylorson and Hendricks (153). Ethrel caused marked stimulation of germination in fall panicum (Panicum dichotomiflorum), witchgrass (P. capillari), smooth crabgrass (Digitaria ischaemum), large crabgrass (D. sanguinallis), barnyardgrass (Echinocloa crus-galli), stinkgrass (Eragrostis cilianesis), and giant foxtail (Setaria faberi). They saw no stimulation of germination in seeds of goosegrass (Eleusine indica), annual bluegrass (Poa annua), yellow foxtail (Setaria glauca), and Johnsongrass (Sorghum halepense) when exposed to ethrel. Ethrel inhibited germination of dicot seeds. Taylorson and Hendricks suggested that there may be an interaction between ethylene, gibberellin and phytochrome in the membrane region which may account for the stimulation they observed. Later Taylorson (147) reported large increases in ethylene being released from seeds during accelerated after-ripening as seed moisture increased. He further suggested that ethylene may overcome dormancy by modifying properties of cellular

or organelle membranes as an active substrate. As such, ethylene may show a preferential distribution to the lipid phase of membrane(s) functioning at some particular site within the membrane(s).

Others have noted the effects of ethylene on seed germination and found that combining ethrel with kinetin resulted in a synergistic stimulation of germination. This was observed by Tao, McDonald and Khan (145) with dormant Indian ricegrass germination resulting when ethrel was present. Rao, Sankhla and Khan (132) observed the additive effect of ethrel and kinetin in overcoming high temperature dormancy in lettuce seed. McDonough (112) reported that kinetin plus gibberellin promoted germination of ten species of mountain range plants. McDonough concluded that gibberellin furthers mobilization of reserves, and is required for initiation especially with species that show embryo dormancy. Khan (89) demonstrated the effect of cytokinins plus gibberellin on alpha amylase synthesis in barley seeds. He noted that gibberellin had an overall stimulation of growth and the effects of cytokinins probably depended on factors within the embryo.

Khan and Tolbert (95) studied the effects of kinetin alone and in combination with red light on wheat seeds. They reported that inhibition of germination was reversed by combining red light and kinetin, but not by either treatment alone. Khan and Tolbert (96) in another study observed the effects of light and kinetin on lettuce seed germination and again noted the interaction of kinetin with light. Later Khan (85) theorized that cytokinin activity induced RNA synthesis and formed part of certain transfer RNA's in the germinating lettuce seed. Khan (87) reported that cytokinins overcome the activity of several inhibitors including ABA. Kinetin was successful in breaking dormancy of the

upper seeds of cocklebur (Xanthium). Khan attempted to explain the activity of kinetin by suggesting that it was able to antagonize endogenous inhibitors present in the embryo. This, however, was dependent on the reversibility of the phytochrome system which suggested that an interplay of endogenous inhibitor participated with kinetin and light as well as with temperature. McDonald and Khan (111) examined the effect of hormones on coat-imposed and embryo dormancy in Indian ricegrass seeds after acid scarification. They found that kinetin enhanced alpha amylase activity and germination. They further suggested that kinetin removed the block formed by ABA.

Phaneendranath and Funk (127) observed the ability of gibberellin, kinetin, ethephon, ABA and fusicoccin to overcome dormancy in Kentucky bluegrass seed. The authors found that germination speed and total germination were increased when gibberellin was used in combination with kinetin and/or ethephon, while kinetin or ethephon used singularly or in combination had little effect on germination of Kentucky bluegrass seed.

MANUSCRIPT I

KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) VARIETAL RESPONSES TO SEED GERMINATION METHODS

ABSTRACT

The Rules for Testing Seeds require that seed germination tests for all varieties of Kentucky bluegrass (Poa pratensis L.) be conducted in light at 15-25 C with 0.2% KNO_3 for 28 days. These methods were largely developed for common Kentucky bluegrass before the advent of the many improved varieties. The objective of this study was to determine if the methods recommended in the current Rules are still applicable for germination testing of a wide selection of modern varieties.

Two-, 6- and 18-month-old seed from nine varieties were evaluated for their germination response to light and dark, KNO_3 and water, and constant and alternating temperature. Multiple lots of several varieties of 2- and 6-month-old seed were tested.

Maximum germination was not obtained at constant temperature (15, 20, or 25 C), indicating that the requirement for alternating temperature has not been eliminated during the development of these varieties.

Alternating temperature, light and KNO_3, the Association of Official Seed Analysts' method, provided the highest germination percentage for the largest number of seed lots. No differential varietal response to these germination methods occurred.

Germination rates of 18- and 6-month-old seed were faster than those of 2-month-old seed, but the data do not support the shortening of the test period from the present 28-day duration.

The current AOSA methods for germinating Kentucky bluegrass seed appear adequate for the representative sampling of varieties included

in the study, regardless of their age.

Additional index words: Light, Alternating temperature, Potassium nitrate, Germination rate.

KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) VARIETAL RESPONSES TO SEED GERMINATION METHODS

INTRODUCTION

The Rules for Testing Kentucky bluegrass (*Poa pratensis* L.) seeds (6) require that germination tests of all varieties be conducted in the light at 15-25 C with 0.2% KNO_3. Fresh and dormant seed should be prechilled at 10 C for 5 days. The test duration is 28 days, in addition to the prechill period. These general germination requirements have evolved slowly over the years. The 1937 Rules (1) recommended 20-30 C with water and light to be used for fresh seed. In 1949 (2), the temperature was changed to 15-30 C, and 0.1% KNO_3 and light were added to the general requirements. In addition, the provision was added for prechilling dormant seed at 10 C for 5 days. In 1954 (3), the concentration of KNO_3 was increased to 0.2%, and a temperature of 10-30 C was added as an alternative to 15-30 C. In 1965 (4), 10-30 C was replaced by 15-25 C as an alternative temperature. In 1970 (5), 15-25 C became the only recommended temperature.

The change in germination methods in the Rules reflect the findings of various researchers who have studied the germination requirements of this species. Toole (17) reported that the use of alternating temperature was an important factor for germination of Kentucky bluegrass seed. Andersen (7) considered 15-30 C to be the most favorable alternating temperature. Sprague (16) found 10-30 C to be superior to 15-30 C and 20-30 C for germination of freshly harvested seed from greenhouse-grown plants.

Prechilling is necessary for maximum germination of immature (11),

but not mature seeds (8, 10). In 1923, Toole (17) suggested the use of dilute KNO_3 for enhancing germination. Bass (8) reported that immature seed was more sensitive to KNO_3 than mature seed. Maguire (13, 14) found that 0.2% KNO_3 increased germination of 'Newport' Kentucky bluegrass, but not that of 'Cougar'. Toole (17) reported that light was not important for complete germination, while Bass (8) concluded that some light is essential for best germination of freshly harvested seed, but not for fully after-ripened seed. No single light intensity between 8-18 and 120-140 ft. c gave consistently highest germination of all samples tested (8). Toole and Borthwick (18) found that brief intermittent periods of 1800 ft. c of light promoted germination of Kentucky bluegrass at 20 C, but prolonged exposures were inhibitory. This intensity of light is much higher than that found in seed germinators.

Delouche (11) reported that most of the seeds in his studies lost their dormancy after 8 weeks, and other workers have reported similar findings. Bass (10) found that immature seeds were more sensitive to germination conditions than seeds that were mature at harvest.

Most studies of germination requirements of Kentucky bluegrass seed have not involved varietal comparisons. The early Rules were developed for common Kentucky bluegrass, but these same methods are now used for over a hundred improved varieties. The objective of this study was to determine if the methods recommended in the current Rules are still applicable for germination testing of a wide selection of modern varieties.

MATERIALS AND METHODS

Seed from nine varieties of Kentucky bluegrass was obtained from the 1977, 1978, and 1979 harvests. All were stored in cloth or paper containers at uncontrolled temperature and humidity conditions prior to testing. Seed from 1977 was stored 18 months, seed from 1978 was stored for 6 months, and seed from 1979 was stored for 2 months.

One seed lot of 'Park', 'Bristol', 'Touchdown', 'Parade', 'Kenblue', 'Merion', 'Baron', 'Newport', and 'Victa' was tested from each harvest. Two additional lots of Touchdown, Newport, Park and Merion from the 1978 and 1979 seed harvests were also tested.

For each germination test, 50 seeds were placed in a 4 5/8 x 4 5/8 x 1 1/8 in. clear plastic box on a blotter saturated with distilled water or 0.2% KNO_3. For each treatment, three boxes were placed at random in the germinators. Germination percentage was obtained by counting the number of seeds germinated after 5, 10, 20, 30, and 45 days. Seeds were considered to have germinated when either roots or shoots appeared. Germinated seeds were removed after counting.

A germination rate index (12) was calculated for each treatment according to the following formula:

$$GRI = \frac{\text{no. of seedlings}}{\text{days to first count}} + \ldots + \frac{\text{no. of seedlings}}{\text{days to final count}}$$

RESULTS AND DISCUSSION

Germination responses to temperature, KNO_3 and light, averaged over varieties and lots, are shown in Figure 1. Maximum germination was not obtained for any of the seed lots at constant temperatures, regardless of the use of light or KNO_3. This is in agreement with the conclusions of Toole (17) and others, and indicates that the requirement for alternating temperatures has not been eliminated during the development of these new varieties. KNO_3 increased germination at each of the constant temperatures, both in light and dark, but this effect was not apparent at alternating temperatures.

Varietal response to KNO_3 and light at 15-25 C are shown in Tables 1-3. With 18-month-old seed (Table 1), the Association of Official Seed Analysts (AOSA) method (light + KNO_3) provided the highest germination in six of the nine varieties and the fastest rate of germination in eight of the nine varieties. In the three varieties in which highest germination was obtained with light + water, the difference was only 2 to 4%.

With 6-month-old seed (Table 2), the AOSA method frequently provided the lowest germination percentage of the four methods. Tests conducted with water in the dark frequently provided higher germination percentages than tests with KNO_3 and light. Thus, it is not possible to demonstrate a consistent requirement for KNO_3 and light for maximum germination.

With 2-month-old seed (Table 3), the AOSA method again provided the highest germination percentage in the largest number of seed lots. When results were summarized over all three ages of seed, the

AOSA method also provided the highest germination percentage in the largest number of seed lots.

It is not possible to make reliable varietal comparisons on the basis of only one lot per variety since factors such as environmental preconditioning may alter the germination requirements of the seed (19). Therefore, tests were made to compare the germination responses of three lots each of Park, Touchdown, Merion, and Newport. When this was done, no consistent varietal response patterns were evident. In 2-month-old seed of Touchdown, for example (Table 3), each lot reached its highest germination level under a different germination regime.

Germination rates varied considerably among varieties, ages of seed, and germination conditions. The 18- and 6-month-old seed germinated much faster than the 2-month-old seed. However, the data do not support the shortening of the test period from the present 28-day duration. These results indicate that the current AOSA method for germinating Kentucky bluegrass seed produces favorable results for the representative sampling of varieties included in this study, regardless of seed age.

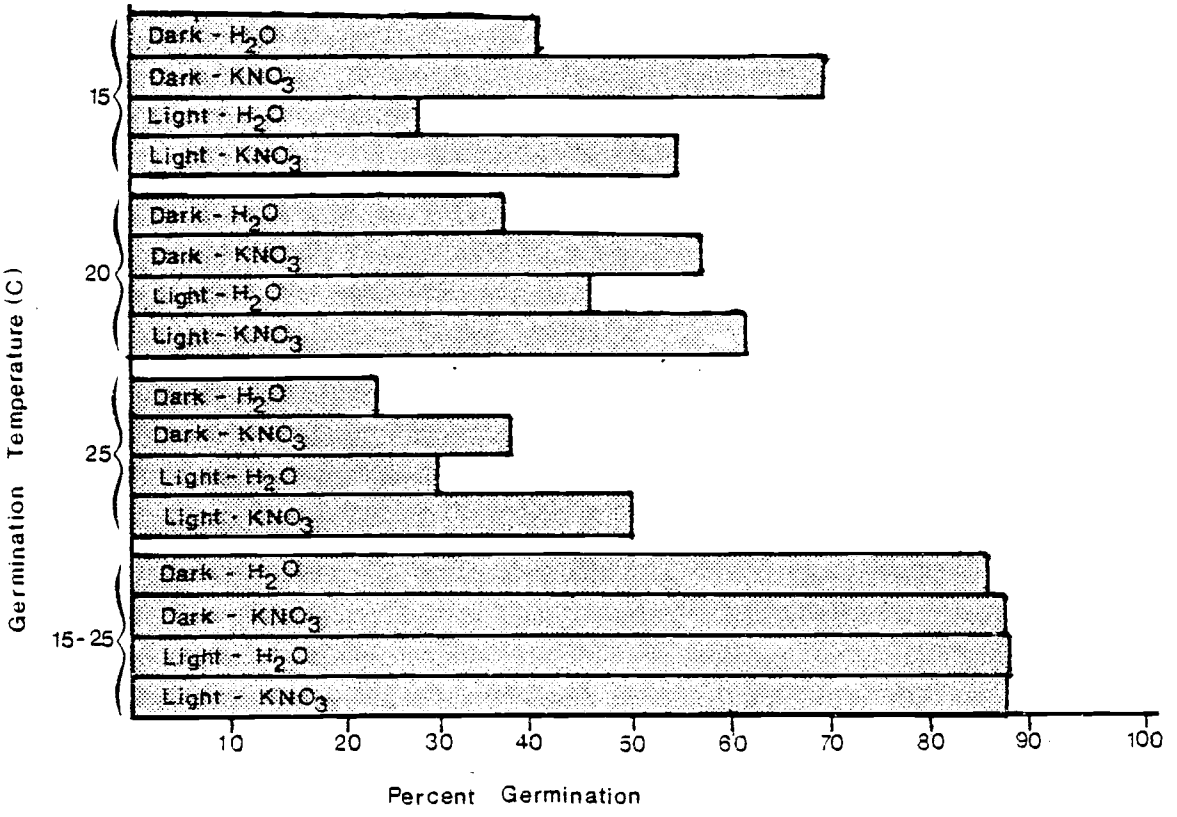

Figure 1. Effect of light, temperature and KNO_3 on germination of Kentucky bluegrass seed. Results averaged over nine varieties and three years.

Table 1. Percentage germination after 30 days and Germination Rate Index for nine varieties of 18-month-old Kentucky bluegrass, germinated at 15-25 C temperature.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park	86	76	89	85	2.4	7.69	9.72	4.92	10.98	0.44
Bristol	95	96	95	97	0.6	13.04	13.38	11.46	13.62	0.44
Touchdown	80	87	88	86	1.1	10.22	12.14	7.78	9.39	1.45
Parade	82	87	89	86	1.7	8.74	9.94	5.80	10.45	0.91
Kenblue	85	79	87	87	1.6	8.38	9.53	5.41	9.63	2.55
Merion	75	85	79	86	2.4	7.95	10.10	5.50	10.41	1.65
Baron	86	94	93	96	1.1	10.71	13.10	6.28	13.26	1.24
Newport	92	89	89	95	1.5	11.57	12.07	6.62	12.79	1.26
Victa	97	97	96	99	2.5	13.50	13.74	10.78	13.86	1.12

Table 2. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 6-month-old Kentucky bluegrass seed, germinated at 15-25 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	99	93	97	96	1.7	11.81	7.83	10.86	11.73	1.04
Lot 2	93	93	89	85	2.5	13.08	12.60	10.08	12.96	1.01
Lot 3	94	95	94	96	1.3	13.14	12.45	12.12	11.60	0.40
Touchdown										
Lot 1	95	92	97	82	2.5	12.52	9.90	11.54	9.67	0.88
Lot 2	91	87	85	93	3.3	12.22	11.13	11.08	11.43	1.00
Lot 3	89	89	93	77	2.3	11.93	9.78	10.31	5.96	0.79
Merion										
Lot 1	90	87	79	75	6.1	6.92	6.30	6.09	6.36	0.83
Lot 2	84	85	85	90	2.4	11.42	10.01	10.37	10.42	N.S.
Lot 3	88	90	87	98	3.4	11.54	10.97	11.70	11.33	N.S.
Newport										
Lot 1	86	90	85	76	4.2	5.84	6.14	4.89	4.51	0.75
Lot 2	97	94	71	88	7.9	12.95	8.62	5.16	6.58	0.97
Lot 3	91	89	89	82	3.2	11.83	9.21	4.33	5.63	0.87
Bristol	83	89	83	87	3.2	10.93	10.91	10.92	11.16	0.96
Parade	85	89	87	84	4.0	9.10	7.68	9.59	9.53	0.80
Kenblue	94	93	96	89	1.8	12.10	10.63	11.73	11.97	0.29
Baron	85	83	91	75	4.8	11.81	7.79	12.21	10.01	1.05
Victa	96	95	100	95	2.0	13.27	10.29	13.08	12.42	1.19

Table 3. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 2-month-old Kentucky bluegrass seed, germinated at 15-25 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	89	67	86	74	6.8	4.26	5.76	4.99	5.46	0.70
Lot 2	90	79	85	87	3.3	7.07	7.84	5.72	7.74	0.48
Lot 3	83	84	80	86	1.0	6.44	7.94	5.77	7.75	0.82
Touchdown										
Lot 1	93	77	92	95	2.4	6.14	6.53	6.12	7.21	0.38
Lot 2	73	56	71	66	6.7	4.38	3.05	4.56	3.13	1.13
Lot 3	87	84	89	87	2.4	5.96	7.37	6.09	7.19	0.39
Merion										
Lot 1	77	86	82	89	5.1	3.40	5.85	4.37	5.26	0.74
Lot 2	85	80	89	81	2.5	5.90	5.70	5.73	5.59	N.S.
Lot 3	87	86	79	80	3.3	7.51	6.64	5.62	5.64	0.51
Newport										
Lot 1	83	87	87	89	3.9	3.72	5.04	4.93	5.05	0.58
Lot 2	90	91	54	95	4.7	5.28	9.95	3.39	6.53	0.53
Lot 3	89	64	87	93	3.1	7.92	9.80	5.62	7.43	0.71
Bristol	74	90	82	82	3.6	7.80	8.22	8.24	6.36	0.83
Parade	67	82	77	83	3.1	3.42	6.94	4.61	5.94	0.48
Kenblue	79	94	79	92	3.3	6.47	8.47	5.34	7.07	1.23
Baron	86	89	88	90	3.6	5.83	7.64	6.06	6.99	0.67
Victa	97	82	96	85	4.1	5.48	8.83	6.00	6.06	0.74

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MANUSCRIPT II

STIMULATION OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED GERMINATION BY HORMONE APPLICATIONS

ABSTRACT

The time required for germination testing of Kentucky bluegrass (Poa pratensis L.) seed is traditionally time consuming and a handicap to efficient marketing of seed. The objective of this study was to determine the feasibility of obtaining accurate germination results for Kentucky bluegrass seed in a shorter period of time by applying growth regulators to the germination media. If successful, these procedures could be adapted for faster germination tests with this crop. Five varieties of 2-month-old Kentucky bluegrass seed were used in this study. Growth regulators were applied to the seed either by pre-soaking or by planting the seeds on blotters saturated with the appropriate solution. Seeds were pre-soaked in solutions of 800 and 1200 ppm GA_3 or ethephon for 12 hours before planting them on blotters saturated with distilled water. Blotters were saturated with the following solutions: kinetin (100 and 200 ppm), GA_3 (100, 200, 400, and 800 ppm), ethephon (100, 200, and 400 ppm), indole butyric acid (100 and 200 ppm), GA_3 + kinetin, GA_3 + ethephon, kinetin + ethephon, GA_3 + kinetin + ethephon, 0.2% KNO_3, GA_3 + KNO_3, ethephon + KNO_3, and distilled water.

Dormant seed lots treated with KNO_3 (AOSA method) did not germinate completely during 31 days if a prechill was not used. Complete germination of the dormant lots occurred in 21 days with KNO_3 + GA_3, KNO_3 + ethephon, and 100 ppm ethephon. These three treatments were among the most effective for the non-dormant lots as well. Kentucky bluegrass seed has a strong requirement for alternating temperature to promote germination, and none of the growth regulators were able to completely

overcome this requirement. Light had little effect on germination at 15-25 C in the presence of water, KNO_3, GA_3, and higher ethephon concentrations. Kinetin and lower concentrations of ethephon, however, showed reduced germination in the dark. When growth regulators were used in combination at 100 ppm, GA_3 counteracted the inhibitory effects of ethephon in the dark, but not that of kinetin.

It appears that the use of KNO_3 in combination with either GA_3 or ethephon would result in faster germination than by the AOSA method, and that the 5-day prechill period is unnecessary if these growth regulator treatments are applied.

Additional index words: Gibberellin, Ethephon, Indole butyric acid, Kinetin, Potassium nitrate, Growth regulators, Dormancy.

STIMULATION OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED GERMINATION BY HORMONE APPLICATIONS

INTRODUCTION

The time required for germination testing of Kentucky bluegrass (*Poa pratensis* L.) seed is notoriously long and a handicap to efficient marketing of seed. The current method of testing dormant Kentucky bluegrass seed, according to the Association of Official Seed Analysts (AOSA, 1), includes placing the seeds at 5 C for 5 days, followed by 28 days at 15-25 C. Additional conditions include the use of 0.2% KNO_3 in the germination media, as well as exposure of the seed to light for 8 hours a day. For non-dormant seeds, the 5-day period at 5 C can be eliminated.

The percentage and rate of germination of many species can be increased by application of growth regulators. Ethylene improves germination of dormant seed lots of peanut (25), subterranean clover (4), Indian ricegrass (3), cocklebur (6), fall panicum (23), and reed canarygrass (16), among others. Gibberellic acid improves germination of dormant seeds of many species including wild oat (6, 18, 21), reed canarygrass (14, 16), and barley (18). Cytokinins, primarily kinetin, increases germination of seeds of Indian ricegrass (22), wheat (13), barley (11), lettuce (12, 26), and others. Phaneendranath and Funk (20) showed that permeation of Kentucky bluegrass seeds with gibberellic acid, alone or in combination with kinetin and/or ethephon dissolved in acetone, generally increased the speed of and/or total germination.

The main intent of germination testing is to obtain and report germination of seeds as quickly and accurately as possible. People

originally involved with seed testing recognized the benefits of chemical stimuli to enhance seed germination. However, at that time, only KNO_3 was known to effectively stimulate seed germination. Since then, many growth regulators other than KNO_3 have been shown to accelerate seed germination. With the lone exception of ethephon or ethylene for dormant peanut seed, no chemical other than KNO_3 has been approved by the AOSA for use in testing germination of seeds.

The objective of this study was to determine the feasibility of obtaining accurate germination results for Kentucky bluegrass seed in a shorter period of time by the application of growth regulators to the germination media. If successful, these procedures could be adapted for quicker germination tests with this crop.

MATERIALS AND METHODS

Two-month-old seed of five varieties of Kentucky bluegrass harvested in 1979 were used in this study. 'Touchdown' and 'Kenblue' were chosen to represent non-dormant varieties, while 'Merion', 'Park', and 'Newport' represent the more dormant varieties. For the purpose of this study, varieties were classified as dormant and non-dormant according to their germination speed using standard germination procedures.

Growth regulators were applied to the seed either by pre-soaking or by planting the seeds on blotters saturated with the appropriate solution. Seeds were pre-soaked in solutions of 800 and 1200 ppm GA_3 or ethephon for 12 hours before planting them on blotters saturated with distilled water.

Blotters were saturated with the following solutions: kinetin (100 and 200 ppm), GA_3 (100, 200, 400 and 800 ppm), ethephon (100, 200 and 400 ppm), indole butyric acid (100 and 200 ppm), GA_3 + kinetin, GA_3 + ethephon, kinetin + ethephon, GA_3 + kinetin + ethephon, 0.2% KNO_3, GA_3 + KNO_3, ethephon + KNO_3, and distilled water. Combinations of growth regulators were prepared with equal amounts of 200 ppm stock solutions. Combinations involving KNO_3 were prepared by making a 0.2% KNO_3 solution using the appropriate 200 ppm growth regulator stock solution.

Germination tests were conducted by planting 50 seeds on top of blotters in closed 4 5/8 x 4 5/8 x 1 1/8 in. clear plastic boxes. Three boxes per variety per treatment were placed in germinators in a completely randomized design. Germinator conditions were 15-25 C,

dark; 15-25 C, light; and 25 C, dark. Germination counts were made after 7, 14, 21, 31, and 45 days. Seeds were counted as germinated if either a radicle or shoot could be observed.

A germination rate index (16) was calculated for each treatment according to the following formula:

$$GRI = \frac{\text{number of seedlings}}{\text{days to first count (7 days)}} + \ldots + \frac{\text{number of seedlings}}{\text{days to final count (45 days)}}$$

RESULTS AND DISCUSSION

Total germination of the seed lots was achieved in 31 days with most of the growth regulators at 15-25 C in the light (Tables 1 and 2).

Dormant seed lots treated with KNO_3 (AOSA method) did not germinate completely during this period if they were not prechilled. Growth regulators caused large differences in germination rates as indicated by the Germination Rate Indexes of the various treatments. The most rapid germination of the dormant lots (Table 1) occurred with KNO_3 + GA_3, KNO_3 + ethephon, and 100 ppm ethephon. These three treatments were among the most effective for the non-dormant lots as well, together with three additional ethephon treatments, two of the GA_3 treatments, GA_3 + ethephon, and GA_3 + kinetin + ethephon (Table 2). In non-dormant lots, germination averaged 93% in KNO_3, KNO_3 + ethephon, and KNO_3 + GA_3. The main effect of adding ethephon and GA_3 was to increase the speed of germination, as evidenced by increases in the Germination Rate Index from 7.10 to 12.86 and 12.93. It appears that the use of KNO_3 in combination with either GA_3 or ethephon would result in faster germination than does the AOSA method, and that the 5-day prechill period is unnecessary if these growth regulator treatments are applied. Of the two combinations, KNO_3 + ethephon might be preferred because of the greater ease of preparing the solutions.

Phaneendranath and Funk (20) used acetone to carry growth regulators into the seed. However, in this study, pre-soaking the seed with growth regulators dissolved in distilled water gave results as favorable as those reported by Phaneendranath and Funk when they used acetone. Since acetone can kill the seed unless managed with extreme

Table 1. Effect of growth regulators on average seed germination and germination rate of three dormant varieties of Kentucky bluegrass (Merion, Park, and Newport) after 31 days at three combinations of temperature and light.

Treatment		Germination			Germination rate		
Growth regulator	Concentration	15-25 C dark	15-25 C light	25 C dark	15-25 C dark	15-25 C light	25 C dark
	ppm	%			Germination rate index		
GA_3	100	93 abc*	93 abc	8 mno	11.71 a-e*	8.95 klm	0.98 v-y
GA_3	200	91 a-d	86 a-f	13 m	10.03 g-k	7.90 no	1.37 vwx
GA_3	400	89 a-e	94 ab	30 l	10.85 e-i	10.95 c-h	4.05 stu
GA_3	800	89 a-e	93 abc	52 ij	10.25 f-j	10.95 c-h	7.03 p
GA_3, pre-soak	800	86 a-f	91 a-d	71 gh	9.44 j-m	8.62 mno	7.88 nop
GA_3, pre-soak	1200	87 a-f	91 a-d	69 h	8.82 lmn	8.40 j-m	6.90 p
Ethephon	100	35 kl	93 abc	1 o	3.26 u	11.55 a-e	0.15 y
Ethephon	200	35 kl	89 a-e	1 o	3.77 tu	10.85 e-i	0.07 y
Ethephon	400	89 a-e	91 a-d	5 mno	10.35 f-j	10.56 e-i	0.64 v-y
Ethephon, pre-soak	800	93 abc	95 a	4 no	10.62 e-i	10.99 c-g	0.50 wxy
Ethephon, pre-soak	1200	83 def	90 a-e	43 jk	8.03 mno	7.71 op	4.05 stu
Kinetin	100	37 kl	94 ab	1 o	3.70 tu	9.84 h-l	0.07 y
Kinetin	200	48 j	91 a-d	0 o	4.05 stu	9.41 j-m	0.00 y
IBA	100	89 a-e	88 a-f	0 o	7.85 nop	9.75 i-l	0.00 y
IBA	200	53 ij	59 i	0 o	5.23 qr	6.80 p	0.00 y
GA_3 + kinetin	100 + 100	50 ij	89 a-e	6 mno	4.92 qrs	10.71 e-i	0.63 v-y
GA_3 + ethephon	100 + 100	95 a	93 abc	12 m	11.88 a-d	11.31 b-f	1.57 vw
Kinetin + ethephon	100 + 100	44 jk	92 a-d	2 no	4.17 r-u	10.88 d-i	0.29 xy
GA_3 + kinetin + ethephon	67 + 67 + 67	91 a-d	93 abc	11 mn	11.09 b-g	11.31 b-f	1.55 vw
GA_3 + KNO_3	200 + 0.2%	87 a-f	94 ab	31 l	11.62 a-e	12.64 a	4.26 q-u
Ethephon + KNO_3	200 + 0.2%	92 a-d	95 a	13 m	12.07 abc	12.22 ab	1.71 v
KNO_3	0.2%	79 fg	84 c-f	0 o	5.32 q	5.09 qrs	0.00 y
Distilled water		81 ef	85 b-f	0 o	3.75	4.57 q-t	0.00 y

*Means within germination percentage and within germination rate index not followed by the same letter differ significantly at 0.05 level of probability as determined by Duncan's multiple range test.

Table 2. Effect of growth regulators on average seed germination and germination rate of two non-dormant varieties of Kentucky bluegrass (Kenblue and Touchdown) after 31 days at three combinations of temperature and light.

Treatment		Germination			Germination rate		
Growth regulator	Concentration	15-25 C dark	15-25 C light	25 C dark	15-25 C dark	15-25 C light	25 C dark
	ppm	%			Germination rate index		
GA_3	100	87 d-i*	83 h-k	47 p	11.72 c-f*	11.01 e-i	3.67 qr
GA_3	200	78 j-m	74 lm	46 p	10.57 g-k	9.03 n	6.12 p
GA_3	400	91 a-g	94 a-d	77 k-m	12.38 a-d	13.07 a	10.71 f-j
GA_3	800	93 a-e	96 ab	85 f-j	12.71 a-d	13.21 a	11.79 b-e
GA_3, pre-soak	800	93 a-e	90 a-g	88 c-h	12.38 a-d	11.63 d-g	11.70 c-f
GA_3, pre-soak	1200	91 a-g	91 a-g	85 f-j	10.38 h-m	9.87 j-n	9.75 k-n
Ethephon	100	84 g-k	96 ab	24 qrs	9.97 j-n	12.27 a-d	3.13 rs
Ethephon	200	85 f-j	95 abc	23 rs	9.97 j-n	12.37 a-d	2.96 rs
Ethephon	400	94 a-d	97 a	58 o	12.94 a	13.12 a	7.82 o
Ethephon, pre-soak	800	94 a-d	95 abc	57 o	12.37 a-d	12.65 a-d	7.71 o
Ethephon, pre-soak	1200	88 c-i	92 a-f	81 i-l	10.53 h-m	10.88 e-j	9.37 mn
Kinetin	100	77 klm	89 b-h	25 qr	9.59 lmn	8.08 o	3.50 qr
Kinetin	200	73 m	89 b-h	21 rst	7.42 o	9.94 i-n	2.43 st
IBA	100	78 j-m	82 h-k	13 u	9.70 k-n	10.42 h-l	1.57 t
IBA	200	73 m	87 d-i	15 tu	9.50 lmn	11.03 e-i	1.82 t
GA_3 + kinetin	100 + 100	91 a-g	93 a-e	31 q	10.92 e-j	11.14 e-h	3.20 qrs
GA_3 + ethephon	100 + 100	95 abc	94 a-d	41 p	12.51 a-d	12.72 abc	4.10 q
Kinetin + ethephon	100 + 100	86 e-j	91 a-g	27 qr	10.04 i-n	11.29 e-h	3.20 qrs
GA_3 + kinetin + ethephon	67 + 67 + 67	95 abc	93 a-e	47 p	12.58 a-d	12.57 a-d	5.93 p
GA_3 + KNO_3	200 + 0.2%	92 a-f	93 a-e	65 n	12.93 a	12.86 a	3.86 qr
Ethephon + KNO_3	200 + 0.2%	93 a-e	93 a-e	60 no	12.79 ab	12.93 a	8.07 o
KNO_3	0.2%	85 f-j	93 a-e	17 stu	7.48 o	7.10 o	1.60 t
Distilled water		85 f-j	85 f-j	2 v	5.53 pq	5.49 pq	0.12 u

*Means within germination percentage and within germination rate index not followed by the same letter differ significantly at 0.05 level of probability as determined by Duncan's multiple range test.

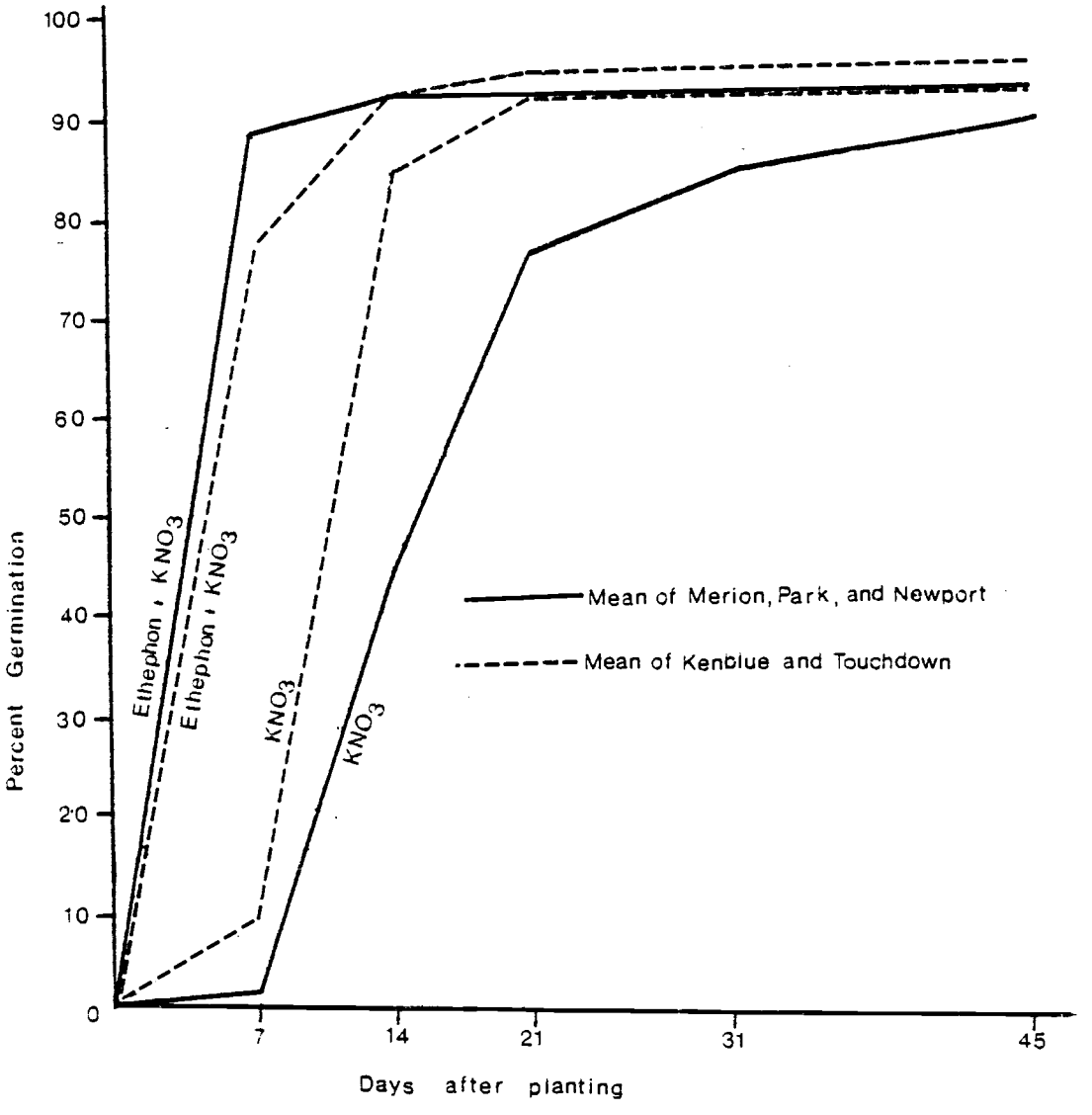

Figure 1. Germination rate of Kentucky bluegrass seed comparing KNO_3 (AOSA method) with ethephon + KNO_3 as moistening agents. Seeds were germinated at 15-25 C with light.

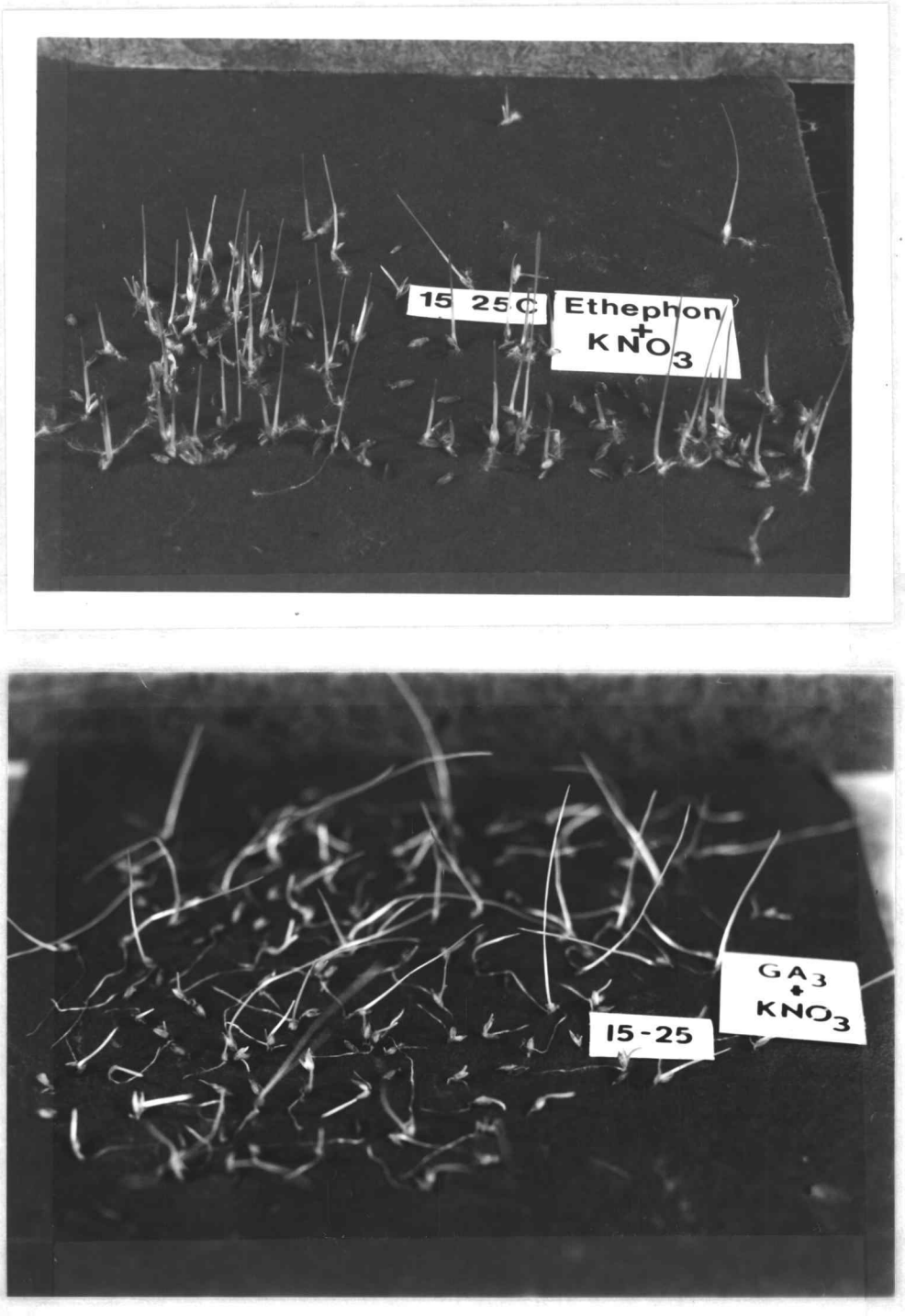

Figure 2. 'Newport' Kentucky bluegrass seed germinated in light at 15-25 C, 7 days after planting. The upper plate shows the effects of ethephon + KNO_3. The lower plate shows the effects of GA_3 + KNO_3.

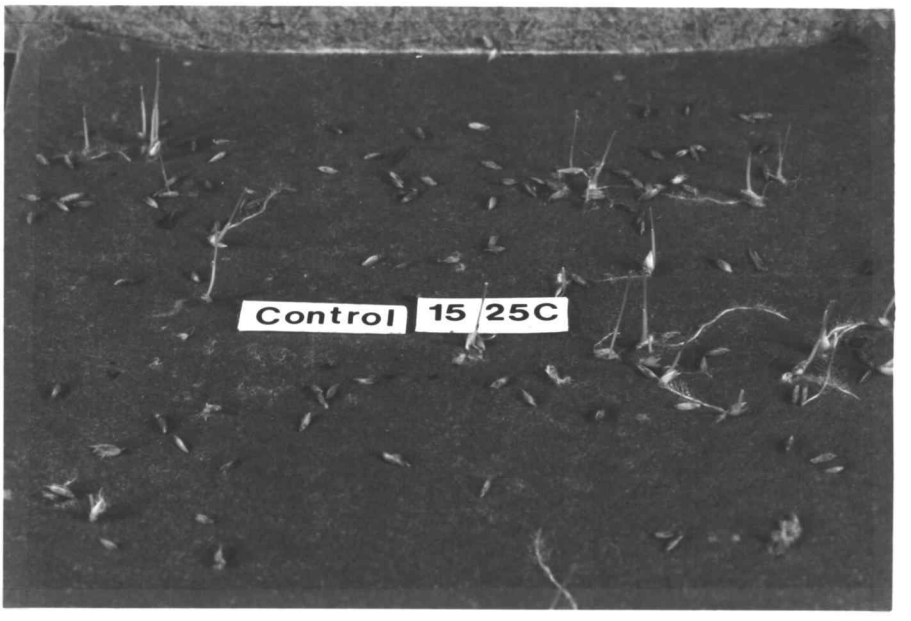

Figure 3. 'Newport' Kentucky bluegrass seed germinated in light at 15-25 C, 7 days after planting.

care, the use of acetone as a growth regulator carrier seems a needless risk.

The germination rates obtained by using KNO_3 + ethephon are compared with those from KNO_3 (AOSA method) in Figure 1. No new germinants were observed in dormant seed lots after 21 days or in non-dormant lots after 14 days when KNO_3 + ethephon was used. According to these results, it may be possible to terminate the germination test for many lots of Kentucky bluegrass seed after 21 days.

Kentucky bluegrass seed has a strong requirement for alternating temperature to promote germination. Germination was reduced from over 80% to nearly 0% at a constant 25 C in the dark in the absence of growth regulators. None of the growth regulators were able to completely overcome the requirement for alternating temperature. Highest germination of both dormant and non-dormant lots at 25 C in the dark occurred after pre-soaking in 800 ppm and 1200 ppm GA_3.

Light had little effect on germination at 15-25 C in the presence of water, KNO_3, GA_3 and higher concentrations of ethephon. Kinetin and lower concentrations of ethephon, however, reduced germination in the dark. When growth regulators were used in combination at 100 ppm, GA_3 counteracted the inhibitory effects of ethephon in the dark, but not that of kinetin. Khan (8), Khan and Tolbert (13) and others (6, 15, 17), have also reported kinetin to be more effective in the light than in the dark.

Dormant and non-dormant seed lots reacted similarly to each of the growth regulators, differing only in degree of response. Germination percentage of non-dormant lots was greater in the presence of

growth regulators and alternating temperatures, and was inhibited less by kinetin and ethephon.

The results of this study indicate that germination tests of Kentucky bluegrass seed may be shortened as much as 12 days by the addition of either GA_3 or ethephon to KNO_3 in the germination medium. These growth regulators obviate the need for a 5-day prechilling period and promote complete germination in 21 days. Results were consistent on seed lots from dormant and non-dormant varieties. If these findings are substantiated by other laboratories on a wider range of seed lots, it is recommended that the use of growth regulators for germination testing of Kentucky bluegrass be incorporated in the Rules for Seed Testing.

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MANUSCRIPT III

STIMULATION OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED GERMINATION BY HULLING

ABSTRACT

One characteristic of Kentucky bluegrass (*Poa pratensis* L.) that makes it less desirable for turf purposes is the relatively long period of time required for germination and establishment. The objective of this study was to determine the feasibility of hulling the seed to enhance speed of germination. Two-month-old seed of five varieties was subjected to three physical treatments: (1) Lemmas and paleas (hulls) were removed by lightly rubbing the seeds with sandpaper. (2) Small portions of the distal ends of the seeds were removed with a razor blade. (3) Seeds were punctured with a needle. Following these treatments, the seeds were germinated under several conditions of temperature, light and substrate.

Hulling the three dormant seed lots resulted in complete germination in 7 days at 15-25 C with light. Seedling growth was sufficient for evaluation of normal and abnormal seedlings in 14 days. Hulling did not completely remove the requirements for alternating temperature and light for germination of dormant lots, although hulling removed the requirement for light and KNO_3. (Alternating temperature was still required for complete germination of non-dormant seed lots.) Puncturing and removing the distal end of seeds was not as effective as completely removing the hulls.

The hulling technique may be useful as a quick viability test, for studying specific problems of dormancy and verifying results of tests conducted by official methods. The potential for hulling Kentucky bluegrass seed as a commercially useful procedure for faster establishment needs further investigation.

Additional index words: Inhibitor, Dormancy, Speed of germination.

STIMULATION OF KENTUCKY BLUEGRASS (*POA PRATENSIS* L.) SEED GERMINATION BY HULLING

INTRODUCTION

One characteristic of Kentucky bluegrass (*Poa pratensis* L.) that makes it less desirable for turf purposes is the relatively long period of time required for germination and establishment. Numerous attempts have been made to hasten seed germination, primarily through the use of various pre-treatments. One pre-treatment procedure is to soak the seed with daily changes of water until the water is clear, after which the seed is bagged. This soaked seed must then be planted within 3 or 4 days since the seed is not dried following the pre-plant treatment. Wetting, followed by drying, enhances germination of orchardgrass (10), crested wheatgrass (8), and Kentucky bluegrass (18). The degree of stimulation, however, is dependent upon the degree of seed dormancy as well as the condition of the field where the seed is planted. Yeam, Murray, and Portz (22) developed a procedure for increasing germination speed of Kentucky bluegrass and zoysia which involves soaking the seed in 30% KOH solution, followed by a light treatment and drying. The seed is then soaked in water prior to seeding.

"Hulling" (removing the lemma and palea) enhances germination of several grasses and is a commercial practice for Bermudagrass and Bahiagrass seed. Other grasses that benefit from removal of the outer coverings include *Poa compressa* (7), beardless ryegrass (14), Bermudagrass (6), Kentucky bluegrass (11), Indian ricegrass (16), orchardgrass (12), several species of *Paspalum* (19), bluestem (2, 4) and buffalograss (3, 5). Several workers have shown or suggested that inhibitors

within the hulls of grass seed contribute to delayed germination (2,3,11,12).

The objective of this study was to determine the feasibility of "hulling" Kentucky bluegrass seed to enhance germination under several conditions of temperature, light and substrate. If successful hulling could provide a commercially useful procedure for faster establishment of Kentucky bluegrass turf.

MATERIALS AND METHODS

Two-month-old seed of five varieties of Kentucky bluegrass harvested in 1979 were used in this study. 'Touchdown' and 'Kenblue' were chosen to represent non-dormant varieties, while 'Merion', 'Park', and 'Newport' represented the more dormant varieties. For the purpose of this study, varieties were classified as dormant and non-dormant according to their germination speed using standard germination procedures.

Three physical treatments were applied to the seed: (1) Lemmas and paleas (hulls) were removed by lightly rubbing the seeds between sheets of 400-mil sandpaper and separating them from the caryopses with a South Dakota-type blower. (2) Small portions of the distal ends of the seeds were removed with a razor blade. (3) Seeds were punctured through the hulls into the endosperm with a needle.

Naked caryopses were germinated with distilled water or 0.2% KNO_3. The punctured and cut seeds were germinated with distilled water. In addition, intact caryopses were soaked 12 hours in 800 ppm solutions of GA_3 or ethephon and germinated with distilled water.

Germination tests were conducted by planting 50 seeds on top of blotters in closed 4 5/8 x 4 5/8 x 1 1/8 in. clear plastic boxes. Three boxes per variety per treatment were placed in germinators in a completely randomized design. Germinator conditions were 15-25 C, dark; 15-25 C, light; and 25 C, dark. Germination counts were made after 4, 7, 14, 21, and 31 days. Seeds were counted as germinated if either a radicle or shoot could be observed.

A germination rate index (11) was calculated for each treatment according to the following formula:

$$GRI = \frac{\text{number of seedlings}}{\text{days to first count}} + \ldots + \frac{\text{number of seedlings}}{\text{days to final count}}$$

RESULTS AND DISCUSSION

Hulling the three dormant seed lots resulted in complete germination in 7 days at 15-25 C with light (Table 1). Seedling growth was sufficient for evaluation of normal and abnormal seedlings in 14 days (Plates 1 and 2). Hulling did not completely remove the requirements for alternating temperature and light. KNO_3 increased germination of hulled seeds in the dark but was not required in the light. When hulled seeds were pre-soaked in GA_3 and germinated in the dark, full germination occurred at 15-25 C, but not at 25 C.

Hulling the two non-dormant lots resulted in germination problems generally parallel to those of dormant seed lots (Table 2). Alternating temperatures were still needed for complete germination, but hulling removed the requirement for light and KNO_3 at alternating temperatures.

Puncturing and removing the distal ends of all dormant and non-dormant seed lots improved the 7-day germination somewhat, but neither technique was as effective as completely removing the hulls (Tables 1 and 2).

The germination rate indexes indicate that hulled seeds of all the varieties germinated at nearly the same rate at each of the three temperature-light combinations. KNO_3 and pre-soaking in GA_3 did not increase the speed of germination. The germination rates of hulled and unhulled seed over a 31-day period are shown in Figure 1.

The improvement in seed germination after removing the hulls has been reported for numerous other grasses. The data indicate that germination is prevented or delayed by factors associated with the

Table 1. Effects of physical treatments and growth regulators on germination and germination rate of three dormant varieties of Kentucky bluegrass (Merion, Park and Newport) after 7 days at three combinations of temperature and light.

Pregermination treatment	Moistening agent	Germination			Germination rate		
		15-25 C dark	15-25 C light	25 C dark	15-25 C dark	15-25 C light	25 C dark
		%			Germination rate index		
Hulls removed	0.2% KNO_3	85 b*	92 a	57 e	12.14 a*	13.00 a	8.29 cd
Hulls removed	distilled water	80 c	91 a	44 f	11.79 a	13.07 a	6.36 ef
Hulls removed 800 ppm GA_3 pre-soak	distilled water	92 a	94 a	80 c	13.21 a	13.57 a	11.43 ab
Hulls removed 800 ethephon pre-soak	distilled water	59 e	66 d	30 g	8.64 cd	9.57 bc	4.48 fg
Hulls present seed punctured	distilled water	24 h	19 i	2 k	5.51 efg	5.36 efg	0.36 h
Hulls present distal end removed	distilled water	32 g	22 hi	10 j	6.86 de	6.02 efg	1.50 h
Hulls present	0.2% KNO_3	7 j	2 k	0 k	5.32 efg	5.09 efg	0.00 h
Hulls present	distilled water	1 k	0 k	0 k	3.75 g	4.57 fg	0.00 h

*Means within germination percentage and germination rate index not followed by the same letter differ significantly at 0.05 level of probability as determined by Duncan's multiple range test.

Table 2. Effects of physical treatments and growth regulators on germination and germination rate of two non-dormant varieties of Kentucky bluegrass (Kenblue and Touchdown) after 7 days at three combinations of temperature and light.

Pregermination treatment	Moistening agent	Germination			Germination rate		
		15-25 C dark	15-25 C light	25 C dark	15-25 C dark	15-25 C light	25 C dark
		%			Germination rate index		
Hulls removed	0.2% KNO_3	93 a*	91 ab	79 c	13.29 a*	13.14 a	11.29 abc
Hulls removed	distilled water	91 ab	93 a	79 c	13.00 a	12.29 ab	11.39 abc
Hulls removed 800 ppm GA_3 pre-soak	distilled water	92 ab	89 ab	85 bc	13.21 a	12.75 ab	12.21 ab
Hulls removed 800 ppm ethephon pre-soak	distilled water	66 de	70 d	47 f	9.50 a-d	10.19 abc	6.77 b-f
Hulls present seed punctured	distilled water	42 f	28 g	17 hij	8.12 a-d	6.69 b-f	2.98 ef
Hulls present distal end removed	distilled water	61 e	49 f	24 gh	10.21 abc	8.33 a-d	3.93 def
Hulls present	0.2% KNO_3	21 ghi	10 j	16 ij	7.48 a-d	7.10 c-f	2.43 ef
Hulls present	distilled water	11 j	2 k	1 k	5.53 c-f	5.57 c-f	1.29 f

*Means within germination percentage and germination rate index not followed by the same letter differ significantly at 0.05 level of probability as determined by Duncan's multiple range test.

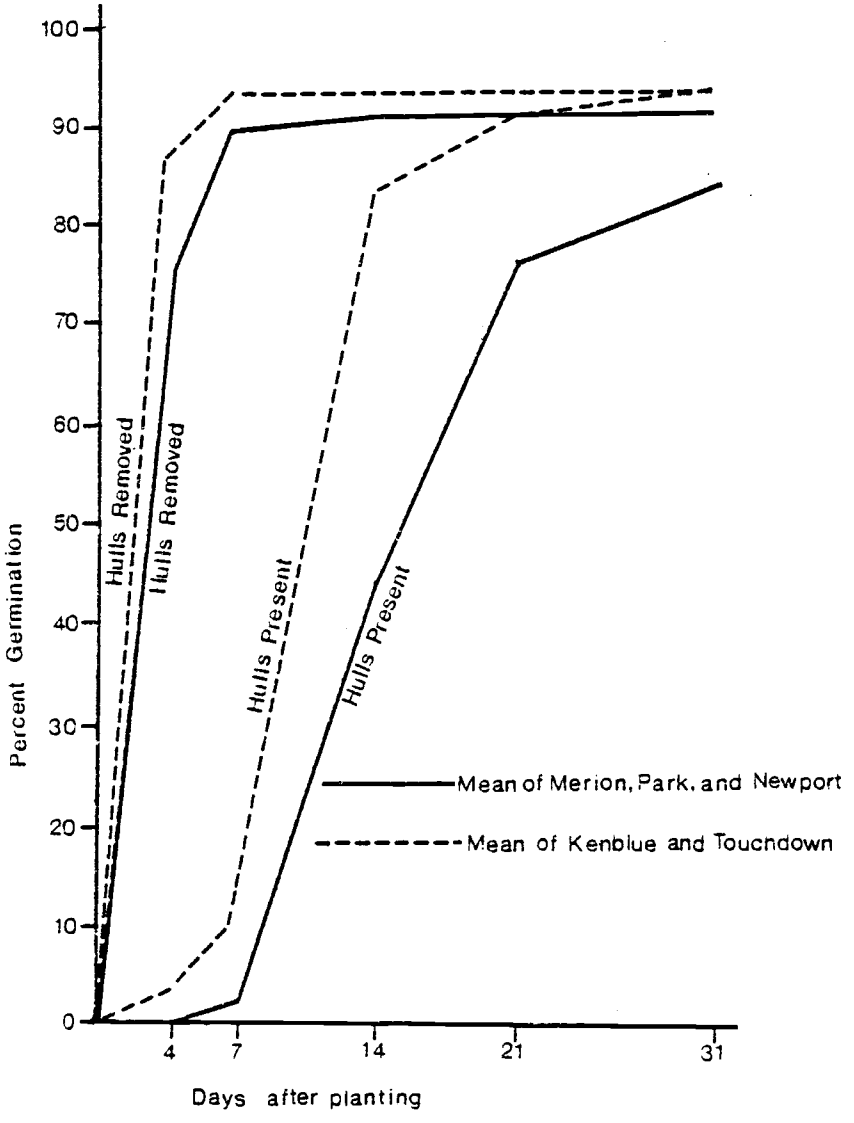

Figure 1. Germination rate of Kentucky bluegrass seed comparing the AOSA method (hulls present) with removal of the hulls. Seeds were germinated at 15-25 C with light and KNO_3.

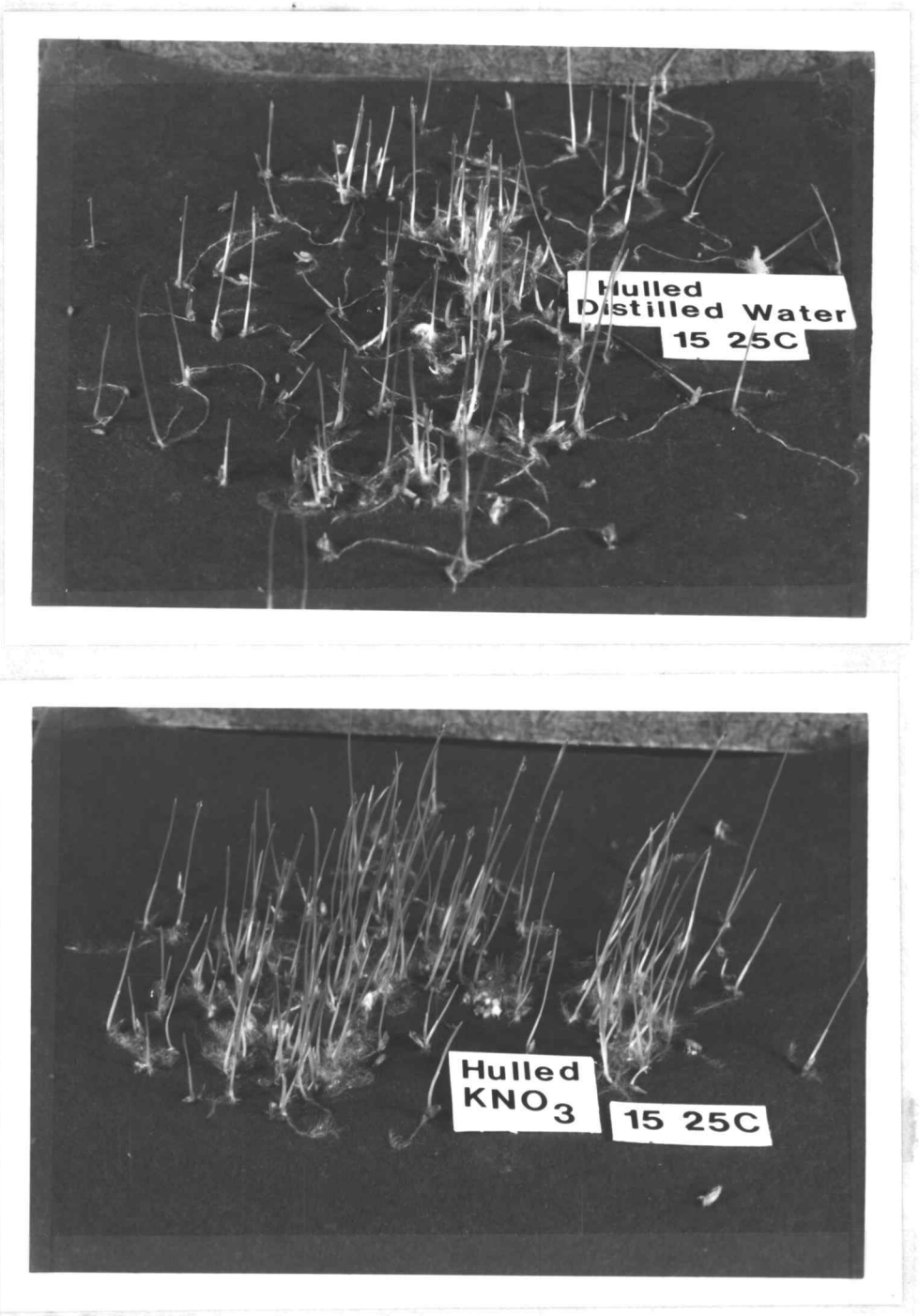

Figure 2. Hulled 'Newport' Kentucky bluegrass seed germinated in light at 15-25 C, 7 days after planting. The upper plate shows the effects of distilled water. The lower plate shows the effects of KNO_3.

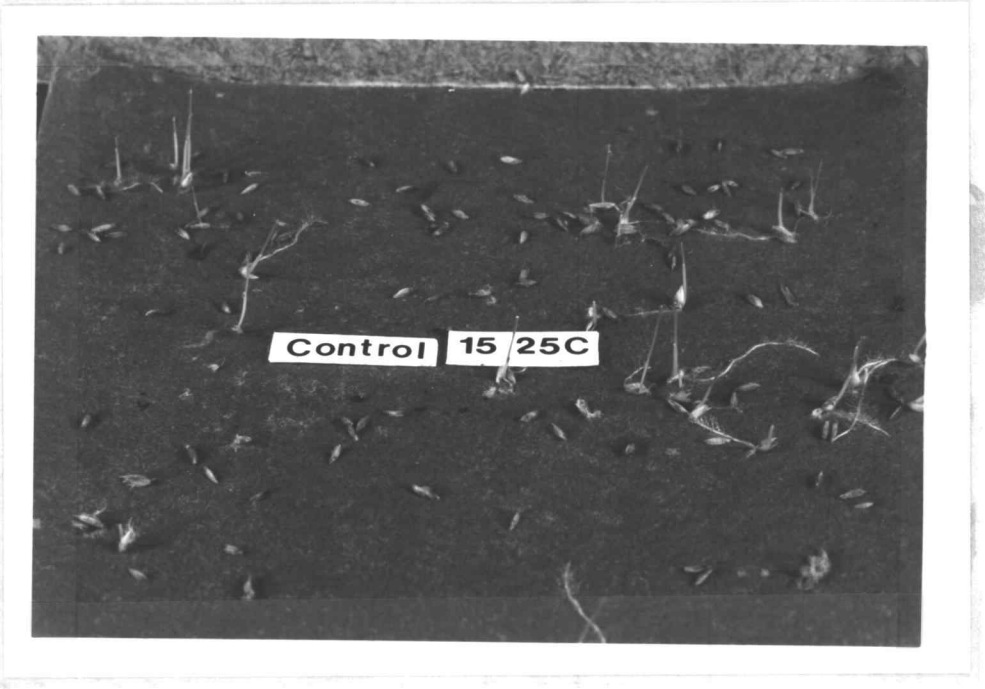

Figure 3. 'Newport' Kentucky bluegrass seed germinated in light at 15-25 C, 7 days after planting.

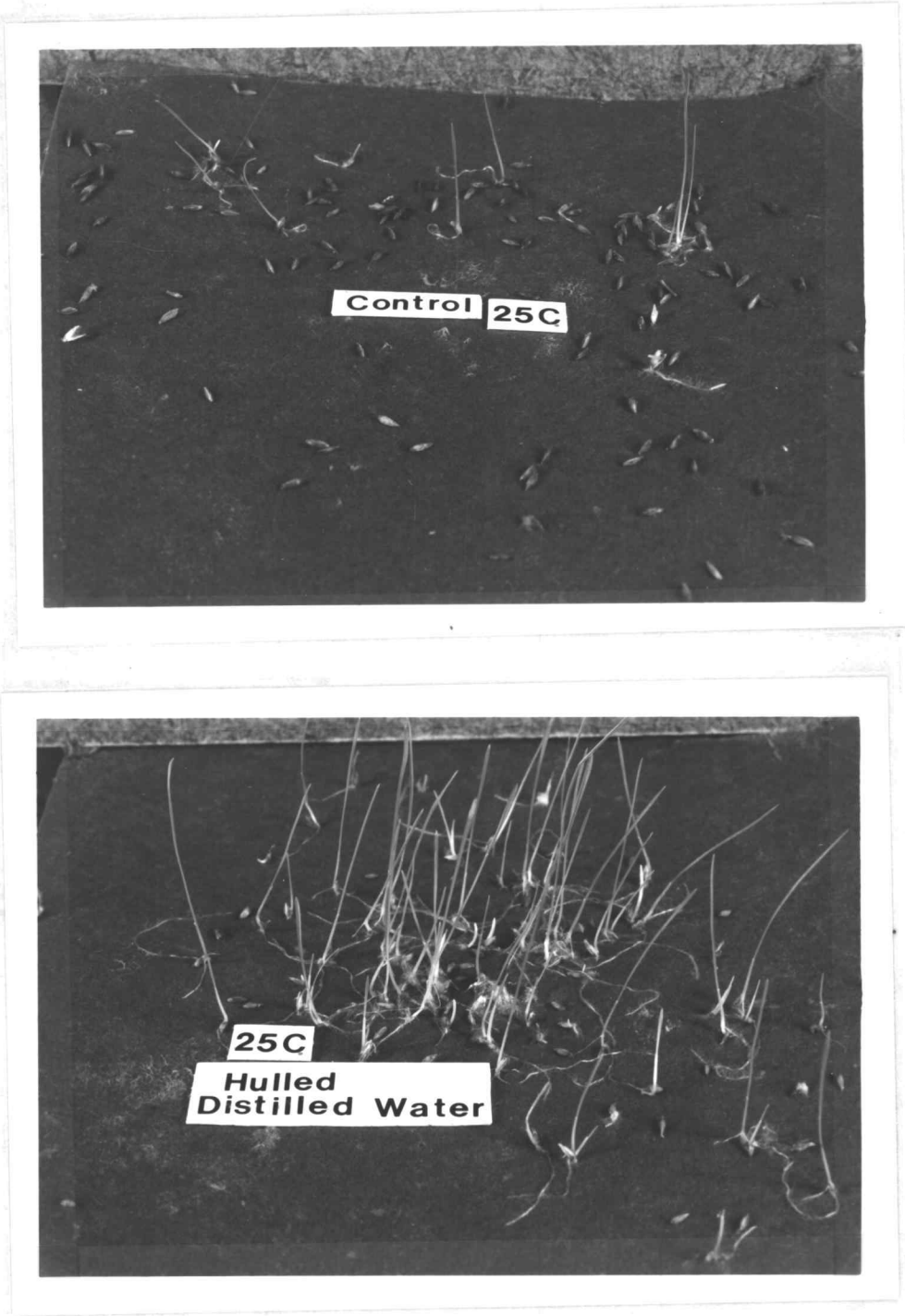

Figure 4. 'Newport' Kentucky bluegrass seed germinated in light at 25 C, 7 days after planting. The upper plate shows the effects of this environment on intact seed. The lower plate shows the effects of hulling in this environment.

hulls. The results are clearly in agreement with previous reports attributing delayed germination to the presence of inhibitors in the hulls (2,3,11,12). Further studies are planned to determine the effects of pre-chilling, alternating temperature, light and growth regulators on these inhibitor levels.

The hulling technique may be useful as a quick viability test, for studying specific problems of dormancy, verifying results of tests conducted by official methods, and obtaining rapid estimates of seed viability to substantiate tetrazolium test results.

The potential for hulling Kentucky bluegrass seed as a commercially useful procedure for faster establishment of turf needs further investigation.

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APPENDICES

Appendix Table 1. Percentage germination after 30 days and final Germination Rate Index for nine varieties of 18-month-old Kentucky bluegrass seed, germinated at 15 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park	37	69	27	53	3.8	1.98	5.40	1.33	3.22	0.43
Bristol	89	95	92	96	3.9	6.09	11.09	5.13	10.68	1.22
Touchdown	85	81	84	81	3.4	5.58	9.73	5.35	10.54	0.92
Parade	44	53	42	37	2.1	2.52	3.90	2.04	3.10	1.15
Kenblue	73	81	68	75	3.5	4.29	7.90	3.96	6.03	0.87
Merion	37	77	27	55	4.1	1.80	5.84	1.20	4.04	0.73
Baron	90	93	61	96	3.2	5.17	9.92	2.89	8.36	0.65
Newport	54	91	61	77	4.2	3.53	9.76	3.55	5.64	0.67
Victa	62	98	23	95	6.0	2.93	1.09	9.33	6.74	0.78

Appendix Table 2. Percentage germination after 30 days and final Germination Rate Index for nine varieties of 18-month-old Kentucky bluegrass seed, germinated at 20 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park	42	60	22	71	6.9	3.71	5.81	1.17	6.65	1.53
Bristol	82	95	93	87	1.4	11.32	12.43	11.47	11.84	0.28
Touchdown	83	83	91	83	3.2	10.92	10.62	9.50	10.73	1.33
Parade	53	51	76	51	6.0	6.05	4.61	5.72	5.15	1.37
Kenblue	71	65	59	65	7.5	9.16	7.57	4.16	6.85	1.09
Merion	33	57	21	81	2.5	3.92	5.50	1.37	7.40	0.63
Baron	63	92	55	89	4.0	6.76	11.07	3.53	10.45	0.98
Newport	81	83	54	77	5.2	10.38	10.28	4.78	8.31	0.88
Victa	93	95	89	91	3.3	13.10	10.00	8.31	10.57	1.62

Appendix Table 3. Percentage germination after 30 days and final Germination Rate Index for nine varieties of 18-month-old Kentucky bluegrass seed, germinated at 25 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park	29	61	35	59	6.3	2.35	6.80	2.33	5.26	0.90
Bristol	92	93	95	98	1.8	11.71	12.95	11.95	12.71	0.78
Touchdown	89	86	85	88	3.7	11.55	11.86	11.05	12.25	0.72
Parade	37	61	62	73	5.6	3.03	6.19	4.14	7.26	1.19
Kenblue	50	66	55	67	6.5	4.58	6.81	5.26	5.85	1.27
Merion	23	33	39	75	5.1	1.94	3.65	2.42	5.58	1.17
Baron	67	93	92	98	2.5	7.05	10.13	7.18	8.21	0.99
Newport	39	75	36	61	6.6	3.99	9.78	2.24	5.72	1.62
Victa	31	67	78	88	4.8	3.60	9.10	6.58	8.98	1.20

Appendix Table 4. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 6-month-old Kentucky bluegrass seed, germinated at 15 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	39	77	15	66	5.5	3.59	4.89	1.53	4.83	0.83
Lot 2	93	98	43	84	3.6	11.61	9.04	4.98	10.11	1.46
Lot 3	96	93	31	77	5.3	10.78	7.20	3.84	8.99	1.65
Touchdown										
Lot 1	72	91	31	83	3.0	5.80	6.14	2.35	7.91	0.77
Lot 2	77	89	63	90	6.7	8.21	5.59	6.74	9.16	1.99
Lot 3	90	91	44	70	3.8	8.84	5.57	4.16	4.20	0.87
Merion										
Lot 1	17	48	10	68	3.6	0.98	2.39	0.53	3.47	0.38
Lot 2	36	50	24	54	8.5	3.28	2.94	2.50	4.59	1.57
Lot 3	51	52	18	67	2.9	4.18	3.07	1.49	5.34	0.46
Newport										
Lot 1	12	35	3	12	3.6	1.06	1.79	0.19	0.76	0.60
Lot 2	29	85	42	67	4.6	4.19	5.05	3.55	4.70	0.91
Lot 3	71	60	13	23	5.0	4.97	3.56	1.14	1.86	0.67
Bristol	41	79	40	86	7.0	5.02	6.15	4.41	10.41	2.97
Parade	10	43	3	57	4.9	0.97	2.97	0.15	5.00	0.96
Kenblue	43	67	25	79	11.0	4.81	4.69	2.24	8.00	2.02
Baron	27	81	35	74	6.0	2.13	4.98	3.06	5.26	1.08
Victa	64	94	25	71	6.5	6.22	6.89	1.73	5.18	1.37

Appendix Table 5. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 6-month-old Kentucky bluegrass seed, germinated at 20 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	28	59	34	79	3.3	2.93	4.35	2.41	6.49	1.31
Lot 2	63	97	62	82	8.3	7.43	13.39	7.68	9.98	1.88
Lot 3	69	95	49	89	6.1	9.44	12.24	5.60	10.53	1.16
Touchdown										
Lot 1	59	89	83	91	6.3	5.38	8.78	8.19	11.04	1.10
Lot 2	85	87	88	84	2.7	9.78	11.79	11.40	8.85	0.88
Lot 3	60	85	73	87	4.1	6.14	9.93	8.23	6.42	1.09
Merion										
Lot 1	13	57	52	85	5.8	0.85	3.09	2.87	5.63	0.71
Lot 2	11	54	39	57	6.2	1.40	6.76	3.85	4.23	1.40
Lot 3	8	59	37	72	3.9	0.95	6.46	2.42	4.92	1.04
Newport										
Lot 1	2	19	5	11	3.8	0.22	0.94	0.46	0.53	0.50
Lot 2	21	79	34	65	3.8	1.77	7.41	3.05	4.00	0.57
Lot 3	5	37	31	35	3.3	0.57	3.15	2.75	2.58	0.83
Bristol	26	64	69	81	5.1	3.44	6.75	8.02	10.03	1.19
Parade	10	60	43	77	6.9	0.91	4.31	2.67	6.44	1.82
Kenblue	30	69	70	86	3.9	3.38	6.21	6.97	9.65	0.72
Baron	21	71	89	81	5.6	2.10	4.75	7.50	6.56	1.21
Victa	45	87	91	95	8.7	4.07	6.43	6.09	8.39	1.20

Appendix Table 6. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 6-month-old Kentucky bluegrass seed, germinated at 25 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	4	17	5	28	2.6	0.51	1.41	0.67	2.97	0.63
Lot 2	7	91	36	90	4.6	0.76	12.61	4.00	10.30	1.12
Lot 3	19	85	43	81	5.9	2.37	10.90	4.95	7.47	1.53
Touchdown										
Lot 1	53	85	73	92	3.3	6.37	9.88	8.44	12.32	0.98
Lot 2	63	81	84	79	6.5	8.32	11.02	11.21	10.30	1.75
Lot 3	53	85	71	73	4.3	6.44	10.87	9.11	7.78	1.27
Merion										
Lot 1	13	37	15	64	2.0	1.51	3.21	1.18	4.90	0.44
Lot 2	11	61	52	84	3.6	1.52	7.64	5.02	11.15	0.62
Lot 3	11	60	41	89	4.2	1.44	7.78	3.79	10.70	0.86
Newport										
Lot 1	0	1	0	1	N.S.	0.00	0.07	0.00	0.05	N.S.
Lot 2	6	64	45	73	5.5	0.52	5.94	2.88	5.10	1.02
Lot 3	0	23	2	12	1.6	0.00	2.22	0.11	0.78	0.32
Bristol	30	54	27	71	4.5	4.19	6.43	3.05	9.23	1.29
Parade	12	34	19	73	4.0	1.56	3.16	2.10	7.52	0.64
Kenblue	24	53	31	73	6.1	3.05	6.00	3.28	8.91	1.01
Baron	14	17	22	51	2.6	1.76	1.69	2.52	5.79	0.65
Victa	19	39	19	55	5.0	2.51	4.21	1.92	5.70	1.38

Appendix Table 7. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 2-month-old Kentucky bluegrass seed, germinated at 15 C.

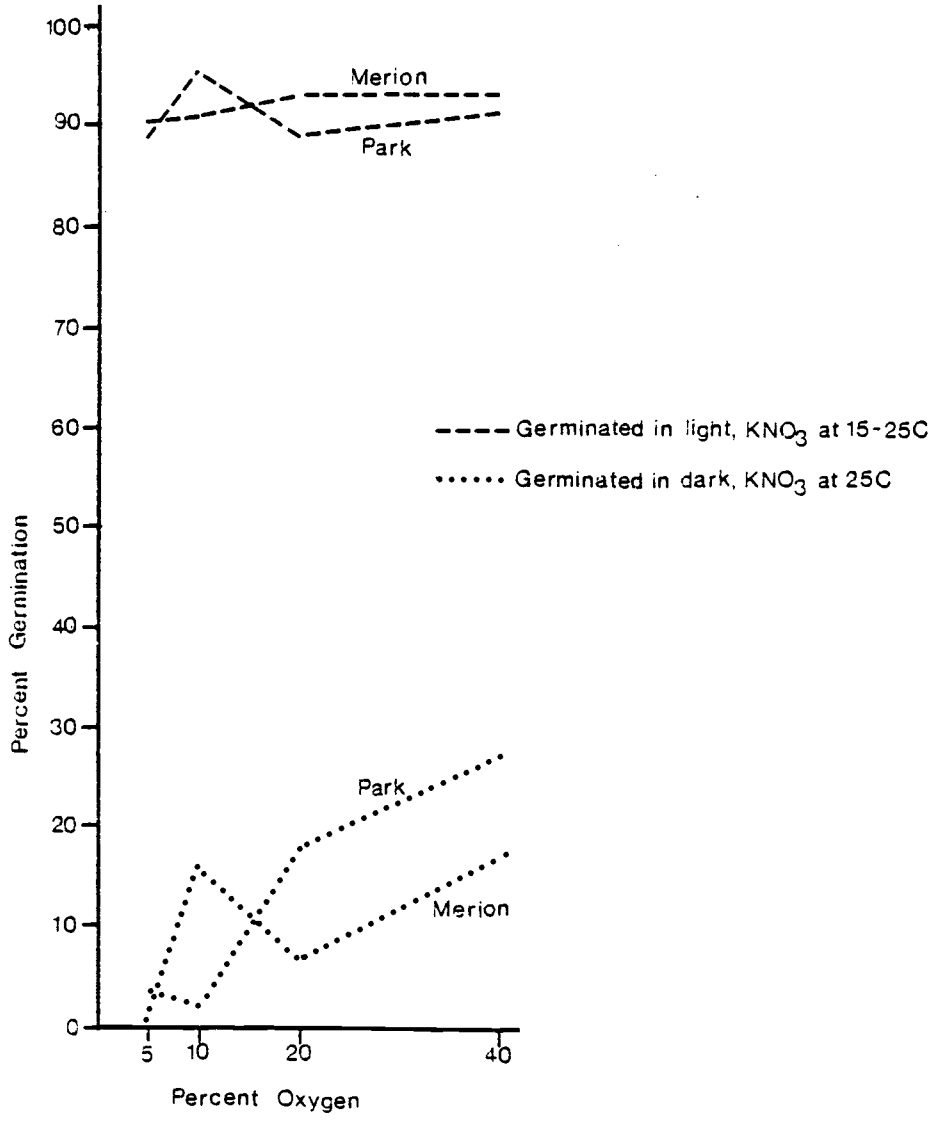
Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	15	77	3	5	6.4	1.05	5.46	0.16	0.63	0.88
Lot 2	67	81	9	40	8.3	4.50	5.47	0.62	2.72	0.59
Lot 3	37	71	9	31	5.2	2.48	4.54	0.57	2.29	0.69
Touchdown										
Lot 1	35	69	5	32	4.5	2.21	4.87	0.33	2.06	0.66
Lot 2	45	71	11	51	3.5	4.45	4.38	0.71	3.22	0.27
Lot 3	61	82	11	55	4.5	3.25	4.32	0.65	3.74	0.68
Merion										
Lot 1	1	33	1	7	4.3	0.10	1.94	0.05	0.63	0.67
Lot 2	14	32	1	5	1.8	0.94	1.91	0.10	0.33	0.24
Lot 3	22	38	2	34	3.8	1.38	2.34	0.13	2.20	0.43
Newport										
Lot 1	1	29	1	2	3.3	0.32	2.14	0.02	0.10	0.45
Lot 2	47	85	3	73	3.0	2.65	5.64	0.14	6.42	0.48
Lot 3	45	85	9	42	7.7	2.90	5.68	0.59	3.44	1.23
Bristol	79	35	74	35	5.4	4.92	2.28	5.95	2.46	0.87
Parade	5	35	7	8	2.3	0.36	2.94	0.51	0.66	0.43
Kenblue	49	85	7	52	5.1	3.65	6.55	0.53	4.99	1.61
Baron	15	77	11	32	3.2	0.10	5.07	0.84	1.99	0.56
Victa	12	72	17	61	3.0	0.76	4.85	0.29	1.14	0.40

Appendix Table 8. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 2-month-old Kentucky bluegrass seed, germinated at 20 C.

Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	7	27	2	2	2.0	0.69	3.10	0.24	0.24	0.50
Lot 2	21	69	8	33	4.2	1.91	6.75	0.54	2.51	0.52
Lot 3	21	34	19	17	2.8	2.02	3.49	1.28	1.64	0.65
Touchdown										
Lot 1	38	50	4	43	7.6	3.48	4.82	0.32	3.12	1.35
Lot 2	13	49	6	53	2.9	2.00	5.76	0.28	5.33	0.41
Lot 3	28	75	9	48	2.9	2.33	6.54	0.57	3.79	0.34
Merion										
Lot 1	0	0	9	5	2.1	0.00	0.00	0.64	0.41	0.34
Lot 2	1	16	3	13	2.3	0.05	1.15	0.19	0.78	0.31
Lot 3	11	6	4	28	3.6	1.10	0.65	0.29	1.84	0.52
Newport										
Lot 1	1	7	1	6	1.7	0.19	0.50	0.05	0.32	0.23
Lot 2	15	67	0	53	3.8	1.71	6.50	0.00	3.99	0.85
Lot 3	31	51	4	22	3.6	3.20	4.96	0.29	1.56	0.93
Bristol	56	27	59	37	4.9	6.48	2.91	5.33	2.64	1.04
Parade	7	11	7	15	2.2	0.78	1.18	0.75	1.40	0.47
Kenblue	41	67	12	53	10.7	4.81	8.00	0.86	4.59	2.32
Baron	11	6	4	28	2.1	1.10	0.65	0.29	1.84	0.24
Victa	6	19	10	24	4.1	0.44	1.78	0.31	1.44	0.49

Appendix Table 9. Percentage germination after 30 days and final Germination Rate Index for nine varieties and several lots of 2-month-old Kentucky bluegrass seed, germinated at 25 C.

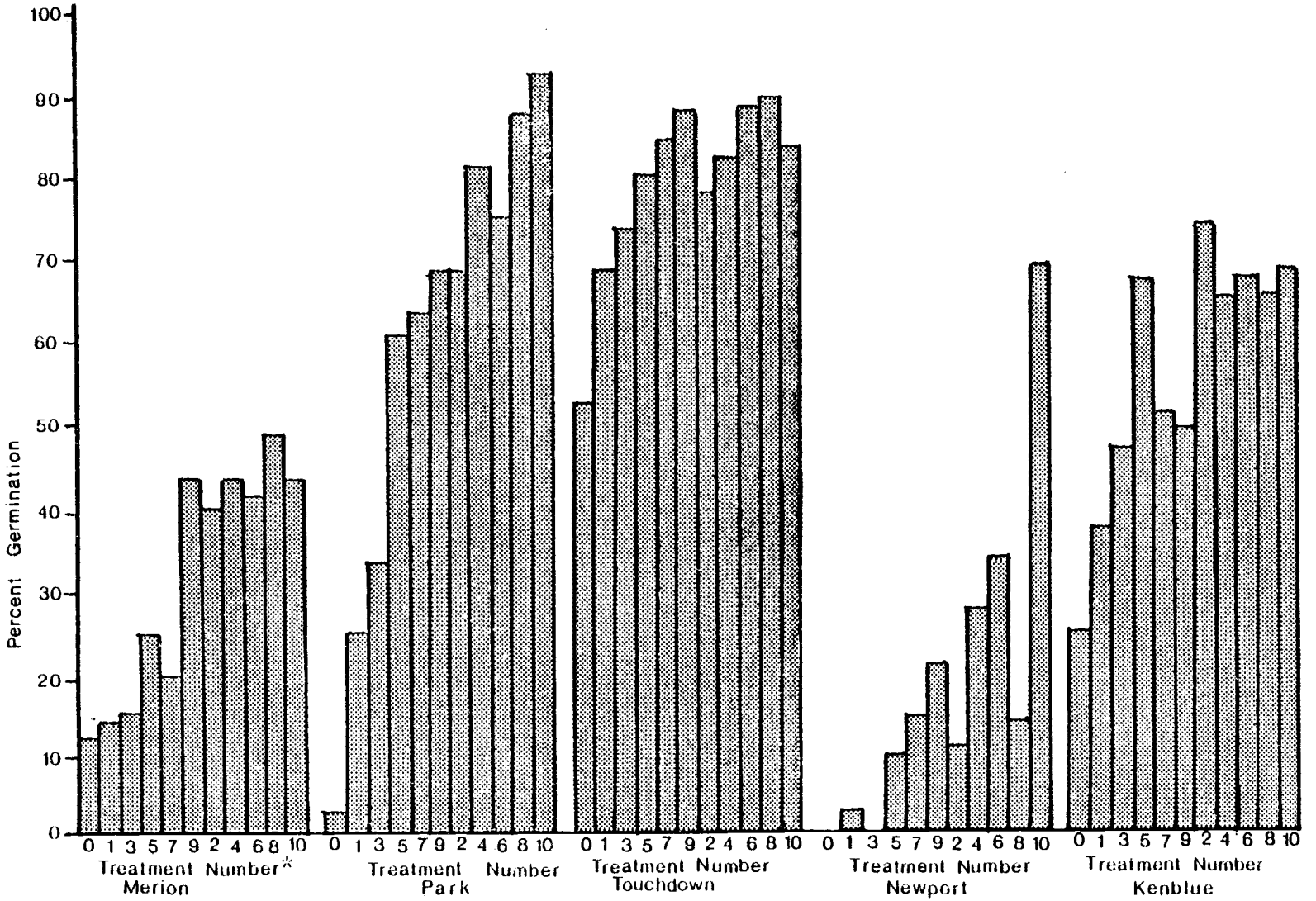
Variety	Germination					Germination rate				
	Dark		Light			Dark		Light		
	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05	Distilled water	KNO_3	Distilled water	KNO_3	LSD .05
	%					Germination rate index				
Park										
Lot 1	0	1	0	1	N.S.	0.00	0.13	0.00	0.10	N.S.
Lot 2	0	14	1	5	4.2	0.00	0.81	0.03	0.33	0.52
Lot 3	2	12	0	21	2.8	0.34	2.02	0.00	1.33	0.65
Touchdown										
Lot 1	1	29	2	69	7.6	0.13	2.43	0.21	8.40	1.35
Lot 2	29	40	34	45	2.9	3.36	5.76	3.45	5.33	0.41
Lot 3	24	62	33	69	2.9	1.96	4.82	2.23	5.04	0.34
Merion										
Lot 1	0	0	1	2	N.S.	0.00	0.00	0.05	0.13	N.S.
Lot 2	0	1	0	11	2.3	0.00	0.06	0.00	0.46	0.31
Lot 3	1	10	2	44	3.6	0.10	2.37	0.13	4.86	0.52
Newport										
Lot 1	0	0	0	3	1.7	0.00	0.00	0.00	0.33	0.23
Lot 2	0	2	0	4	N.S.	0.00	0.15	0.00	0.30	N.S.
Lot 3	5	11	1	12	3.6	0.57	1.67	0.06	0.88	0.93
Bristol	35	15	32	29	4.9	3.14	1.33	2.35	2.18	1.04
Parade	0	1	0	1	N.S.	0.00	0.10	0.00	0.10	N.S.
Kenblue	2	7	1	17	8.6	0.14	0.86	0.05	1.95	N.S.
Baron	1	3	5	8	2.1	0.10	0.25	0.46	0.57	0.24
Victa	0	1	2	5	4.1	0.00	0.05	0.13	0.35	N.S.



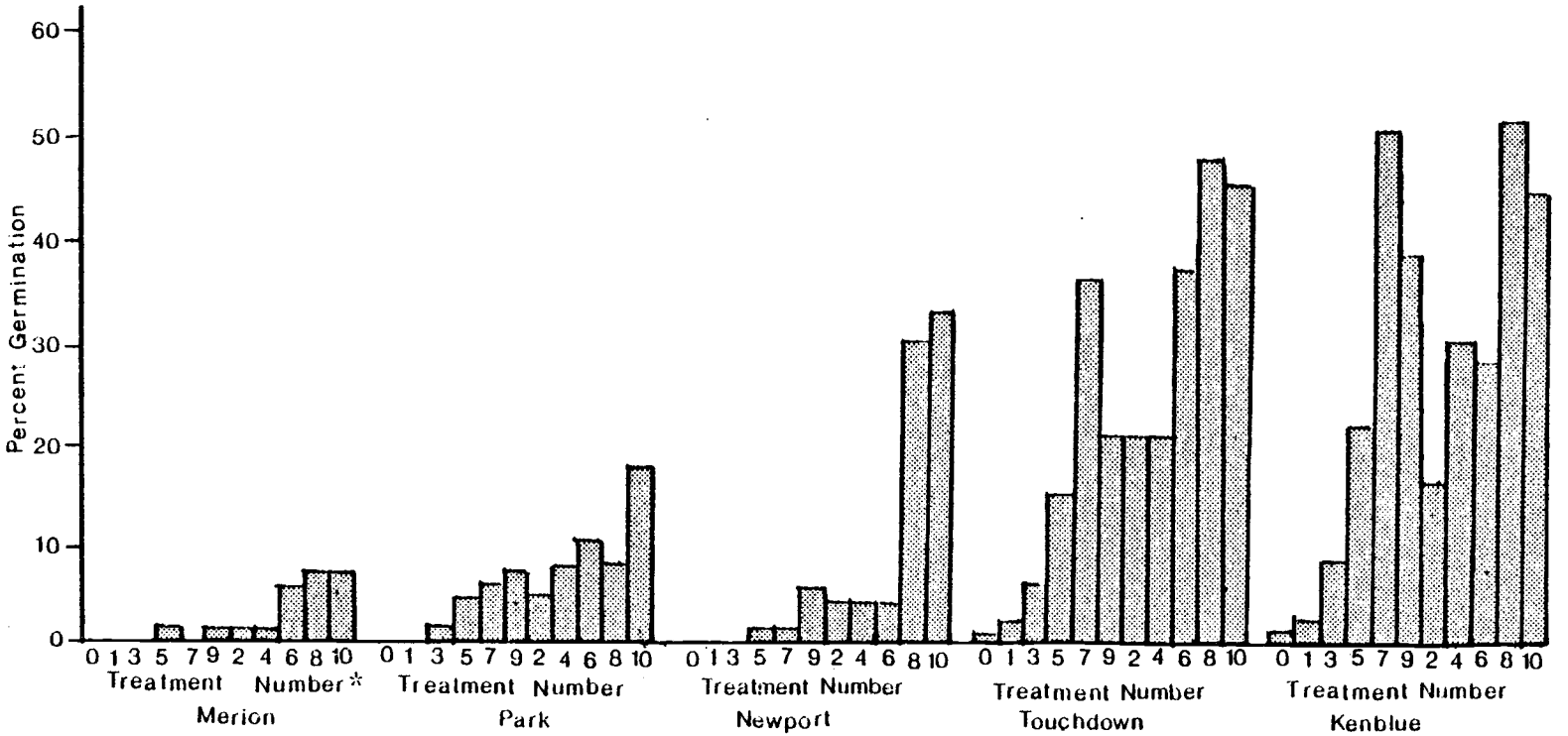
Appendix Figure 1. Effect of different concentrations of oxygen on germination of two varieties of Kentucky bluegrass seed.

Appendix Table 10. Sequence of exposure of the different treatments to the chill environment.

Treatment number	Initial days of chill (5 C)	Days at 25 C	Days of second chill (5 C)	Total days of chill
0				0
1	1			1
2	1	1	1	2
3	2			2
4	2	1	1	3
5	3			3
6	3	2	1	4
7	4			4
8	4	2	2	6
9	5			5
10	5	3	3	8



Appendix Figure 2. Percent germination of six-month-old Kentucky bluegrass seed stratified for varying periods of time and germinated at 25 C in the dark, after 30 days.



*Treatments shown in Appendix Table 10

Appendix Figure 3. Percent germination of two-month-old Kentucky bluegrass seed stratified for varying period of time and germinated at 25 C in the dark, after 30 days.