The Effect of Seed Priming on the Germination, Emergence, and Development of Five Different Grass Species

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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THE EFFECT OF SEED PRIMING ON THE GERMINATION, EMERGENCE, AND DEVELOPMENT OF FIVE DIFFERENT GRASS SPECIES

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ACKNOWLEDGEMENTS

Thanks to my advisor, Dr. S. Ray Smith, for not only guiding me through the writing of this thesis, but for also showing me the value of good organization and communication.

Thanks to my committee members, Dr. Martin Entz and Dr. Isobel Waters, for all their helpful suggestions and comments.

Thanks to Matt Fruehm for his invaluable technical support and to all my fellow graduate students who gave me feedback when I needed it.

Thanks to Ducks Unlimited Canada, the Canadian Seed Growers Association, and the Manitoba Forage Seed Association for their financial support.

Thanks to Michelle and the rest of my family and friends for all their support over the years.

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ABSTRACT

Watts, Jason Christopher. M.Sc., The University of Manitoba, April, 2001. The Effect of Seed Priming on the Germination, Emergence, and Development of Five Different Grass Species. Major Professor; S. Ray Smith.

The production and use of many native grasses is often limited because of high seed dormancy and poor seedling development which result in poor stand establishment. The objective of this study was to develop a simple seed priming method that incorporated the use of stratification. The effect this priming methodology had on the germination and emergence of several grass species was tested in a series of indoor and field experiments. Seeds of little bluestem (*Schizachyrium scoparium* [Michx.] Nash), blue grama (*Bouteloua gracilis* [Wild. ex Kunth] Lag. Ex Griffiths), green needlegrass (*Nassella viridula* [Trin.] Barkworth), prairie sandreed (*Calamovilfa longifolia* [Hook.] Scribn.), and Kentucky bluegrass (*Poa pratensis* L.) were placed into cloth bags and soaked for 24 hours in water or a 0.5% KNO_3 solution. The seeds were chilled at 4°C for either 1 week or 3 weeks, then dried on screens for 48 hours at 25°C. Two controls were used to determine the effects of the priming treatments, one was completely untreated, the other was soaked in water for 1 hour, then dried without any chilling period. All little bluestem priming treatments had equally higher germination and emergence than untreated control seed. Priming green needlegrass for 3 weeks in a 0.5% KNO_3 solution produced the greatest % germination and quickest emergence. Priming prairie sandreed for 3 weeks in water produced the highest emergence counts and fastest germination rate of all treatments. There was little advantage gained from priming blue grama and Kentucky bluegrass. The results of this research showed that seed priming has great potential for improving the germination and emergence of native grasses. However, the results will vary widely depending on species and environmental conditions.

1. INTRODUCTION

There is increasing interest for using native grasses in commercial, aesthetic, and ecological applications. Homeowners are interested in using native grasses for lawns and gardens, schools are interested in using them for educational purposes, and golf courses are interested in using them as turfgrasses. Park and wildlife managers, mining and power companies, and conservation organizations all need an adequate supply of native grass seed for restoration projects in disturbed areas. Governments are interested in native grasses for use in roadsides, since they will last longer than introduced grass species, and since they require much less maintenance during adverse conditions. Native grasses are indigenous to North America and have been naturally selected to withstand the regional environmental conditions. The majority of forage and turfgrass species currently used in North America are indigenous to Europe and Asia and are much less adapted for this environment.

A number of native grass species have potential for production in Manitoba. Each of these native grasses has a number of unique limitations, especially in the areas of seed production and seed quality. Research focused on understanding and overcoming these limitations will not only allow greater use of these native grasses, but will also be applicable in overcoming similar problems with introduced grass species. Since seed quality limitations are usually greater for native grasses than they are for introduced grasses, it is probable that much more information can be obtained by studying these species.

The production and use of native grass seed in Manitoba is limited by two major factors. First, there is an economic limitation. The high cost of native seed prohibits its

use in many applications. Second, there is an establishment limitation. Native grasses are notoriously slow to germinate, and often have long seed dormancies. This makes it extremely hard to consistently establish healthy, productive stands.

Seed priming can be an effective way to overcome the economical and establishment problems associated with native grasses. Seed priming involves the use of any one of a number of different techniques which act to stimulate embryonic root growth. Growth will continue within the seed until it reaches a point just prior to emergence through the seed coat. The primed seeds are then re-dried and planted. Much of the previous work with seed priming has been conducted on vegetable seeds, but recent studies have also been conducted on some native grasses. Slow germinating introduced grasses, such as Kentucky bluegrass *(Poa pratensis* L.*)*, could also benefit from priming.

The establishment of native grasses is enhanced by seed priming in several ways. Firstly, the emergence rate of the seedlings will increase. The seeds have already initiated the germination process, so when they are planted in the field less time will be needed for the plants to emerge. This will allow the seeds to use the spring water much more efficiently, and stand establishment will be much quicker and more robust. As well, the native seeds will be able to compete more effectively with the earlier germinating weeds, thus improving the overall health of the stand, and reducing the need for expensive herbicides.

Secondly, priming may result in native seedling emergence that is more uniform. Native grasses must be able to adapt to many different conditions in order to be successful in restoration situations. Consequently, it is preferred that only a minimum amount of genetic selection be used to develop them for commercial production (in contrast, cultivars

of introduced species have been highly selected for specific traits, such as seed yield and uniformity). While this allows native grasses to retain their genetic diversity, it also acts to increase the variation within seed lots, and many of the seeds will be in various states of dormancy. Some of these seeds will germinate, but others will remain dormant indefinitely. Seed priming can be used to initially break seed dormancy and to stimulate embryonic root growth until the same level of germination is reached by all of the seeds. Thus, a genetically diverse population of seeds will act, in terms of emergence, as a more uniform population.

Finally, seed priming may help the seeds germinate in drier and colder soils. Since germination has already been initiated, less moisture will be needed by the emerging seedling. Also, native seeds which typically need warmer temperatures to begin germination will be able to begin growth at lower soil temperatures. Thus, the emerging seedlings will have an advantage in less than ideal environmental conditions, and will be in a much better position to compete with weeds.

Any establishment benefits gained by priming native grass seeds should improve the economics associated with the seed industry. For one thing, the amount of seed needed to establish a stand will decrease, causing the price associated with it to decrease as well. Improved competitiveness with weeds will reduce the amount and cost of herbicides needed during the establishment phase.

The most popular methods of seed priming are osmotic and solid matrix priming. These techniques use internal and external water potentials to prevent radicle emergence from occurring. The procedures are often complicated and only involve laboratory

conditions. These methods are not practical for most seed companies and it would be beneficial if a simpler method was conceived.

Stratification is another seed treatment method that is commonly referred to as seed priming. It occurs when seeds are exposed to a cool, moist environment for an extended length of time. This often helps break the dormancy associated with the seed since it acts as a signal that spring is on its way. Stratification will also prevent radicle emergence if imbibition occurs in temperatures below the germination range of the seed. Thus, stratification is a simple technique that could be used during seed priming to stimulate germination, but prevent radicle emergence in a seed. Stock Seed Farms in Nebraska have successfully used a modified stratification technique for seed priming of buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.) burs. Preliminary work involving several seed priming treatments was conducted with little bluestem (*Schizachyrium scoparium* [Michx.] Nash) at the University of Manitoba in 1995.

The objective of this research was to develop and apply a simple seed priming system that would enhance the establishment of little bluestem, blue grama (*Bouteloua gracilis* [Wild. ex Kunth] Lag. Ex Griffiths), prairie sandreed (*Calamovilfa longifolia* [Hook.] Scribn.), green needlegrass (*Nassella viridula* [Trin.] Barkworth), and Kentucky bluegrass. The effects of the length of priming, use of KNO_3, and drying were investigated to determine their effect on each species' germination percentage, germination rate, emergence percentage, emergence rate, and plant development.

2. LITERATURE REVIEW

2.1 Grassland Restoration

Grasses from Europe and Asia have been traditionally used to reclaim disturbed sites. They were favoured over the native or indigenous grasses because they grew much more aggressively, and therefore, gave good protection against erosion (Gerling *et al.*, 1996). However, once established, these introduced species often prevented the native plants from re-establishing onto the disturbed areas and often invaded the neighbouring natural areas (Gerling *et al.*, 1996). Introduced grasses have even been known to alter the chemical quality of native rangeland soils (Dormaar *et al.*, 1994).

Recently, there has been an increasing shift away from the use of introduced grass species and more toward the use of native species for restoration projects throughout North America. This interest stems from several different factors. Native grasses evolved under climatic conditions of a continental climate and are adapted to wide fluctuations in moisture and temperature. More and more people are recognizing the intrinsic beauty of the native plants and are using them for their aesthetic values (Morgan *et al.*, 1995). As well, there has been a renewed interest by society for preservation of all things natural (McDonald and Copeland, 1997). Governments have recognized the importance of native species and there are now many provincial, state, municipal, and city programs and laws that require the use of native plants and wildflowers (McDonald and Copeland, 1997). The government of Alberta has required the use of native species for reclamation of public lands in its southern and eastern regions since the early 1990s (Gerling *et al.*, 1996).

There are many limitations that prevent the widespread use of native grasses and forbs. Since native plants are valued for their genetic diversity, it may be self-defeating to artificially select them for higher yielding and easier harvesting characteristics, as is the case with introduced species. Invariably, seed of native plants is not readily available. It must often be collected by hand from existing native stands (McDonald and Copeland, 1997), which is very time consuming and expensive. Seed production of native grasses in cultivated stands also has its challenges. Native grasses are typically poor seed producers, exhibit a high degree of seed shatter, and have lower seed quality than introduced grasses (Smith and Smith, 1997). Native grasses are typically hard to establish because of high seed dormancy and slow seedling establishment (Hsu *et al.*, 1985; Beckman *et al.*, 1993). Native grass seed is also hard to clean and store (Gerling *et al.*, 1996). All of these factors contribute to the high cost of native grass seed, which further limits its use.

In the last 20 years, tremendous progress has been made in the seed production agronomy of native grasses by the USDA-NRCS and by individual producers in the USA and Canada. Recently, a *Native Grass Seed Production Manual* (Smith and Smith, 1997) was published. It summarizes the recommended agronomic practices for many different native grass species. This manual, along with other initiatives, has encouraged producers to grow native grass seed. Some producers are now able to produce seed of species like green needlegrass and switchgrass (*Panicum virgatum* L.) for only slightly more than many traditional introduced species (S.R. Smith, pers. comm.).

2.2 Seed Water Relations

Seed water relations have been discussed fully by Bewley and Black (1994). Basically, water uptake by seeds involves three phases: imbibition, lag, and radicle elongation. The water relations between the seed and the external medium will determine the extent of water uptake because water will diffuse along an energy gradient from high to low water potential (ψ).

The water potential of the cells of a seed can be expressed as follows:

$$\psi_{cell} = \psi_s + \psi_m + \psi_p$$

where ψ_s is the osmotic potential, ψ_m is the matric potential, and ψ_p is the pressure potential. The ψ_s is determined by the concentration of dissolved solutes found in the cell. As solute concentration increases, the ψ_s will decrease. The ψ_m is defined by the hydration of matrices (e.g. cell walls, starch, protein bodies) and their ability to bind water. The ψ_p is caused by the inflow of water into the cell, which causes internal pressure to build-up, thus creating a force on the cell wall and causing ψ_p to increase. ψ_p has a positive value, but ψ_s and ψ_m have negative values because they have a lower potential than pure water.

The water potential of the external medium ($\psi_{external}$) can be expressed using the same components. However, in a soil environment, only ψ_m plays a significant role due to the large concentration of soil particles. If the external medium is pure water, the

water potential will be zero. If the water contains solutes, the water potential will be lower ($\psi<0$).

Mature dry seed has a much lower ψ (more negative) than the surrounding substrate. A dry seed at equilibrium with air that has a relative humidity of 50% has a ψ_{cell} equal to -100 MPa (Nobel, 1970). A saturated soil containing a very dilute concentration of ions usually has a ψ of about -0.03 MPa (Salisbury and Ross, 1992), which is much higher. Thus, a strong energy gradient is created and water will flow into the seed. This is Phase I of water uptake (imbibition). It will occur whether or not the seed is dormant or non-dormant (Bewley and Black, 1994).

When Phase I is reached, the main contributing component to the low ψ is the ψ_m. Since all the water in the seed is bound-up, the ψ_s and ψ_p are negligible, while the matric forces caused by the interfacial interactions of the water with the molecular constituents of the seed cause the ψ_m to be very low (Bradford, 1986). As the seed imbibes water, the ψ_m will become less important and the seed ψ will depend more and more on ψ_s for water uptake. The incoming water will dissolve solutes within the cells, causing the ψ_s to decrease, thus providing the driving force for water uptake. However, as more and more water enters the cells the internal pressure will increase, thus increasing the ψ_p. Eventually, a water content equilibrium will be reached where there is no net water movement and the $\psi_{external}$ is equal to the ψ_{cell}. Water is no longer being taken up and major metabolic events begin to take place in preparation for radicle emergence. This is Phase II of water uptake (lag) and it will occur in both dormant and non-dormant seeds (Bewley and Black, 1994).

Commencement of Phase III (radicle elongation) is dependent on the attainment of a threshold seed water content rather than being dependent on a specific ψ (Bradford, 1986). However, if the $\psi_{external}$ is reduced (as in seed priming), the water content plateau falls and the onset of germination is delayed. Thus, in seed priming, various methods are used to regulate the osmotic or matric components of the $\psi_{external}$, or to ensure that the seeds are removed from the priming mixture during the lag phase. This will allow the seeds to imbibe water and begin their pre-metabolic activity without allowing radicle emergence to occur. The seed will usually only attain 80 to 90% of full imbibition (Pill, 1995).

2.3 Seed Germination

Major metabolic events within the seed are triggered by Phases I and II of water uptake. The seed begins to respire using three different respiratory pathways: glycolysis, the pentose phosphate pathway, and the citric acid cycle (Bewley and Black, 1994). These oxidative pathways produce energy in the form of ATP and reducing power in the form of NADH and NADPH (Desai *et al.*, 1997). Enzymes are also activated during these phases. Giberellins are released from the scutellum and migrate to the aleurone layer where they trigger hydrolytic enzyme synthesis (Copeland and McDonald, 1995). The enzymes are released into the endosperm where they begin to break down the endosperm starch into sugars and amino acids. These soluble and diffusible sugars and amino acids are then absorbed by the scutellum and transported to the shoot and root for use in growth (Raven *et al.*, 1986).

Germination is considered complete once the radicle has expanded and penetrated through the seed coat (Bewley and Black, 1994). In the past, it had been thought that this was accomplished through either cell elongation or division, but it is now thought that cell elongation is the driving force (Bewley and Black, 1994, Desai *et al.*, 1997). As mentioned in the previous section, radicle elongation is dependent on the attainment of a threshold seed water content (Bradford, 1986). The increased water uptake creates an increase in the turgor pressure, and this causes cell elongation to begin (Bewley and Black, 1994). In addition, the tissues surrounding the radicle tip are weakened by the hydrolytic enzymes, which digest and separate their cell walls (Bewley and Black, 1994). Physical loosening of the cell wall can also occur when the cell absorbs water (Desai *et al.*, 1997). Thus, the radicle is able to emerge from the seed and germination is complete.

2.4 Types of Seed Priming

There have been many different priming methods employed in the past, including imbibition, drought hardening, osmopriming, solid matrix priming, and stratification.

2.4.1 Imbibition

This priming method involves soaking the seeds in water for a period of time, followed by removal and drying. This should allow the seeds to become adequately imbibed, without promoting radicle emergence. Haferkamp and Jordan (1977) found that the germination percentage of Lehmann lovegrass (*Eragrostis lehmanniana* Nees.) seeds

that had been imbibed in the dark at 10°C was significantly higher than that for control seeds. Bleak and Keller (1974) imbibed Russian wildrye (*Psathyrostachys juncea* [Fisch] Nevski), Siberian (*Agropyron fragile* [Roth] P. Candargy), desert (*A. desertorum* [Fisch. Ex Link] J.A. Schultes), intermediate (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey), beardless (*Pseudoroegneria spicata* [Pursh] A. Löve), and crested (*A. cristatum* [L.] Gaertn.) wheatgrasses with water until approximately 5% of the seeds had visible radicles. When the seeds were air dried and used in field planting, all of the wheatgrass species had a faster seedling emergence rate. The Russian wildrye did not show any improvement.

Most of the previous studies have allowed the seeds to take up water gradually from a moist substrate. This practice, however, makes it hard to prime large quantities of seed. It is much easier to let the seeds soak for a limited time period. Soaking seeds for extended periods of time can be harmful because the germinating seed is unable to get a sufficient oxygen supply (Orphanos and Heydecker, 1968). In most cases, benefits have only been realized when soaking time is short (Hakozoki, 1973; Kano, 1968), or when other mechanical methods have been used to provide aeration (Heydecker and Coolbear, 1977).

The seeds must also be removed from the water during the lag phase (prior to radicle emergence) and must then be dried to prevent any further radicle development. This is the most limiting factor when using this method. Each seed will initiate radicle emergence at different times, making it hard to determine the proper soaking time. Constant monitoring is needed because once radicle emergence is initiated, the seeds cannot be dried without injury, and the advantages gained by priming will decrease dramatically (Berrie and Drennan, 1971).

Water imbibition simply advances the germination process within the seed and when it is planted there is a reduction in the time it takes to germinate. Unlike most other priming methods, however, there is no effect on the uniformity of germination.

2.4.2 Drought Hardening

Drought hardening is very similar to imbibition. Limited amounts of water are used to hydrate seeds followed by drying them back down to about 30-70% of their initial dry weight (May *et al.*, 1962; Henckel, 1964). This process is then repeated several times. The term drought hardening was used originally because it was once thought that the main advantage was improving the drought tolerance of the ensuing plants (Henckel, 1964; Henckel *et al.*, 1968). However, later research has not always been able to confirm that the germination benefits obtained by this method also improved mature plant drought tolerance.

Hanson (1973) and Berrie and Drennan (1971) showed that several imbibition-dehydration cycles enhanced wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.) germination, respectively. Woodruff (1969; 1973) found that drought hardened wheat had subsequently greater root area and thicker leaves with thicker cell walls and more bound water (i.e. more resistance to cell dehydration). In contrast, Evenari (1964) used three wet-dry cycles on sorghum (*Sorghum bicolor* [L.] Moench), but was unable to find any advantages.

2.4.3 Osmopriming

Osmotic priming is a process that allows seeds to imbibe in an aerated osmotic solution which contains various osmotica, such as polyethylene glycol (PEG) or various salts (Osburn and Schroth, 1989). Water uptake by the seeds is regulated by the osmotic potential of the external solution.

Each species or seed lot will vary when it comes to the correct osmotic potential to use during osmopriming. If the osmotic potential is too high, water uptake will not be adequately regulated and radicle emergence may occur. In addition, the type of solute used will cause variation in the amount of water absorbed by seeds (Parmar and Moore, 1968). Brocklehurst and Dearman (1984) found that –1.5, –3.0, and –2.0 MPa for PEG, glycerol, and KH_2PO_4, respectively, were needed to produce similar effects in regulating water uptake in several vegetable seeds. This shows that a standard osmotic potential cannot be used for every type of solute. The optimal water potential for a seed lot is estimated to be the least negative water potential that does not result in radicle elongation during priming (Evans and Pill, 1989). In general, the lower the molecular weight, the more negative the osmotic potential necessary to restrict water uptake by the seed (Brocklehurst and Dearman, 1984) and the osmotic potential used in seed priming usually ranges from between -0.8 to -1.6 MPa (Khan, 1992).

PEG is a relatively inert, non-toxic compound that is unable to enter seed tissue at molecular weights above 4000 (Heydecker and Coolbear, 1977). It acts to lower the water potential of the priming solution by increasing the solution's solute concentration. Water will still enter the seed, but at levels low enough that radicle emergence will not occur.

PEG has been shown to increase the rates of seed germination and seedling emergence of carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), leek (*Allium porrum* L.), and onion (*Allium cepa* L.) seeds (Brocklehurst and Dearman, 1984). Rivas *et al.* (1984) found that priming jalapeno pepper (*Capsicum annuum* L.) and tabasco pepper (*Capsicum annuum* L. var. *annuum*) in a -4 bar PEG-6000 solution for 120 hours increased germination rates. Wheat, maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and sorghum displayed enhanced germination rates when primed with PEG-6000/7500 solution (Bodsworth and Bewley, 1981).

A major problem with using PEG occurs because of its viscous nature, which acts as a barrier to oxygen movement (Mexal *et al.*, 1975). This requires that the priming solution is vigorously aerated and that the seed is adequately rinsed off prior to seeding. These obstacles must be overcome before large amounts of seed can be primed using PEG. Bubble-column and stirred-bioreactor technology have been used to prime up to 10 kg of seed (Bujalski *et al.*, 1989). However, this is nowhere near the level needed for commercial use.

Salts also act to lower the external water potential. In contrast to PEG, salt solutions have comparable oxygen availability to that of water (Heydecker and Coolbear, 1977) and are relatively inexpensive. However, salts are able to penetrate seed tissues and this could cause adverse toxicity effects. The influx of ions could also act to lower the seed osmotic potential, thus affecting the water content equilibrium between the seed and the priming solution. The result would be a greater inflow of water, causing a reduction in the radicle growth suppression effects of the priming solution (Brocklehurst and Dearman, 1984). Many different salts have been used in the past with varying degrees of success (Bradford,

1986), but potassium nitrate (KNO_3) appears to have the greatest potential as an osmotic priming solute.

Early studies on buffalograss (Wenger, 1941), vine mesquite (*Panicum obtusum* Kunth) and plains bristlegrass (*Setaria vulpiseta* [Lam.] Roemer & J.A. Schultes) (Toole, 1940), and poverty oatgrass (*Danthonia spicata* [L.] Beauv. Ex Roemer & J.A. Schultes) (Toole, 1939) showed that dilute concentrations of KNO_3 increased seed germination. Rivas *et al.* (1984) tested three different concentrations of KNO_3 and found that a 3% solution was most effective in preventing radicle emergence and stimulating the germination rate of pepper seeds. Bradford (1985) dramatically increased the rate of germination of several cantaloupe (*Cucumis melo* L.) varieties by using a 3% KNO_3 solution.

KNO_3 has several advantages aside from its osmotic properties. First, it may have a nutritional function. Ells (1963) attributed the increased germination rate of tomatoes (*Solenum lycopersicum* L. var. *lycopersicum*) at low temperatures (below 20°C) to the nutritional affects of priming in K_3PO_4 and KNO_3 solutions. Second, KNO_3 may have dormancy breaking powers. Hargurdeep *et al.* (1986) found that nitrate-deficient lambsquarters (*Chenopodium album* L.) seeds required a combined application of KNO_3 and ethylene to break seed dormancy. Egley (1984) found that pre-incubation of purslane (*Portulaca oleracea* L.) seeds in 20 mM KNO_3 solutions at 35°C for 4 to 7 days before ethylene applications significantly increased germination. This combination produced better results than those obtained by either stimulus alone. Dormant seed of field pennycress (*Thlaspi arvense* L.) achieved 100% germination after being treated with a combination of 10 mM KNO_3, light, and a temperature shift from 12 to 22°C (Hargurdeep

et al., 1987). However, no germination was observed when water was used instead of the KNO_3 solution. Dormant wild oat (*Avena fatua* L.) seeds that were placed in 0.0002, 0.002, and 0.02 M concentrations of KNO_3 in the light experienced a higher level of germination than seeds that were placed in distilled water (Hilton, 1984). The use of a 0.2% KNO_3 solution is recommended by the Association of Official Seed Analysts (AOSA) for breaking seed dormancy of several native grasses (Maxon, 1995).

2.4.4 Solid Matrix Priming (SMP™)

SMP is a relatively new process where seed is mixed with an inorganic or organic carrier. It is then provided aeration and just enough water to allow seed imbibition, but not radicle emergence (Kubik *et al.*, 1988). Originally, it was thought that only the matric potential of the carrier matrix was responsible for the regulation of seed water uptake. Recently, however, it has been determined that the benefits gained from using moist semi-solid or solid carriers can sometimes be attributed to the osmotic, not the matric component of the external water potential (Khan, 1992). This will occur when the solid carrier contains salts that can be readily dissolved (e.g. Agro-Lig [Leonardite shale]). Unfortunately, many of the earlier studies (e.g. Taylor *et al.*[1988]) did not make this distinction and it is unknown which of the components (matric or osmotic) were being used.

'True' matripriming controls seed hydration through the matric potential generated by the adsorptive, interfacial tension, attractive, and adhesive forces found between the carrier matrix, matrix-air, and matrix-water interfaces (Hadas, 1982). As such, the solute component of the water potential is negligible (Khan, 1992). Inorganic hydrous silicate

clay, Celite (diatomaceous silica), Micro-Cel (produced by hydrothermal reaction of diatomaceous silica, hydrated lime, and water), and Zonolite vermiculite all use the matric component rather than the osmotic (Rush, 1991; Khan 1992).

By using a 1:1:1 ratio of sugar beet (*Beta vulgaris* L.) seed, dry hydrous silicate clay, and water, Rush and Vaughn (1993) found that two days after planting, seed germination increased from 1% to 34%, and by the seventh day, the mean radicle length had increased from 0.1 mm to 1.2 mm. Rush (1991) found that using solid matrix primed sugar beet seeds significantly increased the rate of emergence and uniformity over the control, as well as other seeds that had been osmotically primed with PEG-8000 or with NaCl. Beckman *et al.* (1993) found that SMP treatments increased both big bluestem (*Andropogon gerardii* Vitman) and switchgrass emergence over the control. However, the seeds were not dried prior to planting, and it was not stated what the SMP carrier actually was.

Hardegree and Emmerich (1992a) found that matric priming at -1.6 MPa frequently caused increases in the germination percentage of side-oats grama (*Bouteloua curtipendula* [Michx.] Torr.), buffelgrass (*Pennisetum ciliare* [L.] Link var. *ciliare*), Lehmann lovegrass, and klinegrass (*Panicum coloratum* L.) when germinated at reduced water potentials. They also found that there were reduced or detrimental effects on germination if matric priming occurred at a water potential more negative than -1.6 MPa. Hardegree (1994a) found that optimal matric priming water potentials (i.e. the least negative water potential that prevented radicle emergence during treatment) ranged from -1.0 MPa to -2.5 MPa for bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve), thickspike wheatgrass (*Elymus macrourus* [Turcz.] Tzvelev), basin wildrye (*Leymus cinereus* [Scribn. & Merr.]

A. Löve), sheep fescue (*Festuca ovina* L.), canby bluegrass and sandberg bluegrass (*Poa secunda* J. Presl.), and bottlebrush squirreltail (*Sitanion hystrix* [Raf.] Swezey ssp. *elymoides*). These studies are further evidence that it is impossible to develop a generic priming treatment for different native grass species.

2.4.5 Stratification

Stratification occurs when seeds are exposed to low temperatures and moist conditions for extended periods of time before any attempt is made to germinate them at warmer temperatures (Raven *et al.*, 1986). Many native grasses need stratification to break the physiological dormancy of their seeds. Chilling has been shown to enhance germination rate and percentage of several native perennial grasses. For example, switchgrass had an increase in germination of 32% when stratified for 30 days at 4°C (Sautter, 1962), and 338% when stratified for 14 days at the same temperature (Hsu *et al.*, 1985).

It is possible to prevent radicle emergence if imbibition of water occurs in temperatures outside the range of germination for the seeds being treated (Heydecker and Coolbear, 1977). This can have important consequences for commercial priming endeavors. First, many of the grass species require a period of stratification anyway, so this method would serve two functions, thus reducing the amount of time and money needed. Second, since the cold temperature acts to prevent radicle emergence, there is no need to be as careful in regulating the amount of water the seeds receive, which is the case with simple water imbibition.

Stratification can be combined with osmopriming to further improve the germination of certain species. The stratification treatment will break seed dormancy, while the osmopriming treatment will act to reduce germination time (Khan, 1992). Since the cold temperature is preventing radicle emergence, a low osmotic potential is not needed. This will allow KNO_3 to be added in lower concentrations, thereby alleviating the toxicity hazards and allowing the seeds to take advantage of its nutritional and dormancy-breaking characteristics. Khan and Karssen (1980) found that osmopriming moist-chilled goosefoot (*Chenopodium bonus-henricus* L.) seeds with a -0.86 MPa PEG solution for seven days in the light reduced the time to germination.

2.5 Advantages of Seed Priming

2.5.1 Increased Germination and Emergence

Seed priming has been shown to increase the germination and emergence success of several different grasses. Beckman *et al.* (1993) found that SMP treatments increased big bluestem emergence by 18% when seeds were planted in a greenhouse setting. They also found that switchgrass emergence was increased between 35% and 150% over dry, untreated seed. Hardegree (1994b) found that the final seed germination percentage of three native perennial bunchgrasses was increased by seed priming if the seeds were not dried after the priming process.

S.R. Smith (unpublished data) found that little bluestem (*Schizachyrium scoparium* [Michx.] Nash) seed also benefited from seed priming. Seedlots were soaked

in a 0.2% KNO_3 solution or distilled water for 2 hours at 4°C. The seed was drained for 1 to 2 hours and stored for 1.5 weeks at 4°C. The bags were then removed and the seeds were allowed to air dry at approximately 20°C. A third treatment involved storing dry seed at 4°C for 1.5 weeks. Seed used for a control was stored at approximately 20°C. The seeds from the various treatments were planted in plots at the Carman Research Station. Stand density measurements were taken on July 13, 1995 and the results are shown in Table 1.

Table 1. Mean density (no. m^{-1} row) of little bluestem (*Schizachyrium scoparium* [Michx.] Nash) planted at Carman, MB in 1995.

Seed Treatment	Mean Density
Control	29.2 b[z]
Chill	23.6 b
H_2O Stratification	52.0 b
KNO_3 Stratification	83.6 a

[z]Means with the same letter are not significantly different according to an LSD test (p=0.05).

2.5.2 Increased Emergence Rate and Uniformity

Regardless of the method, the usual result of seed priming is increased germination rate and uniformity (Bodsworth and Bewley, 1981; Hardegree and Emmerich, 1992a, 1992b; Hardegree, 1994a; Beckman *et al.*, 1993; Frett and Pill, 1995). The advantages of having an increased germination rate are obvious. Seeds will be able to take advantage of the existing spring moisture and will be able to better compete with weeds. As such, it will allow for greater establishment in the field.

Seed priming enables the seed to remain in the priming solution for an extended period of time. This allows their germination processes to advance to the point just prior

to radicle emergence and then stop. If the seeds are kept in the priming solution for a long enough time, they will all reach the same stage, and when they are subsequently planted, they will germinate at roughly the same time (Durrant *et al.*, 1983). This ability to synchronize the germination of native grasses is very important. Native grasses must be able to adapt to many different conditions in order to be successful in restoration situations. Consequently, it is preferred that only a minimum amount of selection is used to develop them for commercial production, unlike cultivars of introduced species, which have been highly selected for specific traits. While this allows them to retain their genetic diversity, it also acts to increase the variation within seed lots, and it is unlikely that the seeds will germinate uniformly on their own.

2.5.3 Improved Performance Under Sub-Optimal Conditions

Seed priming has been shown to help germination in such adverse seedbed conditions as low and high temperatures, and reduced water availability. Bleak and Keller (1970) found that primed crested wheatgrass seed produced better stands as the soil moisture at planting decreased. Hanson (1973) showed that imbibing and re-drying wheat seeds increased the rate of coleoptile emergence of treated seeds that were grown under low temperature and osmotic stress. Frett and Pill (1995) conducted tests on four fescue species and found that SMP at -1.5 MPa and 20°C for four days resulted in increased germination synchrony and germination percentage at reduced water availability (-0.6 MPa) and higher temperatures (35°C). Rivas *et al.* (1984) found that priming promoted germination of jalapeno seeds at temperatures in which untreated seeds

would not germinate. Bradford (1985) found that cantaloupe varieties that were primed with 3% KNO_3 solution showed enhanced germination and emergence at less than optimal temperatures.

2.6 Some Factors That Affect Seed Priming

There are many different variables that will affect the outcome of the priming treatment, regardless of the method used. They include the following:

2.6.1 Priming Temperature

The temperature at which seeds are primed appears to influence their subsequent germination. Hardegree (1994a) found that the majority of grass seeds that he tested had a much higher germination rate when matriprimed at 25°C as opposed to 10°C. Frett and Pill (1995) found that when four fescue species were primed in PEG, $NaNO_3$, and exfoliated vermiculite No. 5 (SMP) at 20°C, the general result was a lower germination rate than at 10°C.

The temperature of the priming solution can affect the length of priming (Parera and Cantliffe, 1994a). Frett and Pill (1995) found that if the priming temperature at a given water potential was raised, there was a greater chance that germination would occur during priming. However, the probability of germination was reduced when the priming duration was shortened.

2.6.2 Priming Duration

The length of time that seeds are primed will have a profound effect on whether or not the treatment is successful. If seeds are primed in an osmotic salt solution for extended periods of time, toxicity might become a factor.

Bodsworth and Bewley (1981) tested maize, wheat, barley, and sorghum and found that in order for the priming method to achieve the maximum promoting effect, different priming durations (ranging from one to ten days) were needed, even though a -1 MPa PEG solution at 10°C was used for each crop species. Haferkamp and Jordan (1977) found that Lehmann lovegrass seeds which had been imbibed in water and in the dark at 10°C had an increased germination percentage as the priming duration increased from 24 to 72 hours. Hardegree and Emmerich (1992b) found that matric priming at high water potentials for shorter periods of time generally produced optimal germination, while seeds primed at lower water potentials and for longer periods of time had a reduced germination response. Based on the findings of these studies, it appears that the optimal length of priming can vary depending on the type of species being treated.

2.6.3 Seed Drying

Many of the studies done in the past have tested the effects of priming without any drying prior to germination or emergence testing (Hardegree and Emmerich, 1992a, 1992b; Hardegree, 1994a; Beckman *et al.*, 1993). In practical use, however, it is

essential that the primed seeds are dried. This will allow them to be transported, planted, and stored with ease.

Drying primed seeds often causes a reversal of the priming effect (Heydecker and Coolbear, 1977). Rivas *et al.* (1984) found that drying KNO_3 primed jalapeno and tabasco seeds decreased the beneficial effects gained, although germination was still much faster than that found in un-primed seeds. Bodsworth and Bewley (1981) found that air drying of several different osmotically primed crop seeds reduced the advantages of priming, and the longer the period of drying, the greater the reduction of priming advantages. Crested wheatgrass seeds that underwent superficial drying after water imbibition had a much greater reduction in seedling germination and rate of emergence than did the moist primed seeds, but still had considerable advantages over the un-primed seeds (Bleak and Keller, 1969). Hardegree (1994b) matric primed thickspike wheatgrass, bluebunch wheatgrass, canby bluegrass, sandberg bluegrass, bottlebrush squirreltail, sheep fescue, and basin wildrye, and found that the primed seeds had significantly faster germination rates than the non-primed seeds, even after drying.

Many of the previous studies have not specified all the factors used in the drying process. For example, Alvarado *et al.* (1987) dried back tomato seeds at 30°C after priming, but did not specify the relative humidity (RH) or drying duration. These omissions make it very hard to decide on the best drying regime to use. Recent studies have indicated that a higher drying temperature produces better results. Parera and Cantliffe (1994b) found that four sh2 sweet corn cultivars had better seed vigour and greater field seedling emergence when dried at 30 and 40°C than at the slower dehydration rates found at 15 and 20°C. Khan and Ptasznik (1992) found that the

advantages of priming snap bean seeds were maintained by drying in 40% RH, 34-36°C, and 0.7-1.4 m/s air flow velocity. These seeds were dried to approximately 15% seed moisture content, which is higher than the normal moisture level of stored seed.

2.7 Grass Species Descriptions

2.7.1 Little Bluestem

Little bluestem is a warm-season, perennial bunchgrass with short scaly rhizomes (Looman, 1982). It produces many pith-filled stems and can grow to a height of about 60 cm. A single, branched panicle tops each stem and is made up of hair-covered and awned seeds (Looman, 1983).

Little bluestem is a relatively drought tolerant grass of the mixed grass prairie (Haas *et al.*, 1994). At one time it was found throughout much of North America ranging from the Rockies to the Atlantic Coast and from the Yukon to the Gulf of Mexico (Looman, 1983). Some of the densest stands in the northern Great Plains are found in Manitoba's Interlake, the Manitoba-Saskatchewan border, and throughout the Missouri Coteau. Other remnants can be found throughout the prairies on sandy and gravelly soils that have adequate moisture or a high water table (Looman, 1982).

2.7.2 Blue Grama

Blue grama is a warm-season perennial bunchgrass that has fibrous roots and occasionally short-scaly rhizomes (Looman, 1982). Its roots can extend down to 50 cm

and a few secondary roots have been known to extend to a depth of 1 m (Looman, 1983). It grows to a height of about 50 cm tall and has a dense mat of short, narrow leaves. Reproductive tillers or culms usually have two dark brown, sickle-shaped spikes with all the flowers found along the upper sides (Looman, 1983). Seeds are small and hairy with numerous awns.

Blue grama is the most abundant grass found in the short grass prairie (Looman, 1983). It ranges from South America through Mexico and into North America. It becomes sparser as it moves north, but is found as far north as the tree-line of Saskatchewan and Alberta (Looman, 1983). It is one of the most water-use efficient grasses under moderate and low precipitation levels and performs best in medium to heavy textured soils in the brown and dark brown soil zones (Abouguendia, 1995).

2.7.3 Prairie Sandreed

Prairie sandreed is a warm-season, strongly rhizomatous and sod-forming grass (Haas *et al.*, 1994). Its roots can extend to a depth of 1.5 m, but the majority are found within 50 cm of the surface (Johnson, 1997). It can grow up to 2 m tall and has course, leafy stems (Johnson, 1997). The seed head is a panicle and the seeds are relatively smooth with many callus hairs (Looman and Best, 1987).

Prairie sandreed can be found throughout the prairies on sandy soils, sand dunes, along lakeshores, and in open forests. It is one of the most important species of the Great Sand Hills and is extremely drought tolerant (Johnson, 1997).

2.7.4 Green Needlegrass

Green needlegrass is a cool season bunch grass that has a dense fibrous root system that extends down to a depth of 2 to 3 m. Its abundant basal leaves are long and narrow and its stems can reach heights of 1.25 m. It has a panicle-type seed head with small, black and hair-covered seeds (Looman, 1983). A weak awn is two to three cm long (Johnson, 1997). There is a high level of dormancy associated with the seed of this species (Abouguendia, 1995).

Green needlegrass ranges throughout the central interior plains of North America (Looman, 1983). Although it can be found everywhere on the prairies, it is not typically found in dense stands. It does extremely well in loamy to clayey soils that are well drained (Abouguendia, 1995).

2.7.5 Kentucky Bluegrass

Kentucky bluegrass is a cool season sod-forming grass that has dense creeping roots. The short leaves are mostly basal, but reproductive tillers can reach heights of 1 m (Looman, 1983). It has an open panicle and has seeds with numerous cobweb-like hairs at its base (Johnson, 1997).

Kentucky bluegrass was introduced from Europe prior to 1700 and is now found throughout North America (Looman, 1983). It grows best in fertile areas that have a high level of moisture. It is slow to establish, but once it has a firm foothold in an area, it will quickly form a dense sod.

3. MATERIALS AND METHODS

Experiments were conducted using little bluestem (Minnesota point of origin), blue grama (Minnesota point of origin), green needlegrass ('Lodorm'), prairie sandreed ('Goshen'), and Kentucky bluegrass ('Welcome') grass seed. The first four species are native and are currently being used in reclamation plantings. Seed of these species is often cost prohibitive and shows more inherent seed dormancy than most introduced species. Thus, they are ideal candidates for seed priming. Kentucky bluegrass is an introduced species that was included in the experiment because it is typically slow to establish and it was felt that this species may also benefit from seed priming. A series of experiments were conducted in germination cabinets, growth rooms, and in small plot field experiments to explore the effects of several simple seed priming treatments on these five species.

3.1 Seed Priming Protocol

One of the main objectives of this study was to design a simple seed priming protocol that could be used on a practical level. Many priming studies in the past have used laboratory techniques that were not easily extrapolated to a field scale level (Hardegree, 1994a, 1994b; Frett and Pill, 1995; Pill *et al.*, 1997; Pill and Korengel, 1997). The seed priming protocol was developed with this in mind. Different priming treatments will produce varying results when conducted on different grass species (Frett and Pill, 1995; Beckman *et al.*, 1993; Yamamoto *et al.*, 1997a; Pill *et al.*, 1997),

cultivars of the same species (Yamamoto *et al.*, 1997b), and even seed lots of the same cultivar (Brocklehurst and Dearman, 1983a, 1983b). Therefore, this study incorporated various factors that have been shown to have an effect on the priming procedure. Specifically, the use of an osmotic priming solution and chilling duration were thought to warrant further investigation.

Stock Seed Farms in Nebraska have successfully used a modified stratification technique for seed priming of buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.) burs. Their procedure involved placing the burs in burlap sacks and immersing them in a 0.5% KNO_3 solution for 24 hours. No aeration was provided during this time. The burlap sacks were then removed, drained, and stored on racks in a cooler for 3 to 4 weeks at approximately 1°C. Following this, the burs were emptied into drying bins equipped with drying floors. They were stirred periodically and allowed to air dry at approximately 10°C. Drying at this temperature prevented germination because buffalograss seed requires about 15°C to germinate.

The seed priming protocol developed in this study was based on the method used by Stock Seed Farms. Seed from each of the five species was prepared for the *Field Experiment* using the following procedure. Five - 250 gram samples were selected from the original seed lots of each species and placed in 20 x 30 cm cloth bags. One sample was stored at 20°C as a control, while the other samples were subjected to different seed treatments (Table 2). Two samples were prepared for a 3 week chilling period. One of these was placed in 4 L of distilled water, while the other was placed in 4 L of a 0.5% KNO_3 solution. A 0.5% KNO_3 solution was used because it is the standard for commercial priming of buffalograss (Stock Seed Farms, pers. comm.). The distilled water and KNO_3

solutions were maintained at 4°C in a commercial refrigerator and the seeds were allowed to soak for 24 hours.

It was important to incorporate the use of the low temperature and KNO_3 into the seed priming protocol because of the high degree of dormancy associated with some native grasses. Priming at low temperatures would also allow the seeds to imbibe, essentially starting the germination process, but would prevent them from completing the germination process. This allowed us to avoid the use of higher temperature osmotic or matric priming methods that work well in a laboratory setting, but are impractical for larger scale applications.

Table 2. Seed treatment regimes used for five grass species for Field and Indoor Seed Priming Experiments at the University of Manitoba.

Treatment	Abbreviation[z]	Solution	Temp. (°C)	Soaking Duration	Chilling Duration	Thiram 75WP	Drying
1	C	n/a	n/a	n/a	n/a	no	no
2	CT[y]	distilled H_2O	20	1 hour	n/a	yes	yes
3	H1	distilled H_2O	4	24 hours	1 week	yes	yes
4	H3	distilled H_2O	4	24 hours	3 weeks	yes	yes
5	K1	0.5 % KNO_3	4	24 hours	1 week	yes	yes
6	K3	0.5 % KNO_3	4	24 hours	3 weeks	yes	yes

[z]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.
[y]Not used in the Field Experiment.

Following the 24 hour imbibition period, each cloth bag was removed, squeezed dry, and the seed emptied into a plastic pan. Thiram 75WP was sprinkled onto the seed with a metal strainer at the recommended rate (0.09 kg Thiram 75WP per 25 kg grass seed) to prevent fungal growth and seed decay during the chilling period. The container was then covered and the seed shaken to evenly distribute the fungicide. The seed was then removed

from the container and placed back into the cloth bag. The cloth bags were then placed into plastic bags and sealed to prevent any moisture loss. The bags were placed back into a commercial refrigerator and allowed to chill for 3 weeks at 4°C. The plastic bags were opened and the seed mixed every day. The entire procedure was repeated two weeks later on the two remaining untreated samples. These samples were allowed to chill for 1 week only.

Upon completion of the 3 and 1 week chilling periods, the seed from each of the priming treatments was removed from the bags and placed on 75 x 75 cm drying screens. All the screens were then placed in a controlled environment chamber (growth room) on an elevated metal screen bench that allowed unobstructed air movement. The growth room was maintained at 25°C with maximum wind and in complete darkness. The seed was allowed to air dry for 48 hours and then placed in new cloth bags. Seed priming for the *Field Experiment* was completed on April 26, 1997, twenty-four days after it had started. The seed was stored at 20°C in a laboratory until it was planted at the Carman and Winnipeg field sites.

The protocol described for the field experiment was also used to prime seeds in the *Indoor Experiments* (Table 2) with the following exceptions. First, although seed from the same seed lots was used for these experiments, only 75 g of seed of each species was placed in a 13 x 17 cm cloth bag. Second, each sample was pre-soaked in 2 L of 0.5% KNO_3 solution or distilled water. Third, the seeds were dried on 30 x 30 cm screens.

An additional treatment was also added to the *Indoor Experiments* to test the effects of drying the seed. This treatment was added since any practical priming method

would have to include drying the seed after priming to facilitate planting in the field. It allowed the seed to imbibe water without exposing the seed to the effects of the 1 or 3 week chilling period. The seed of this treatment was then dried under the same conditions as the primed and chilled seeds, and therefore, should provide an indication on whether or not the drying process was detrimental. Thus, after the other samples had completed their 1 and 3 week chilling periods, a sixth sample from each species was placed in a cloth bag and soaked in 2 L of distilled water at 20°C for one hour. This seed was then removed from the cloth bag and Thiram 75 WP was added in the same manner as in the previous treatments. This seed was then spread-out on the screens along with the primed treatments and allowed to dry according to the aforementioned drying scheme. The seed priming protocol for the *Indoor Experiments* began on September 30, 1997 and ended on October 24, 1997.

Two important components included in our seed priming protocol were the use of a fungicide and drying the seed after priming. Several experiments were conducted to further explore the effects of these components.

3.1.1 Fungicide Experiment

In preliminary priming experiments it was discovered that fungus had developed on the seed during the chilling period. This experiment was included to determine if the use of Thiram 75WP in the seed priming protocol would have a detrimental effect on the germination of the 5 grass species.

One hundred seeds were placed on three 90 mm Whatman No. 1 filter papers which were placed inside a 100 x 15 mm plastic petri dish. Two petri dishes were prepared for each species. The first petri dish was used as a control and did not have any fungicide applied to it. Thiram 75WP was sprinkled onto the seeds of the second petri dish at the recommended rate of 0.09 kg Thiram 75WP per 25 kg of grass seed. This procedure was repeated for each of the 5 species. The seeds were maintained in a 0.2% KNO_3 solution for 14 days at 5°C. The petri dishes of blue grama, prairie sandreed, and little bluestem were then placed in an Econaire germination cabinet (Model #SG-30) and allowed to germinate for 14 days at a 20 - 30°C night/day cycle. Eight hours of light were provided during the day. Green needlegrass and Kentucky bluegrass were germinated in an Econaire Ecological chamber (Model #TC-19) at the same time. In this case a 15 - 25°C night/day cycle was used, and the green needlegrass petri dishes were covered to prevent any light from penetrating. These chilling and germination regimes were based on the 1995 AOSA guidelines shown in Table 3. The petri dishes were kept moistened throughout the experiment with distilled water. The number of germinated seeds were counted on the 7th and 14th days of the experiment. A seed was considered germinated if the radicle had protruded 2 mm out of the seed coat (Hardegree, 1994b).

Table 3. AOSA laboratory germination recommended methods of testing for 5 different grass species (Maxon, 1995).

Species	Temp. (°C)[z]	Specific Requirements[y]	Fresh and Dormant Seed
Blue grama	20 - 30	light	KNO_3
Little bluestem	20 - 30	KNO_3;light	Prechill at 5 °C for 2 weeks
Prairie sandreed[x]	-	-	-
Green needlegrass	15 - 30	KNO_3; dark	-
Kentucky bluegrass	15 - 25	KNO_3;light	Prechill at 10 °C for 5 days

[z]Test to be held at 1st temperature for 16 hours and at 2nd temperature for 8 hours per day.
[y]Light to be provided for 8 out of 24 hours if called for. KNO_3 to be added as a 0.2% solution.
[x]There are no known guidelines for this species.

The experiment was analyzed as a Randomized Complete Block Design (RCBD) with 4 replications in a split plot arrangement. Main plot was species and subplot was seed treatment. The 7 and 14 day data were analyzed separately using analysis of variance (ANOVA). At 7 days, the germination percentage data had heterogeneous variances for species, but homogeneous variances for seed treatment. The data were square transformed to correct this problem. The 14 day data had homogeneous variances for both species and seed treatment, so the data were not transformed. F-protected least significant difference ($LSD_{0.05}$) tests were calculated for species within a seed treatment for both Day 7 and Day 14 data. T-tests were conducted on Day 7 seed treatments within species. No t-tests were conducted on the Day 14 data because the F-test was not significant for seed treatment or the species x seed treatment interaction.

3.1.2 Seed Moisture Experiment

Since the seed priming protocol involved seed imbibition and drying, it was important to assess the impact that these processes had on the seed moisture content of the species being investigated. With this in mind, seed that had been primed for the *Indoor Experiments* was used to conduct another experiment designed to explore the effects of imbibing and drying the seeds during the priming process.

Two samples were taken from each of the six treatments of each species immediately after the chilling period (Pre-Dry), and immediately after drying (Post-Dry).

The samples were placed into #2 Coin paper envelopes and dried in a Fisher Isotemp Oven (200 Series Model 255G) at 70°C for 24 hours. A 5-decimal (± 0.01 mg) Sartorius Research R200D Electronic Semi-Microbalance was used to weigh the samples before and after drying. Seed moisture content was determined on a wet weight basis using the following formula: (mass before drying - mass after drying)/ mass before drying x 100 = % moisture content.

These data were analyzed as a RCBD with 2 replications in a split plot arrangement. Main plot was species and subplot was seed treatment. The Pre-Dry and Post-Dry seed moisture data were analyzed separately using ANOVA. Bartlett's tests indicated that each data set had homogeneous variances, so the data were not transformed. F-protected least significant difference ($LSD_{0.05}$) tests were calculated for seed treatments within a species for Pre-Dry and Post-Dry data.

3.2 Field Experiment

The purpose of this experiment was to test the effectiveness of the priming protocol under field conditions. The experiment was planted in two locations. The first was on a Hochfeld series loamy sand soil at the University of Manitoba Carman Field Station on May 20, 1997. This area had flax (*Linum usitatissimum* L.) grown on it the previous year. The second location was planted at the University of Manitoba Winnipeg Field Station on May 29, 1997. This was on a Riverdale series cumulic regosol clay-loam soil that had been summer-fallowed the previous year. Soil analyses were taken of each location and are presented in Table 4.

Table 4. Characteristics of Winnipeg and Carman soils used in Field and Growth Room Experiments involving little bluestem, blue grama, green needlegrass, prairie sandreed, and Kentucky bluegrass seed.

	Field Experiment[z]		Growth Room Experiment[y]	
Characteristics	Winnipeg	Carman	Winnipeg	Carman
Soil Quality				
pH	7.1	6.7	7.6	5.8
E.C. (Salinity)	0.6	0.6	0.8	0.2
Available Nutrients (kg ha^{-1})				
Nitrate	45	57	22	76
Phosphate	129	74	76	76
Potassium	1207	533	1108	694
Sulphate	64	51	40	45

[z]Based on 15 soil samples taken to a depth of 30 cm in May 1997.
[y]Based on 15 soil samples taken to a depth of 30 cm in September 1997.

The seed was planted using a cone seeder at a rate of 430 pure live seed (PLS) per m^2 (40 PLS per ft^2) in 6 x 1 m plots. Each plot consisted of 6 seed rows with 15 cm row spacing. The plots were arranged in a split plot arrangement within a RCBD. The main plots were species and the subplots were seed treatments. Six replications were used. The germination percentage of the fungicide-treated seeds of the *Fungicide Experiment* was used to calculate the PLS of each seed lot (Table 5). One hundred grams of cracked and autoclaved wheat was added to the grass seed in order to maintain even seed distribution during planting. The seeds were planted at a depth of 1 cm.

Table 5. Pure seed, germination percentage, and pure live seed (PLS) of 5 grass species used to determine the weight of seed needed for planting in both the Field and Growth Room Experiments.

Species	Pure Seed	Germination[z]	PLS of Seed Lot[y]	Number of Seeds per Gram	Weight of Seeds planted in a 6 x 1 m field plot[x]	Weight of Seeds planted in a 30 cm growth room row[w]
	----------%----------				----------g----------	
Little bluestem	90.4	54.0	44.8	573	8.58	0.1431
Blue grama	67.3	49.5	33.3	1597	4.51	0.0752
Green needlegrass	98.9	47.8	47.3	399	12.73	0.2123
Prairie sandreed	98.9	78.5	77.6	604	5.12	0.0854
Kentucky bluegrass	98.0	87.5	85.8	4796	0.58	0.0097

[z]Taken from the Fungicide Experiment
[y]PLS = Pure Seed x Germination / 100
[x]Field Expt. Based on a planting rate of 430 PLS per m^2 (40 PLS per ft^2)
[w]Growth Room Expt. Based on a planting rate of 40 PLS per 30 cm row

Measurements of seedling number and number of tillers per plant were made 3 times throughout the growing season (Table 6). Sampling began at the first sign of seedling emergence. Subsequent sampling was conducted 2 and 4 weeks after this initial date. Four 1-m lengths of row were randomly chosen in each plot and marked with flags. The number of seedlings found in each 1-m sample length were counted to get a measure of seedling emergence. In addition, on the 1st sampling date, a seedling in each 1-m sample row was randomly chosen and a coloured plastic ring placed around the plant to mark it. The development of these seedlings was recorded by counting the number of tillers found on each marked plant. Twenty-four soil samples were randomly taken from each site during seeding and sampling and used to determine the soil moisture content of the top 15 cm. These samples were weighed before and after drying for 7 days at 70°C.

The data were subjected to ANOVA tests for a RCBD using a split plot arrangement with data from 4 samples per plot and 6 replications. Main plots were species and subplots were seed treatments. Each location and sampling date was analyzed separately. In all cases, the data had to be square root transformed because the

data was not normally distributed. The two locations were not combined in this analysis because a Bartlett's test on the data showed that the error variances were not homogeneous and because the C.V.'s were still greater than 20. The R^2 values obtained were all quite low, indicating that much of the variability in the data was not accounted for by the experimental design. F-protected least significant difference ($LSD_{0.05}$) tests were calculated on the transformed data.

Table 6. Important dates in which little bluestem, blue grama, green needlegrass, prairie sandreed, and Kentucky bluegrass were seeded and sampled for the Field Seed Priming Experiment conducted in 1997 at the Winnipeg and Carman field stations.

Description	Winnipeg	Carman
Seeding Date	May 29	May 20
1st Sampling Date	June 26	June 10
2nd Sampling Date	July 10	June 24
3rd Sampling Date	July 24	July 8

3.3 Indoor Experiments

The *Indoor Experiments* were initiated because of the huge variability involved in the field experiments. It was thought that the variability associated with harsh environmental conditions experienced in the field experiment could be reduced and the differences between treatments could be examined in much greater detail.

3.3.1 Petri Dish Experiment

The purpose of this experiment was to test the effectiveness of the seed priming protocol within the controlled environment of a germination cabinet. Specifically, the effect of the final germination percentage and rate were investigated.

Three 90 mm Whatman No. 1 filter papers were placed inside a 100 x 15 mm plastic petri dish and soaked with distilled water. One hundred seeds were counted out from each species - treatment combination and placed on the filter papers in the petri dish. All of the treatments were replicated four times.

Germination was conducted in an Econaire germination chamber that was set at a 20 - 30°C night - day cycle. White florescent lights were activated for the day period of 8 hours. Counts were made of germinated seeds every day for 2 weeks beginning on November 7, 1997. A seed was considered germinated when the radicle had protruded 2 mm from the seed coat (Hardegree, 1994b). Distilled water was added to the petri dish each day to ensure the filter papers did not dry out. The final germination percentage was determined by the number of germinated seeds on the 14^{th} day of the experiment. Germination rate was calculated by taking the number of days it took to reach 50% of the final germination percentage.

Both variables were subjected to ANOVA tests for a RCBD using a split plot arrangement. Main plots were species and subplots were seed treatments. A Bartlett's test on the petri dish data showed that in all cases the variances were not homogeneous for species, but were homogeneous for seed treatments. Therefore, arc sine transformed data were used for the final germination percentage and inverse log transformed data

were used for the germination rate. In all cases, the C.V. was decreased and the N-probability plot showed more normality when the data were transformed, although the species variances remained heterogeneous. F-protected $LSD_{0.05}$ tests were calculated on the transformed data. In the case of the germination rate, there were missing data because some of the petri dishes had no germination at all, making it impossible to know when 50% of the final germination had taken place. Thus, the $LSD_{0.05}$ values were calculated using the missing data formula technique as outlined in Gomez and Gomez (1984).

3.3.2 Growth Room Experiment

The purpose of this experiment was to test the effectiveness of the seed priming protocol in two different soil types within the controlled environment of a growth room. Specifically, the effect of the final emergence count, emergence rate, and growth stage were investigated.

Wooden boxes (30 x 122 x 10 cm) were filled with soil and partitioned into two halves. For each half box, all 6 seed treatments of a species were planted at a depth of 1 cm. The treatments were planted side-by-side in two 30-cm long rows. The spacing between the parallel rows was 3.8 cm. A 30 cm border row of green needlegrass was planted on either side of the first and last rows in each half box. The seeds were planted at a rate of 40 PLS per 30 cm row based on the purity and germination of each seed lot (Table 5). Two types of soil were used in this experiment. The first type was a clay loam soil collected from the University of Manitoba Winnipeg Field Station, while the other

type was a sandy loam soil taken from the University of Manitoba Carman Field Station (Table 4).

The wooden boxes were placed onto elevated metal screen benches in a growth room. All the boxes were watered until the soil was saturated. The bottom of each wooden box was made out of metal screening, so the water was able to drain freely. The temperature within the growth room was maintained at 25°C for 16 h and at 15°C for 8 h. Light was provided to simulate daytime conditions and fans were activated to maintain air circulation. The soil was saturated every 2 days and allowed to dry in between, thus allowing the unique characteristics of each soil to become fully exhibited (e.g. crusting in the Winnipeg soil). Emergence counts were made every 2 days and continued for 20 days after planting (DAP). The data collected from the counts were used to calculate the emergence at 10 DAP, the emergence at 20 DAP, and the time it took to reach 50% of the highest emergence number reached. Six seedlings from each treatment were randomly selected at 28 DAP and the Haun Scale was used to determine their growth stage (Haun, 1973).

All 4 variables were subjected to ANOVA tests for a RCBD arranged as a split split plot with soil type as the main plot, species as the sub-plot, and seed treatment as the subsubplot. Four replicates were used. Bartlett's tests were conducted on all four variables in the growth room experiment. Seedling emergence 10 days after planting (DAP), seedling emergence 20 DAP, and seedling growth stage at 28 DAP had heterogeneous species variances, but homogeneous soil and seed treatment variances. Arc sine, square root, square, and log transformations were conducted on these data, but they were unsuccessful in making the species variances homogeneous, so the original

data were used in the ANOVA tests. The emergence rate had heterogeneous soil variances in addition to species variances. The arc sine transformation was used to make the data more normal and soil variances homogeneous. Species variances remained heterogeneous after the transformation. F-protected $LSD_{0.05}$ tests were calculated on all the data.

4. RESULTS AND DISCUSSION

4.1 Seed Priming Protocol

One of the main objectives of this study was to develop a simple seed priming protocol that could be used in field scale applications. However, in developing this protocol, several questions came to light concerning the use of fungicide and the effect that drying would have on the primed seed. The following experiments were conducted to address those concerns.

4.1.1 Fungicide Experiment

The *Fungicide Experiment* was undertaken to determine if the addition of Thiram 75 WP would have detrimental effects on seed germination. Beckman *et al.* (1993) observed that a decrease in final germination percentage of big bluestem seeds may have been caused by a fungal growth that developed when the seeds were being solid matrix primed at 4°C over a 14 day duration. The incorporation of Thiram 75 WP into the seed priming protocol was important since it is recommended for the control of seed decay, seedling blight, and damping off (Manitoba Agriculture and Food, 2000). However, it would be unwise to use Thiram 75 WP if it had an inhibitory effect on the seed germination and seedling growth.

At both 7 and 14 days after starting (DAS) the experiment there were significant differences between species (Table 7). The majority of the germination potential for

prairie sandreed, little bluestem, and blue grama was reached at 7 days, while it took the full 14 days for Kentucky bluegrass and green needlegrass. At 14 DAS, Kentucky bluegrass had the highest germination percentage (84.8%) followed by prairie sandreed (76.4%). Little bluestem, blue grama, and green needlegrass germination percentages were statistically the same (52.3, 49.6, and 51.5% respectively). The ANOVA test did not show any significant difference between seed treatments for either 7 or 14 DAS.

Table 7. Fungicide Experiment: effects of using Thiram 75WP during seed priming and germination of 5 grass species counted on the 7th and 14th day after starting (DAS).

	% Germination[z]					
	7 DAS[y]			14 DAS		
Species	Control	Fungicide[x]	t-test[w]	Control	Fungicide	t-test
	%			%		
Kentucky bluegrass	71.8 *a*	67.0 *b*	NS	82.0 *a*	87.5 *a*	NS
Prairie sandreed	72.3 *a*	76.5 *a*	NS	74.3 *a*	78.5 *b*	NS
Little bluestem	49.8 *b*	52.8 *c*	NS	50.5 *b*	54.0 *c*	NS
Blue grama	49.3 *b*	49.3 *c*	NS	49.8 *b*	49.5 *c*	NS
Green needlegrass	44.0 *b*	9.0 *d*	**	55.3 *b*	47.8 *c*	NS
mean	57.4	50.9		62.4	63.5	

ANOVA	df	Significance	Significance
Species (SP)	4	**	**
Seed Treatment (ST)	1	NS	NS
SP*ST	4	**	NS
CV (%)		15.12	10.30
R^2		0.970	0.935

** = significant at 0.01 probability level; NS = not significant

[z]Average of 4 replications, each containing 100 seeds. In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

[y]All tests were based on square transformed means, but original means are presented. $LSD_{0.05}$ values are based on square transformed means.

[x]Fungicide was Thiram 75WP applied at a rate of 0.02 g per 5 g of seed.

[w]t-tests made between seed treatment within each species.

There were significant species x seed treatment interactions at 7 DAS. T-tests showed that only green needlegrass had a significant difference between treated and non-treated seed. This difference was quite pronounced with the untreated seeds having five times the germination percentage of the treated seed.

At 14 DAS the ANOVA test showed that the species x treatment interaction was no longer significant. This is likely because the fungicide-treated green needlegrass seed had caught up to the germination of the non-treated green needlegrass seed.

The results for green needlegrass were interesting because it appeared that Thiram 75 WP actually inhibited the germination in the early stages of the germination process. This was an unexpected result since Thiram 75 WP is recommended for use in green needlegrass germination tests by AOSA (Maxon, 1995). One possible explanation is that the green needlegrass seed, which has quite a hard seed coat (Frank and Larson, 1970), benefits from the fungal invasion. The seed coat of green needlegrass is such a problem for germination that one of the AOSA recommended germination methods involves acid scarification of the seed using 98% H_2SO_4 for 10 minutes prior to beginning the germination test (Maxon, 1995). Thus, the fungal growth may actually act to deteriorate the seed coat and aid in the penetration of water and oxygen into the seed, thereby increasing the germination percentage. Given enough time, the fungicide treated seed will imbibe enough water to germinate, causing this seed to achieve the same germination percentage as the untreated seed by day 14. This argument is strengthened because at 7 DAS, 100% of the untreated seeds were observed to be infected with fungus while none of the fungicide-treated seeds were contaminated. This data was not quantified in this study.

The results of this experiment indicated that the addition of Thiram 75WP did not affect the final germination percentage of any of these species. However, because some of the seed would be subjected to a moist-chill period of up to 3 weeks, it was thought that the Thiram 75 WP should be used as a precautionary measure. Several other seed priming experiments have incorporated the use of fungicides into their priming and testing procedures (Hardegree, 1994a, 1994b; Beckman *et al.*, 1993; Hsu *et al.*, 1985).

4.1.2 Seed Moisture Experiment

The *Seed Moisture Experiment* was undertaken to investigate the effects of imbibition and drying on the various seed priming treatments. For both the Pre-Dry and the Post-Dry data, the species and seed treatment main effects were significant, as was the interaction between them (Table 8).

Pre-Dry Data

The Pre-Dry data showed that all treatments absorbed water, regardless of the soaking or chilling durations (Table 8). One of the control treatments included in the seed priming protocol involved soaking the seed for only 1 hour before it was dried. This treatment was included in the experiment so that the effects of the drying procedure could be investigated. To do this properly, the seed would have to be imbibed to the same level as that of the seeds being primed before they were dried down. These seeds could not be exposed to any low temperatures because if they did, any differences seen in germination

or emergence could have been a result of the cold treatment, not the drying process. Therefore, it was decided that the seeds would be only soaked for 1 hour at 20°C. One hour was chosen instead of 24 hours because the soaking would be done at 20°C instead of 4°C. Since germination would not be suppressed by a low temperature, it would be important to allow water imbibition to occur without triggering Phase III (radicle emergence) (Bewley and Black, 1994). This would prevent damage to the seed when it was subsequently dried back down (Berrie and Drennan, 1971). Thus, one of the objectives of this experiment was to determine if 1 hour was long enough for the seeds to imbibe the same amount of water as the seeds that had been soaked for 24 hours and chilled for 1 or 3 weeks.

Table 8. Seed Moisture Experiment: seed moisture percentage of 5 grass species taken after the soaking and/or chilling period, and after drying.

	Seed Moisture									
Seed	Pre-Dry					Post-Dry				
Treatment	LB[z]	BG	GN	PS	KB	LB	BG	GN	PS	KB
C[y]	8.79 *a*[x]	9.94 *a*	9.85 *a*	9.26 *a*	6.78 *a*	8.18 *a*	8.76 *a*	8.78 *a*	8.68 *a*	9.50 *a*
CT	65.47 *b*	72.26 *bc*	34.33 *b*	48.20 *b*	53.07 *b*	5.74 *bc*	5.46 *b*	5.72 *bc*	6.34 *b*	5.83 *b*
H1	66.02 *b*	72.39 *bc*	41.16 *d*	55.93 *d*	59.79 *c*	5.91 *b*	5.52 *b*	5.64 *bc*	6.21 *bc*	5.91 *b*
H3	63.96 *b*	72.13 *bc*	39.48 *cd*	54.47 *cd*	57.51 *c*	5.40 *c*	5.30 *b*	5.27 *c*	5.62 *d*	5.95 *b*
K1	66.07 *b*	70.02 *b*	39.71 *cd*	53.66 *cd*	58.45 *c*	5.42 *c*	5.34 *b*	5.77 *b*	5.94 *bcd*	5.49 *b*
K3	65.79 *b*	73.31 *c*	38.05 *c*	52.65 *c*	59.36 *c*	5.53 *bc*	5.25 *b*	5.64 *c*	5.85 *cd*	5.65 *b*

ANOVA	df	Pre-Dry Significance	Post-Dry Significance
Species (SP)	4	**	**
Seed Treatment (ST)	5	**	**
SP*ST	20	**	*
CV (%)		2.78	3.62
R^2		0.998	0.986

* = significant at 0.05 probability level; ** = significant at 0.01 probability level

[z]LB = little bluestem; BG = blue grama; GN = green needlegrass; PS = prairie sandreed; KB = Kentucky bluegrass

[y]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[x]Average of 2 replications. In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

Soaking the seeds for 1 hour prior to drying allowed sufficient time for both little bluestem and blue grama seeds to attain the same moisture content as the seeds that were soaked for 24 hours and then chilled for 1 and 3 weeks (Table 8). However, soaking for 1 hour did not allow Kentucky bluegrass, prairie sandreed, and green needlegrass enough time to gain the same seed moisture level as any of the seed priming treatments. One possible explanation for this involved the amount of hairs and awns associated with the seeds of the different species. Both blue grama and little bluestem are commonly referred to as having seed dispersal units, rather than actual seeds. This is because blue grama "seeds" consist of florets and spikelets and little bluestem "seeds" consist of the fertile floret, the joint of the raceme, and the stalk of the sterile spikelet (McDonald and Copeland, 1997). This effectively gives them a much greater surface area than other species such as Kentucky bluegrass, prairie sandreed, and green needlegrass (McDonald and Copeland, 1997). The increased surface area would permit these seeds to have access to a greater volume of water and enable them to absorb it more rapidly (Copeland and McDonald, 1995). Thus, it appears that the 1 hour soaking period was not enough time to bring these seeds up to the correct moisture content. In the future, it may be necessary to conduct a preliminary experiment to more closely pinpoint the time needed for the seeds to complete imbibition.

Another objective of this experiment was to determine if the seeds of the priming treatments had maintained the same moisture content regardless of the chilling duration. This should have been the case since all these treatments were soaked for 24 hours and then drained of excess moisture prior to beginning the chilling period. The seeds were

put into plastic bags, so there should have been no moisture loss. If the seeds had lost moisture during the chilling period any resulting germination and emergence data might be attributed to this factor, rather than the actual length of chilling. In all species, with the exception of blue grama, there was no difference in the moisture content of seed chilled for 1 week versus 3 weeks (Table 8). In blue grama, the 1 week KNO_3 treatment did not achieve as high a seed moisture percentage as the 3 week KNO_3 treatment. It is possible that this treatment did not absorb as much water during the 24 hour soaking treatment. This could have an impact on the final results of the germination and emergence tests. However, the 1 week KNO_3 treatment did absorb the same amount of moisture as both water based priming treatments.

The final objective of the seed moisture experiment was to determine if the seeds chilled in distilled water would absorb more water than those in the 0.5% KNO_3 solution. This would occur if the 0.5% KNO_3 solution was strong enough to affect the osmotic balance between the seed and the external solution. This would be important because the KNO_3 was being used for its dormancy breaking properties (Hargurdeep *et al.*, 1987; Hilton, 1984; Maxon, 1995), not its osmotic ones. Bradford (1985) used a 3% KNO_3 priming solution to improve the germination rate of cantaloupe seed. A 0.2% KNO_3 solution is often used in germination testing and is recommended for a variety of species by AOSA (Maxon, 1995). However, it was not clear what effect a slightly higher concentration of 0.5% KNO_3 would have on the osmotic balance between the priming solution and the seed. The results of this analysis showed that there was no difference between the water and KNO_3 primed seeds at either 1 or 3 weeks of chilling (Table 8).

This is an indication that the concentration of KNO_3 was not high enough to alter the amount of water that was imbibed by the seed.

Post-Dry Data

Drying the seed after priming is essential to facilitate mechanical planting on a field scale level. However, there is the possibility that the drying process would be counteractive to the benefits achieved by the rest of the priming process. Evans and Pill (1989) associated the slower germination of primed and dried seeds compared to those that had not been dried with the reduced seed moisture found in the dried seed. This reduction in germination rate could be a factor of the increased time needed for the primed and dried seeds to re-imbibe (Brocklehurst *et al.*, 1984). The Post Drying data of this experiment indicated that the drying process caused all the primed seed to be dried to a lower moisture content than the control seed which was not put into the drying room (Table 8). Thus, even though the drying process in these experiments occurred at 25°C with air movement and no additional heat, it is possible that it could still have affected the final outcome of the experiments.

Since all the treatments were dried to a seed moisture percentage below that of the undried seed, it is important to investigate the differences between the primed seed treatments. Only blue grama and Kentucky bluegrass exhibited no difference between the seed moisture percentage of any of the soaked and dried treatments. However, differences were observed in the other three species.

Little bluestem seed that had been chilled for 3 weeks in water was dried to a lower seed moisture percentage than seed that had only been chilled for 1 week in water

(Table 8). Bodsworth and Bewley (1981) found that osmopriming for 8 days at 10°C caused disintegration of the testa in soybean (*Glycine max* L.) seeds, whereas the same treatment for only 6 days did not. Deterioration of the little bluestem seed coat would have allowed more moisture to be removed during the drying process and may have been the reason that the seeds chilled for a longer duration had a lower moisture percentage.

Little bluestem seeds chilled for 1 week in KNO_3 were dried to a lower seed moisture content than little bluestem seeds that had been chilled in distilled water for the same length of time (Table 8). This suggests that the KNO_3 may have had a deteriorating effect on the seed coat.

The only difference found in the green needlegrass treatments after drying was between the 3 week KNO_3 chilled seed and the 1 week KNO_3 chilled seed (Table 8). The 3 week treatment produced a lower seed moisture content than the 1 week one, similar to the little bluestem. There was no similar difference shown between the water-based treatments. This indicated that the longer duration of chilling was not enough by itself to cause deterioration of the seed coat which would result in greater seed moisture loss. Rather, it was both the presence of the KNO_3 combined with the longer chilling duration that produced this effect. This corresponds to the AOSA testing requirements for green needlegrass which recommend the use of acid scarification to promote germination because of its hard seed coat (Maxon, 1995).

Prairie sandreed was the only species where the soaked and dried control seed did not have the same seed moisture content as that of all the other priming treatments after drying (Table 8). In this case, it was the 3 week chilling treatments that produced seed with a lower seed moisture content after drying. This was an interesting finding since

this control treatment had not originally imbibed the same amount of water as the other 3 week priming treatments. This indicated that even though a lesser amount of water was imbibed, it would not always result in a lower seed moisture content after drying. This was not the case for green needlegrass and prairie sandreed. Seed from these species also did not imbibe as much water during the 1 hour soaking treatment as was imbibed by seed from the priming treatments. However, the seed of these species was dried down to the same level as the other treatments.

The 3 week water chill treatment of prairie sandreed seeds also produced a lower seed moisture percentage after drying than the 1 week water chill treatment. Although a similar difference was not found in the KNO_3 based treatments, these results do suggest that, similar to little bluestem, the longer chilling period somehow caused the seed to lose more moisture during drying.

4.2 Field Experiment

The field experiment was designed to test whether or not the seed priming treatments applied would improve the emergence and growth of the seed under field conditions. The split plot arrangement with species as the main plot factor and seed treatment as the subplot factor was used in this experiment. The split plot arrangement allowed the precision of the species measurements to be sacrificed so that the precision of the seed treatment measurements was increased (Gomez and Gomez, 1984). It was assumed that there would already be a strong difference between the species, so a high level of precision would not be needed to detect it. Also, it was not the intent of this

study to compare species, but rather, to assess the priming protocol on a number of different species.

Growing conditions in the field experiments were not ideal. The Winnipeg site had serious problems with surface crusting because of the clay based soil and the lack of soil structure caused by the previous year's summerfallow. The plots were planted on May 29th and at the time the soil moisture was quite good at 24.4% (Figure 1). However, 3 days later there was a substantial rainfall event (21.8 mm) which was followed by about 2 weeks of warm, dry weather. The amount and intensity of the rainfall caused the top 2 cm to become heavily crusted. Since the seeds were planted shallowly, they were trapped in the soil crust and unable to access the soil moisture present at greater soil depths. Any seedlings not found in the crust were unable to break through it and could only emerge between the cracks. The soil was quite black and the seedlings that did emerge were subjected to extreme heat that caused many of them to wither and die. Emergence did not occur until about June 26 which was preceded by a period in which it rained 8 days out of 13. This kept the soil surface relatively moist and allowed some of the remaining seed to germinate and emerge.

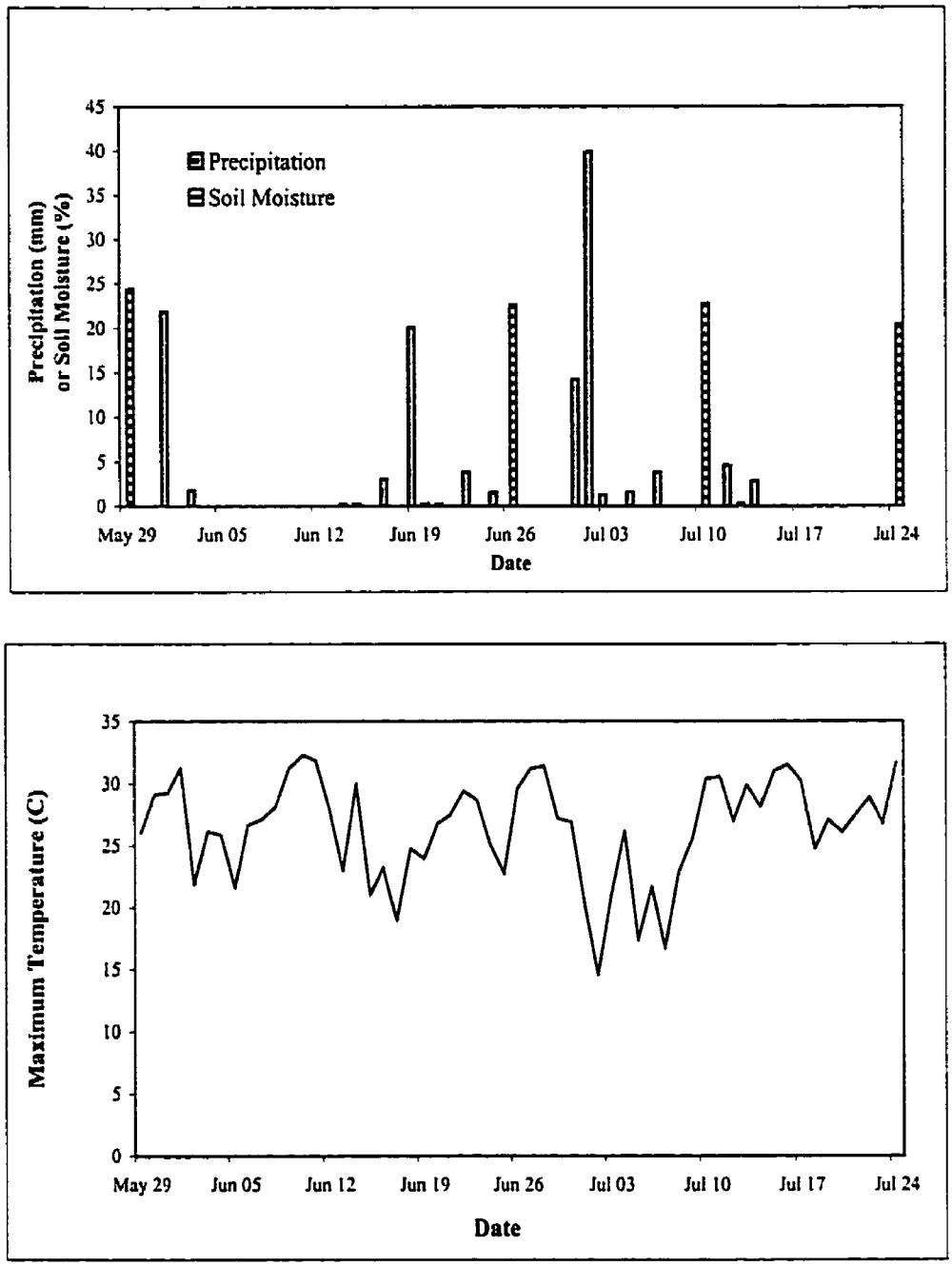


Figure 1. Rainfall events (mm), soil moisture (%), and maximum ambient temperature (°C) taken at the Winnipeg Field Station during the summer months of 1997.

The grasses were planted in Carman on May 20th, 9 days earlier than at Winnipeg. The first emergence occurred approximately 16 days earlier than Winnipeg. When this site was planted, the soil moisture was 14.5% (Figure 2). There was no rainfall after planting until May 31st and June 1st. However, the sandy loam soil found here was not prone to crusting and about a week after the June 1st rainfall the first emergence was noted. By this date soil moisture had decreased to 9.6% (Figure 2), caused by lack of precipitation and high daytime temperatures. A serious problem was encountered at this site because of a heavy infestation of weeds. The sample areas within each plot were hand weeded throughout the experiment to expose 15 cm of bare soil on either side of each sample area, but it is still possible that competition for moisture and light was detrimental to seedling development. Weeds will compete for space, nutrients, moisture, and sunlight (McDonald and Copeland, 1997).

The major result of these harsh environmental conditions was a data set that had much uncontrolled variability. In many cases, the overall emergence was very low, and no Kentucky bluegrass seedlings emerged in either location. Kentucky bluegrass is well known to be extremely hard to establish and several priming studies in the past had shown that priming increased the rate of germination and seedling emergence (Pill *et al.*, 1997; Yamamoto *et al.*, 1997b). It is probable that the benefits of the priming treatments were not enough to overcome the severe crusting and weed infestation problems associated with this experiment. The previous two experiments cited were conducted in the controlled confines of a greenhouse or growth chamber. Unfortunately, because of the lack of emergence, Kentucky bluegrass was excluded from the analysis of the field experiment data.

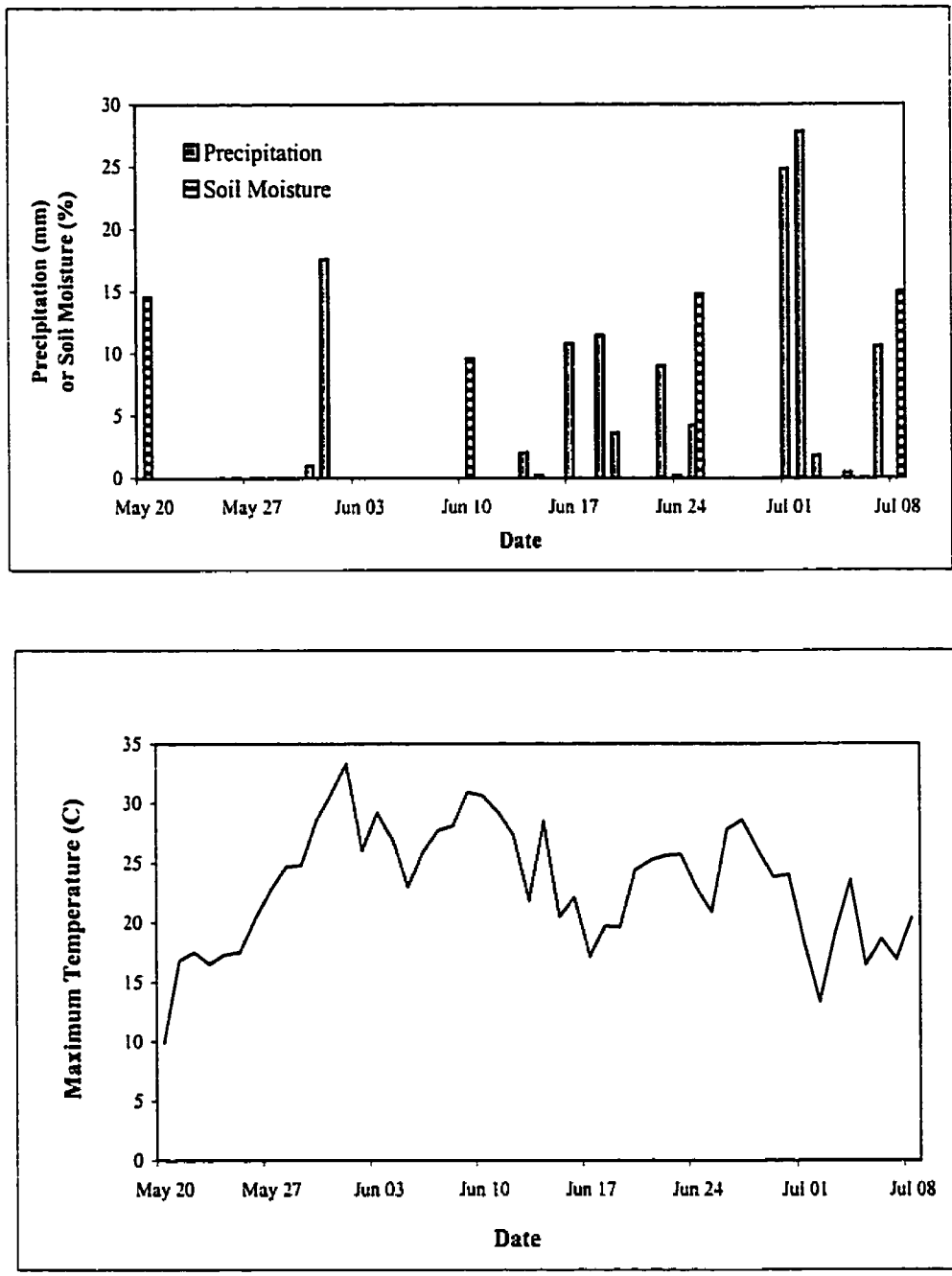


Figure 2. Rainfall events (mm), soil moisture (%), and maximum ambient temperature (°C) taken at the Carman Field Station during the summer months of 1997.

The variability in the data of this experiment was not accounted for by the experimental design. As well, although F-protected least significant difference ($LSD_{0.05}$) tests were calculated on the transformed data where possible, in most cases no significant differences were detected. Consequently, the results obtained from this preliminary field study data must be interpreted carefully.

Seedling Number – Carman Site

The seed treatment main effects for seedling number were only significant at Carman on the 1st sampling date ($p<0.001$) (Table 9). However, on the 2nd sampling date at Carman, the seed treatment main effect were significant at the increased confidence level of $p=0.063$. Both of these data sets were subjected to an $LSD_{0.05}$ test. By the 3rd sampling date, no differences were detected between seed treatments. There were no species x seed treatment interactions, so only the seed treatment main effects will be discussed.

With the first sampling date at Carman, all seed priming treatments had significantly higher plant counts than the control (Table 10). There was no difference between any of the 4 priming treatments. Two weeks later, the priming treatments were still noticeably higher than the control. The water-based treatments were not statistically different from the control, although they both trended higher in real numbers. The KNO_3-based priming treatments were still significantly higher than the control. By 4 weeks after the 1st count, there was no longer any difference between treatments. This occurred because the control continued to increase in emergence, the water-based priming

treatments did not change, and the KNO_3-based priming treatments declined to levels actually below that of the control between the 2nd and 3rd sampling dates. This suggested that only seedlings from the KNO_3 priming treatments were dying. However, this trend could have been caused by experimental error since the data were not statistically significant. It could also have been caused by variations in seed dormancy. The untreated control seed may have been still quite dormant, causing the seeds to emerge over a longer period of time. The primed seed should have had less dormancy, and therefore, the majority would have emerged right away.

Table 9. Significance, CV, and R^2 values for the number of grass seedlings per m or row in Seed Priming Field Experiments conducted at Carman and Winnipeg in 1997. ANOVA based on square root transformed data.

Sampling Date[z]	ANOVA	df	Carman	Winnipeg
1	Significance			
	Species (SP)[y]	3	**	**
	Seed Treatment (ST)[x]	4	**	NS
	SP*ST	12	NS	*
	CV (%)		29.4748	30.4162
	R^2		0.5340	0.6139
2	Significance			
	Species (SP)	3	**	**
	Seed Treatment (ST)	4	NS (p=0.063)	NS
	SP*ST	12	NS	*
	CV (%)		21.2474	28.7327
	R^2		0.5626	0.6983
3	Significance			
	Species (SP)	3	**	**
	Seed Treatment (ST)	4	NS	NS
	SP*ST	12	NS	**
	CV (%)		22.2672	27.8466
	R^2		0.4721	0.7162

** = significant at 0.01 probability level; * = significant at 0.05 probability level; NS = not significant

[z]For Carman, 1 = June 10, 2 = June 24, 3 = July 8; For Winnipeg, 1 = June 26, 2 = July 10, 3 = July 24

[y]Species = blue grama; green needlegrass; little bluestem; prairie sandreed

[x]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried

Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

There are several possible explanations for the decrease in seedling survival in the KNO_3 treatments. One, the KNO_3 had an effect that weakened the seedlings and caused them to die off after a period of time. Two, both water and KNO_3 priming treatments produced seedlings that were dying off, but only the water-based treatments had enough new emergence to counter the decline. The water-based priming treatments might not have broken the dormancy on the seeds as effectively as the KNO_3 priming treatments. KNO_3 is known to have dormancy breaking properties and is recommended for use in germination tests of many different species (Maxon, 1995).

Seedling Number – Winnipeg Site

In Winnipeg, there were no significant seed treatment differences observed at any of the sampling dates (Table 9). However, seedling numbers in Winnipeg were much lower than in Carman and there was very little change in numbers from the 1st through to the 3rd sampling date (Table 10).

There was a significant species x seed treatment interaction for the first two sampling dates at the Winnipeg location, and a highly significant interaction for the third sampling date. However, when F-Protected $LSD_{0.05}$ tests were conducted on all 3 Winnipeg sampling dates, only the last date showed differences between treatments at the species level.

Table 10. Seed Priming Field Experiment: number of plants found per metre of row on three separate sampling dates in the sandy-loam soil of Carman, MB and the clay-loam soil of Winnipeg, MB in 1997. Each sampling date and location was analyzed independently of the others and was based on square root transformed data (original means shown here). Seed treatments were compared within species.

Location	Sampling Date	Seed Treatment[z]	Species[y] BG	GN	LB	PS	All
Carman	June 10	C	12.25	4.71	5.38	9.58	7.98 *b*[x]
		H1	19.13	9.04	10.83	10.25	12.31 *a*
		H3	17.25	7.46	12.33	9.04	11.52 *a*
		K1	16.63	12.13	12.38	11.38	13.13 *a*
		K3	16.25	13.17	10.17	13.46	13.26 *a*
	June 25	C	15.46	22.04	13.25	16.21	16.74 *b*
		H1	23.96	27.00	17.08	12.79	20.21 *ab*
		H3	21.42	20.38	18.54	13.33	18.42 *ab*
		K1	20.54	28.79	17.92	17.08	21.08 *a*
		K3	18.92	31.21	15.79	17.63	20.89 *a*
	July 08	C	16.21	23.79	13.71	16.50	20.07
		H1	24.00	25.67	18.04	12.54	20.06
		H3	21.04	21.88	17.42	12.29	18.66
		K1	21.00	23.29	18.63	17.38	18.16
		K3	18.38	25.25	14.46	16.54	17.55
Winnipeg	June 26	C	1.75	7.17	2.29	2.63	3.46
		H1	1.63	7.17	3.33	2.54	3.67
		H3	1.42	5.67	4.33	3.21	3.66
		K1	0.92	8.63	4.25	2.38	4.04
		K3	1.79	7.75	3.33	1.88	3.69
	July 10	C	1.67	11.29	2.46	2.42	4.46
		H1	1.71	9.13	3.17	2.54	4.14
		H3	1.25	7.92	3.88	3.04	4.02
		K1	0.83	9.58	4.17	2.42	4.25
		K3	1.58	8.13	3.29	1.79	3.70
	July 24	C	1.58	13.04 *a*	2.63	2.25	4.88
		H1	1.54	9.83 *ab*	3.33	2.46	4.29
		H3	1.17	7.21 *b*	4.00	2.71	3.77
		K1	0.88	9.00 *ab*	4.00	2.33	4.05
		K3	1.33	7.79 *b*	3.38	1.88	3.59

[z]Control treatment: C = untreated seed. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[y]BG = blue grama; GN = green needlegrass; LB = little bluestem; PS = prairie sandreed

[x]Average of 6 replications. In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

At the 3rd sampling date in Winnipeg, there was no difference observed in seedling number for blue grama, little bluestem, or prairie sandreed (Table 10). In these cases, the priming treatments did not have any effect on the seedling number. However, it was a different story for green needlegrass. In this case, all the priming treatments produced the same results as the untreated control except for the two treatments that involved a 3 week chilling duration. Each of these treatments had seed emergence counts that were at least 5 seedlings per metre less than that of the untreated control (Table 10). It appeared from this result that the longer the chilling period, the less emergence that would occur, regardless of the priming solution.

Number of Tillers/Plant – Carman and Winnipeg

The number of tillers per plant was taken as a measure of plant development. At the 1st sampling date, no tillers were observed, but by the 2nd sampling date tillers had begun to form. There were no significant seed treatment main effects or species x seed treatment interactions for any of the data sets (Table 11).

Table 11. Seed Priming Field Experiment: significance, CV, and R^2 values for the number of tillers found at the Carman and Winnipeg sites. ANOVA based on square root transformed data.

Sampling Date[z]	ANOVA	df	Carman	Winnipeg
1	Significance			
	Species (SP)[y]	3	**	**
	Seed Treatment (ST)[x]	4	NS	NS
	SP*ST	12	NS	NS
	CV (%)		23.85198	39.54295
	R^2		0.713142	0.557567
2	Significance			
	Species (SP)	3	**	**
	Seed Treatment (ST)	4	NS	NS
	SP*ST	12	NS	NS
	CV (%)		27.90131	33.31796
	R^2		0.773389	0.711394

** = significant at 0.01 probability level; NS = not significant

[z]For Carman, 1 = June 24, 2 = July 8; For Winnipeg, 1 = July 10, 2 = July 24

[y]Species = blue grama; green needlegrass; little bluestem; prairie sandreed

[x]Control treatment: C = untreated seed. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

It appeared from this data that there was no increase in tiller number caused by the priming treatments (Table 12). However, it is important to note that there was a lot of variability associated with this experiment.

There are some general observations that can be made. Although the Winnipeg soil produced much lower plant counts than that of Carman, it appears that the surviving plants had a greater number of tillers than those of the Carman plants. Tiller number was generally 2 to 3 times higher in Winnipeg than in Carman (Table 12). This was probably a reflection of the shading and cool conditions caused by all the weeds at the Carman location. Generally, the tillers of all species in both locations increased slowly from the 2nd to the 3rd sampling period as their roots developed. By the time of the 3rd measurement date, the tiller number had increased at a much faster rate. These plants had become established and were growing at a much faster rate.

Table 12. Seed Priming Field Experiment: number of tillers per plant found on two separate sampling dates in the sandy-loam soil of Carman, MB and the clay-loam soil of Winnipeg, MB. Each sampling date was analyzed independently of the others and was based on square root transformed data (original means shown here).

Location	Sampling Date	Seed Treatment[z]	Species[y] BG	GN	LB	PS	All
Carman	June 25	C	1.38 [x]	0.04	0.09	0.00	0.38
		H1	1.48	0.00	0.09	0.00	0.39
		H3	1.58	0.00	0.09	0.00	0.42
		K1	1.21	0.09	0.20	0.00	0.37
		K3	1.42	0.04	0.08	0.00	0.39
	July 08	C	5.17	1.24	1.83	0.04	2.07
		H1	4.48	1.14	2.04	0.00	1.91
		H3	5.63	0.70	1.73	0.00	2.01
		K1	4.23	1.00	2.05	0.00	1.82
		K3	4.91	0.95	1.67	0.00	1.88
Winnipeg	July 10	C	3.38	1.05	1.04	0.06	1.38
		H1	3.92	1.18	1.74	0.05	1.72
		H3	3.95	1.18	2.22	0.00	1.84
		K1	3.73	1.96	1.41	0.00	1.77
		K3	2.55	1.63	1.83	0.05	1.51
	July 24	C	17.86	4.32	4.77	0.82	6.94
		H1	18.13	5.50	7.74	0.84	8.05
		H3	18.89	7.36	8.10	0.60	8.74
		K1	17.45	6.33	6.23	1.95	7.99
		K3	15.81	7.24	7.45	1.24	7.93

[z] Control treatment: C = untreated seed. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[y] BG = blue grama; GN = green needlegrass; LB = little bluestem; PS = prairie sandreed

[x] Average of 6 replications. There was no species x seed treatment interaction observed.

4.3 Indoor Experiments

Although there was a high degree of variability found in the data obtained from the *Field Experiment*, there were still some positive observations made. For instance, at each site and sampling date, all of the priming treatments produced a higher little bluestem seedling emergence than the untreated control (Table 10). This same trend was observed for blue grama at the Carman location (Table 10). After these observations

were made, it was decided that the priming protocol still had merit, but that further testing should be conducted in a more controlled environment. With that in mind, the *Indoor Experiments* were initiated.

4.3.2 Petri Dish Seed Priming Experiment

The purpose of this experiment was to test whether or not the seed priming treatments that were developed would improve final germination percentage and germination rate.

Final Germination Percentage

There were significant species and seed treatment main effects found when testing the final germination percentage in this experiment (Table 13). The species x seed treatment interaction was also significant, which implied that the difference in final germination percentage found among the species depended on the type of seed treatment. Thus, the following discussion will deal with how the various seed treatments affected each individual species.

There was no significant difference exhibited between the germination percentages of the blue grama untreated control seed or any of the primed seed (Table 13; Figure 3). On the surface, this indicated that there was no advantage gained by any of the priming treatments. However, the 1 hour soaked and dried control treatment produced a statistically lower germination percentage (51.3%) than the untreated control seed

(60.0%). Although the priming treatments had statistically the same germination percentage as the soaked and dried control seed, they were all numerically higher. This suggested that drying was detrimental to the priming process and may have been masking any benefits produced. Hardegree (1994b) found that when bottlebrush squirreltail seeds were osmotically primed in a solution of PEG 8000 and then air dried for 7 days, the resulting germination percentage was lower than untreated seed. Further investigation showed that it was actually the drying process that caused the germination to decrease, not the priming. Primed seed that was germinated without drying had a germination percentage of 78% which was higher than the primed and dried seed which had a germination percentage of 57%.

Table 13. Petri Dish Seed Priming Experiment: final germination percentage of seed germinated in petri dishes for 14 days. Seed treatments compared within species. All tests based on arc sine transformed means, but original means are presented.

Seed	Species[y]					
Treatment[z]	BG	GN	KB	LB	PS	All
C	60.0 *a*[x]	9.5 *a*	86.8 *b*	40.5 *b*	68.3 *bc*	53.0 *a*[x]
CT	51.3 *b*	1.0 *b*	86.8 *b*	46.8 *ab*	64.8 *c*	50.1 *b*
H1	56.3 *ab*	3.3 *b*	87.0 *b*	50.3 *a*	76.8 *a*	54.7 *a*
H3	55.8 *ab*	2.0 *b*	87.3 *b*	53.3 *a*	73.5 *ab*	54.4 *a*
K1	54.3 *ab*	13.8 *a*	83.3 *b*	52.8 *a*	68.0 *bc*	54.4 *a*
K3	57.0 *ab*	9.0 *a*	85.0 *b*	52.5 *a*	69.8 *abc*	54.7 *a*

ANOVA	df	Significance
Species (SP)	4	**
Seed Treatment (ST)	5	**
SP*ST	20	**
CV (%)		7.518871
R^2		0.976606

** = significant at 0.01 probability level

[z]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[y]BG = blue grama; GN = green needlegrass; KB = Kentucky bluegrass; LB = little bluestem; PS = prairie sandreed

[x]Average of 4 replications. In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

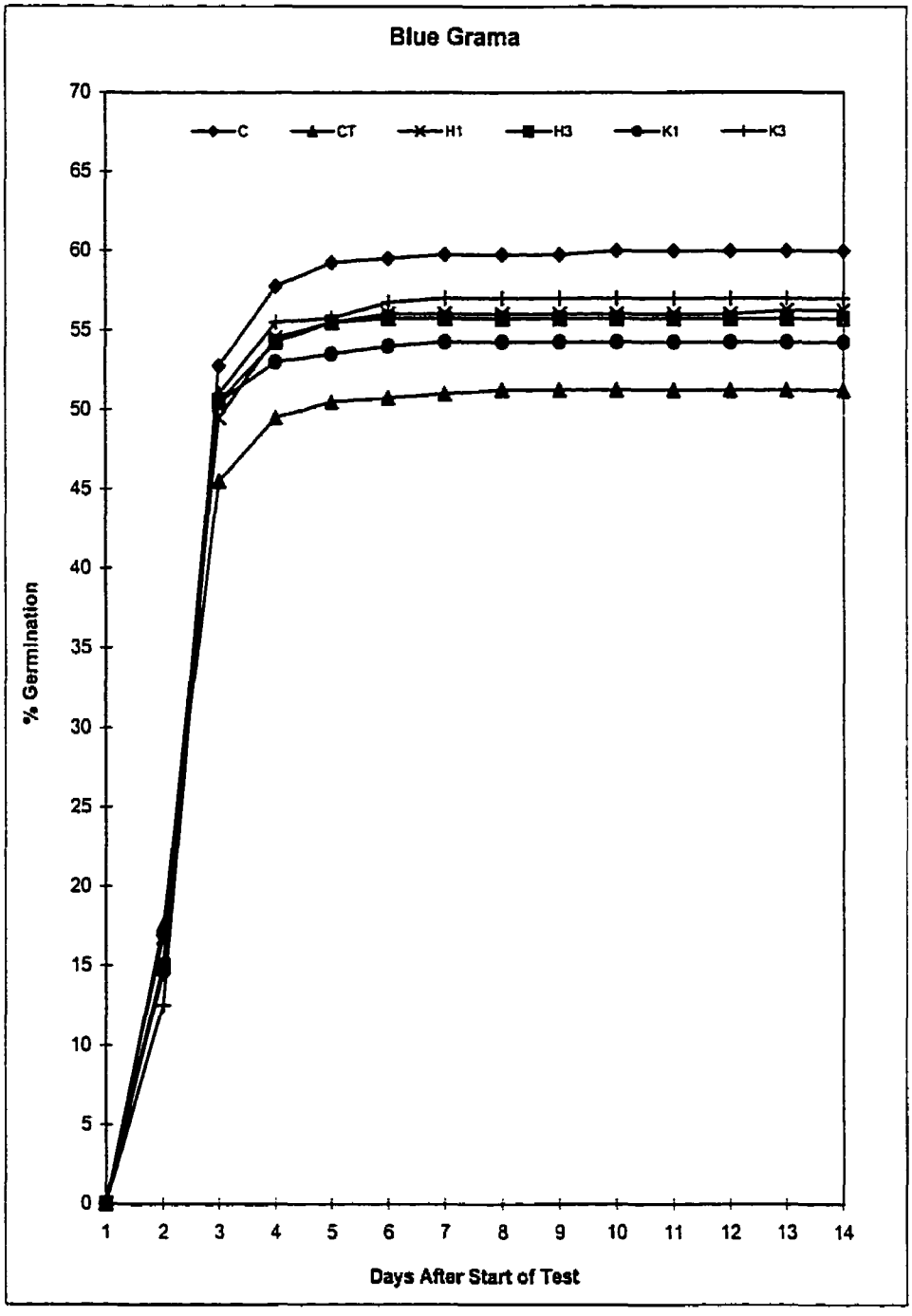


Figure 3. Seed Priming Petri Dish Experiment: germination percentage of blue grama seeds germinated in petri dishes for 14 days (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

The lack of advantage gained from priming the blue grama seeds could also be explained by a lack of seed dormancy. The seed used in this experiment could have had a very low dormancy associated with it, so the priming treatments would not have produced an advantage in the petri dish. The AOSA seed testing guidelines do not require a prechilling period to break seed dormancy, although they do require that 0.2% KNO_3 be used for fresh and dormant seed (Table 3) (Maxon, 1995). This indicated that blue grama seeds do not have a high level of dormancy. In fact, this species was included in the study not for its dormant seed qualities, but rather, as a hard to establish grass that may benefit from seed priming. It is well known that blue grama will generally emerge within 4 to 5 days, but will die off after 6 to 8 weeks if adventitious roots do not develop (Van der Sluijs and Hyder, 1974). Therefore, any advantages gained from the priming treatments may be better highlighted in the growth room experiment where the seed was planted under more natural conditions.

Green needlegrass had extremely low final germination percentages for all treatments (Table 13; Figure 4). There are several possible explanations for this. First, germination of green needlegrass can be difficult because of its high dormancy levels (Abouguendia, 1995) and AOSA recommends a Tetrazolium test for determining the final germination percentage (Maxon, 1995). Second, during the germination test all the species were subjected to the same conditions, and therefore, the green needlegrass was not germinated in the dark as is recommended by AOSA (Maxon, 1995). This was the most realistic explanation since the germination percentage of green needlegrass seeds in

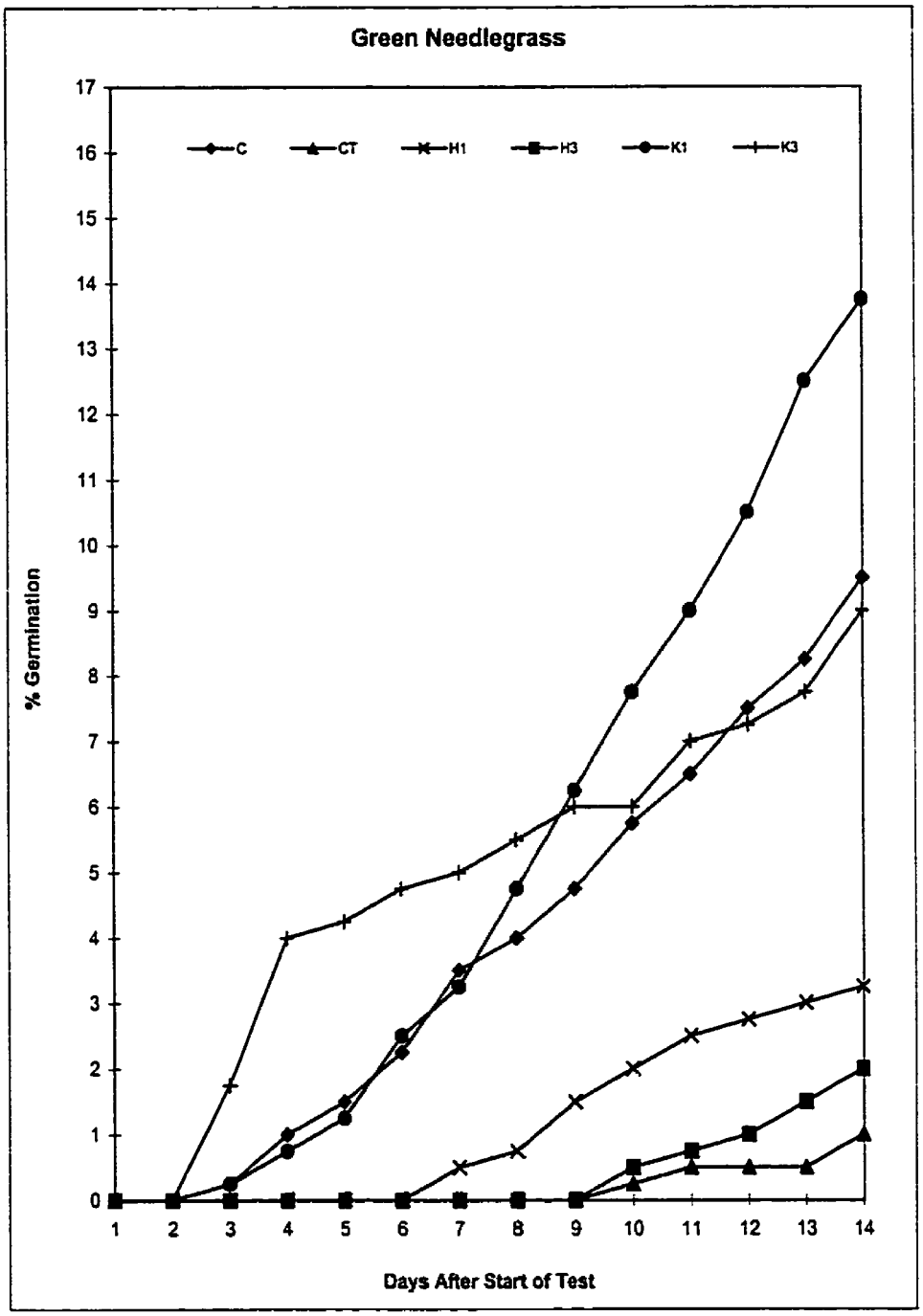


Figure 4. Seed Priming Petri Dish Experiment: germination percentage of green needle seeds germinated in petri dishes for 14 days (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

the *Fungicide Experiment* was found to be 47.8% (Table 7). These seeds were incubated in the dark and produced a much higher germination percentage than any of the treatments in this experiment.

In green needlegrass, as with blue grama, there appeared to be damage caused by the drying process. The 1 hour soaked and dried control seed only had 1.0% germination, while the untreated control seed had a higher germination of 9.5%. The water based priming treatments had the same germination percentage as the soaked and dried control, indicating that these priming treatments were not able to overcome the detrimental effects of the drying process. In contrast, the two KNO_3 based treatments had significantly higher germination than the water based treatments and the soaked and dried control treatment. This indicated that the addition of 0.5% KNO_3 to the priming solution produced an increased germination percentage. This was consistent with the AOSA Rules For Seed Testing which require green needlegrass seeds to be germinated on blotters moistened with a 0.2% KNO_3 solution in order to break seed dormancy and stimulate germination (Maxon, 1995). However, since there was no difference between the KNO_3 based priming treatments and the untreated control, it appeared that the benefits of these priming treatments were still being tempered by the drying process. The length of priming in either solution did not make a difference to the final germination percentage.

There was no difference found between the final germination percentages of any of the Kentucky bluegrass seed treatments (Table 13; Figure 5). In fact, all treatments had a germination of 83.3% or better. This would indicate that the full germination

potential of the seeds was achieved when they were subjected to the ideal conditions of the germination test. This is logical given the fact that seed from a commercial cultivar (Welcome) was used. Plants with a long history of domestication generally show less dormancy than recently domesticated or wild species (Copeland and McDonald, 1995). Maguire and Steen (1971) found that using a 0.2% KNO_3 solution caused seeds of a highly dormant Kentucky bluegrass variety to increase germination in a petri dish, while no increase was noted for seeds of a non-dormant variety. They also indicated that the seed dormancy was mainly a result of the freshness of the seed, and it had been lost after 9 months. The seed that was used in this study had been stored for longer than 1 year and presumably did not have any post harvest dormancy associated with it. However, Kentucky bluegrass was included in the study to provide a comparison of a hard-to-establish introduced species vs. the native species, not because it has highly dormant seeds. It can take at least 4 weeks to emerge and even longer to establish (Yamamoto *et al.*, 1997). The results found in this experiment are reasonable and any differences between priming treatments may become evident under more natural conditions like those found in the *Growth Room Experiment*.

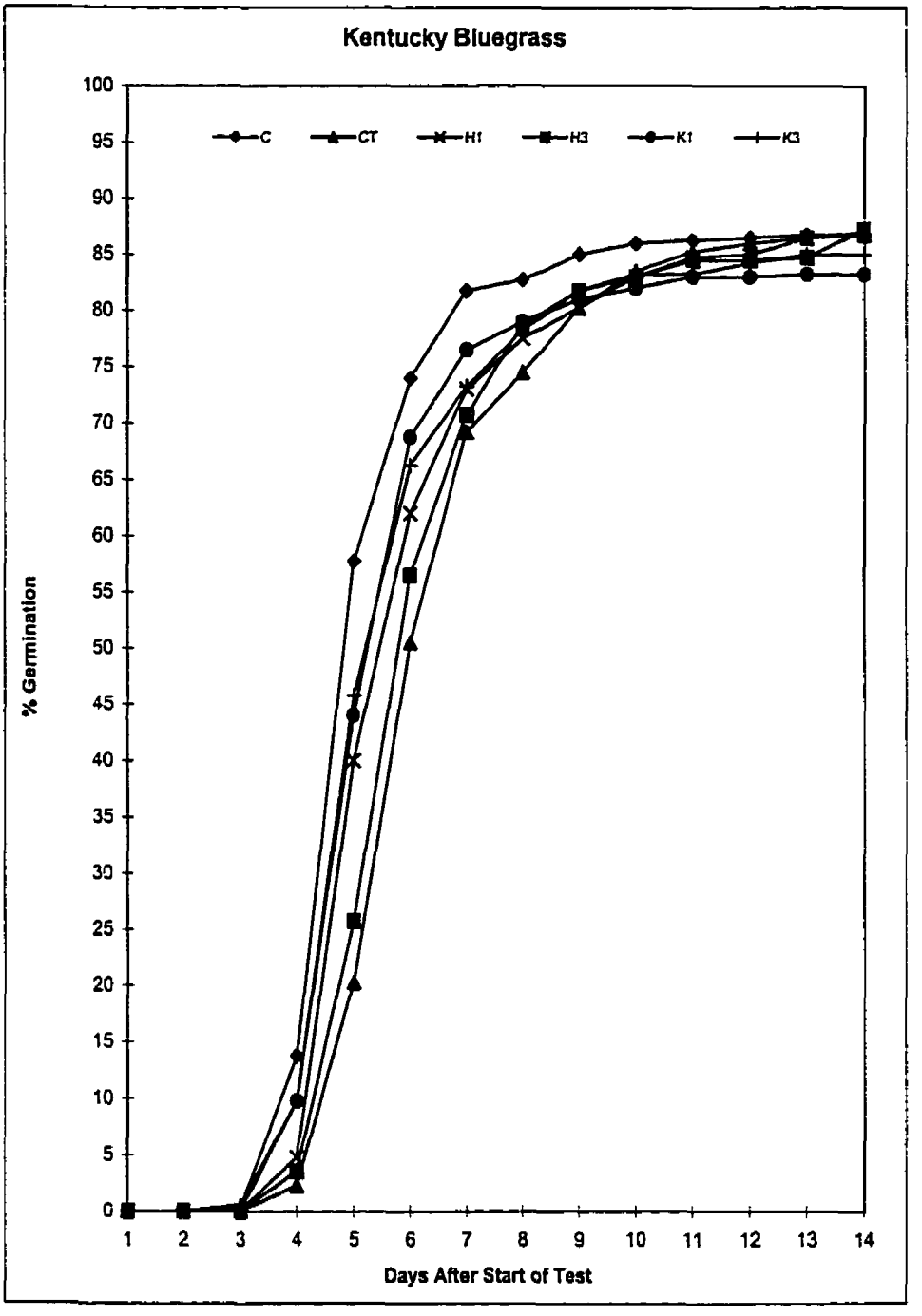


Figure 5. Seed Priming Petri Dish Experiment: germination percentage of Kentucky bluegrass seeds germinated in petri dishes for 14 days (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

All the little bluestem priming treatments produced significantly higher final germination percentage than the untreated control (Table 13; Figure 6). This advantage was at least 25% higher than the untreated control seeds' final germination of 40.5%. This result corresponded with an earlier experiment conducted at the University of Manitoba which found that priming little bluestem seeds using water or a 0.2% KNO_3 solution resulted in 78% and 186% greater germination than untreated seed, respectively (S.R. Smith, unpublished data). There was no difference found between any of the little bluestem priming treatments in this study, indicating that adding 0.5% KNO_3 to the priming solution or chilling for the longer duration of 3 weeks had no added benefit. As well, the untreated control seed did not have a significantly different final germination than the 1 hour soak and dry treatment. This indicated that there was no damage caused by drying. The fact that the soaked and dried control seed and all the primed seeds also had statistically the same germination percentage indicated that this treatment might have actually acted to increase the final germination.

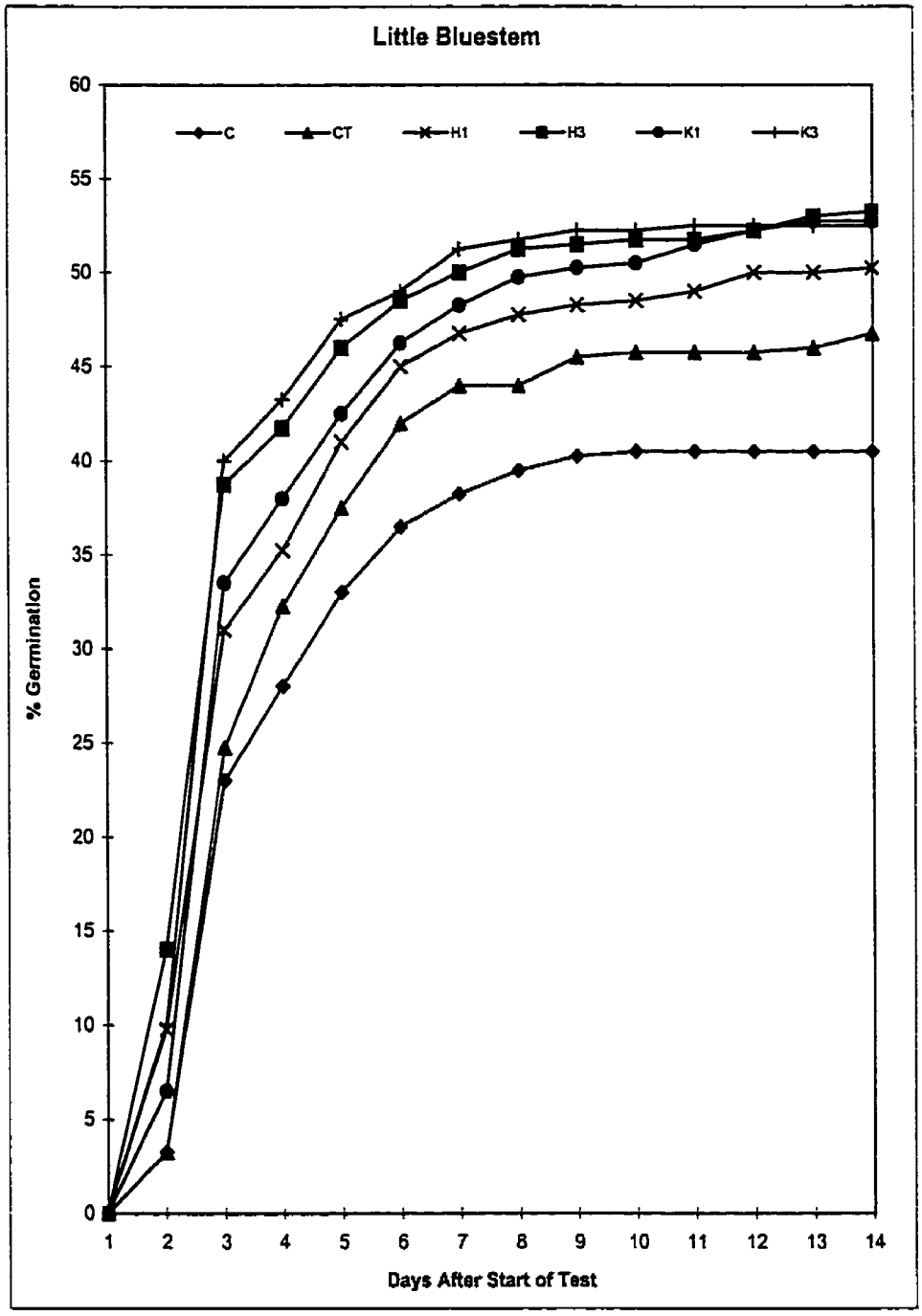


Figure 6. Seed Priming Petri Dish Experiment: germination percentage of little bluestem seeds germinated in petri dishes for 14 days (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

There was no difference between prairie sandreed soaked and dried control seed and the untreated control seed, indicating that drying had no detrimental effect on final germination (Table 13; Figure 7). All the seed priming treatments produced germination percentages that were at least as high as the untreated control. The exception was the 1 week water priming treatment which had significantly higher germination than the untreated control. This seed also had a significantly higher germination than the seed primed in a 0.5% KNO_3 solution for the same length of time. This indicated that the use of KNO_3 had an inhibitory effect and it may have actually cancelled out the benefit of chilling for 1 week. Similar results have been found in experiments with lettuce. Lettuce seed germination has been reported to be inhibited by the use of KNO_3 (Copeland and McDonald, 1995) and AOSA recommends using a prechilling period of 3 days at 10°C to break the dormancy without the addition of a 0.2% KNO_3 solution (Maxon, 1995). The chilling duration of the prairie sandreed seeds was not important for either priming solution since in both cases, 1 week of chilling produced the same germination results as 3 weeks.

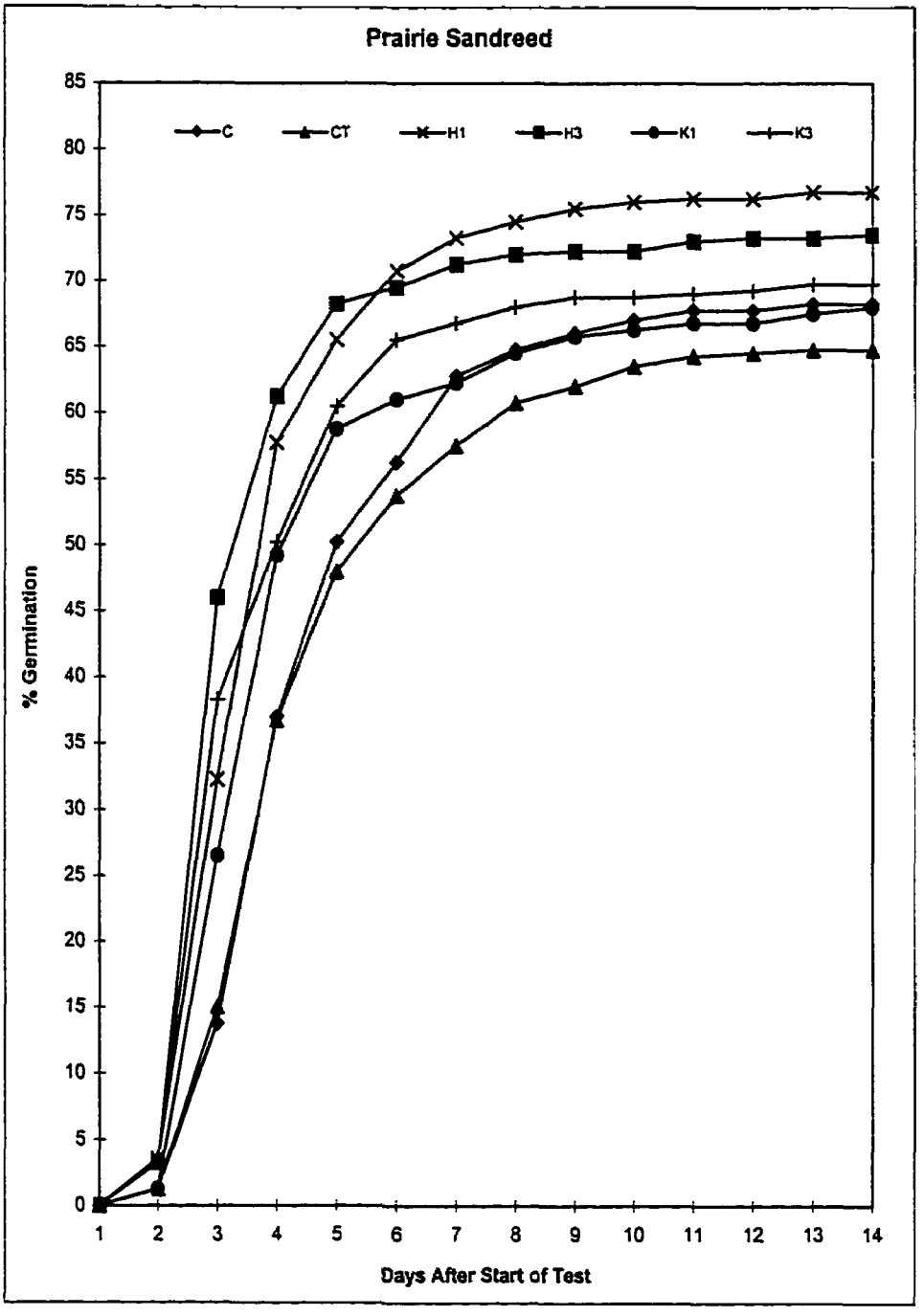


Figure 7. Seed Priming Petri Dish Experiment: germination percentage of prairie sandreed seeds germinated in petri dishes for 14 days (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

Germination Rate

There were significant species and seed treatment main effects found when testing for the germination rate in the *Petri Dish Experiment* (Table 14). Furthermore, the interaction between these two factors was also significant, which will allow the discussion to focus on how each species was affected by the various seed treatments.

Similar results were found for the germination rate as were found for the final germination percentage of blue grama. There was no difference found between the untreated control and any of the seed priming treatments, indicating that no advantage was gained by the use of the priming treatments (Table 14). The only real difference was exhibited by the soaked and dried control seed which germinated faster than the untreated control seed. This not only indicated that drying the seed was not detrimental to the germination rate, but also that chilling might not be necessary to increase the germination rate of blue grama. Simple imbibition for 1 hour followed by drying improved the germination rate where the more complex seed priming treatments did not. Since the soaked and dried control seed was imbibed to the same seed moisture percentage as the priming treatments (Table 8), it is possible that it was the priming method itself (i.e. the use of chilling and KNO_3) that cancelled out the rate improvement. This argument is further strengthened by the fact that the KNO_3 based seed treatments had significantly slower germination than the soaked and dried control seed. Bleak and Keller (1974) found that five wheatgrass species had faster emergence when they were allowed to imbibe water at 17°C and then air dried. This increase in emergence rate did not translate

into a more successful plant establishment. Their result was similar to that found in the *Petri Dish Experiment* which found that the final germination percentage of the soaked and dried control seed was not better than the untreated control seed.

Table 14. Petri Dish Seed Priming Experiment: number of days it takes to reach 50% of the final germination percentage (G_{50}) of 5 grass species exposed to different priming treatments and germinated in petri dishes. Seed treatments compared within species. All tests based on $\log^{-1}$ transformed means, but original means presented.

Seed Treatment[x]	Species[y] BG	GN	KB	LB	PS	All
C	2.5 *b*[w]	8.5 *b*[z]	4.6 *a*	2.9 *bc*	3.9 *c*	4.5 *d*[v]
CT	2.3 *a*	10.5 *bc*	5.9 *b*	3.0 *c*	3.8 *c*	4.5 *bcd*
H1	2.4 *ab*	9.3 *bc*	5.1 *ab*	2.8 *abc*	3.3 *b*	4.3 *abc*
H3	2.4 *ab*	12.2 *c*	5.5 *ab*	2.5 *a*	2.9 *a*	4.7 *ab*
K1	2.5 *b*	9.2 *bc*	5.0 *ab*	2.8 *abc*	3.4 *bc*	4.6 *cd*
K3	2.5 *b*	5.3 *a*	5.0 *ab*	2.6 *ab*	3.0 *ab*	3.7 *a*

ANOVA	df	Significance
Species (SP)	4	**
Seed Treatment (ST)	5	**
SP*ST	20	**
CV (%)		4.813628
R^2		0.964187

** = significant at 0.01 probability level

[x]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[y]BG = blue grama; GN = green needlegrass; KB = Kentucky bluegrass; LB = little bluestem; PS = prairie sandreed

[z]$LSD_{0.05}$ values used to compare some of the seed treatment means within Green Needle were adjusted due to missing data.

[w]Average of 4 replications. In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

[v]$LSD_{0.05}$ values for these seed treatment means (compared over all species) were adjusted due to missing data.

Soaking and drying the untreated green needlegrass seeds resulted in no difference in germination rate from that of the untreated control seed (Table 14). This indicated that the drying process was not detrimental to the germination rate. This is in contrast to what was found for the germination percentage. However, it is important to note that although

there were no significant differences found in germination rate between these two treatments, the soaked and dried seed did take 2.0 days longer to reach 50% of the final germination percentage than the untreated control seed took. The only seed treatments that produced different results from that of the untreated control were the ones that involved a 3 week priming duration. In this case, if water had been used as the priming solution, the resulting germination rate was slower than the untreated seed. However, if 0.5% KNO_3 was added the germination rate became faster than that for the untreated control. The 3 week KNO_3 priming treatment was also statistically faster than the 1 week KNO_3 priming treatment, indicating that a longer chilling duration was necessary in order for the KNO_3 to be effective. These results reconfirm the importance of KNO_3 for green needlegrass seed germination as observed in the final germination percentage results of this experiment and in the AOSA seed testing guidelines (Maxon, 1995).

There was no significant difference found between the germination rate of Kentucky bluegrass untreated control seeds and the primed seeds (Table 14). However, the slowest germination was produced by the seeds that had been soaked for 1 hour and then dried. This suggested that drying was harmful to germination rate. Pill and Korengel (1997) found that matrically and osmotically primed Kentucky bluegrass seeds had a faster germination rate than untreated seeds, and that drying the primed seeds for 2 days at 65% relative humidity and 21°C caused the germination rate to decrease slightly. Since the germination rate of the untreated seed was not slower than that of the primed seed, but was slower than that of the soaked and dried seed, the advantages of the priming may have been counteracted by the drying process.

Little bluestem had no significant difference between germination rate of the control seed soaked for 1 hour and the untreated control seed (Table 14). This indicated that the germination rate was not affected by the drying process. This was the same result that was found with the final germination percentage data (Table 13). Three of the four priming methods had the same germination rate as the controls. However, the seed that was primed for 3 weeks in water germinated faster than the unprimed seed. This also supported the conclusions reached from the final germination data which indicated that the use of KNO_3 may not have been necessary for seed priming of little bluestem. In fact, the simple act of chilling may have been most important. Romo (1990) found that stratification at 5°C for 48 hours followed by drying at 30°C for 8 hours improved the germination rate of Altai wildrye (*Leymus angustus* (Trin.) Pilger).

Prairie sandreed had the same germination rate for the untreated control and the soaked and dried control (Table 14) which indicated that drying was not detrimental to the germination rate. The same results were found for the germination percentage (Table 13). As well, all the seed treatments produced a germination rate that was as good or better than that of the controls. In this case, both water based treatments and the 3 week KNO_3 treatment produced seed with faster germination rates. It has been shown that the 1 week water treatment produced the highest germination percentage for prairie sandreed seeds (Table 13). This treatment was also found to have a better germination rate than the untreated seed. However, increasing the chilling period to 3 weeks actually produced an even faster germination. The advantage gained by increasing the length of the chilling period to 3 weeks was also shown to be true with the KNO_3 based treatments. This indicated that the longer the chilling period, the faster the rate of germination. Although

it was found in the final germination percentage data that KNO_3 had an inhibitory effect, this was not evident in the germination rate data since the 3 week KNO_3 treatment had the same germination rate as the water based treatments.

4.3.3 Growth Room Experiment

The purpose of this experiment was to test whether or not the treatments that were developed in the seed priming protocol would improve the number of seedlings emerged, the emergence rate, and the growth stage.

Emergence At 10 and 20 DAP

There were no significant main effects for soil type at either 10 or 20 DAP (Table 15). This indicated that the soil type did not influence the emergence results and that the same trends occurred regardless of soil type. The species and seed treatment main effects were significant for both counts. However, since there were also significant interactions involving these two factors, these main effects will not be interpreted.

The 3-way interaction between soil type, species, and seed treatment was not significant at either 10 or 20 DAP (Table 15). As well, there was no significant interaction between soil and species at 10 or 20 DAP, indicating that the effect of the soil was not dependent on the species. However, the interaction between soil type and seed treatment was significant at 10 DAP, and the interaction between species and seed

treatment was significant at both 10 and 20 DAP. These interactions will be the focus of the following discussion.

Table 15. Seed Priming Indoor Experiments: significance, CV, and R^2 values for seedling emergence at 10 (E_{10D}) and 20 (E_{20D}) days after planting, emergence rate (E_{50}), and growth stage (E_{GS}) of seeds of 5 grass species exposed to several seed priming treatments and planted into clay-loam and sandy-loam soils in a controlled environment.

ANOVA	df	E_{10D}	E_{20D}	E_{50}[z]	E_{GS}
Significance					
Soil (SO)	1	NS	NS	NS	*
Species (SP)	4	**	**	**	**
SO*SP	4	NS	NS	**	**
Seed Treatment (ST)	5	**	**	**	NS
SO*ST	5	**	NS	NS	NS
SP*ST	20	**	**	**	*
SO*SP *ST	20	NS	NS	NS	NS
CV (%)		16.7077	12.2076	3.8919	8.5397
R^2		0.9695	0.9601	0.9546	0.9346

** = significant at 0.01 probability level; * = significant at 0.05 probability level; NS = not significant
[z]Based on arc sine transformed data.

The soil x seed treatment interaction was only significant at 10 DAP (Table 15). This indicated that the effect of the seed treatment was dependent on the soil type at 10 DAP, but not at 20 DAP.

At 10 DAP, there was no difference between the untreated control seed and the soaked and dried control seed in either the Winnipeg or Carman soils (Table 16). This inferred that any differences between seed treatment in the same soil type were a result of the priming process, not the drying process. All priming treatments produced higher emergence counts than the controls regardless of soil type. In the Carman soil, the seed from the 3 week water and KNO_3 treatments had the highest emergence at 69.6 and 67.3 seedlings, respectively. This indicated that the longer chilling duration produced better

results than the 1 week chilling duration on a sandy loam soil. In the Winnipeg soil, it was a different effect, with the KNO_3 based treatments having a higher emergence count than the water based treatments.

Table 16. Seed Priming Indoor Experiment: number of seedlings emerged at 10 and 20 days after planting seed of 5 grass species exposed to several priming treatments and planted into clay-loam (Winnipeg) and sandy-loam (Carman) soils in a controlled environment.

DAP	Factor	Species[z]					Soil Type[y]		All
		BG	GN	KB	LB	PS	Carman	Winnipeg	
10	Seed Trtmnt[x,w]								
	C	120.6 *c*[v]	19.5 *cd*	18.8 *a*	17.3 *b*	54.0 *b*	41.3 *d*	50.8 *c*	46.0 *c*
	CT	129.5 *bc*	14.3 *d*	20.4 *a*	21.4 *b*	57.1 *ab*	44.5 *d*	52.6 *c*	48.5 *c*
	H1	142.8 *a*	27.6 *c*	20.8 *a*	56.4 *a*	61.8 *ab*	60.1 *c*	63.6 *b*	61.9 *b*
	H3	144.3 *a*	41.0 *b*	21.8 *a*	64.4 *a*	64.9 *a*	69.6 *a*	65.0 *b*	67.3 *a*
	K1	135.3 *ab*	56.1 *a*	20.0 *a*	64.6 *a*	58.5 *ab*	61.3 *bc*	72.5 *a*	66.9 *a*
	K3	138.1 *ab*	65.4 *a*	21.8 *a*	66.3 *a*	56.8 *ab*	67.3 *ab*	72.1 *a*	69.7 *a*
	Soil Type[u]								
	Carman	129.6	32.1	10.0	54.8	60.2			57.3
	Winnipeg	140.5	42.5	31.2	42.0	57.5			62.7
20	Seed Trtmnt								
	C	113.5 *b*	69.2 *c*	35.4 *a*	27.5 *c*	61.4 *b*	63.6	59.2	61.4 *d*
	CT	122.4 *b*	68.6 *c*	38.3 *a*	37.1 *b*	69.5 *ab*	67.6	66.8	67.2 *c*
	H1	131.9 *a*	88.9 *b*	36.0 *a*	76.5 *a*	68.4 *ab*	81.6	79.1	80.3 *b*
	H3	134.9 *a*	87.4 *b*	33.8 *a*	82.1 *a*	74.0 *a*	88.1	76.8	82.4 *ab*
	K1	136.6 *a*	101.9 *a*	33.9 *a*	85.1 *a*	65.4 *ab*	87.4	81.8	84.6 *a*
	K3	131.6 *a*	96.0 *ab*	33.3 *a*	79.4 *a*	67.3 *ab*	83.6	79.5	81.5 *ab*
	Soil Type								
	Carman	129.5	92.1	35.0	68.8	67.7			78.6
	Winnipeg	127.5	78.5	35.2	60.4	67.6			73.8

[z]BG = blue grama; GN = green needlegrass; KB = Kentucky bluegrass; LB = little bluestem; PS = prairie sandreed

[y]Seed treatments averaged over all species and compared within soil type.

[x]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[w]Seed treatments averaged over both soil types and compared within species.

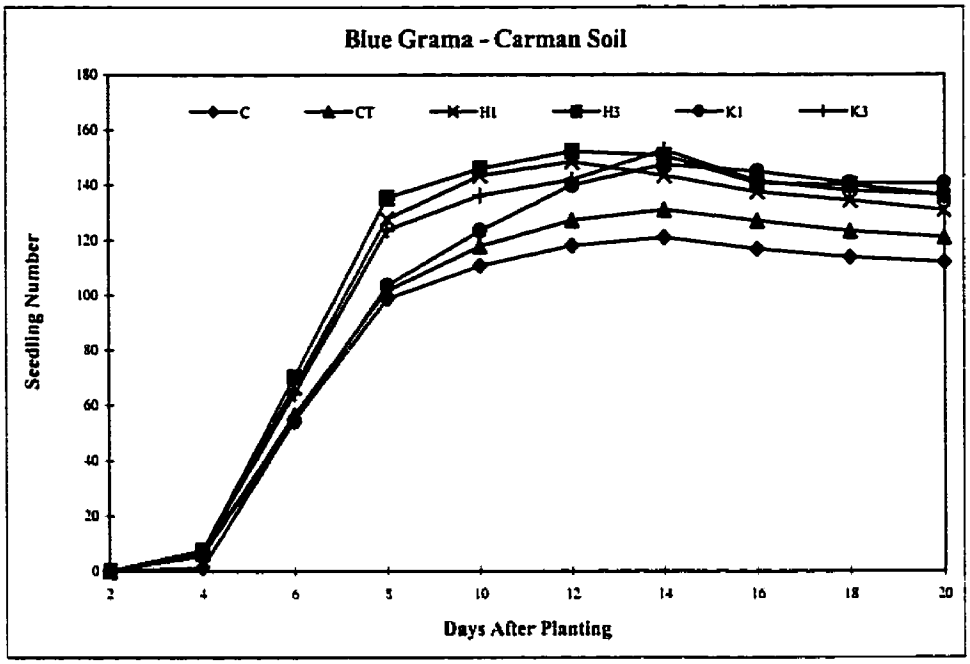
[v]In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

[u]Soil types averaged over all seed treatments and compared within species.

There was no difference between soil types at the same seed treatment level (averaged over all species) at 10 DAP. This was a result of a very high $LSD_{0.05}$ value of 21.37 plants. In general, however, the Winnipeg soil had higher emergence counts than the Carman soil at 10 DAP.

There were significant species x seed treatment interactions at both 10 and 20 DAP (Table 15), indicating that the effect of the seed treatment was dependent on the type of species.

Blue grama showed no difference between the untreated control seed and the soaked and dried control seed at either 10 or 20 DAP (Table 16; Figure 8). This indicated that drying did not have a detrimental effect. This was contrary to the final germination percentage results found in the *Petri Dish Experiment* (Table 13). All the priming treatments produced a higher emergence count than the controls at both 10 and 20 DAP. This advantage ranged from 15 to 23 seedlings, but no priming treatment had an advantage over the other (Table 16). Again, this is different from the germination results in which the untreated control had the same germination percentage as all the priming treatments.



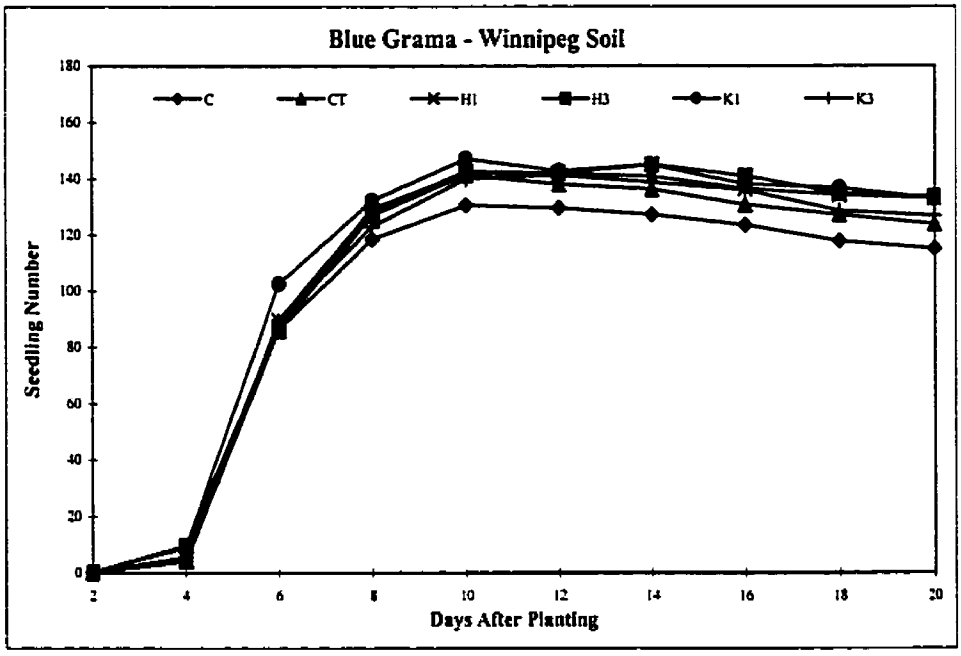


Figure 8. Seed Priming Growth Room Experiment: emergence of blue grama seeds exposed to several seed priming treatments and planted into clay loam (Winnipeg) and sandy loam (Carman) soils in a controlled environment (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

The petri dish experiment was used to test the effectiveness of the seed priming treatments because it was much easier and faster to do than a growth room experiment. However, the results obtained from such a test are not always realistic, and we felt that it was important to see how the results obtained from both testing methods compared. Many of the priming studies done in the past have used petri dishes (Bodsworth and Bewley, 1981), germination boxes (Pill and Korengel, 1997; Frett and Pill, 1995), or specialized priming/germination cups (Hardegree, 1994a, 1994b; Hardegree and Emmerich, 1992a, 1992b) to test priming results. However, the results found from these types of tests may not provide accurate information about the potential field performance of a seed lot. This is because the criteria for germination was only based on the identification of essential structures that may allow a seedling to become a healthy plant, because the tests are conducted in conditions that are extremely synthetic and seldom relate to field conditions, and because there is no distinction between weak and strong seedlings in the final germination percentage (Copeland and McDonald, 1995). It would be unfortunate if certain seed priming procedures were disregarded because of an unfavourable result obtained in a germination test.

Green needlegrass also showed no difference between the controls for either 10 or 20 DAP (Table 16; Figure 9), indicating that the drying process was not detrimental. Similar to what was found for blue grama, the results of the *Petri Dish Experiment* were contrary to those found in this experiment. All of the green needlegrass priming treatments produced higher seedling emergence counts than the two controls except for the 1 week water priming treatment at 10 DAP. At that time, this treatment had the same emergence as the untreated control, but by 20 DAP it had produced 19.7 more seedlings.

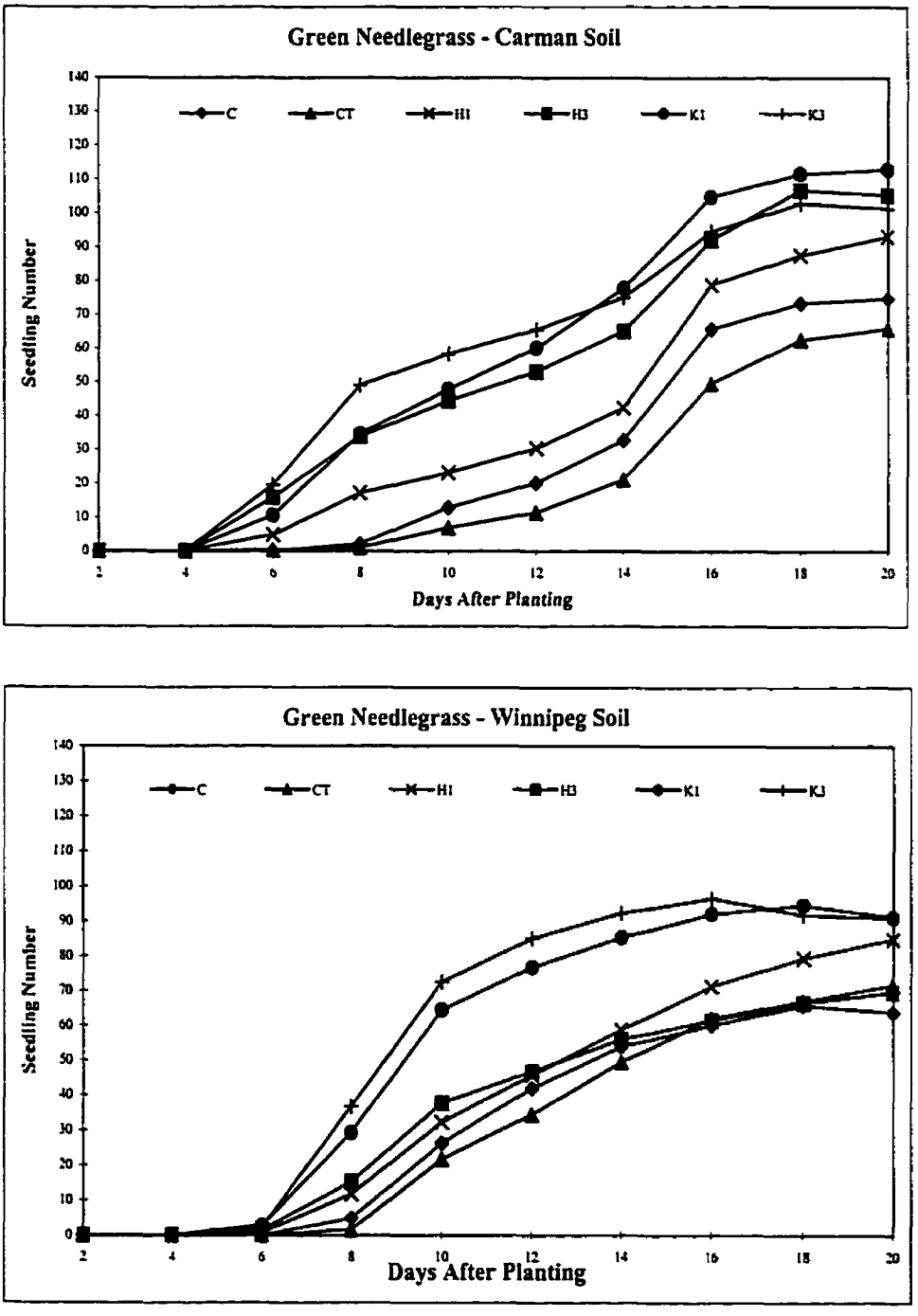


Figure 9. Seed Priming Growth Room Experiment: emergence of green needlegrass seeds exposed to several seed priming treatments and planted into clay loam (Winnipeg) and sandy loam (Carman) soils in a controlled environment (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

The 3 week water primed seed emergence of green needlegrass was 13.4 seedlings higher than the 1 week water treatment at 10 DAP, but by 20 DAP the difference had evened out (Table 16). This indicated that if water was used as the priming solution, an early advantage may be obtained by chilling for a longer period. The AOSA recommendations for seed testing require at least a 2 week prechilling treatment prior to the germination of many grass seeds (Maxon, 1995), so it is likely that the 1 week treatment used in this experiment was not long enough. The highest emergence counts were obtained by the KNO_3 based treatments at both 10 and 20 DAP. With the exception of the 3 week KNO_3 priming treatment at 20 DAP, all of these treatments produced greater seedling numbers than the water-based treatments. This corresponded to the results of the *Petri Dish Experiment* which also showed that the KNO_3 based treatments had better germination than the water based ones (Table 13). There was no advantage gained by chilling using KNO_3 for 3 weeks as opposed to 1 week. This indicated that a priming treatment of 1 week with 0.5% KNO_3 was better than a 3 week priming treatment with water. This has important implications for commercial systems since it would be more economical to only have to prime the seed for one week instead of three.

The difference in results between the green needlegrass *Petri Dish and Growth Room Experiments* could be because the seeds in the petri dishes were germinated in 8 hours of light each day. The AOSA guidelines for seed testing of green needlegrass require incubation in the dark (Maxon, 1995) and this could be the reason that the overall germination percentage for the entire petri dish experiment was extremely low (ranging from 1.0 to 13.8%) even though the viability of the seed was much higher as witnessed in the *Growth Room Experiment*. The seed for the *Growth Room Experiment* was planted

into soil and allowed to germinate in the dark and the results obtained were much better. This provides another example of why the results from a standard germination test may be misleading.

Kentucky bluegrass had the same emergence for all 6 seed treatments at 10 and 20 DAP (Table 16; Figure 10). This was the same result that was observed in the *Petri Dish Experiment* (Table 13). It appeared that seed priming would not increase the germination percentage or emergence count of Kentucky bluegrass. This was similar to results found in other studies. Pill *et al.* (1997) found that matrically priming Kentucky bluegrass seeds in fine, exfoliated vermiculite at 20°C for 4 days did not increase the germination percentage. Pill and Korengel (1997) found that Kentucky bluegrass seeds osmotically primed in a –1.5 MPa KNO_3 solution for 4 days at 20°C did not produce a greater germination percentage than untreated seeds.

It was apparent that there was no dormancy associated with the Kentucky bluegrass seed used in this experiment (i.e. germination of untreated seed in the *Petri Dish Experiment* was 86.8%). This might be a reason that the priming treatments were not as effective in increasing germination or emergence numbers on the Kentucky bluegrass seeds as they were with some of the native grass seeds. Maguire and Steen (1971) had found in a previous experiment that using a 0.2% KNO_3 solution on non-dormant Kentucky bluegrass seeds had no effect on the germination percentage.

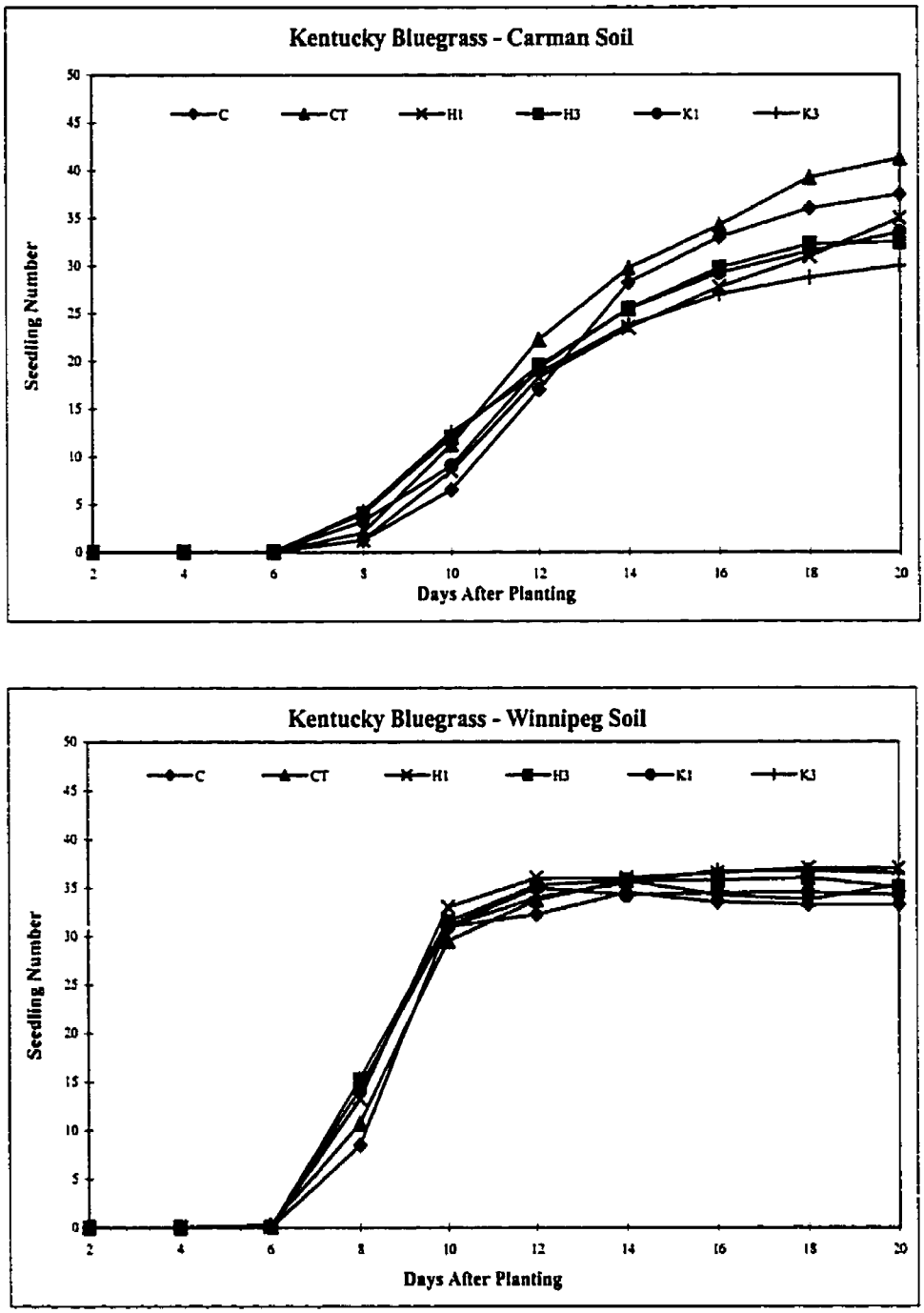
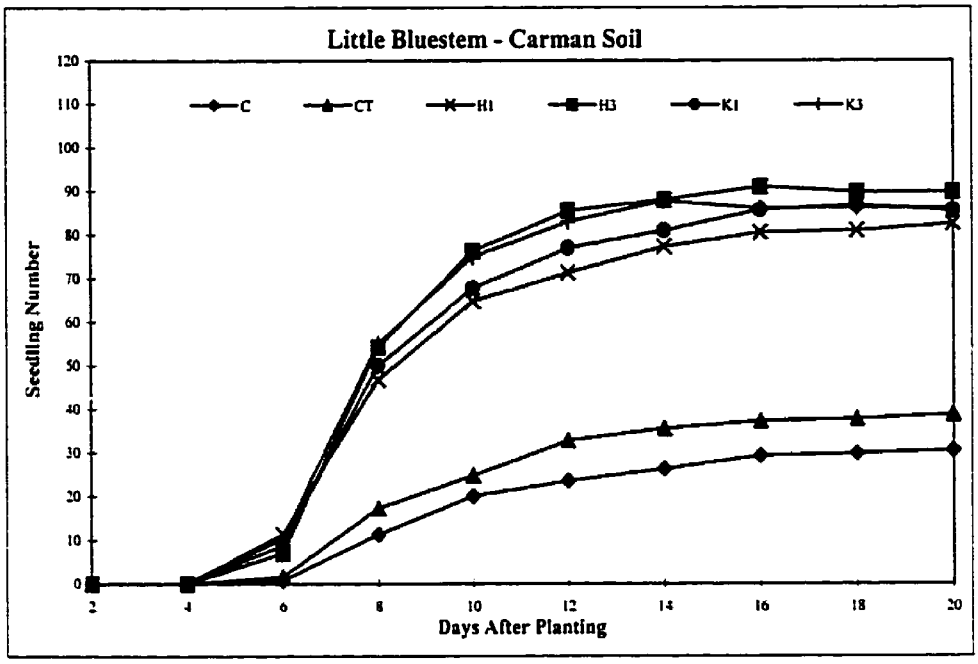


Figure 10. Seed Priming Growth Room Experiment: emergence of Kentucky bluegrass seeds exposed to several seed priming treatments and planted into clay loam (Winnipeg) and sandy loam (Carman) soils in a controlled environment (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

Little bluestem did not exhibit a significant difference between the controls at 10 DAP, but by 20 DAP, the soaked and dried control seed had surpassed the emergence of the untreated control seed by 9.6 seedlings (Table 16; Figure 11). This indicated that not only was the drying process not detrimental, but that an advantage was gained even with a short soaking period of 1 hour. Since the emergence of the seeds that were soaked and dried was between that of the untreated control and the seed treatments that included a chilling period, it is possible that some of the seeds were dormant while others were not. Dormancy is defined as a state in which seeds are prevented from germinating even though favourable germination conditions exist (Copeland and McDonald, 1995). Since the soaked and dried seed did not have as high an emergence as the primed seed (i.e. the full emergence potential was not realized), it is realistic to assume that there were some dormant seeds in this seed lot. That may have been why the emergence of this treatment was not as high as that of the primed seed. The priming treatments involved a moist chilling period, which allowed the dormant seeds to break the dormancy associated with them and the overall emergence increased. This practice (i.e. stratification) has been documented to break seed dormancy of some species by altering the inhibiter-promotor balance of the seed coat (Copeland and McDonald, 1995). The difference observed in this experiment could not have been a result of different imbibition rates since all the treatments absorbed the same amount of moisture (Table 9).



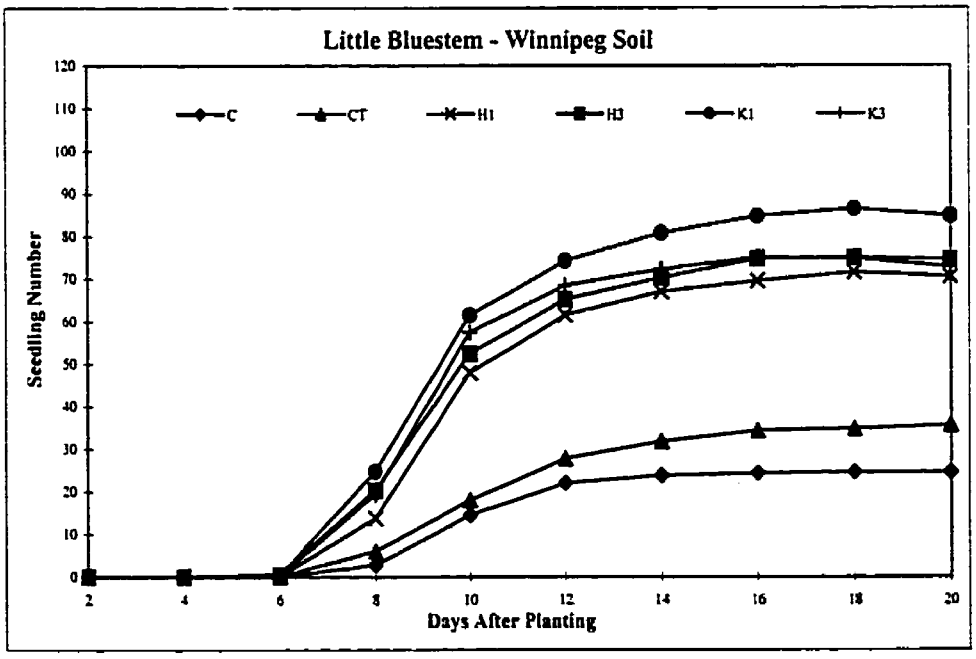


Figure 11. Seed Priming Growth Room Experiment: emergence of little bluestem seeds exposed to several seed priming treatments and planted into clay loam (Winnipeg) and sandy loam (Carman) soils in a controlled environment (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

Similar to the *Petri Dish Experiment*, all of the little bluestem seed priming treatments had better results than the controls (Table 16). The seed emergence of the priming treatments in this experiment was at least 3 times better than that of the controls at 10 DAP, and 2.5 times better at 20 DAP. There was no difference between any of these treatments. Other studies have also documented the positive effects of seed priming. Beckman *et al.* (1993) found that solid matrix priming big bluestem for 14 days at 4°C and for 2 days at 17°C resulted in an 18% higher emergence than untreated seed. Hsu *et al.* (1985) found that big bluestem germination in petri dishes increased by 24% over the control as a result of chilling the seed for 14 days at 4°C.

The two prairie sandreed control treatments produced the same emergence count at both 10 and 20 DAP (Table 16; Figure 12), indicating that the drying process did not have a detrimental effect. All the priming treatments produced a seedling emergence that was equal to the emergence of the controls with the exception of the seed chilled for 3 weeks in water. It had a higher emergence count at both 10 and 20 DAP. This was a different result than that found in the *Petri Dish Experiment*. In that experiment, it was the 1 week water chilled seed that had a higher germination than the untreated control seed (Table 13). Regardless, it appeared that the use of KNO_3 was not beneficial to advancing the germination or emergence of prairie sandreed, and in fact, it may have actually masked the benefits obtained from the water based priming treatments. Other studies have observed that KNO_3 may be detrimental to the seed germination of some species (Copeland and McDonald, 1995).

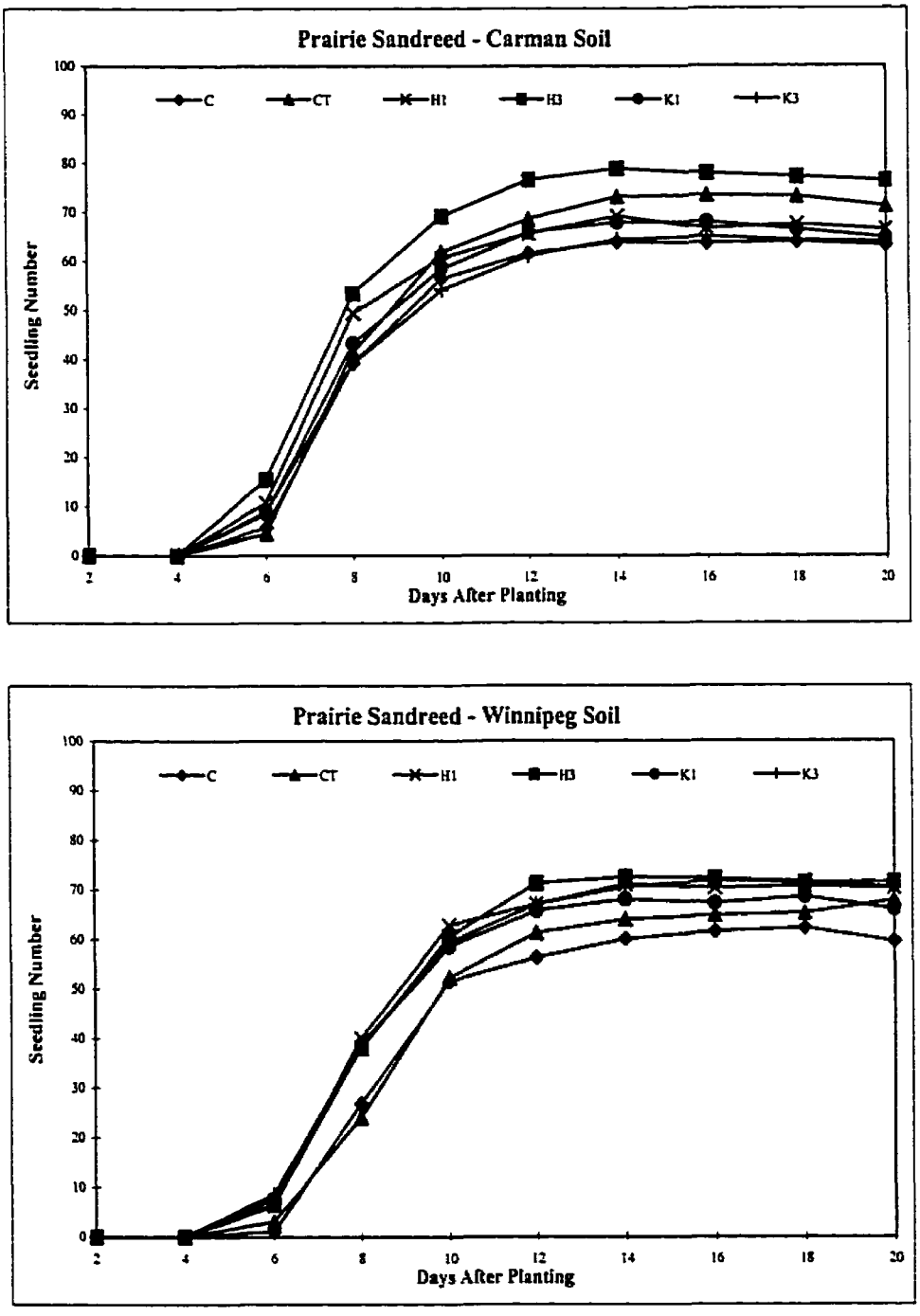


Figure 12. Seed Priming Growth Room Experiment: emergence of prairie sandreed seeds exposed to several seed priming treatments and planted into clay loam (Winnipeg) and sandy loam (Carman) soils in a controlled environment (Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.).

Emergence Rate

The soil main effect for emergence rate was not significant, nor was the soil x seed treatment interaction effects (Table 15). This indicated that the same trends were observed between the seed treatments in both the Carman and Winnipeg soils. However, since the soil x species interaction was significant, it appeared that the type of soil did have an influence on the performance of the various species.

Both green needlegrass and Kentucky bluegrass emerged faster in the Winnipeg soil than they did in the Carman soil (Table 17). This could be attributed to the fact that they are both C_3 grasses and their seeds are not able to germinate as quick in the drier, sandier Carman soil. Prairie sandreed, little bluestem, and blue grama are all C_4 grasses which are especially adapted to dry conditions (Raven et al., 1986). The emergence rate of these species was the same in both the Winnipeg and Carman soils (Table 17).

Both the species and seed treatment main effects were significant (Table 15). However, since the interaction between these two factors was also significant, the focus of the discussion will be based on how the seed treatments influenced emergence rate within each species.

There was no difference found in the emergence rate of any of the five blue grama seed treatments (Table 17). This is very similar to the results found in the *Petri Dish Experiment* (Table 14). Blue grama is known to emerge extremely fast (Briske and Wilson, 1977), and it appeared that the priming methods of this study did not increase its emergence rate.

Table 17. Seed Priming Indoor Experiment: number of days to reach 50% of the highest emergence number (E_{50}) of 5 grass species exposed to several priming and drying treatments and planted into clay-loam (Winnipeg) and sandy-loam (Carman) soils in a controlled environment. Based on arc sine transformed data.

	Species[z]					Soil Type[y]		
Factor	BG	GN	KB	LB	PS	Carman	Winnipeg	All
Seed Trtmnt[x][w]								
C	6.1 *a*[v]	12.7 *d*	10.8 *a*	9.6 *b*	8.0 *a*	10.1	8.8	9.4 *de*
CT	6.1 *a*	13.4 *d*	10.6 *a*	9.3 *ab*	8.1 *a*	10.0	9.0	9.5 *e*
H1	6.0 *a*	13.1 *d*	10.5 *a*	8.6 *a*	7.5 *a*	9.6	8.7	9.2 *cd*
H3	6.0 *a*	11.8 *c*	10.1 *a*	8.6 *a*	7.8 *a*	9.1	8.6	8.9 *bc*
K1	6.0 *a*	10.8 *b*	10.5 *a*	8.6 *a*	7.9 *a*	9.3	8.3	8.8 *ab*
K3	6.0 *a*	9.8 *a*	10.3 *a*	8.6 *a*	7.9 *a*	8.9	8.1	8.5 *ab*
Soil Type[u]								
Carman	6.5 *a*	13.0 *b*	12.1 *b*	8.3 *a*	7.6 *a*			9.5
Winnipeg	5.6 *a*	10.8 *a*	8.8 *a*	9.5 *a*	8.1 *a*			8.6

[z]BG = blue grama; GN = green needlegrass; KB = Kentucky bluegrass; LB = little bluestem; PS = prairie sandreed

[y]Seed treatments averaged over all species and compared within soil type.

[x]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[w]Seed treatments averaged over both soil types and compared within species.

[v]In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

[u]Soil types averaged over all seed treatments and compared within species.

Green needlegrass showed no significant difference between untreated control and soaked and dried control seed (Table 17), indicating that drying the seeds after priming was not harmful. The results obtained here are somewhat similar to those found in the *Petri Dish Experiment* (Table 14) in that the emergence rate was as good or better for the priming treatments than it was for the untreated control. The results were not exactly the same because the 3 week water treatment of the *Petri Dish Experiment* actually took longer to germinate than the untreated control. Since the seeds used for the *Growth Room Experiment* were planted into soil, and therefore, allowed to germinate in the dark as is

recommended by AOSA (Maxon, 1995), it is likely that these emergence rate results were more realistic than those found for the germination rate in the *Petri Dish Experiment.*

All the green needlegrass priming treatments produced faster emergence than the controls, with the exception of the 1 week water treatment which had the same emergence rate (Table 17). Seeds primed for 3 weeks in water emerged 1.3 days faster than seeds that were only primed for 1 week in water. The same trend was observed for the KNO_3 based priming treatments where the 3 week chilling period produced seeds which emerged 1.0 day faster than those with a 1 week chilling period. These results indicated that a longer chilling period will produce a faster seed emergence than a shorter chilling period. Both the KNO_3 based treatments produced seed with faster seedling emergence than seed from the water-based treatments. Priming for 3 weeks using KNO_3 produced the fastest emergence rate of any of the treatments, 2.9 days faster than the untreated control. This species' emergence rate benefited the most of all the other species from the priming treatments. This was logical since it has such a hard seed coat and at least 50% of its dormancy is considered to be associated with the lemma and palea (Fendall and Carter, 1965). Its seed coat does not restrict water uptake, but rather, restricts oxygen utilization by the embryo, and physically prevents coleoptile and radicle emergence (Frank and Larson, 1970). The longer the seed can be stratified, the more likely it is that these physical barriers will be overcome and the seed will germinate.

Kentucky bluegrass showed no change in rate of emergence with any of the seed treatments (Table 17). This was essentially the same result as found in the *Petri Dish Experiment* (Table 14) which was interesting because many recent studies have found

that priming was very effective in increasing the germination or emergence rate of Kentucky bluegrass (Yamamoto *et al.*, 1997a, 1997b; Pill and Korengel, 1997; Pill *et al.*, 1997). In the *Petri Dish Experiment*, the drying process was thought to be masking the positive effects of the priming treatments. However, there does not appear to be the same effect happening in this experiment.

Little bluestem also showed no difference between the emergence rate of the controls (Table 17). This was the same finding as that of the *Petri Dish Experiment*, which indicated that any advantage gained from the priming treatments was not lost after drying the seeds. As well, in the *Petri Dish Experiment*, only the 1 week water priming treatment produced seed with a faster germination time than the controls, and all the other priming treatments germinated at the same time as the untreated control. In this experiment, all of the priming treatments emerged 1.0 day faster than the untreated control seed. There was no advantage gained by using any particular priming method since each had an emergence rate of 8.6 days.

There was no difference in emergence rate for any of the prairie sandreed seed treatments (Table 17). This was very different than the results found in the *Petri Dish Experiment* which found that both water based priming treatments and the 3 week KNO_3 priming treatment produced seed that had a faster germination rate than the untreated control (Table 14). It is possible that the advantages of the priming treatments were not expressed under the more realistic conditions of the *Growth Room Experiment*, although none of the treatments were detrimental to the emergence rate.

Development Stage At 28 DAP

The ANOVA test conducted on the development stage at 28 DAP showed that there were significant main effects for soil and species (Table 15). The interaction between these two factors was also significant, indicating that the species exhibited different effects depending on what type of soil they were grown in. The soil x seed treatment interaction and the 3-way interaction between soil, species, and seed treatment were not significant, so the seed treatment effects of each species could be averaged together when discussing the effect the soil type had on each species.

Seedlings grown in the Winnipeg soil generally had a higher development stage than those grown in the Carman soil (Table 18). When the actual species x soil relationships were considered, however, only Kentucky bluegrass exhibited a higher development stage in the Winnipeg soil. There was no difference between soil types for any of the other species. The advantage that Kentucky bluegrass seedlings had in the Winnipeg soil could be a reflection of its higher water holding capacity. Kentucky bluegrass is known to require a lot of moisture to get it established (Smoliak, 1981) and this may not have been available in the sandier soil of Carman since it tended to dry much faster than the Winnipeg soil. Kentucky bluegrass is also not tolerant to acidic soils and will perform best in the pH range of 5.8 to 8.2 (Smoliak, 1981). The Carman soil used in this experiment had a pH of 5.8 while the Winnipeg soil had a pH of 7.6 (Table 4). The higher acidity of the Carman soil may have acted to slow the development of the Kentucky bluegrass seedlings.

The seed treatment main effect and the soil x seed treatment interaction effect were not significant (Table 15). However, the species x seed treatment interaction was significant, meaning that the seed treatments affected each individual species differently. This effect was consistent regardless of soil type.

Table 18. Seed Priming Indoor Experiment: development stage 28 days after planting of 5 grass species exposed to several priming and drying treatments and planted into clay-loam (Winnipeg) and sandy-loam (Carman) soils in a controlled environment. Haun Scale used to determine development stage.

	Species[z]					Soil Type[y]		
	BG	GN	KB	LB	PS	Carman	Winnipeg	All
Seed Trtmnt[x w]								
C	3.00 *a*[v]	1.69 *ab*	3.16 *ab*	3.29 *a*	2.30 *a*	2.63	2.74	2.69 *a*
CT	2.94 *ab*	1.51 *bc*	3.21 *ab*	3.22 *a*	2.15 *a*	2.54	2.67	2.61 *ab*
H1	2.61 *c*	1.46 *c*	3.09 *b*	3.28 *a*	2.33 *a*	2.51	2.59	2.55 *b*
H3	2.75 *bc*	1.56 *abc*	3.31 *a*	3.09 *a*	2.24 *a*	2.59	2.59	2.59 *ab*
K1	2.64 *c*	1.72 *ab*	3.15 *ab*	3.21 *a*	2.34 *a*	2.48	2.74	2.61 *ab*
K3	2.76 *bc*	1.74 *ab*	3.35 *a*	3.25 *a*	2.26 *a*	2.60	2.74	2.67 *a*
Soil Type[u]								
Carman	2.91 *a*	1.59 *a*	2.94 *b*	3.16 *a*	2.19 *a*			2.56 *b*
Winnipeg	2.65 *a*	1.63 *a*	3.48 *a*	3.29 *a*	2.34 *a*			2.68 *a*

[z]BG = blue grama; GN = green needlegrass; KB = Kentucky bluegrass; LB = little bluestem; PS = prairie sandreed

[y]Seed treatments averaged over all species and compared within soil type.

[x]Control treatments: C = untreated seed; CT = soaked in water for 1 hour, then dried. Priming treatments: H1 = soaked in water, chilled 1 week, then dried; H3 = soaked in water, chilled 3 weeks, then dried; K1 = soaked in 0.5% KNO_3, chilled 1 week, then dried; K3 = soaked in 0.5% KNO_3, chilled 3 weeks, then dried.

[w]Seed treatments averaged over both soil types and compared within species.

[v]In a column, means followed by a common letter are not significantly different at the 5% level according to an LSD test.

[u]Soil types averaged over all seed treatments and compared within species.

Blue grama development stage did not differ between the two controls (Table 18). However, each priming treatment produced a lower development stage than the untreated control seed and there was no difference found in development stage between these four priming treatments. This indicated that the priming treatments actually slowed the seedling development of this species. Blue grama development and establishment is

known to be dependent on its ability to develop adventitious roots (Briske and Wilson, 1977). This is because blue grama forms these roots at or very near to the soil surface where moisture conditions are rarely conducive to root development. Beckman *et al.* (1993) found that solid matrix priming treatments of big bluestem did not affect the average number of adventitious roots per plant. Thus, priming may not be beneficial to the overall development of blue grama because it did not promote adventitious root development. In fact, blue grama is the only species in our study that exhibited a drop in emergence numbers from the 10 DAP count to the 20 DAP count (Table 16). This does not explain why the development stage of the primed seedlings is lower than that for the untreated seedlings.

There was no difference between the untreated control and the soaked and dried control seed for green needlegrass (Table 18). In fact, almost all seed treatments produced identical development stages as that achieved by the untreated control seed. The exception was the 1 week water primed seed which produced a lower development stage than the untreated control seed. Studies of other grasses have found that more rapid seedling emergence of primed seed than from nonprimed seed resulted in a greater seedling shoot fresh and dry mass (Frett and Pill, 1995; Pill and Korengel, 1997; Pill *et al.*, 1997) and advanced seedling growth (Yamamota *et al.*, 1997a). Brocklehurst *et al.* (1984) observed that the greater plant growth exhibited by primed leek seed as opposed to nonprimed leek seed was a result of earlier seedling emergence, not increased relative growth rate. Since the green needlegrass emergence rate of the primed seed was increased greatly from that of the untreated seed, it was thought that the development stage would also increase. This was not the case in this study. A similar result was found

by Yamamoto *et al.* (1997b) for Kentucky bluegrass. They found that the rate of second and third leaf emergence and seedling size 28 days after emergence did not differ between primed and non primed seedlings even though the number of days required for 50% emergence was shortened by 5 to 14 days for the primed seedlings.

All the seed treatments of Kentucky bluegrass produced the same development stage as the untreated control seed (Table 18). This was expected since the emergence rate of Kentucky bluegrass also did not change because of priming. Yamamoto *et al.* (1997a) found that quicker emergence from solid matrix primed Kentucky bluegrass seed resulted in seedlings with more advanced growth. Pill and Korengel (1997) found that Kentucky bluegrass seedling shoot mass was greater for primed seed than for nonprimed seed because of an advancement in germination, not because of the stimulation of growth. Since the emergence rate of Kentucky bluegrass did not increase in this study, it was unlikely that the development stage would increase either.

Both little bluestem and prairie sandreed showed no difference between any of the six seed treatments (Table 18). This would seem to be a logical result for prairie sandreed, considering the fact that there was also no difference observed in its emergence rate (Table 17). In contrast, the emergence rate of little bluestem was greater for the primed seed than it was for the nonprimed seed and the explanation for this could be similar as that discussed for green needlegrass.

5. GENERAL DISCUSSION AND CONCLUSIONS

There have been many seed priming methods developed in the past that have sought to improve the germination and emergence of grasses. Most have involved complex methods that promote the start of germination, but prevent its completion. This complexity makes it very difficult to extrapolate success obtained in the laboratory to success in the field. In this study, a priming protocol was developed that incorporated the use of temperature as a means to control the germination process. It was used successfully to increase germination and emergence of several different grass species.

Little bluestem seed showed the most positive responses to treatments tested in this study for all the species tested. Each of the priming treatments increased the final germination percentage, emergence count, and emergence rate of little bluestem. Green needlegrass seed also showed a positive response to some of the priming treatments. Both of the KNO_3 based treatments produced a greater germination percentage and emergence count than the untreated control. The results for blue grama were also positive, but not to the same degree as they were for little bluestem and green needlegrass. There was no advantage observed for the priming treatments in the final germination percentage test, although each of the priming treatments had an advantage in the emergence count test.

The use of KNO_3 did not appear to be beneficial to prairie sandreed seeds and might have actually had a detrimental effect. The best results were obtained by the water based treatments. Essentially, none of the priming treatments produced a germination or emergence advantage for the Kentucky bluegrass seeds. This was the one species that

showed no benefit from any of the priming treatments. It was also the only introduced species in the study and the one with the least level of seed dormancy.

Although the priming protocol developed here was effective for some species, there are still some adjustments that could be made to it. For example, the effect of drying the seed after the chilling period could be investigated further. The results of this study indicated that the drying process was generally not detrimental to germination or emergence of the species tested. However, that is not to say that the process was not damaging. In fact, it could be that comparing the soak and dry control treatment to the untreated control was not a good indicator of the drying effect. A better method of assessment may have been to include a treatment of seed that was primed, but not dried. This was not done in this study because it would have doubled the number of treatments, making the experiment quite unwieldy. The number of species being evaluated could have been reduced to accommodate the extra treatments, but we felt it was more valuable to test as many species as we could.

It might also have been beneficial to measure the seed moisture percentage immediately after the soaking period. This would have allowed a better assessment of moisture loss during the chilling period. It would have been interesting to know if more moisture was lost during the 3 week chilling period than was lost during the 1 week chilling period.

In the future, much more extensive testing should be completed to ensure that the best possible priming method has been determined for a particular species. Positive results have been observed in this study, and the following framework is suggested for future research. The priming methodology developed here could be used as a screening

process. Specifically, the *Petri Dish and Growth Room Experiments* could be used for preliminary screening of different species, cultivars, and seed lots. The successful priming treatments could then be selected and tested under field conditions to make sure they actually work. This would also allow a much more detailed examination of specific priming treatments since there would be fewer of them to evaluate in the field setting. Once it had been determined what particular priming treatment worked the best, efforts could be made to apply it onto a commercial scale. The simplicity of the priming protocol developed here should allow this transition to occur without too much trouble.

One of the biggest limitations priming methods are faced with is the complexity of developing a system that will work consistently for different species, cultivars of the same species, and seed lots of the same cultivar. The methodology developed in this study is simple enough to be extrapolated to a larger scale and can also be used as a screening method to determine the parameters that will be most effective for that particular seed lot, cultivar, or species.

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