Methods for enhancing seed germination of eastern gamagrass

Carla Jeanne Rogis

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Recommended Citation
Rogis, Carla Jeanne, "Methods for enhancing seed germination of eastern gamagrass" (2002). Retrospective Theses and Dissertations. 21306.
https://lib.dr.iastate.edu/rtd/21306

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Methods for enhancing seed germination of eastern gamagrass

by

Carla Jeanne Rogis

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:
Lance R. Gibson (Major Professor)
Allen Knapp
Robert Horton

Iowa State University
Ames, Iowa
2002

Graduate College
Iowa State University
This is to certify that the master’s thesis of

Carla Jeanne Rogis

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
# TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION ................................................................. 1

   Thesis organization .......................................................................................... 2

CHAPTER 2. LITERATURE REVIEW ...................................................................... 3

   Potential of eastern gamagrass as a forage crop ............................................... 3
   Use of eastern gamagrass for conservation purposes ......................................... 5
   Problems limiting the use of eastern gamagrass ............................................... 5
   Reproductive anatomy of eastern gamagrass .................................................... 6
   Seed dormancy .................................................................................................. 7
   Importance of temperature to seed dormancy ............................................... 10
   Seed stratification .............................................................................................. 10
   Respiration and gas exchange effects on seed dormancy .............................. 12
   Stratification effects on seed hormones .......................................................... 13
   Pre-sowing conditioning of seed ...................................................................... 15
   Conclusion ........................................................................................................ 19

CHAPTER 3. ENHANCING GERMINATION OF EASTERN GAMAGRASS WITH 
MOIST-PRECHILLING AND GA₃ ......................................................................... 21

   Abstract ........................................................................................................... 21
   Introduction ....................................................................................................... 21
   Materials and methods .................................................................................... 23
      Statistical analysis ......................................................................................... 24
   Results .............................................................................................................. 25
      Total seed germination ................................................................................. 25
      Germination rate .......................................................................................... 26
      Abnormal seedlings, dormant seed, and dead seed .................................... 27
CHAPTER 1. GENERAL INTRODUCTION

Eastern gamagrass (*Tripsacum dactyloides*, L.) is a perennial grass species found from the northeastern and north central United States and south into Mexico, Central America, the Caribbean, and into Bolivia and Paraguay in South America (Newell and deWet, 1974). High productivity and palatability by grazing animals relative to other warm-season grasses make it attractive as a forage crop. Fine et al. (1990) found forage dry-matter production to be 5.0 Mg ha\(^{-1}\) in the first year of establishment with an increase to 22.6 Mg ha\(^{-1}\) by the third year. The quality of eastern gamagrass hay has been found to be more than adequate to meet the energy and protein requirements of many ruminants (Burns et al., 1996). The hay also favored higher daily gains by animals in comparison to switchgrass (*Panicum virgatum* L.) and flaccidgrass (*Pennisetum flaccidum* Griseb.) (Burns et al., 1996). Horner et al. (1985) found that eastern gamagrass was nearly equal to alfalfa hay in digestibility. The plant characteristics, high leaf proportion and low dead tissue ratio, made gamagrass nearly ideal for grazing and lead to high daily gain by animals (Burns et al., 1992).

Eastern gamagrass also has high potential for use in conservation practices. One way to slow runoff and prevent soil erosion is to plant narrow grass hedges (Dewald et al., 1996). Because of gamagrass’s natural tolerance to both drought and soil wetness, its perennial nature, and its growth from a strong crown, it is very suitable for grass hedges. A densely uniform stand of eastern gamagrass would slow concentrated runoff. Also, the bunched crowns of the grass would cause backwaters that would allow deposition of sediment. This deposition would even out low spots and eventually disperse run-off so that it is less erosive.

Even though eastern gamagrass is highly productive, has sufficient forage quality, and can be used to reduce soil erosion from croplands, it is grown on a limited land area in the U.S. Problems with stand establishment have been a major restraint to its adoption. Stand establishment problems in eastern gamagrass result, at least partially, from a very strong seed dormancy. Ahring and Frank (1968) reported that it was not uncommon to find only 3 to 5%
germination of newly harvested seed. The seed dormancy mechanisms of this species are not yet fully understood. A better understanding of the inhibitors to gamagrass seed germination and development of methods for breaking the seed dormancy could lead to management strategies that result in more widespread use of this promising warm-season grass.

This thesis is a report of methods tested for breaking seed dormancy in eastern gamagrass and new insight into the dormancy mechanisms in this species. In one study, eastern gamagrass seed germination after treatment with a combination of two known germination promoters – moist, pre-chilling and gibberellic acid (GA) – was compared to germination after moist, pre-chilling alone and no pre-chilling. In a second study, solid matrix priming methods for eastern gamagrass seed were developed for three solid materials. Water uptake into eastern gamagrass seeds was determined at various water potentials in each material. Solid matrix priming systems were then designed to hydrate eastern gamagrass seed to levels that would stimulate the early phases of germination while restricting advancement into later stages of germination. Germination of eastern gamagrass seed was observed after 7 d of conditioning in each of the three solid materials hydrated at either high or low water potentials with water, buffered GA$_3$ solution, unbuffered GA$_3$ solution, or buffer solution alone to determine if any of these systems could break dormancy.

**Thesis organization**

This thesis is organized in journal manuscript format. Chapter 1 is a general introduction and a description of the thesis contents. Chapter 2 is a review of literature pertinent to the research questions and studies. Chapter 3 contains a paper on the combined effects of moist, pre-chilling and GA$_3$ on eastern gamagrass germination that will be submitted to a scientific journal. Chapter 4 contains a second manuscript that describes systems for solid matrix priming of eastern gamagrass seed. Chapter 5 summarizes the research and discusses implications of the results. The literature cited in Chapters 1, 2, and 5 is listed in a section that follows Chapter 5.
CHAPTER 2. LITERATURE REVIEW

Eastern gamagrass (*Tripsacum dactyloides*, L.) is a perennial grass species found from the northeastern and north central United States and south into Mexico, Central America, the Caribbean, and into Bolivia and Paraguay in South America (Hitchcock and Chase, 1950; Newell and de Wet, 1974). Polk and Adcock (1964) describe historical accounts of eastern gamagrass being highly valued for its grazing potential. Early settlers grazed milk cows and horses on it and claimed it produced better tasting milk and longer working horses. Because eastern gamagrass was preferred by many animal species for grazing and early settlers allowed animals to graze this grass intensely for extended periods of time, most natural stands of eastern gamagrass no longer exist (Polk and Adcock, 1964). Interest in eastern gamagrass for grazing, hay, silage, and conservation uses has resurfaced in recent years due to research studies that have identified the high productivity (Aiken, 1998) and quality of eastern gamagrass compared to other species (Horner et al., 1985; Burns et al., 1992, 1996) and traits that make it desirable for soil conservation (Dewald et al., 1996).

**Potential of eastern gamagrass as a forage crop**

Ruminant livestock production in much of the northern U.S. and other temperate regions throughout the world relies on grazing of cool-season grasses. About 60-70% of most cool-season grass’s growth occurs by July 1 in the northern U.S. These grasses grow very little and often become dormant during the hot, dry summer months of July and August (Matches and Burns, 1995). During this lag in productive grass growth, grazing animals can suffer in performance due to the grazing of poor quality grasses (Matches and Burns, 1995). Some additional growth in cool-season grasses occurs during the autumn months with adequate rainfall. One solution to the gap in summer production of cool-season forages is to plant warm-season grasses, which have their greatest growth in June through September in the northern hemisphere (Volenc and Nelson, 1995). Warm-season grasses perform better with warm, summer temperatures than they do in cooler temperatures (Kephart et al., 1995).
Incorporation of these warm-season grasses into a rotational grazing system can even the forage supply throughout the entire growing season. Eastern gamagrass (*Tripsacum dactyloides* L.) is a warm-season grass that could be used to enhance summer forage production in the U.S.

High productivity and palatability relative to other warm-season grasses makes eastern gamagrass attractive as a grazing and forage crop. Fine et al. (1990) found forage dry-matter production to be 5.0 Mg ha$^{-1}$ in the first year of establishment with an increase to 22.6 Mg ha$^{-1}$ by the third year. Eastern gamagrass has plant characteristics, such as a high leaf to stem ratio and leaves positioned well above the heavy stem bases, that make it conducive to providing high daily animal rate of gain (Burns et al., 1992). Eastern gamagrass also maintained a low proportion of dead tissue compared to flaccidgrass (*Pennisetum flaccidum* Griseb) and bermudagrass (*Cynodon dactylon* [L.] Pers.). The quality of eastern gamagrass hay has been found to be more than adequate to meet the energy and protein requirements of ruminants and gave higher daily gains in wethers when compared to switchgrass (*Panicum virgatum* L.) and flaccidgrass (Burns et al., 1996).

Horner et al. (1985) found that eastern gamagrass was nearly equal to alfalfa hay in digestibility, but lower in feeding value. Dry matter intake of eastern gamagrass when fed to mature wethers was found to be similar to that of alfalfa hay. However, first cutting eastern gamagrass was significantly higher in dry matter digestibility (DMD) with a DMD of 57.3% compared to alfalfa’s 53.6%. The neutral detergent fiber (NDF) and acid detergent fiber levels of eastern gamagrass were higher than those of alfalfa, but were more digestible than these fractions in alfalfa hay. The lower feeding value was attributed to the chemical composition of the eastern gamagrass hay. Alfalfa hay contained higher levels of crude protein and lower levels of NDF than first and second-cuttings of eastern gamagrass hay. Eastern gamagrass met the maintenance and lactation requirements for crude protein outlined
by the National Research Council indicating that it could be useful in livestock feeding systems (Horner, 1985).

Eastern gamagrass hay compared favorably to hay of other high quality warm-season grasses (Burns et al., 1996). There was higher nitrogen retention by animals consuming eastern gamagrass when compared to switchgrass and flaccidgrass. Dry matter intake of both vegetative and reproductive eastern gamagrass by wethers was greater than that for flaccidgrass, but there was no intake difference between eastern gamagrass and switchgrass (Burns et al., 1996). There was no difference in intake of vegetative and reproductive eastern gamagrass differing in maturity by four weeks.

Use of eastern gamagrass for conservation purposes

Eastern gamagrass also has great potential for use in conservation practices. Eastern gamagrass’s natural tolerance to drought and soil wetness, its perennial nature, and its growth from a strong crown, make it a good species for controlling soil erosion. One, highly underutilized, way to slow runoff and prevent soil erosion is to plant narrow grass hedges (Dewald et al., 1996). Grass hedges are narrow strips of erect grasses planted in parallel lines perpendicular to slopes. These hedges slow concentrated runoff and cause backwaters that result in the deposition of eroded sediments. This, in turn, results in berms that fill in low spots and help disperse future runoff (Dewald et al., 1996). The crown structure of eastern gamagrass provides a sturdy base and its vegetative material has the capacity to grow through sediment, so the plant will continue to survive despite sediment inundation. The bunched crowns of the grass trap sediment, which produces a natural terracing effect.

Problems limiting the use of eastern gamagrass

The merits of eastern gamagrass as a grazing material, as well as a conservation tool, make it an attractive option for producers. However, there are several reasons eastern gamagrass is not commonly used by farmers and ranchers. It was preferentially grazed from native range early in the settlement of the U.S. (Polk and Adcock, 1964). Because of an
inability to withstand overgrazing, eastern gamagrass was killed when extensive grazing or hay production reduced the vegetative growth below 15 cm for an extended period of time (Polk and Adcock, 1964). However, eastern gamagrass can persist under a variety of animal stocking rates when grazing is deferred for the remainder of the growing season when canopy height falls to 30 to 38 cm (Aiken and Springer, 1998).

Difficulty of stand establishment has severely limited the reseeding of eastern gamagrass into native ranges and mechanical seeding by forage producers and conservationists. Like many warm-season grasses, eastern gamagrass exhibits an extremely high level of seed dormancy. Seed testing laboratories commonly find only 3 to 5% germination in newly harvested eastern gamagrass seed lots (Ahring and Frank, 1968). This dormancy can be an impediment to obtaining a full and immediate stand in commercial plantings. Therefore, eastern gamagrass use is likely to lag behind that of other forage and conservation species until suitable methods of breaking the seed dormancy are found.

**Reproductive anatomy of eastern gamagrass**

The inflorescence of eastern gamagrass is described as a spike. The inflorescence on the main stem of the plant consists of 2 to 4 spikes and the branch stems usually contain a single spike (Chase, 1950). The lower portion of the spike consists of cupules stacked one on top of another. The staminate floral structures, which make up about one-half of the eastern gamagrass spike, are located above the jointed cupules. The cupule, which serves as the seed dispersal unit, is an indurate covering that adheres to the mature caryopsis (Hitchcock and Chase, 1950). It consists of a rigid, hollow, modified rachis and two glumes. Together, the rachis and glumes surround the caryopsis. A lemma and the very thin palea lie between the glumes and the caryopsis.

Some eastern gamagrass cupules contain no caryopsis or a caryopsis of poor, underdeveloped quality. Ahring and Frank (1968) found that only 52 to 68% of cupules actually contained a seed. It is impossible to distinguish these empty or underdeveloped
seeds visually because of the thick coverings. Not only does this covering make quality
determination difficult, but has also been attributed as a cause of the species' high level of
seed dormancy (Anderson, 1985; Tian et al., 2002a).

**Seed dormancy**

The definition of dormancy as "a physical or physiological condition of a viable seed that
prevents germination even in the presence of otherwise favorable germination conditions"
provided by Copeland and McDonald (1995) is representative and easily understood. Seed
dormancy is a survival characteristic of plants that allows seed germination to occur during
the most favorable conditions available for germination and seedling development (Copeland
and McDonald, 1995).

Seed dormancy is a mechanism through which plants are able to adapt and survive their
environment (Copeland and McDonald, 1995). Dormancy is a desirable quality in seeds
because it prevents preharvest sprouting and maintains seed quality. Dormancy also gives
seeds a time period in which they can take advantage of other adaptive benefits. Examples of
these benefits are: dispersal by wind, water, and organisms, avoidance of temporary
conditions that cannot support growth of the vegetative phase of the seedling, and avoidance
of competition from other plants (Simpson, 1990). However, if the dormancy is not
overcome before exposure to environmental conditions favorable for germination, it creates
difficulties in crop establishment and management (Copeland and McDonald, 1995).

Many researchers have attempted to classify seed dormancy. Simpson (1990) explained
that primary dormancy, or the dormancy most seeds experience, can be renewed in a seed
after it is shed from the parent plant and the dormancy is then termed "secondary dormancy".
Nikolaeva (1977) maintains that there are three types of primary dormancy - exogenous,
endogenous, and mixed. Exogenous dormancy can be some kind of physical or chemical
property of the seed coat that prevents the seed from germinating. Physical dormancy is also
called hardseededness and is often a factor of seed coats being impermeable to water. It
could also be a result of some kind of mechanical resistance to a germinating embryo. Under natural conditions, hardseededness is removed by repeated exposure to thermal and biological influences. Chemical dormancy is often seen in plants of tropical origin in which pericarps or other structures of the seed release some kind of inhibitor that prohibits germination. In a natural system these inhibitors are usually leached from the seed during a rainy season.

Endogenous dormancy also has several subgroups (Nikolaeva, 1977). Overall, endogenous dormancy results from some anomaly of the embryo itself. One category of endogenous dormancy is morphological dormancy. This is most commonly the underdevelopment of the embryo. Another example of endogenous dormancy is physiological dormancy, which is attributed to a decreased functionality of the embryo to complete processes necessary for germination. Physiological dormancies are often overcome by low temperatures, dry storage, or altering and controlling light levels (Nikolaeva, 1977). Nikolaeva (1977) also goes on to further classify physiological dormancy into the relatively vague divisions of non-deep, intermediate, and deep physiological dormancy. Another line of thinking divides dormancy into three simple categories of endo-dormancy, para-dormancy, and eco-dormancy (Simpson, 1990). Endo-dormancy is the term for dormancy caused by physiological factors inside the seed. Para-dormancy is the dormancy caused by physiological factors outside the affected seeds. Finally, eco-dormancy is the term given to the dormancy controlled by environmental factors.

The classification of seed dormancy based on the seed’s reaction to outside forces can confuse the true concept of dormancy. The classifications above often point to environment, genetics, or location of the seed on the mother plant as creators of seed dormancy. In actuality, these factors may control the expression of dormancy, but processes within the seed determine whether germination occurs or not. Ultimately, most classifications systems for dormancy are inadequate because the definitions used do not separate the effect
(dormancy or breaking of dormancy) from the physiological factors involved. Eliminating the confusion created by the dormancy classification based on the seeds reaction to outside forces could lead to more effective communication between scientists.

Dormancy is an amazing phenomenon controlled by factors intrinsic to the seed. It must be understood that seed dormancy often cannot be traced to one control or cause. A seed, like any organism, is a complex, dynamic, biological system in a changing environment. Many controls of dormancy overlap and even contribute to each other (Simpson, 1990).

Although seed dormancy in eastern gamagrass is not well understood, there may be several possible controls. The cupule could be a physical restriction to water and/or oxygen uptake or a mechanical constraint to embryo expansion. Springer et al. (2001) studied a synthetic cupule's effect on caryopsis germination by placing caryopsis extracted from eastern gamagrass cupules into plastic tubes. “Banded” caryopses had a germination of 4 to 8% and the un-banded or naked caryopses achieved 38 to 50% germination levels. Because no chemical germination inhibitors could be excreted by the synthetic cupule or “band”, the lower germination levels were attributed to mechanical restriction of the germinating seed by the band.

Tian et al. (2002a) tested the roles of the cupule and pericarp on seed dormancy of eastern gamagrass. Cupule removal resulted in 16 to 48% germination depending on the seed lot tested, compared to 5 to 18% germination for seeds with intact cupules. Cupule removal followed by pericarp scarification resulted in germination of all viable seeds. It was concluded that cupule removal and pericarp scarification overcame the seed dormancy in eastern gamagrass. However, establishing a mechanical system to remove the cupule and scarify the pericarp without damaging the caryopses would be difficult because the cupule shape and size of eastern gamagrass is not uniform, the embryo extends the length of the caryopsis, and the embryo is not recessed relative to the surface of the caryopsis.
Importance of temperature to seed dormancy

In some species, dormant conditions are only seen at certain temperatures. For example, barley and wheat exhibit dormancy of varying levels at temperatures greater than 15°C. However, once exposed to temperatures colder than 15°C, 100% germination of these species is observed (Bewley and Black, 1994). Probert (1994) claims that temperature is “arguably the most important environmental variable responsible for the synchronization of germination with conditions suitable for seedling establishment.” Three separate physiological processes have been recognized in seeds as being affected by temperature (Probert, 2000). First, temperature and moisture content determine the rate of deterioration in all seeds. Second, temperature affects the rate of dormancy loss in seeds. Finally, temperature affects germination rate of non-dormant seeds. One hypothesis as to why these physiological processes occur is that abrupt changes of temperature are known to cause a phase transition in cell membranes (Bewley and Black, 1994). This phase transition causes a decrease in membrane activity while increasing the permeability of the membrane. This may allow for the transport of germination promoters in or inhibitors out of cells required for inducing germination in the seed.

Seed stratification

Pre-chilling of seeds, sometimes referred to as stratification, has been developed as a method for manipulating temperatures to break seed dormancy and encourage uniform germination. The designation as “stratification” comes from early studies on horticultural crops where seed was placed between layers or “strata” of sod (Probert, 2000). The seeds were allowed to imbibe moisture from the sod layers and were left outside over winter for exposure to cold temperatures. These treatments resulted in better germination of fruit seeds. In fact, as early as 1664, Evlyn reported that before spring sowing of forestry species, such as Acer and Fagus, seeds should first be placed in moist sand or soil and held out-doors for the winter (Probert, 2000). Stratification can be done without the sod layers. Most methods
involve allowing seeds to imbibe water to a certain percentage of water by weight and then exposing them to temperatures between 3°C and 10°C (Copeland and McDonald, 1995). Duration of exposure varies considerably from days to years of cool temperature between species (Probert, 2000).

Stratification has been identified as one of the few suitable seed dormancy breaking mechanisms in eastern gamagrass (Ahring and Frank, 1968; Anderson, 1985). Ahring and Frank (1968) concluded that 6 to 8 weeks of moist pre-chilling at 4°C was necessary to promote germination of eastern gamagrass seeds to within 60 to 80% of all live seeds. Anderson (1985) found that a cold, moist treatment period of at least 60 days was necessary for germination of eastern gamagrass seeds with cupules intact.

In Nikolaeva’s (1969) review of the physiology of deep dormancy in seeds, he states that stratification occurs in a series of stages. The first of these stages, which is not dependent on temperature, is the swelling of the seeds that occurs during water uptake in both living and dead seeds. Enzymatic activity is aroused and the main food reserve substances like proteins and fats are broken down and their products flow toward the embryo. This prompts the beginning of embryo growth. Together, the water uptake and nutrient mobilization make up the preparatory phase before germination. Cold temperatures begin to have the greatest effect after embryo growth is initiated. In the presence of cold temperatures, the processes of germination continue and accelerate greatly just before protrusion of the radicle. In contrast, when seeds continue the germination process in constant warm temperature, these processes or phases of germination gradually slow or even stop (Nikolaeva, 1969).

Bewley and Black (1994) acknowledge a phase transition in membranes but also continue the theory of how chilling affects germination. They speculate that this chilling process retards some metabolic response that inhibits germination to the point that other germination promoting processes can prevail despite these inhibiting processes. They concluded that the individual processes required for germination, such as promoter stimulation and inhibitor
deactivation, must respond to temperature differently. Another theory is that chilling affects enzyme concentration or production; however, no experimental evidence has been found to support these theories.

**Respiration and gas exchange effects on seed dormancy**

Limitations to gas exchange between the atmosphere outside the seed and the embryo has been suggested as a dormancy mechanism because temperature changes and the integrity of structures covering the seed can affect the dormancy level of seeds (Nikolaeva, 1977). The pericarp, the endosperm, and floral structures that remain with the seed are all covering structures that could limit gas exchange. Oxygen is more soluble in cold water, so the oxygen requirements of the embryo may be better satisfied when seeds are moist pre-chilled (Probert, 2000). The cold temperatures cause the imbibed moisture to be a better carrier of oxygen through impeding structures and into the caryopsis.

Nikolaeva (1977) isolated embryos of *Acer tataricum* L., exposed them to different stratification temperatures, and measured oxygen concentration in the embryo. **O₂** concentration was consistently higher in embryos stratified at 0° to 3° C when compared to stratification at 15 to 20° C. Respiration rates tested at 25°C temperatures were 122 µL O₂ h⁻¹ g⁻¹ wet weight for immature embryos stratified in 15° to 20° C and 150 µL O₂ h⁻¹ g⁻¹ for embryos stratified at 0° to 3° C. There was a parallel relationship in intact seeds although respiration rates were much lower in intact seeds than in isolated embryos. The respiration of intact seeds was 37 µL O₂ h⁻¹ g⁻¹ wet weight when stratified at 15° at 20° C and 60 µL O₂ h⁻¹ g⁻¹ for those stratified at 0° to 3° C. Nikolaeva concluded that coverings on the seed were a passive barrier to gas exchange, which limited embryo respiration rates.

Seed covering structures could also be an obstruction to gas diffusion from the seed. Anderson (1985) hypothesized that dormancy of eastern gamagrass caryopses with intact cupules could be related to a buildup of **CO₂** within the seed as a result of respiration. The high **CO₂** levels would not have an inhibitory effect on seed germination. He thought that
stratification might enhance gas permeability of the cupule so that CO₂ could readily diffuse from the seed. This hypothesis proved false when tested. Anderson found that enriched CO₂ levels had insignificant effects on germination of seed with or without covers. Cold, moisture stratification significantly increased germination when compared to cold, dry treatment or storage at room temperature. However, there were no significant differences in germination between seeds stratified in ambient and 5% (enriched) CO₂ environments indicating no CO₂ inhibition. In one population the enriched CO₂ actually increased germination.

**Stratification effects on seed hormones**

The activity and balance of growth hormones are another factor associated with temperature-controlled seed dormancy. Nikolaeva (1977) found the activity of various enzymes to remain low under imbibition in warm temperatures. However, the activity of these enzymes increased under cooler temperatures. Abscisic acid (a known inhibitor of germination) levels drop during the stratification of apple, ash, walnut, and hazelnut seeds while endogenous levels of gibberellins (a promoter) increased. It has been suggested that the activation temperatures of enzymes required for hormone synthesis may control shifts in hormone concentrations within the seed (Bewley and Black, 1994).

Several hormones, including gibberellins, cytokinins, and ethylene, regulate germination processes. Species with seeds that require chilling, light, or after-ripening to stimulate germination often exhibit strong germination responses when exposed to these hormones. The success of these hormones in promoting germination varies greatly among species and many species do not respond at all (Bewley and Black, 1994).

Gibberellins are one of the most widely studied hormones because they stimulate embryo growth and germination (Simpson, 1990). Gibberellic acid (GA) stimulates and regulates α-amylase production after radicle emergence from the germinating seed (Jones and Stoddart,
The \(\alpha\)-amylase is an important stimulant of substrate mobilization for continuation of the germination process (Jones and Stoddart, 1977).

Hilhorst (1998) developed a model for temperature-mediated release of seed dormancy based on a membrane-receptor occupancy theory. The model assumes that a number of active membrane receptors for phytochrome, a receptor pigment for light responses, and nitrate increase or decrease as dormancy is released or induced by chilling. In a non-dormant seed, the activated receptor will bind the physiologically active form of phytochrome, Pfr, and initiate a signal transduction chain, leading to Gibberellic acid (GA) synthesis and germination. The effect of chilling in Hilhorst's model is not to increase GA levels, but to initiate the GA producing mechanism, which results in greater GA production when the seed experiences temperatures suitable for germination.

Gibberellic acid may be a stimulant of eastern gamagrass seed germination. GA applied to eastern gamagrass caryopses removed from the cupule significantly increased germination levels above those for stratified seed and seed stored at room temperature (Anderson, 1985). Tian et al. (2002b) reported that GA\(_3\) solution buffered below it's pKa significantly increased germination of caryopses removed from the cupule to within 9 to 10 percentage points of all viable seeds. Treatment of intact cupules with both buffered and unbuffered GA\(_3\) solution was much less effective and germination only increased to 16% for seed treated with unbuffered GA\(_3\) compared with 9% for soaking in water alone. Germination response from treatment with buffered GA\(_3\) solution was similar to soaking in water. There were significant differences among the seed lots tested in this study suggesting dormancy mechanisms were stronger or weaker due to differences in genetics or production environment. Together the studies of Anderson (1985) and Tian et al. (2002b) demonstrated that GA\(_3\) could influence eastern gamagrass seed dormancy, but the response was heavily influenced by the presence of the cupule structures.
Pre-sowing conditioning of seed

Young seedlings are exposed to many extremely harsh environmental stresses, such as fluctuations of moisture and temperature. In addition to these stresses, delayed germination or uneven stands can open the door to competition from other plant species (Simpson, 1990). In order to create rapid and synchronized germination in a crop with dormancy; the seed must be treated to overcome the dormancy within most of the seeds that will be planted. This treatment is often referred to as pre-sowing conditioning. This concept of applying seed treatments is not a new one. In ancient Greece, farmers would soak seeds in water or milk and honey before planting in hopes of "awakening" the seed and increasing germination rate and emergence (Parera and Cantliffe, 1994). Russian farmers of the 17th century would use salt solutions to treat seeds in attempts for similar results (Parera and Cantliffe, 1994). More advanced methods of pre-conditioning, such as seed coat scarification, exposure to fluctuating temperatures or light, and controlled hydration, have been developed in modern times.

In order to determine the amount of moisture that is adequate to induce germination it is necessary to understand how moisture contents react during germination. Water uptake by the seed during imbibition and germination follows a triphasic pattern (Copeland and McDonald, 1995). The first phase, or Phase I, is a short period of rapid uptake of water by the seed. During this time, enzyme activation occurs in order to prepare the seed for germination. Phase II is often referred to as the lag phase. There is little water uptake, but many processes necessary for germination are occurring. Examples of these processes are increased respiration and loss of dry weight. Also, hydrating to Phase II allows early DNA replication, increased RNA and protein synthesis, and greater ATP availability. These effects are irreversible even after desiccation of the imbibed seed. Phase III occurs as the root elongates from the seed. Because the root is functional at this time, water uptake is increased again (Copeland and McDonald, 1995). Once radicle protrusion occurs the seed is
considered to be germinating and desiccation after this time will likely lead to death (Taylor et al., 1998).

An understanding of this process allows manipulation of seed through controlled hydration. The primary goal of pre-sowing conditioning is to induce the seed to undergo Phase I and Phase II of the water uptake pattern, but prevent seeds from entering Phase III (Kahn, 1992). The seed is then “primed” for germination and the pre-sowing conditioning is stopped before the visible signs of germination. The ultimate goal of conditioning is an ability to dry the seed down again without losing the benefits of the treatment. Water availability or water potentials can be regulated to control the rate and amount of water uptake by seeds (Taylor et al., 1998). Water potential is the sum of the pressure potentials asserted by matric and osmotic forces in a system. Seeds have a critical physiological water potential for germination, usually between 0 and –2 MPa (Harris, 2000).

Matricconditioning and osmoconditioning are grouped within a set of techniques that use matric or osmotic forces to control seed hydration. Collectively, these techniques are referred to as “priming” because they stimulate the early phases of seed germination (Taylor et al., 1998). A matricconditioning system, often referred to as solid matrix priming (SMP), utilizes various ratios of solid carrier and water to establish an equilibrium moisture content and water potential that controls seed hydration (Khan, 1992). Matric forces hold the water in these highly porous, non-soluble materials with high water holding capacity. Benefits of matricconditioning are reduction in the time to germination and seeding emergence, synchronized emergence, and improved stand establishment and yield (Khan, 1992). The slower hydration that occurs during matric priming relative to hydration under field conditions has been shown to reduce the damage caused to seeds that are prone to imbibitional damage in cold temperatures (Khan et al., 1995; Dawidowicz-Grzegorzewska, 1997).
Osmoconditioning is a process of regulating osmotic forces by placing seed in solutions of inorganic salts, such as KNO₃ and K₃PO₄, dissolved in water. Another popular substance used for osmoconditioning is the chemically inert, polyethylene glycol (PEG). The PEG exerts osmotic pressure when dissolved in water, but the large molecules cannot penetrate the seed coat. PEG could also be considered as a matric conditioning substance because the large molecules exert matric forces that influence water uptake into seeds (Parera and Cantliffe, 1994). Agro-Lig, a shale derived solid carrier with a low saturation water content (30-40% by weight), also exerts both osmotic and matric forces (Kahn, 1992). Agro-Lig saturated with water has a matric potential of zero. Since conditioning with Agro-Lig usually involves addition of water equal to 60-95% of Agro-Lig’s weight, osmotic forces control the hydration of the seed added to this system (Khan, 1992).

Certain characteristics are necessary for carriers to be effective for matric conditioning (Kahn et al., 1990; Khan, 1992). They are: (1) high matric potential and therefore negligible solute or osmotic potentials, (2) negligible water solubility, (3) chemical inertness, (4) high water holding capacity in addition to high flowability when dry (5) various particle sizes, structures, and porosities, (6) high surface area, (7) high bulk value and low bulk density, which will provide results at low levels of water addition, and (8) an ability to adhere to the seed surface. Two materials that meet these criteria are Vermiculite #5 and MicroCel E. Vermiculite #5 is a very fine grade of expanded vermiculite (hydrated magnesium-aluminum-silicate) created as by-product of exfoliating larger grades (Khan, 1990). It has a bulk density of 64 to 160 kg m⁻³ and can hold up to 410% of its weight in water. MicroCel E is a synthetic calcium silicate that resembles the natural silicates of diatomaceous earth. This fine powder has a bulk density of 86 kg m⁻³ and holds up to 550% of its weight in water.

Matric conditioning has increased rate of emergence and final emergence (Parera and Cantliffe, 1991, 1992; Kahn et al., 1992, 1995). Khan et al. (1992) examined the effectiveness of matric conditioning in improving stand establishment of several vegetable
seeds. Field emergence of carrot (*Daucus carota* var. *sativus* Hoffm.) was much improved after conditioning with MicroCel. Time to 10% of final emergence (*T*$_{10}$) was reduced by 2.6 to 2.8 d and time to 50% emergence (*T*$_{50}$) was decreased by 2.1 to 3 d. Not only was germination rate increased, but final emergence over the non-conditioned seed was increased by 150% for ‘Nantes’ and by 39% for ‘Long Imperator’. Matriconditioning of tomato (*Lycopersicon esculentum* Mill.) seeds provided little improvement in emergence rate, but increased final percent emergence by 86%. Pepper (*Capsicum annuum* L.) seeds had a 1.5-d reduction in *T*$_{10}$ and *T*$_{50}$ and a 30% increase in total emergence. Conditioning of snap beans (*Phaseolus vulgaris* L.) reduced *T*$_{10}$ and *T*$_{50}$ by 0.8 d; however, total emergence was decreased from 75% to 55%. The explanation for the decrease in emergence was that the high initial water content at the time of planting might have made the snap bean seeds more susceptible to mechanical damage or soilborne disease.

Matriconditioning also provides an easy media for incorporating nutrients, pesticides, and hormones into the seed conditioning process (Khan, 1992).) Pesticides, gibberellin (GA$_3$), and biocontrol agents have been added to conditioning systems using MicroCel E (Khan et al., 1995). Apron (metalaxyl) and thiram were applied through the conditioning liquid to table beet, sugar beet, and watermelon seeds. GA$_3$ was applied to snap beans. Actively growing culture of *Pseudomonas aureofaciens* was suspended in the conditioning water during sweet corn seed conditioning. Matriconditioning with or without these additions increased the rate of emergence and final emergence percentages in early field plantings at suboptimal temperatures. Conditioning alone decreased time to *T*$_{50}$ anywhere from one to three days and final emergence was increased by 10 to 30% depending on the species. Germination was greater when the chemicals were added. Snap bean emergence was improved from 38 to 58% just from conditioning. When fungicides were used in addition to conditioning with GA$_3$, percent emergence was 92%. Conditioning sweet corn with
fungicides in MicroCel E reduced T50 from 13.8 d to 12.5 d and total emergence from 50 to 60% of seeds planted.

Matricconditioning has been studied as a method of improving establishment of seven perennial grasses native to the U.S. Great Basin, which is located between the Rocky, Sierra Nevada, and Cascade mountains (Hardegree, 1994). Improvement of the rate and amount of stand establishment in the native grasses would allow better competition with cheatgrass (*Bromus tectorum* L.), an undesirable, non-native, annual grass. The conditioning system consisted of a PEG solution separated from the seed by a cellulose membrane that only allowed water to pass. The membrane excluded the PEG from contact with the seeds and a matric-potential control surface was created on top of the membrane where the water potential was in equilibrium with the osmotic potential of the solution under the membrane. This conditioning technique increased rate of germination at 10°C and 25°C in all seven perennial grasses.

Beckman et al. (1993) studied the effects of matricconditioning on germination of two species of warm-season grasses. The big bluestem (*Andropogon gerardii* Vitman) cultivars Kaw and Pawnee and Switchgrass (*Panicum virgatum* L.) cultivars Cave-in-Rock and Pathfinder were used in their study. The conditioning of seeds in a solid matrix priming medium moistened with Captan fungicide solution increased emergence of both species when planted in a greenhouse (Beckman, 1993).

**Conclusion**

Dormancy is a complex phenomenon that allows dispersal of seeds in time and germination during conditions that ensure species survival. However, seed dormancy presents considerable challenges to rapid and uniform stand establishment required when cultivating domesticated crops. While seed dormancy was a desirable trait for the survival of native stands of eastern gamagrass, it is a significant impediment to wide adoption of this promising grass for forage and conservation plantings.
The physiological mechanisms that release seed dormancy, which appear to be highly species dependent, are not well understood in eastern gamagrass. Moist, prechilling has been identified as one of the few methods for breaking seed dormancy in eastern gamagrass with intact cupules. However, moist, prechilling is labor intensive and complicates the handling and marketing of eastern gamagrass seed. Cupule removal and scarification can remove nearly all of the seed dormancy in eastern gamagrass. However, a large-scale method of cupule removal that performs uniform cupule extraction without risking the integrity of the caryopsis has not been found.

Additional methods for breaking seed dormancy and enhancing germination could lead to greater use of eastern gamagrass. This thesis contains a report on several systems tested for their ability to enhance eastern gamagrass seed germination. Moist, cold prechilling in combination with gibberellic acid application to seed was evaluated in one study. Systems for solid matrix priming eastern gamagrass in Vermiculite #5, MicroCel E, and Agro-Lig were developed in a second study. Gibberellic acid, and citric-diphosphate buffered gibberellic acid were added to the solid matrix priming systems to determine their ability to stimulate seed germination.
CHAPTER 3. ENHANCING GERMINATION OF EASTERN GAMagrass WITH MOIST-PRECHILLING AND GA₃

A paper to be submitted to *Crop Science*

C. Rogis, L.R. Gibson, A.D. Knapp, and R. Horton

Abstract

Eastern gamagrass (*Tripsacum dactyloides*, L.), a warm season, perennial grass with great potential for forage and conservation uses, has a high level of seed dormancy, making establishment difficult. Wet, prechilling up to six weeks is a standard method for providing germinable gamagrass seed. Earlier research showed that gibberellic acid (GA₃) increased germinability of decupulated gamagrass, but was less effective when the caryopsis remained in the cupule. We hypothesized that gibberellic acid together with prechilling may increase germination of cupulated seed above levels obtained by prechilling or gibberellic acid alone. This study assessed the germination of three lots of cupulated eastern gamagrass to 0.001M GA₃ and exposure to 4°C for 0 to 7 weeks. Seeds soaked in GA₃ solution averaged 43% germination of all live seeds during the first three weeks of prechilling and was significantly higher than the germination of water soaked seeds averaging 35% germination. Seed reached peak germinability after four weeks of prechilling and remained at this level during the final weeks. After four weeks of moist prechilling, germination levels of GA₃ and water treated seed were similar at 64 to 70%. The most pronounced effect of GA₃ was more rapid germination of seed in all of the prechilling durations tested.

Introduction

Eastern gamagrass (*Tripsacum dactyloides* L.) is a warm-season, perennial grass species found from the northeastern and north central United States and south into Mexico, Central America, the Caribbean, and into Bolivia and Paraguay in South America (Newell and deWet, 1974). High productivity (Fine et al., 1990) and quality (Horner et al., 1985; Burns et al., 1996) relative to other warm-season grasses make it attractive as a forage crop. Plant
characteristics of high leaf proportion and low dead tissue ratio can lead to high daily gains for animals grazing eastern gamagrass (Burns et al., 1992). Gamagrass also has high potential for use in conservation practices. Its natural drought and wetness tolerance, perennial nature, and growth from a strong crown, make it a good species for grass hedges used to control water runoff and soil erosion from row-crop fields (Dewald et al., 1996). It can tolerate herbicides used on the adjacent crops, shading from cultivated crops, and climatic extremes.

Despite its potential, problems with stand establishment have limited the use of eastern gamagrass. Seed dormancy is very strong in this species and less than 10% of newly harvested seeds typically germinate (Ahling and Frank, 1968; Tian et al., 2002a). Ahling and Frank (1968) reported that cold, wet stratification of seeds for six weeks increased germination to 58 to 66%. Anderson (1985) also noted that a cold, moist period of 60 days in 4°C enhanced eastern gamagrass seed germination. It is unclear from these reports if dormant seeds remained at the end of the prechilling period.

Gibberellin plant hormones have also been shown to overcome seed dormancy in eastern gamagrass. Low molar solutions of gibberellic acid increased the germination of gamagrass caryopses removed from the encompassing cupule structures, but had only small effects on dormancy when caryopses remained in the cupules (Anderson, 1985; Tian et al., 2002b). Removal of the cupule and application of GA₃ increased germination to within a few percentage points of the total amount of viable seed (Tian et al., 2002b).

Because cupule removal is labor intensive and risks the integrity of the caryopsis, it is necessary to find methods of increasing germination of intact seeds. In the following study we tested whether combinations of known germination promoters, cold, moist prechilling and GA₃, could more completely break seed dormancy and promote seed germination in eastern gamagrass than the individual application of these promoters.
Materials and methods

Two seed lots of “Iuka” eastern gamagrass produced in 1998 and 2000 and one lot of “Pete” produced in 2000 were used for this study. Seeds were received in November of their year of production and stored at 4 °C and 40% relative humidity until needed. Seed lots were immediately returned to cold storage after being sampled for experimental use.

Seeds were imbibed for 24 hours with either 0.001M gibberellic acid (GA3) solution or de-ionized water. Two sheets of blue blotter paper (Anchor Paper Co., St. Paul, MN) were placed in 12.07 cm by 12.07 cm plastic boxes and saturated with 27 mL of GA3 solution or de-ionized water. After the liquid had been applied to the blotter paper, 9 g of seed were placed in the boxes and the lids were sealed. The boxes were maintained at 25 °C for 24 hours before being placed in cold temperatures.

After the 24 h imbibition period, the seeds were placed in cold temperatures for a 1 to 7 week stratification period. The plastic boxes with blotter paper, liquid, and seeds were placed in the cold temperature room at 4 °C and 40% relative humidity. One set of seeds was randomly selected for a 0 week stratification treatment and germinated immediately after the imbibition period.

A box for each imbibition treatment was removed from the cold room at weekly periods to test the effect of stratification period length on germination. New plastic boxes were prepared with two sheets of blue blotter paper and saturated with 24 mL of de-ionized water. Fifty seeds with intact cupules were randomly selected from each imbibition treatment and placed in a box for germination testing. Fifty seeds of dry (unimbibed) seed were randomly sampled from the original lots for germination testing as well. Seeds not included in the germination test were weighed, dried in an oven at 130 °C for 6 h and weighed to determine moisture content.

The germination tests were carried out at 30/20 °C alternating temperature (Ahring and Frank, 1968) with light (four 40W cool-white fluorescent lights vertically oriented on each
the left and right sides of the germinator) and 30 °C for 8 h daily. Darkness and 20 °C temperature were combined for the other 16 h of a daily cycle. Germination counts were made every 7 d for 28 d. Seeds were considered germinated if the coleoptile exceeded the seed in length and the seedling was normal according to the seedling evaluation criteria of AOSA for comparable grasses (AOSA, 1992). Normal seedlings were removed as they were counted. Water was added to each germination box as needed to maintain optimum moisture levels. After 28 d of incubation, abnormal seedlings were counted and ungerminated seeds were examined by tetrazolium (TZ) tests and classified as dormant or dead.

**Statistical analysis**

The study was conducted as a randomized factorial design with three replications. The experimental unit was 9 g of seed that were imbibed with gibberellic acid or de-ionized water and placed in cold temperature from 0 to 7 weeks. Dry seed was germinated each week and used as a control. The Proc GLM procedure of SAS (SAS Institute, Cary, NC) was used to analyze final germination (day 28 of the germination period), abnormal seedlings, dormant seeds, and dead seeds. The Proc Mixed procedure of SAS was used with an auto-regressive co-variance structure to analyze the repeated measurements of germination over the 28 d germination period. Significance of main effects and interactions of seed treatment, seed lot, prechilling duration, and germination time were determined with an F test. Tukey's test (Steel and Torrie, 1980a) was used to make mean comparisons of main effects. Statistically significant interactions were subjected to further ANOVA using the slice command of Proc GLM. Germination for each week of the prechilling period was compared to germination at seven weeks of prechilling with Dunnett's procedure (Steel and Torrie, 1980b) to determine the amount of prechilling required for peak germination. The significance level for all comparisons was P <0.05.
Results

Total seed germination

The main effects of seed treatment, seed lot, and prechilling duration influenced the amount of seed germination at the conclusion of the 28 d germination period (Table 1). There was a significant interaction between seed treatment and prechilling duration, which was caused by the inclusion of the dry control, which had virtually no change in final germination percentages depending on weeks of exposure (Fig. 1). Because the comparison of interest was between the GA3 and water treatments, germination data of the dry seeds were removed and the data set was reanalyzed (Table 1). The week by treatment interaction was no longer present in this analysis and the main effects of seed treatment and prechilling duration could be more clearly interpreted. The Iuka 2000 seed lot had 3 percentage points less germination than the Iuka 1998 and Pete 2000 seed lots when analyzed with and without the dry seed. However, this difference was only significant when the dry seeds were included in the analysis.

Germination of both GA3 and water treated seed peaked at four weeks of prechilling exposure and was maintained at these levels through the remaining four weeks of prechilling. Total germinations increased for both GA3 and water treated seed with each week of additional prechilling from week 0 until week 4. The final germination ranged from 64 to 70% with four to seven weeks of moist pre-chilling, compared to 8 to 16% for dry seed.

Analysis of 0 through 3 weeks of exposure to moist-prechilling (Table 2) revealed significant differences in final germination due to seed treatment, lot, and prechilling duration. GA3 treated seeds averaged 8 percentage points higher final germination than water-treated seeds during this period (Fig. 1). The differences among lots occurred because the final germination counts of Iuka seed produced in 2000 were 7 percentage points less than the germination of Iuka produced in 1998 and Pete seed produced in 2000. Differences
in seed treatment, lot and prechilling duration did not occur for weeks four through seven of the moist-prechilling treatments.

**Germination rate**

Analysis of variance was performed on the data set that included the normal seedling counts made every 7 d of the germination period to determine if there were differences in rate at which seed germinated (Table 3). Seed treatment by germination time and prechilling duration by germination time interactions occurred when this analysis was performed both with and without dry seed. In addition there were three-way interactions between seed treatment, prechilling duration, and germination time and seed lot, prechilling duration, and germination time. The interactions that included seed treatment resulted from more rapid germination in GA3 treated seed than the water treated seed (Fig. 1). During the first three weeks of prechilling, the GA3 stimulated greater amounts of germinating seed at days 7, 14, 21, and 28 of the germination period. There was little difference in germination between GA3 and water treated seed at 14, 21, and 28 d of the germination period for 4 to 7 weeks of prechilling. But, the number of seedlings at day 7 of the germination period was 43% for GA3 treated seed compared to 25% in water treated seed indicating more rapid germination with GA3 treated seed.

The three seed lots responded differentially to combinations of prechilling duration and germination time. They had similar germination at day 7 of the germination period during the first four weeks of prechilling, but Iuka 2000 had 6% greater germination than the other two lots for 5 to 7 weeks of prechilling. At days 14, 21, and 28 of the germination period Iuka 1998 had 5 to 6% less germination than the other two seed lots for 0 to 3 weeks of prechilling. There were no differences in germination among lots with at least 4 weeks of prechilling and 14 d of germination time.
Abnormal seedlings, dormant seed, and dead seed

Abnormal seedlings were counted at the conclusion of the 28 d germination period and the remaining seeds were determined to be dormant or dead using a tetrazolium test (Table 4). There were no interactions between main effects when only the GA3 and water prechilling treatments were included in the analysis (Table 5). The number of abnormal seedlings was influenced by both seed treatment and prechilling duration. GA3 treated seed averaged 3 abnormal seedlings compared to 2 for untreated and water treated seeds (Fig 2.) This small difference suggested that prechilling with GA3 did not adversely affect the normal development of the seedlings. No prechilling and 1 week of prechilling produced between 4 and 5 abnormal seedlings compared to an average of 2 abnormal seedlings for 2 to 7 weeks of prechilling.

The amount of dormant seed decreased with each week of prechilling and after seven weeks there was only 3% and 7% dormant seed remaining in the GA3 and water treated seeds, respectively, compared to 73% dormancy in seed that did not receive moist prechill treatment (Fig. 2). Iuka produced in 2000 averaged 6 percentage points more dormant seed over the course of the 7 weeks of prechilling than Iuka 1998 or Pete 2000.

The two moist prechilling treatments (with and without GA3) produced similar amounts of dead seeds, which were greater than dead seed recorded at the completion of the germination for the unchilled seed (Fig 2). The amount of dead seed in dry, water treated, and GA3 treated seed was 13 to 16% for 0 to 4 weeks of prechilling. With 5 to 7 weeks of prechilling, the amount of dead seed in the water treated and GA3 treated seed increased to 20 to 26%. Since there was no increase in germination for 5 to 7 weeks of prechilling, the reduction in seed dormancy over this period was caused by increasing amounts of seed death. Seeds of Iuka from 1998 and Pete from 2000 averaged approximately 17% dead compared to 14% for Iuka harvested in 2000.
Discussion

The results of this study provide several previously unreported insights into the dormancy and germination of eastern gamagrass seed. Combinations of GA$_3$ and moist, prechilling for four or more weeks did not increase final germination of eastern gamagrass seeds above levels attained by moist prechill without GA$_3$. However, GA$_3$ did stimulate more rapid and greater germination when seed was pre-chilled for less than three weeks or less. Once the dormancy was completely broken with four weeks of prechilling, the GA$_3$ was effective at increasing the rate at which the seed germinated. The increased rate of germination could have an impact on the success of eastern gamagrass establishment. More rapid germination could give seedlings an increased ability to compete with weeds, insect pests, and soil pathogens. Mueller et al. (2000) and Aberle et al. (2002) found that eastern gamagrass stand establishment was sensitive to dry soil conditions in the weeks following planting. More rapid germination could increase the chance of generating an acceptable stand if eastern gamagrass seed was planted into moist, soil that dried over the course of the establishment period. Field studies are required to determine if combinations of GA$_3$ and moist prechilling increase field establishment success.

The duration of prechilling required for breaking the seed dormancy in eastern gamagrass was less in our study than in previous reports. Prechilling for four weeks broke nearly all of the seed dormancy and there was no difference among three seed lots tested. The level of germination remained stable with additional prechilling for up to seven weeks. Ahring and Frank (1968) stated “six to eight weeks on moist substrate at 5 to 10°C was sufficient to promote germination to within 60 to 80% of all live seeds”, which has become an industry standard. A closer examination of their data indicated that one lot required six weeks for maximum germination, however another required only two weeks. Our levels of final germination were similar to those reported by Ahring and Frank (1968). Anderson (1985) reported that 30 d of prechilling had very little influence on eastern gamagrass seed.
germination, while 60 and 90 d of prechilling increased germination to about 40% of the live seeds compared to 8% in unchilled seed. Seeds were soaked on moistened substrates in germination boxes in our study and the two previous studies. However, in our studies we soaked the seeds at room temperature for 24 h before exposing the seeds to cold temperatures. Reducing the prechilling duration for eastern gamagrass seed from 6 to 4 weeks could reduce seed costs and stand establishment expenses.

The reports of Ahring and Frank (1968) and Anderson (1985) did not address the fate of the eastern gamagrass seed that remained ungerminated after peak germination was reached with prechilling. A decrease in germination with more than 6 weeks of prechilling as reported by Ahring and Frank (1968) could result from seed death or cycling back into dormancy. Final germination did not increase after four weeks of moist prechilling in our study. But, the number of dead seeds increased with each week of moist prechilling past five weeks until very few dormant seeds remained with seven weeks of prechilling. These trends suggest that the remaining ungerminated seeds were not capable of producing viable seedlings and would die with additional weeks of prechilling.

Literature cited


Table 1. Analysis of variance for final germination (on day 28 of the germination period) of eastern gamagrass seed after treatment with water or GA$_3$ and moist prechilling for 0 to 7 weeks. The analysis was done with and without weekly comparisons to dry seed that was not moist prechilled.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df With Dry Seed</th>
<th>df Without Dry Seed</th>
<th>F-value With Dry Seed</th>
<th>F-value Without Dry Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment (S)</td>
<td>2</td>
<td>1</td>
<td>770.96***</td>
<td>14.04***</td>
</tr>
<tr>
<td>Lot (L)</td>
<td>2</td>
<td>2</td>
<td>5.40**</td>
<td>2.94</td>
</tr>
<tr>
<td>S x L</td>
<td>4</td>
<td>2</td>
<td>1.72</td>
<td>0.71</td>
</tr>
<tr>
<td>Prechilling duration (D)</td>
<td>7</td>
<td>7</td>
<td>98.42***</td>
<td>110.30***</td>
</tr>
<tr>
<td>S x D</td>
<td>14</td>
<td>7</td>
<td>25.70***</td>
<td>1.01</td>
</tr>
<tr>
<td>L x D</td>
<td>14</td>
<td>14</td>
<td>1.19</td>
<td>1.28</td>
</tr>
<tr>
<td>S x L x D</td>
<td>28</td>
<td>14</td>
<td>1.14</td>
<td>0.83</td>
</tr>
</tbody>
</table>

**, *** Significant at the $P =0.01$ and 0.001 level, respectively.
Table 2. Analysis of variance for final germination (on day 28 of the germination period) of eastern gamagrass seed after treatment with water or GA3 and moist prechilling for 0 to 3 weeks. This analysis compared GA3 and water-treated seed and did not include dry seed that was not moist prechilled.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment (S)</td>
<td>1</td>
<td>16.33***</td>
</tr>
<tr>
<td>Lot (L)</td>
<td>2</td>
<td>5.88**</td>
</tr>
<tr>
<td>S x L</td>
<td>2</td>
<td>0.84</td>
</tr>
<tr>
<td>Prechilling duration (D)</td>
<td>3</td>
<td>109.62***</td>
</tr>
<tr>
<td>S x D</td>
<td>3</td>
<td>0.66</td>
</tr>
<tr>
<td>L x D</td>
<td>6</td>
<td>0.42</td>
</tr>
<tr>
<td>S x L x D</td>
<td>6</td>
<td>0.51</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

**, *** Significant at the $P = 0.01$ and 0.001 level, respectively.
Table 3. Analysis of variance for eastern gamagrass seed germination after treatment with water or GA3 and moist prechilling for 0 to 7 weeks. This analysis includes germination counts made every 7 d of a 28 d period and was done with and without weekly comparisons to dry seed that was not moist prechilled.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>With Dry Seed df</th>
<th>F-value</th>
<th>Without Dry Seed df</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment (S)</td>
<td>2</td>
<td>1264.75***</td>
<td>1</td>
<td>51.40***</td>
</tr>
<tr>
<td>Lot (L)</td>
<td>2</td>
<td>9.90***</td>
<td>2</td>
<td>8.16***</td>
</tr>
<tr>
<td>S x L</td>
<td>4</td>
<td>2.32</td>
<td>2</td>
<td>1.04</td>
</tr>
<tr>
<td>Prechilling duration (D)</td>
<td>7</td>
<td>244.93***</td>
<td>7</td>
<td>262.29***</td>
</tr>
<tr>
<td>S x D</td>
<td>14</td>
<td>58.38***</td>
<td>7</td>
<td>1.25</td>
</tr>
<tr>
<td>L x D</td>
<td>14</td>
<td>1.52</td>
<td>14</td>
<td>1.82*</td>
</tr>
<tr>
<td>S x L x D</td>
<td>28</td>
<td>1.14</td>
<td>14</td>
<td>0.58</td>
</tr>
<tr>
<td>Error 1</td>
<td>215</td>
<td></td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Germination time (T)</td>
<td>3</td>
<td>1251.41***</td>
<td>3</td>
<td>1073.37***</td>
</tr>
<tr>
<td>S x T</td>
<td>6</td>
<td>155.46***</td>
<td>3</td>
<td>19.83***</td>
</tr>
<tr>
<td>L x T</td>
<td>6</td>
<td>1.73</td>
<td>6</td>
<td>0.81</td>
</tr>
<tr>
<td>D x T</td>
<td>21</td>
<td>34.31***</td>
<td>21</td>
<td>35.15***</td>
</tr>
<tr>
<td>S x L x T</td>
<td>12</td>
<td>0.82</td>
<td>6</td>
<td>0.68</td>
</tr>
<tr>
<td>S x D x T</td>
<td>42</td>
<td>10.40***</td>
<td>21</td>
<td>4.48***</td>
</tr>
<tr>
<td>L x D x T</td>
<td>42</td>
<td>2.20***</td>
<td>42</td>
<td>2.14***</td>
</tr>
<tr>
<td>S x L x D x T</td>
<td>84</td>
<td>1.27</td>
<td>42</td>
<td>0.99</td>
</tr>
<tr>
<td>Error 2</td>
<td>648</td>
<td></td>
<td>431</td>
<td></td>
</tr>
</tbody>
</table>

*, *** Significant at the $P = 0.05$ and 0.001 level, respectively.
Table 4. Analysis of variance for abnormal seedlings, dormant seeds, and dead seeds of eastern gamagrass after treatment with water or GA$_3$ and moist prechilling for 0 to 7 weeks. Abnormal seedlings were counted after 28 d of germination testing. Dormant and dead seeds were determined by tetrazolium testing also at the end of the germination-testing period. This analysis includes weekly comparisons to dry seed that was not moist prechilled.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Abnormal seedlings</th>
<th>Dormant seeds</th>
<th>Dead seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment (S)</td>
<td>2</td>
<td>10.32***</td>
<td>1211.78***</td>
<td>24.00***</td>
</tr>
<tr>
<td>Lot (L)</td>
<td>2</td>
<td>0.84</td>
<td>18.25***</td>
<td>5.83**</td>
</tr>
<tr>
<td>S x L</td>
<td>4</td>
<td>0.92</td>
<td>2.14</td>
<td>0.40</td>
</tr>
<tr>
<td>Prechilling duration (D)</td>
<td>7</td>
<td>7.10***</td>
<td>161.76***</td>
<td>10.79***</td>
</tr>
<tr>
<td>S x D</td>
<td>14</td>
<td>2.24**</td>
<td>41.24***</td>
<td>2.71**</td>
</tr>
<tr>
<td>L x D</td>
<td>14</td>
<td>1.92*</td>
<td>1.80*</td>
<td>1.24</td>
</tr>
<tr>
<td>S x L x D</td>
<td>28</td>
<td>1.20</td>
<td>1.28</td>
<td>1.05</td>
</tr>
<tr>
<td>Error</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, **, *** Significant at the $P = 0.05$, 0.01, and 0.001 levels, respectively.
Table 5. Analysis of variance for abnormal seedlings, dormant seeds, and dead seeds of eastern gamagrass after treatment with water or GA$_3$ and moist prechilling for 0 to 7 weeks. Abnormal seedlings were counted after 28 d of germination testing. Dormant and dead seeds were determined by tetrazolium testing also at the end of the germination-testing period. This analysis does not include weekly comparisons to dry seed that was not moist prechilled.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Abnormal seedlings</th>
<th>Dormant seeds</th>
<th>Dead seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment (S)</td>
<td>2</td>
<td>5.78*</td>
<td>27.90***</td>
<td>0.81</td>
</tr>
<tr>
<td>Lot (L)</td>
<td>2</td>
<td>1.95</td>
<td>11.93***</td>
<td>3.84*</td>
</tr>
<tr>
<td>S x L</td>
<td>4</td>
<td>0.31</td>
<td>0.87</td>
<td>0.63</td>
</tr>
<tr>
<td>Prechilling duration (D)</td>
<td>7</td>
<td>8.98***</td>
<td>235.70***</td>
<td>12.86***</td>
</tr>
<tr>
<td>S x D</td>
<td>14</td>
<td>0.70</td>
<td>1.19</td>
<td>1.23</td>
</tr>
<tr>
<td>L x D</td>
<td>14</td>
<td>1.46</td>
<td>1.79</td>
<td>1.08</td>
</tr>
<tr>
<td>S x L x D</td>
<td>28</td>
<td>1.60</td>
<td>1.00</td>
<td>1.45</td>
</tr>
<tr>
<td>Error</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, *** Significant at the $P=0.05$ and 0.001 level, respectively.
Figure 1. Germination of eastern gamagrass seed after treatment with water or GA₃ and moist prechilling for 0 to 7 weeks. Average of three seed lots – ‘Iuka’ produced in 1998 and 2000 and ‘Pete’ produced in 2000. Counts were taken on days 7, 14, 21, and 28 of the germination-testing period.
Figure 2. Abnormal seedlings, dormant seeds, and dead seeds of eastern gamagrass after treatment with water or GA$_3$ and moist prechilling for 0 to 7 weeks. Average of three seed lots – 'Iuka' produced in 1998 and 2000 and 'Pete' produced in 2000. Counts were determined after a 28 d germination-testing period.
CHAPTER 4. SYSTEMS FOR SOLID MATRIX PRIMING OF EASTERN GAMAGRASS SEED

C. Rogis, L.R. Gibson, A.D. Knapp, and R. Horton

Abstract

Eastern gamagrass (*Tripsacum dactyloides*, L.), a warm season, perennial grass with great potential for forage and conservation uses, has a high level of dormancy, making establishment difficult. Methods of overcoming this dormancy could increase the use of eastern gamagrass. Solid matrix priming, the controlled hydration of seed in a system of solid carrier and water, has been used with some success to enhance germination in several vegetable crops. Gibberellic acid (GA), a known promoter of eastern gamagrass germination, can be added to solid matrix priming systems. In this study, three systems were developed for conditioning eastern gamagrass seeds using the solid carriers Agro-Lig, MicroCel E, and Vermiculite #5. In Agro-Lig, the final seed water content was 44, 44, 44, and 28% and days required for complete hydration were 1, 7, 7, and 3 in systems at -0.2, -0.4, -0.7, -1.2 MPa, respectively. In MicroCel E, the final water content of the seed was 39, 32, 27, 28, and 29% at -0.2, -0.6, -0.8, -1.0, and -1.3 MPa, respectively, and seeds were fully hydrated on day 7 of the conditioning period in all water potentials. With Vermiculite #5, the final water content of the seed was 35, 32, 28, 25, and 23% at -0.2, -0.5, -0.7, and -1.2 MPa, respectively, and seeds were fully hydrated on day 3 of the conditioning period in all water potentials. GA₃ buffered to pH 5.2, unbuffered GA₃ (pH 3.0) were added in 0.01 M concentration solutions to systems with water potentials of -0.4 and -0.6 MPa in Agro-Lig and -0.2 and -0.4 in MicroCel E and Vermiculite #5 and compared to systems with deionized water or buffer solution alone. Total germination was only 10% in Agro-Lig compared to 16 to 20% for MicroCel E and Vermiculite #5. Addition of GA₃ increased germination to 18% from 12 to 13% in water or buffer alone.
Introduction

Stand establishment problems resulting from seed dormancy currently limit the use of eastern gamagrass. Seed dormancy is very strong in eastern gamagrass and less than 10% of newly harvested seeds typically germinate (Ahring and Frank, 1968; Tian et al., 2002a). Application of gibberellic acid (GA) may have potential for breaking seed dormancy in eastern gamagrass. Low molar solutions of GA increased the germination of eastern gamagrass caryopses removed from the encompassing cupule structures, but had only small effects on dormancy when caryopses remained in the cupule (Anderson, 1985; Tian et al., 2002b). Removal of the cupule and application of GA3 increased germination to within a few percentage points of the total amount of viable seed (Tian et al., 2002b).

Growth hormones, such as GA, have been incorporated into solid matrix priming systems, which control the rate and level of seed hydration through osmotic or matric forces of water potential (Khan, 1992; Khan et al., 1995; Taylor et al., 1998). Inclusion of 0.001mM GA3 in priming systems with MicroCel E increased the emergence rate for snap beans, but final germination was not different from non-conditioned seeds. When snap bean seeds were condition in 0.002 mM GA3 solution and Vermiculite #5, germination rate was similar to that of the seed conditioned in MicroCel E, but final emergence was less (Khan et al., 1992). Fungicides (Apron, thiram, and demosan) and 2µM GA3 added to solid matrix priming systems for snap beans and table beet had small effects on germination rate, but improved final emergence from 58 to 92% in snap bean and 100 to 119% in the multigerm table beet cultivar (Khan et al., 1995).

Solid matrix priming utilizes carriers possessing characteristics such as high water holding capacities, low osmotic potentials, and low bulk density (Kahn et al., 1990; Khan, 1992). Three solid carriers that have been used in priming are Agro-Lig, MicroCel E, and Vermiculite #5 (Khan, 1992; Parera and Cantliffe, 1994).
Agro-Lig is a Leonardite shale with a high bulk density of 700 kg m\(^{-3}\) (45 lb ft\(^{-3}\)). It is relatively hydrophobic and becomes saturated at only 40 to 50% of its weight in water (Kahn, 1992). Agro-Lig saturated with water has a matric potential of zero, so systems that utilize it exert both osmotic and matric forces depending on the hydration level. Since conditioning with Agro-Lig usually involves addition of water equal to 60-95% of Agro-Lig’s weight, osmotic forces control the hydration of the seed added to this system (Khan, 1992). Khan (1992) used the carrier for priming of pea and sweet corn with inconsistent field emergence effects. Hartz and Caprile (1995) found increased sweet corn emergence in laboratory tests after solid matrix priming with Agro-Lig, but enhancement of field emergence was inconsistent.

MicroCel E and Vermiculite #5 have both been used with success in the pre-conditioning of various vegetable seeds (Khan, 1992; Khan et al., 1995; Dawidowicz-Grzegorzewska, 1997). MicroCel E is a synthetic silicate derived from diatomaceous earth. It has low bulk density, very fine particle size, and a high water holding capacity. Vermiculite #5 is a very fine grade of expanded vermiculite (hydrated magnesium-aluminum-silicate) created as by-product of exfoliating larger grades. Vermiculite #5 has a low bulk density, various particle sizes, and a high water holding capacity. Both MicroCel E and Vermiculite #5 have no detectable osmotic potential (Khan et al., 1992).

Solid matrix priming has been used to enhance the germination of native, warm-season grasses (Beckman et al., 1993). Big bluestem and switchgrass seeds were solid matrix primed with fungicide solution, seed in a 2:1:2 ratio, and a water potential of -6 MPa for 14 d. The priming systems were allowed to either condition at room temperature (17°C) or in chilling conditions (4°C). Priming increased emergence of big bluestem seed in the greenhouse by 7 percentage points and switchgrass by 19 percentage points. No significant differences in emergence were seen at the two temperatures in big bluestem seed. Chilling conditions increased germination of the switchgrass seeds by an average of 23 percentage
points (Beckman et al., 1993). Matric priming also increased the cold temperature germination rate of seven warm-season grasses native to the Great Basin of the U.S. (Hardegree, 1994).

Because removal of the eastern gamagrass cupule is labor intensive and risks the integrity of the caryopsis, it is necessary to find a method of increasing germination of intact seeds. We hypothesized that exposure of intact eastern gamagrass cupules to GA₃ in a solid matrix priming system could be an effective system for breaking seed dormancy. The objectives of the study reported here were two-fold - 1) to develop solid matrix priming systems for eastern gamagrass utilizing Agro-Lig, MicroCel E, and Vermiculite #5 as carriers, And, 2) to determine if addition of low molar concentrations of buffered GA₃ and unbuffered GA₃ to these systems would stimulate germination of eastern gamagrass seed.

Materials and methods

Developing solid matrix priming systems

Conditioning systems were developed for three solid carriers - Agro-Lig, MicroCel E, and Vermiculite #5. The carriers were stored in large airtight containers at room temperature (25°C) for the duration of the study. The moisture contents of the carriers were determined by placing 5 g of Agro-Lig and 3 g of MicroCel E and Vermiculite #5 in aluminum weighing dishes and drying in a convection oven at 100°C for 24 hours. Four replications were randomly selected from different areas of the bulk container. The samples were placed in a desiccator to cool after being removed from the oven and weighed after reaching room temperature.

Adding water in increments of 50% by weight up to 600% created twelve water and carrier mixtures. The amount of water added to each was adjusted to account for the original moisture content of the carriers. The mixtures were thoroughly blended with a spatula and allowed to equilibrate for 16 h. Water potential was determined on 7 ml samples of the mixtures in a Decagon WP4 dew point potentiameter (Decagon Devices, Inc., Seattle, WA).
The fit of curves for the relationship between carrier water content and water potential and the variability between two replications were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS; SAS Institute, NC).

Conditioning systems with water potentials of -0.2, -0.4, -0.6, -0.8, and -1.0 MPa were developed for each carrier. Twenty-five g of seed of the eastern gamagrass variety ‘Pete’ harvested in 1999 (Gamagrass Seed Co., NE) was added to 100 g of carrier hydrated to a targeted water potential. The seeds were stored in a controlled environment of 4°C and 40% relative humidity from the time they were acquired in 2000. Seeds were conditioned for 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 days. The hydrated carriers with seed were placed in airtight containers and kept at room temperature for the duration of the conditioning period. Each container was shaken on a daily basis to keep contents evenly mixed. Seeds were sieved from the carrier at the end of each conditioning period. Moisture was determined on 5 g samples of carrier and 10 g samples of seeds in a convection oven at 100°C for 24 hours. Samples were allowed to cool to room temperature in a desiccator before weighing. Water potentials of the carriers and seed were determined on 7 ml samples of each in the WP4 Potentiometer.

This portion of the study was replicated three times with each replication spaced in time. Means and standard deviations were calculated to determine the actual water potentials obtained and the variability across the duration of the conditioning period and among replications. The general linear model procedure of SAS was used to determine interactions and significant differences among treatments. The response between seed moisture content and duration of conditioning followed a non-linear hyperbola, which levels off at an asymptote. Therefore, the equation for Mitscherlich’s Law of Diminishing Returns was used to fit lines to the weeds seeded data using Proc NLIN of SAS (Snedecor and Cochran, 1989). The form of the equation used was $y = A - B (e^{-cx})$, where $y =$ seed moisture content, $A =$ maximum seed moisture content, $B =$ the difference between the maximum possible seed
moisture content and moisture content on day one of the conditioning period, \( c = \text{constant}, \) and \( x = \text{days of conditioning}. \) Dunnett's multiple comparison test (Steel and Torrie, 1980a) was used to determine when seeds were fully hydrated in each of the water potential systems.

**Solid matrix priming eastern gamagrass seed with \( \text{GA}_3 \)**

Conditioning systems were developed using the two water potentials in each material that provided the greatest seed water content without causing seed germination in the first 7 d of priming. Water potentials of -0.4 and -0.6 MPa were selected for Agro-Lig because the system was saturated at -0.2 MPa. Water potentials of -0.2 and -0.4 MPa were used for MicroCel E and Vermiculite #5. Four treatment solutions were tested at the two water potentials in each carrier – deionized water, \( \text{GA}_3 \) buffered to pH 5.2, unbuffered \( \text{GA}_3 \), and buffering solution without \( \text{GA} \). \( \text{GA}_3 \) was used at a concentration of 0.01M, which had a pH of 3.0 when unbuffered. A citric acid disodium phosphate buffer (Citric acid + Na\(_2\)HPO\(_4\)) was used to bring the buffered \( \text{GA}_3 \) to pH 5.2. Buffering of the \( \text{GA}_3 \) solution was based on the findings that pH’s below 6.0 enhanced the \( \text{GA}_3 \)-response of isolated barley aleurone layers (Singjorgo et al., 1993). An intermediate pH was chosen because buffering \( \text{GA}_3 \) solutions to pH 3.5 was detrimental to eastern gamagrass seed germination in a previous study (Tian et al., 2002b).

The conditioning systems were contained in airtight plastic boxes at ambient room temperature (25°C and 50% RH) for 7 d. The moisture content of the seed and the water potential of the carrier system were tested at the conclusion of the conditioning period using the methods described previously.

Fifty seeds were randomly selected from each treatment solution, water potential, and carrier treatment combination for germination testing. The seeds were placed on two sheets of saturated blotter paper in airtight containers and placed in the germinator. The germination test was carried out at 30/20°C alternating temperature (Ahring and Frank, 1968) with light (four 40W cool-white fluorescent lights vertically oriented on each the left and right sides of
the germinator) and 30°C for 8 h daily. Darkness and 20°C temperature were combined for a 16 h period. Germinations were counted at 7, 14, 21, and 28 d for normal germinations. Germinated seeds were counted as normal if the coleoptile and root were similar in length and were at least as long as the cupule. On day 28, abnormal germinations were counted and ungerminated seeds were visually analyzed to determine the number of empty seeds and tested with tetrazolium solution to determine the amount of dead and dormant seeds. The percent germination, abnormal seedlings, dead seeds, and dormant seeds were calculated after adjusting for the amount of empty seeds in each treatment.

Three replications were performed in time. Seed moisture content, carrier water potential, final germination, abnormal seedlings, dead seeds, and dormant seeds were analyzed using the general linear model procedure of the SAS system. A Tukey’s Studentized Range test was used to determine differences among main effects (Steel and Torrie, 1980b). The mixed procedure of SAS with an auto-regressive co-variance structure was used to analyze the repeated measurements of germination over time.

Results and discussion

Development of conditioning systems

The initial moisture contents of Agro-Lig, Vermiculite #5, and MicroCel-E were 12.91, 0.44, and 4.52%, respectively. Water potential in Agro-Lig and Vermiculite #5 containing 50% water could not be determined by the potentiometer. Similarly, water potential in MicroCel E could not be measured at 50 and 100% water content. Saturation occurred at 450, 500, and 400% water content in Agro-Lig, MicroCel E, and Vermiculite #5, respectively. The relationship between carrier water content and water potential was curvilinear for both Agro-Lig and Vermiculite #5 (Fig. 1). For MicroCel E, this relationship was curvilinear for water contents between 100 and 300% and linear for water contents between 300 and 450%. The conditioning systems created were quite stable as indicated by a lack of significant replication effects for any of the three carriers.
The water potential response to carrier water content for MicroCel E and Vermiculite #5 in this study closely matched those developed by Khan (1992). However, the measurements obtained in this study for the various moisture contents of Agro-Lig were much different than those found by Khan (1992). They found that 0 MPa, or saturation, occurred at approximately 50% water content by dry weight. We were able to obtain readings through 400% moisture by dry weight. However, because of the hydrophobic nature of Agro-Lig, the mixture may not have had evenly distributed moisture throughout the solid. Also, Khan (1992) was using a standard procedure and equation by Olson (1979) to develop the relationship between moisture content and matric potential, whereas this study employed the use of a machine to measure the water potential. Taylor et al. (1988) found that the major contributor to Agro-Lig’s water potential was the solute potential. Matric potential accounted for less than only 1.5% of the total water potential.

The equations of the response of water potential to carrier water content (Fig. 1) were used to hydrate carriers to known water potential for eastern gamagrass conditioning systems. Water potentials of -0.2, -0.4, -0.6, -0.8, -1.0 were targeted for each carrier. The actual water potentials measured are presented in Figure 2. Actual potentials were \(-0.19 \pm 0.13, -0.37 \pm 0.14, -0.65 \pm 0.09, -1.18 \pm 0.10, -30.17 \pm 11.96\) MPa for Agro-Lig; \(-0.24 \pm 0.06, -0.55 \pm 0.08, -0.83 \pm 0.09, -1.05 \pm 0.17, -1.32 \pm 0.19\) MPa for MicroCel E; and \(-0.24 \pm 0.06, -0.47 \pm 0.17, -0.71 \pm 0.12, -1.23 \pm 0.29, -2.27 \pm 0.99\) for Vermiculite #5. The -1.0 MPa systems for Agro-Lig and Vermiculite #5 were unsuitable for conditioning as indicated by the poor relation between the targeted and actual water potentials and the high variability over time and replications. ANOVA indicated that water potentials of the carriers did not differ with duration of the conditioning period. The water losses from the carriers were minimal and illustrate that sufficient amounts of moisture were available for controlled hydration of the seed.
The initial moisture content of the seeds added to the conditioning systems was 5.2%. Seed hydration in Agro-Lig at the various water potentials is presented in Figure 3. There was an interaction between water potential and duration of priming. The hydrophobic nature of Agro-Lig resulted in inconsistent hydration of the material and seed in the -1.0 MPa system. The -0.2 MPa system was primarily liquid in nature, so the seed took up moisture very quickly and there was little control over hydration of the seed. The final water content of the seed was 44, 44, 44, and 28% at -0.2, -0.4, -0.7, -1.2 MPa (actual water potentials corresponding to target water potentials of -0.2, -0.4, -0.6, -0.8). The days required for complete hydration were 1, 7, 7, and 3 for the targeted water potentials, respectively.

Seed moisture content varied with water potential and duration of conditioning in MicroCel E (Fig. 4). The final water content of the seed was 39, 32, 27, 28, and 29% at -0.2, -0.6, -0.8, -1.0, and -1.3 MPa. There was no interaction between water potential and duration. The seeds were fully hydrated on day 7 of the conditioning period in all water potentials.

Seed moisture content also varied with water potential and duration of conditioning in Vermiculite #5 (Fig. 6). The final water content of the seed was 35, 32, 28, 25, and 23% at -0.2, -0.5, -0.7, and -1.2 MPa. There was no interaction between water potential and duration. The seeds were fully hydrated on day 3 of the conditioning period in all water potentials.

It is interesting that it took considerably longer for full seed hydration in MicroCel E than in Vermiculite #5 at similar water potentials and final seed water contents. Wuest (2002) suggested that vapor should be regarded as the primary source of water for seed hydration. Conditioning materials with larger pore spaces would provide a greater proportion of the moisture in the vapor phase than those with smaller pore size. Because MicroCel E has greater bulk density, larger surface area, and smaller particle size (Kahn et al., 1992), it has smaller pore spaces than Vermiculite #5. The small pore space and large surface area of MicroCel E in combination with the high water content needed to attain water potentials of –
0.2 and -0.4 MPa suggest that the amount of seed hydration by the liquid phase would have been greater in it than in Vermiculite #5. However, it is not clear why these conditions would slow moisture uptake by the seed. Wuest (2002) found that wheat seed reached equilibrium water content more quickly when allowed to take up moisture in the liquid phase and more slowly when seed hydration occurred exclusively from water vapor.

The conditioning systems were continued for 28 d to determine if seed germination occurred in any of the various water potential systems. No germination occurred in Agro-Lig during this period and in MicroCel E and Vermiculite #5 until 14 d after conditioning began. Germinations did eventually occur at each matric potential in both MicroCel E and Vermiculite #5, but levels were generally less than 2% of the total seed in each system. Since eastern gamagrass seed reached its maximum moisture content by 7 d with no visible germination, this amount of time appeared to be sufficient for conditioning seeds within the range of water potentials used in this study.

**Solid matrix priming with GA₃**

Actual water potentials attained in the unbuffered solutions were within ± 0.1 MPa of the intended water potentials in all three carriers. However, the buffered solutions had 0.4, 0.3, and 0.6 lower water potentials than intended in Agro-Lig, MicroCel E, and Vermiculite #5, respectively. The lower water potentials in the buffered solutions produced lower final seed moisture contents than those attained in the unbuffered solutions. In Agrolig, the GA₃ and water treatments produced seed with final moisture content of 42% compared to 40% in buffer only and buffered GA₃. There was no difference in seed hydration between the two intended water potentials. With MicroCel E, seed moisture content was 36% at the higher targeted water potential and 31% at the low water potential with no differences due to treatment. In Vermiculite #5, seed moisture content was 33% for the higher water potential and 29% in the lower water potential. When averaged across the two water potentials, GA₃ and water only treatments produced seed with 33% moisture compared to 29% for seeds.
conditioned with buffered GA₃ and buffer only. The lower water potentials and seed moisture contents attained in the buffered solutions of Agro-Lig and Vermiculite #5 suggests that the citric acid disodium phosphate added an osmotic component that lowered the water potential in these systems.

When final germinations, abnormal seedlings, dormant, and dead seeds were analyzed by carrier the largest significant effects were replication effects for dead seed in Agro-Lig, abnormal and dead seedlings in MicroCel E, and dormant seed in Vermiculite #5 (Table 1). Water potential and seed treatment had very little influence on any of the germination and viability measurements. There were no differences in any of the measurements for the main effect of water potential. There was however, an interaction of water potential by treatment in the amount of dead seeds observed in MicroCel E treated seeds. This interaction was due to differences between the -0.2 and -0.4 MPa in the seed treatments of GA₃ only (24% vs. 15%) and buffer only (17% vs. 24%). Abnormal seedlings were affected by seed treatment when treated in Vermiculite #5. Vermiculite #5 with only GA₃ had the highest number of abnormal seedlings (5%) and was significantly higher than abnormal seedlings found in buffer only (1%). Buffered GA₃ and water treatments did not differ significantly from each other or GA₃ and buffer only treatments.

Since there were no differences between water potentials in any of the carriers, a second ANOVA was performed to analyze differences among and treatment effects across the three carriers (Table 2). Carrier had the largest effect on germination and viability. Conditioning in Agro-Lig produced less germination, more abnormal seedlings, fewer dormant seed, and more dead seed than conditioning in the other two carriers. Final germinations were 10% in Agro-Lig compared to 16 to 20% for seed treated in MicroCel E and Vermiculite #5. Agro-Lig produced 5% abnormal seedlings compared to 2-3% when seeds were treated with MicroCel E or Vermiculite #5. Only 55% dormant seed remained at the conclusion of the conditioning in Agro-Lig compared to 62% in Vermiculite #5. MicroCel E had 58%
dormant seed and was not significantly different from Agro-Lig or Vermiculite #5. Conditioning in Agro-Lig resulted in 31% dead seed compared to 20% in MicroCel E and Vermiculite #5.

Seed treatment had significant effects on germination, abnormal seedlings and dormant seeds when averaged over all three carriers. Buffered GA₃ promoted the highest germination, 19%, which was significantly higher than 12 to 13% in seeds treated with water or buffer only. GA₃ alone was not significantly different from any of the other treatments with 18% germination. GA₃ treated seeds had 5% abnormal germinations compared to 2% in water treated seeds. Buffered GA₃ and buffer only treated seeds had 4% and 2% abnormal seedlings, respectively and were not significantly different than the other two treatments. Dormant seed levels were highest in water and buffer only treated seed conditioning systems with 61 to 62% dormant seed remaining at the conclusion of priming and 28 d of germination. GA₃ only treated seed had the least amount of dormant seed with 54% and was not significantly different from the buffered GA₃ treatment, which contained 55% dormant seed. Amount of dormant seeds in buffered GA₃ treatments did not differ statistically from dormancy levels in water and buffer only treated seed.

Analysis of the data over the 28 d germination period for each carrier again revealed that water potential did not influence germination (Table 3). The only significant treatment effect in this analysis was found in MicroCel E and occurred between GA₃ treated seed which averaged 20% germination over time and water treated seed which averaged 12% germination. Percent seed germination increased with the amount of germination time in all three carriers (Fig. 6). Germination continued to increase at each 7 d increment of the 28 d germination period for seeds conditioned in Agro-Lig. The germination of seed primed in MicroCel E and Vermiculite #5 increased up until 21 d of germination time, but did not change between 21 and 28 d. No interaction of germination time with seed treatment in any of the carriers indicated that the germination rate was similar among the four seed treatments.
It appears from these results, that Agro-Lig may not be suitable as a carrier for use in solid matrix priming systems for eastern gamagrass. Seeds treated in Agro-Lig had lower levels of germination, higher numbers of abnormal seedlings, and higher levels of dead seed than both Vermiculite #5 and MicroCel E treated seed. It is evident that GA3 had a slight impact on germination levels of eastern gamagrass in the solid matrix priming systems developed in this study. However, increasing germination to an average of only 18 to 19% will most likely not cause solid matrix priming to become a widely used method for overcoming dormancy in eastern gamagrass seed.

**Literature Cited**


Table 1. Analysis of variance for germination, abnormal seedlings, dormant seeds, and dead seeds of eastern gamagrass after priming for 7 days at \(-0.4\) and \(-0.6\) MPa in Agro-Lig and \(-0.2\) and \(-0.4\) MPa in MicroCel-E and Vermiculite #5. Seeds were conditioned with deionized water, 0.01 M GA$_3$ solution, pH 5.2 citric acid disodium phosphate buffer solution, and 0.01 M buffered GA$_3$ solution. Germinated and abnormal seedlings were counted after 28 d of germination testing. Dormant and dead seeds were determined by tetrazolium testing at the end of the germination-testing period.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Germination</th>
<th>Abnormal seedlings</th>
<th>Dormant seeds</th>
<th>Dead seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Agro-Lig</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>0.99</td>
<td>1.25</td>
<td>2.04</td>
<td>6.64**</td>
</tr>
<tr>
<td>Water potential (P)</td>
<td>1</td>
<td>2.03</td>
<td>0.74</td>
<td>0.00</td>
<td>0.73</td>
</tr>
<tr>
<td>Seed treatment (S)</td>
<td>3</td>
<td>2.09</td>
<td>1.69</td>
<td>2.55</td>
<td>1.00</td>
</tr>
<tr>
<td>P x S</td>
<td>3</td>
<td>0.44</td>
<td>0.50</td>
<td>0.11</td>
<td>0.42</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MicroCel E</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>1.91</td>
<td>8.51**</td>
<td>2.65</td>
<td>18.18***</td>
</tr>
<tr>
<td>Water potential (P)</td>
<td>1</td>
<td>0.04</td>
<td>0.03</td>
<td>0.10</td>
<td>0.94</td>
</tr>
<tr>
<td>Seed treatment (S)</td>
<td>3</td>
<td>2.26</td>
<td>2.14</td>
<td>2.24</td>
<td>5.28**</td>
</tr>
<tr>
<td>P x S</td>
<td>3</td>
<td>0.80</td>
<td>0.38</td>
<td>1.93</td>
<td>5.61**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vermiculite #5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>2.14</td>
<td>2.13</td>
<td>6.90**</td>
<td>1.70</td>
</tr>
<tr>
<td>Water Potential (P)</td>
<td>1</td>
<td>0.05</td>
<td>0.13</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Seed Treatment (S)</td>
<td>3</td>
<td>1.46</td>
<td>3.85*</td>
<td>1.22</td>
<td>0.72</td>
</tr>
<tr>
<td>P x S</td>
<td>3</td>
<td>0.04</td>
<td>0.50</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, **, *** Significant at the P =0.05, 0.01 and 0.001 level, respectively
Table 2. Analysis of variance for germination, abnormal seedlings, dormant seeds, and dead seeds of eastern gamagrass after priming for 7 days in Agro-Lig, MicroCel-E, and Vermiculite #5. Seeds were conditioned with deionized water, 0.01 M GA₃ solution, pH 5.2 citric acid disodium phosphate buffer solution, and 0.01 M buffered GA₃ solution. Germinated and abnormal seedlings were counted after 28 d of germination testing. Dormant and dead seeds were determined by tetrazolium testing at the end of the germination-testing period.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Germination</th>
<th>Abnormal seedlings</th>
<th>Dormant seeds</th>
<th>Dead seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>1.37</td>
<td>1.93</td>
<td>12.03***</td>
<td>16.51***</td>
</tr>
<tr>
<td>Carrier (C)</td>
<td>2</td>
<td>14.80***</td>
<td>6.08**</td>
<td>5.08**</td>
<td>27.85***</td>
</tr>
<tr>
<td>Seed treatment (S)</td>
<td>3</td>
<td>4.80**</td>
<td>3.56*</td>
<td>4.96**</td>
<td>0.94</td>
</tr>
<tr>
<td>C x S</td>
<td>6</td>
<td>0.62</td>
<td>1.16</td>
<td>1.29</td>
<td>1.78</td>
</tr>
<tr>
<td>Error</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*,**,**,* Significant at the P =0.05, 0.01 and 0.001 level, respectively
Table 3. Analysis of variance for eastern gamagrass germination after priming for 7 days at −0.4 and −0.6 MPa in Agro-Lig and −0.2 and −0.4 in MicroCel-E and Vermiculite #5. Seeds were conditioned with deionized water, 0.01 M GA₃ solution, pH 5.2 citric acid disodium phosphate buffer solution, and 0.01 M buffered GA₃ solution. Germinations were measured at 7, 14, 21, 28 d of the germination-testing period.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Agro-Lig</th>
<th>MicroCel-E</th>
<th>Vermiculite #5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>1.12</td>
<td>3.30</td>
<td>4.19*</td>
</tr>
<tr>
<td>Water potential (P)</td>
<td>1</td>
<td>1.25</td>
<td>0.12</td>
<td>0.52</td>
</tr>
<tr>
<td>Seed treatment (S)</td>
<td>3</td>
<td>2.24</td>
<td>3.44*</td>
<td>1.45</td>
</tr>
<tr>
<td>P x S</td>
<td>3</td>
<td>0.36</td>
<td>1.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Error 1</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (T)</td>
<td>3</td>
<td>30.45***</td>
<td>44.80***</td>
<td>50.50***</td>
</tr>
<tr>
<td>P x T</td>
<td>3</td>
<td>1.41</td>
<td>0.82</td>
<td>0.42</td>
</tr>
<tr>
<td>S x T</td>
<td>9</td>
<td>1.95</td>
<td>0.81</td>
<td>0.70</td>
</tr>
<tr>
<td>P x S x T</td>
<td>9</td>
<td>0.58</td>
<td>0.26</td>
<td>0.87</td>
</tr>
<tr>
<td>Error 2</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, *** Significant at the $P = 0.05$ and 0.001 level, respectively.
Figure 1. Water uptake curves for three matric conditioning carriers. Water potentials were measured at various water contents using an electronic potentiometer.

- Agro-Lig: $y = -7E-06x^2 + 0.0061x - 1.485$, $R^2 = 0.9905$
- MicroCel-E: $y = 0.0013x - 0.785$, $R^2 = 0.9899$
- Vermiculite #5: $y = -4E-05x^2 + 0.0275x - 4.9185$, $R^2 = 0.9929$
Figure 2. Water potentials of carrier materials during matric conditioning of eastern gamagrass.
Figure 3. Water uptake of eastern gamagrass seeds during conditioning in Agro-Lig. Water uptake was fit to following equation: $y = A - B \ e^{-cx}$, where $y =$ seed moisture content, $A =$ maximum seed moisture content, $B =$ the difference between the maximum seed moisture content and at seed moisture at 1 d of conditioning, $c =$ constant, and $x =$ days of conditioning.
Figure 4. Water uptake of eastern gamagrass seeds during conditioning in MicroCel E. Water uptake was fit to following equation: \( y = A - B \cdot e^{-cx} \), where \( y \) = seed moisture content, \( A \) = maximum seed moisture content, \( B \) = the difference between the maximum seed moisture content and at seed moisture at 1 d of conditioning, \( c \) = constant, and \( x \) = days of conditioning.
Figure 5. Water uptake of eastern gamagrass seeds during conditioning in Vermiculite #5.

Water uptake was fit to following equation: $y = A - B \, (e^{-cx})$, where $y =$ seed moisture content, $A =$ maximum seed moisture content, $B =$ the difference between the maximum seed moisture content and at seed moisture at 1 d of conditioning, $c =$ constant, and $x =$ days of conditioning.
Figure 6. Germination of eastern gamagrass after conditioning for 7 d in Agro-Lig, MicroCel E, or Vermiculite #5. Moisture sources used in the conditioning systems were deionized water, unbuffered 0.01 M GA₃ solution, 0.01 M GA₃ solution buffered to pH 5.2 with citric acid disodium phosphate buffer, or buffer solution without GA₃.
CHAPTER 6. GENERAL CONCLUSIONS

Many factors appear to influence dormancy in eastern gamagrass seed. The strong indurate cupule that acts as a dispersal unit may be one obstacle in the germination of eastern gamagrass. However, work by Tian et al. (2002 a,b) and Anderson (1985) showed that cupule removal from the caryopsis was not enough to completely overcome eastern gamagrass's seed dormancy. This indicates dormancy controlled, at least partially, by factors inherent to the caryopsis. The cause of this dormancy has yet to be determined. Although the dormancy is partially released with removal of the eastern gamagrass caryopsis from the cupule, there is currently no efficient, uniform, and mechanical way to remove it without injuring the embryo. Methods that break the dormancy of cupulated seed could reduce the stand establishment costs for eastern gamagrass resulting in greater adoption of this promising grass by forage producers and conservationists.

Gibberellins and moist prechilling are known promoters of germination in many species and have increased the rate and percent of seed germination in eastern gamagrass. However, there are no reports of the effect of combinations of these two treatments on eastern gamagrass seed germination. In the research reported in this thesis, final percent germination was positively enhanced by 0.001M GA₃ when seed was prechilled for three weeks or less. However, germination was similar with and without GA when eastern gamagrass was moist prechilled for four or more weeks. GA₃ in combination with prechilling increased the germination rate of eastern gamagrass seed for prechilling durations of 0 to 7 weeks. More rapid germination, provided by treating seeds with GA₃, could give germinating seedlings a competitive advantage against pests and disease.

Obtaining maximum germination after moist prechilling for four weeks is a much shorter time than the six to eight weeks reported by Ahring and Frank (1968) and 60 days reported by Anderson (1985). Decreasing the time needed to effectively prechill eastern gamagrass seeds from the currently practiced six to eight weeks to four weeks could reduce seed costs.
and establishment expenses. In our studies, seeds were allowed to imbibe moisture at room temperature for 24 h before being placed in cold temperatures. Perhaps allowing imbibition to occur before slowing the metabolism with chilling was particularly effective at stimulating germination processes. Proving this idea would require direct testing of seed allowed to imbibe moisture at room temperature to seed imbibed in cold temperatures.

Previous reports on eastern gamagrass prechilling did not address the fate of seeds that remained ungerminated after peak germination was reached. A decrease in germination with more than 6 weeks of prechilling as reported by Ahring and Frank (1968) could result from seed death or cycling back into dormancy. In our study, final germination did not increase after four weeks of moist prechilling. But, the number of dead seeds increased with each week of moist prechilling past five weeks until very few dormant seeds remained with seven weeks of prechilling. These trends suggest that the remaining ungerminated seeds were not capable of producing viable seedlings and would die with additional weeks of prechilling.

The addition of GA3 to solid matrix priming systems using Agro-Lig, MicroCel E, and Vermiculite #5 increased germination of eastern gamagrass seed. However, the germination level of about 20% of all live seed was not great enough to suggest that these systems would be cost effective for enhancing eastern gamagrass germination. Although the solid matrix priming systems did not break much of the eastern gamagrass dormancy in the context they were used in this study, useful information was gained by developing the systems and incorporating hormones into the priming process. We were able to determine the water potential created in each carrier at various water contents and the rate at which the seeds hydrated in each carrier. We also determined the level of seed hydration at various water potentials in each carrier. Further work is needed to determine if solid matrix priming in combination with prechilling and GA3 can further shorten the time required to break the majority of the seed dormancy in eastern gamagrass.
It is clear from our current and past studies (Tian et al., 2002b) that gibberellic acid enhances the germination of eastern gamagrass seed. Perhaps the gibberellic acid was acting as an activator of germination processes within the embryo to encourage rapid germination. Also, it is possible that GA3 was generating germination in seeds with lower levels of dormancy or that were inherently more sensitive to dormancy breaking treatments than other seeds within the seed lot.
LITERATURE CITED


ACKNOWLEDGEMENTS

Thanks to the Leopold Center for Sustainable Agriculture for providing the funding for this research. I would like to thank my committee for making this thesis a possibility. To Dr. Horton who provided the “isolation chamber”, some equipment, and much guidance on water potentials. To Dr. Knapp, thank you for your service as an educator and guidance as a researcher. Thanks for taking me from “verbage” on matricconditioning in Agronomy 338 to understanding the concept and science of it through my thesis work. Most importantly, to my major professor Dr. Lance Gibson, you have been a role model to me professionally and personally. I thank you for your patience, guidance, support, and dedication throughout this project. Also, thank you for giving me the opportunity to study at Iowa State University.

I would also like to thank Bryce Lemke, Trent Siebrecht, Dustin Gleason, and Ezra Aberle. These people helped me in the collection of my data and provided friendship and advice. My office mates provided positive energy in my time at Iowa State University. It has been a pleasure to learn about Texas, Mexico, Argentina, New York, and Illinois as well as to develop friendships with such wonderful people.

Finally I would like to thank my family and Kerry. Thank you mom for instilling in me a love of science at an early age, dad for helping to create an interest in the seed industry I hope to work in, and to Sara for being a wonderful friend and a role model of dedication and loving what you do. Thanks to Kerry for providing much needed and appreciated support and friendship in the past year and a half. I couldn’t have gotten here without all your support and love. It has been truly appreciated and opened my eyes to the blessings in my life.