PUBLIC WORKS TECHNICAL BULLETIN 200-1-109
30 SEPTEMBER 2011

PRE-GERMINATION TO ENHANCE SEEDING SUCCESS AND ESTABLISHMENT OF VEGETATION

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DEPARTMENT OF THE ARMY
U.S. Army Corps of Engineers
441 G Street NW
Washington, DC 20314-1000

CECW-CE

Public Works Technical Bulletin 30 September 2010

No. 200-1-109

FACILITIES ENGINEERING
ENVIRONMENTAL

PRE-GERMINATION TO ENHANCE SEEDING SUCCESS AND ESTABLISHMENT OF VEGETATION

1. Purpose.

a. The purpose of this Public Works Technical Bulletin (PWTB) is to transmit background and guidance for the pre-germination of native species to maximize the function, success, efficiency, and aesthetics of native species establishment while minimizing the associated costs in improved and unimproved areas on military installations.

b. All PWTBs are available electronically (in Adobe® Acrobat® portable document format [PDF]) through the World Wide Web (WWW) at the National Institute of Building Sciences' Whole Building Design Guide web page, which is accessible through URL:

http://www.wbdg.org/ccb/browse_cat.php?o=31&c=215

2. Applicability. This PWTB applies to all U.S. Army facilities engineering activities.

3. References.

a. Army Regulation (AR) 200-1, "Environmental Protection and Enhancement," 13 December 2007.

b. EO 13514, "Federal Leadership in Environmental, Energy, and Economic Performance," 05 October 2009.

4. Discussion.

a. AR 200-1 contains policy for environmental protection and enhancement, implementation of pollution prevention, conservation of natural resources, sustainable practices, compliance with environmental laws, and restoration of previously damaged or contaminated sites.

b. EO 13514, as signed by President Obama in October 2009, has the goal to “establish an integrated strategy towards sustainability in the Federal Government”. Additionally, it requires that management of water quality and stormwater management issues on installation lands are addressed to include predevelopment hydrology. As part of these requirements, the use of vegetation—specifically native vegetation— is critical to help achieve higher water quality and reduce overland runoff from storm events. The use of native landscaping can greatly reduce the use of potable water, improve infiltration, decrease erosion and sedimentation, improve overall soil condition and greatly reduce pest management.

c. This PWTB focused on the use of native grass species commonly used in Military Land Management for erosion and sediment control. Quick, rapid, and successful establishment of vegetation on military training lands is highly desirable for preventing erosion, facilitating training use, sustaining land resources, and other factors. Establishing vegetation can be both difficult and costly due to manpower, equipment, and seed costs as well as the frequent need for multiple seedings. In turf management, a common practice is to start germination outside of the soil prior to seeding. This practice has also been used on areas subjected to wild fires to quickly establish vegetation in areas of high risk, i.e., steep slopes, critical habitat, and sites adjacent to water. Such pre-germination can lend itself to use by the Army in problematic areas such as small/large arms range firing and impact points, firing range burn sites, construction areas, and any area with a need for quick establishment.

With appropriate use of pre-germination techniques and applications, installations would see an increase in success rate for seeding. When used in conjunction with hydro-seeding technology, pre-germination is applicable for difficult to reach areas. Establishment of important and key species would help increase and maintain installation biodiversity. Faster establishment of vegetation would result in better air and water quality; it also may help compliance with CAA, CWA, Endangered Species Act (ESA), and National Environmental Policy Act (NEPA).

d. Appendix A contains an introduction to seed germination processes and current pre-germination treatment technologies. It also summarizes the importance of quick, successful vegetation establishment on military training lands and how pre-germination treatment technologies could aid in achieving this objective.

e. Appendix B explains the studies that were conducted to investigate the applicability and efficacy of pre-germination treatment on native grass seeds. Results from this investigation indicated that knowledge of the varietal dormancy rates was critical to success of establishment. Additionally, species reacted much differently from one to another to the pre-germination treatments. Overall, Big Bluestem preformed best with Gibberillic Acid (GA), Switchgrass reacted best to potassium nitrate (KNO_3), and Little Bluestem was negatively impacted by the pre-germination treatments.

f. Appendix C summarizes lessons learned from the study and gives recommendations for native seed pre-treatment practices.

g. Appendix D provides a list of acronyms and abbreviations used in this PWTB.

5. Points of Contact.

a. Headquarters, US Army Corps of Engineers (HQUSACE) is the proponent for this document. The point of contact (POC) at HQUSACE is Mr. Malcolm E. McLeod, CEMP-CEP, 202-761-5696, or e-mail: Malcolm.E.Mcleod@usace.army.mil.

b. Questions and/or comments regarding this subject should be directed to the technical POC:

U.S. Army Engineer Research and Development Center
Construction Engineering Research Laboratory
ATTN: CEERD-CN-N (Heidi R. Howard)
2902 Newmark Drive
Champaign, IL 61822-1076
Tel. (217) 398-5865
FAX: (217) 373-7266
E-mail: heidi.r.howard@usace.army.mil

FOR THE COMMANDER:

For JAMES C. DALTON, P.E., SES
Chief, Engineering and
Construction Division
Directorate of Civil Works

APPENDIX A:

INTRODUCTION TO SEED GERMINATION PROCESSES AND CURRENT PRE-GERMINATION TREATMENT TECHNOLOGIES

Authors: Heidi Howard, Timothy Cary, Daniel Koch, and Thomas Smith.

Seed Germination

Seed germination is defined as the emergence of the radicle through the seed coat. Common garden or cultivated seeds germinate if given only water and reasonably warm temperatures. Wild species, notably grasses, usually have deeper dormancy to avoid sprouting in late summer or fall when the seeds are commonly dispersed in nature. This dormancy assures that tender seedlings are not frozen at a young age by the approaching winter. Instead, the seedlings do not appear until warm weather arrives in springtime.

The process of seed germination is complex and can be affected at different stages by many interacting factors such as temperature, moisture, oxygen, light, substrate, and the maturity and physiological age of seed. In laboratory germination tests, these factors are optimized or at least standardized, in order to measure the maximum number of seeds capable of producing healthy, well-developed seedlings. A laboratory germination test does not take into account the effects on the seed of non-optimal planting conditions. It is therefore useful to view a laboratory germination test result as an expectation of potential rather than an absolute emergence.

Germination Tests

There are two common determinations that are made from germination tests—viability and germinability (usually both are expressed as a percentage).

Viability simply means that the seed is alive. It does not indicate that the seed will germinate. Viability tests may be as simple as cutting a seed with a knife blade to determine if an embryo is present. More complex viability tests involve the use of a tetrazolium test which aims to determine which seed tissues are alive and have the potential to germinate under optimum conditions. Tetrazolium is a colorless chemical (2,3,5-Triphenyltetrazolium Chloride) that reacts with tissues that are able to respire and stains them red. After the proper sectioning and preparation of the seed, this chemical shows (by the red color) the respiring or living tissue in the seeds. In this way, living tissue in seed embryos can be distinguished from non-living tissue. This test will not detect seedling abnormalities; it only detects which tissues are viable (alive). Because of this, occasionally a tetrazolium test and germination test result for the same sample will not be comparable. This is because any factor that affects the seed as it actually germinates (e.g., chemical damage, dormancy, or disease) is not detected by tetrazolium tests. (The presence of living tissue does not necessarily mean the embryo will germinate.) For seeds of major crop species, standards have been developed that relate the tetrazolium reaction to potential germination. These standards, however, have not been developed for the seeds of most native species.

Germinability is a much more meaningful factor for individuals interested in propagating plants from seeds. To obtain an estimate of germinability, the seeds must be subjected to a germination test. A standard laboratory test is typically based on 200 seeds. Seeds are germinated under optimum environmental conditions for an optimum period of time according to species. However, for many species, including natives, optimal conditions are not well understood. For some species the length of the test can be long. Dormancy breaking measures are typically used because germination tests can only assess the germination of non-dormant seed. Also, distinctions are made between normal, abnormal and dead seeds during germination. Results usually are reported as percentage germinated (or percentage dormant seed where relevant). Major quality problems observed on a sample may be noted, but without further testing, the problems usually are not quantified on the test report. This type of germination test has the advantage of seed industry acceptance and relatively low cost.

More detailed and costly diagnostic germination tests generally follow the standardized testing protocol. The length of time involved and the level of analysis can differ greatly with results being reported as percentage germinated, percentage dead seed, and percentage abnormal seedlings. In addition, a subset percentage is given for damage (e.g., heat, chemical, sprouting, mechanical, and disease) for dead and abnormal seeds.

Seed Collection and Handling

The use of locally or regionally produced seed (ideally from wild sources) results in the advantage of employing plant ecotypes which are pre-adapted to local conditions. While commercial varieties and cultivars may be more readily available and recommended, they may be more costly and will not necessarily result in the best germination and stand establishment for a specific area. Seed harvesting, handling, and cleaning are important seed quality considerations in vegetation reestablishment efforts. In most cases, seed will be purchased from outside sources and these important details will have been addressed by the supplier.

Seed storage following acquisition can be important and needs to be considered by the installation user. A key to seed storage is maintaining proper moisture conditions so that the seeds remain alive but do not germinate. Remember that the amount of water that the storage atmosphere will hold as a vapor is directly related to temperature. The warmer the air, the more moisture it will hold. When the air temperature drops, relative humidity will increase. Droplets of water may then condense and form in storage containers. Consequently, storage in paper or mesh bags in a cool, dry location is satisfactory for most seeds. Once the seeds have reached moisture equilibrium, storage in glass jars or plastic boxes is possible to avoid insect and rodent contamination. Some seeds can be stored easily in small lots, but suffer losses in viability when larger quantities of seeds are stored together. Some seeds have short storage lives, and seed stocks of these species must be renewed annually. Research on the species used and stored is needed to determine storage requirements as well as the length of viability for longer-term storage.

Pre-germination Treatment Considerations

Pre-germination can be used where a rapid establishment of cover is desired and/or to reduce competition/weed pressures. In its simplest form, pre-germination involves soaking seeds in water and, when the first seeds show signs of germination, removing the seeds and planting them. However, there are also other considerations.

After-ripening

Seeds of many species will not germinate immediately after harvesting and must pass through a period of dormancy before germinating. This dormancy requirement, which varies by species, allows for certain physiological changes to occur within the seed that make it capable of germination. A variant of this type of dormancy is called temperature-dependent after-ripening. In this type of dormancy, seeds will germinate only within a certain temperature range. Other variations include responses using alternating temperatures, light, stratification, leaching of growth inhibitors, and other conditions.

Scarification

For the seed to be permeable to water, some seeds require some form of scarification to break the hard seed coats. This scarification can be accomplished with mechanical, thermal, or chemical treatments. If the seeds are large enough, scarification may be accomplished by filing a notch in the coat or by clipping it, taking care not to injure the embryo. Smaller seeds can be mechanically scarified by abrading them in some manner. Such scarification may be as simple as rubbing the seeds between sheets of sandpaper.

Alternatively, mechanical scarifiers have been developed, such as those with rotating drums (lined with an abrasive material) in which the seeds are tumbled. However, any type of mechanical scarification that increases germinability can result in decreased viability. Thermal scarification is obtained by dropping seeds into boiling water and then allowing the water to cool. Such treatment may have many other influences, such as thermal shock to the embryo or leaching soluble inhibitors. In areas that have freezing winter temperatures, thermal cracking of seed coats can also be obtained by fall seeding at shallow depths.

One chemical method of scarification is to use concentrated sulfuric acid to remove hard seed coats. With this and similar chemical approaches, the duration of treatment varies with the species and even with individual seedlots. Heating from the acid reaction along with rinse water and the resulting hydrolysis of the seed tissue may induce germination rather than simply increasing the intake of water as intended. If using this method, always try to control the temperature of the acid-treated seeds in a water bath, use a small amount of acid and seed in a large volume of water, and use a neutralizing solution after the treatment.

Stratification

Seeds that absorb water but fail to germinate are good candidates for stratification. Stratification means placing of seeds in a wet environment at temperatures that are not normally conducive to germination. Such treatment is termed “cool-moist stratification.” The duration requirements of stratification treatments can range from a few days to many months. For prolonged stratification, a substrate must be furnished to retain moisture. Peat is an often used substrate material, but other common materials include sand and vermiculite. Some species of conifers will even respond to simple stratification which is accomplished by soaking the seeds overnight in water and then placing the damp seeds in plastic bags that are sealed for a period of time. Seeds of other species require other specific stratification temperatures. With many native species seeds, germination can be difficult and can require prolonged periods and require extensive experimentation for successful stratification.

Nitrogen

The supply of nitrogen is a very influential factor in enhancing seed germination. Nitrogen is frequently added as fertilizer in the form of potassium nitrate (KNO_3), but also in the form of ammonium nitrate (NH_4NO_3). Lush, rapid growth of seedlings in spring or after rains is associated, in part, with the availability of nitrogen in the seedbed. For some species, nitrogen will not only increase germination, but also can be responsible for breaking dormancy. Nitrogen, in whatever form, can be applied at different concentrations depending not only on the species, but also on inherent or existent soil fertility.

Gibberellic Acid

The seed takes up water from the environment in a process known as imbibition. During imbibition, water passes through the embryo, picking up the germination signal, the hormone gibberellic acid (GA). Scientists don't know exactly how gibberellic acid (giberellin), a growth regulator, works in seed germination, but they do know that very low concentrations can greatly enhance germination. Concentrations of from 1 to 250 parts per million (ppm) are commonly used to improve germination. Combinations of gibberellic acid and potassium nitrate are often more effective than either material alone. These materials can be obtained from chemical suppliers, and application is easy to perform, even for large quantities of seed. Specialized nurseries can treat seed as part of seed orders if inhouse treatment is not desired or cost effective.

Light

In species with thin seed coats, light may be able to penetrate into the dormant embryo. The embryo may then either use the presence of light or the absence of light to trigger its germination process. Small seeds with a thin seed coat, like grasses, are likely to use light as a signal for germination. If these are buried shallowly enough for light to hit the seed, germination will begin and initiate photosynthesis before the seed runs out of reserves. If the small seed is deeply buried, however, it does not germinate. If it were to germinate, it may not make it to the surface before on-board reserves run out. Thus, evolution favors shutting down germination in the dark for those small-seeded species. For large-seeded species, the opposite may be true.

Summary

Although employed in the turf industry, pre-germination techniques are not yet commonly used for native species establishment. Rapid and successful establishment of native vegetation to promote healthy ground cover on training lands can be enhanced through the use of pre-germination techniques and approaches. To reduce post-harvest losses and to enhance plant and cover establishment, seed requires proper selection plus proper timing and care during harvest and storage. Germination of seeds can be stimulated by special treatments. Some of those treatments use chemicals that may be relatively expensive but are used in very small quantities. Military land managers are urged to consider using the techniques described in this PWTB when establishing vegetative cover.

APPENDIX B:

STUDY METHODS AND RESULTS

To promote healthy ground cover on training lands, the methods used for rapid and successful establishment of commercial turf grass species were tested with native species. Our hypothesis was that selecting varietals showing similar dormancy trends and encouraging pre-germination via treatment would increase establishment rates and decrease associated costs. Documenting these methods will allow installation land managers to select proper varietals and pre-germination treatments.

Laboratory Experiment

Preliminary growth chamber studies were used to determine varietals of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and switchgrass (*Panicum virgatum*) that exhibit similar dormancy rates (Table B-1). By subjecting these varietals to treatments of cold, gibberellic acid (GA), potassium nitrate (KNO_3) and a combination of GA and KNO_3, under controlled conditions we were able to determine the optimal treatments to evaluate under field conditions.

Table B-1. Percentage of pure live seed, germination, and dormancy for various commercial varieties of switch grass.

Variety	Release	Pure Seed (%)	Germination (%)	Dormancy (%)
Southlow	MI* Ecotype	98.9	6	90
Dacotah	Commercial	98.6	7	73
Blackwell	Commercial	99.7	15	73
Forestburg	Commercial	99.5	41	54
Alamo	Commercial	97.8	35	45
Carthage	NC* Ecotype	99.7	51	43
Cave-in-Rock	Commercial	99.8	71	24
Sunburst	Commercial	99.7	82	13
Shawnee	Commercial	99.9	88	4
Shelter	Commercial	99.9	94	0

*MI = Michigan; NC = North Carolina

A study design was developed to determine treatment performance under both greenhouse and field conditions. Three cultivars were selected for each native grass species (big bluestem, little bluestem, and switchgrass). The treatments for each grass species are described in Table B-2. The greenhouse experiments were performed in a growth chamber with these conditions: temperature of 15 °C, 16 hours of daylight, and 70% humidity.

Table B-2. Seed pre-treatments for greenhouse and field experiments.

Treatment	**Concentrations (ppm)**	**Seed Treatment**
KNO_3	500; 1,000,; 2,000	20-min soak
Cold	NA	Soaked in DI water overnight at 20 °C
Giberellic Acid	100; 500; 1,000; 2,000	18-hr soak
Control	NA	No treatment
Giberellic Acid & KNO_3	1,000 & 1,000	18-hr soak in Giberellic Acid, 20-min soak in KNO_3

Results from the greenhouse growth chamber experiments for switchgrass are displayed in Table B-3, Table B-4, and Figure B-1. With the exception of the high concentration GA treatments (1,000 and 2,000 ppm), all treatments generally resulted in increased germination rates when compared with the control.

Table B-3. Growth chamber results for percent germination of switchgrass varieties subjected to pre-germination treatments.

Treatment	**Blackwell**	**Carthage**	**Forestburg**	**Shawnee**	**Shelter**	**Southlow**	**LSD @ 0.05**
Cold (20 °C)	33.25	19.50	30.50	24.00	13.00	4.80	10.03
Control	13.25	16.50	30.50	32.50	9.50	3.75	6.29
GA 100	23.50	28.25	32.25	40.75	12.00	9.25	14.58
GA 500	34.00	50.25	41.25	45.25	12.50	9.25	20.25

Treatment	Blackwell	Carthage	Forestburg	Shawnee	Shelter	Southlow	LSD @ 0.05
GA 1000	17.25	12.75	21.75	23.25	6.00	3.25	8.85
GA 2000	6.75	1.75	3.25	1.75	0.50	0.50	ns
GA 1000 + KNO3 1000	32.50	26.00	37.00	41.75	9.00	6.00	12.48
KNO3 500	19.50	31.75	32.25	36.50	7.00	3.00	6.99
KNO3 1000	23.75	27.75	39.25	39.50	8.75	6.50	14.63
KNO3 2000	22.25	29.50	35.50	35.25	11.00	7.75	10.85
LSD @ 0.05	12.06	14.81	10.98	13.12	5.52	5.07	

Table B-4. Growth chamber germination: summary of all switchgrass varietals subjected to pre-germination treatments.

Treatment	% Germination
Cold (20 °C)	20.79
Control	17.67
GA 100	24.33
GA 500	32.08
GA 1000	14.04
GA 2000	2.47
GA 1000 + KNO_3 1000	25.38
KNO_3 500	21.67
KNO_3 1000	24.17
KNO_3 2000	23.54
LSD @ 0.05	4.36

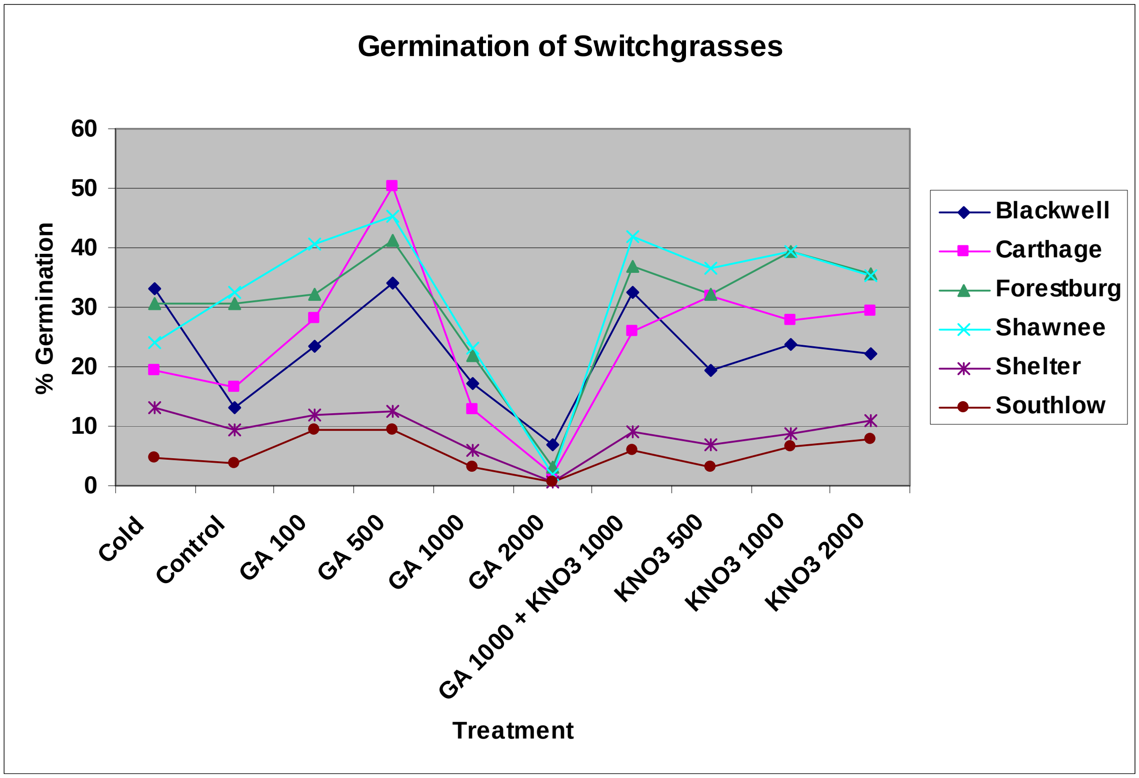

Figure B-1. Effects of pre-germination treatment on switchgrass varieties in growth chamber experiment.

Field Experiment

For the field experiments, Fort Drum (located in Jefferson County, New York) was chosen as the study site. Silt loam soils at a 0-50% slope under mixed forest and grassland make up most of its soils. However, soils used in this project were predominantly sandy silt soils. The same three cultivars for each grass species were subjected to the pre-germination treatments (cold, 100 ppm GA, 1,000 ppm KNO_3, and a control). Each treatment was assigned a 20 x 30 ft plot (Figure B-2). The plots were disced and raked prior to hand-broadcasted planting of the seed (Figure B-3). To ensure good seed-to-soil contact, all plots were rolled after planting.

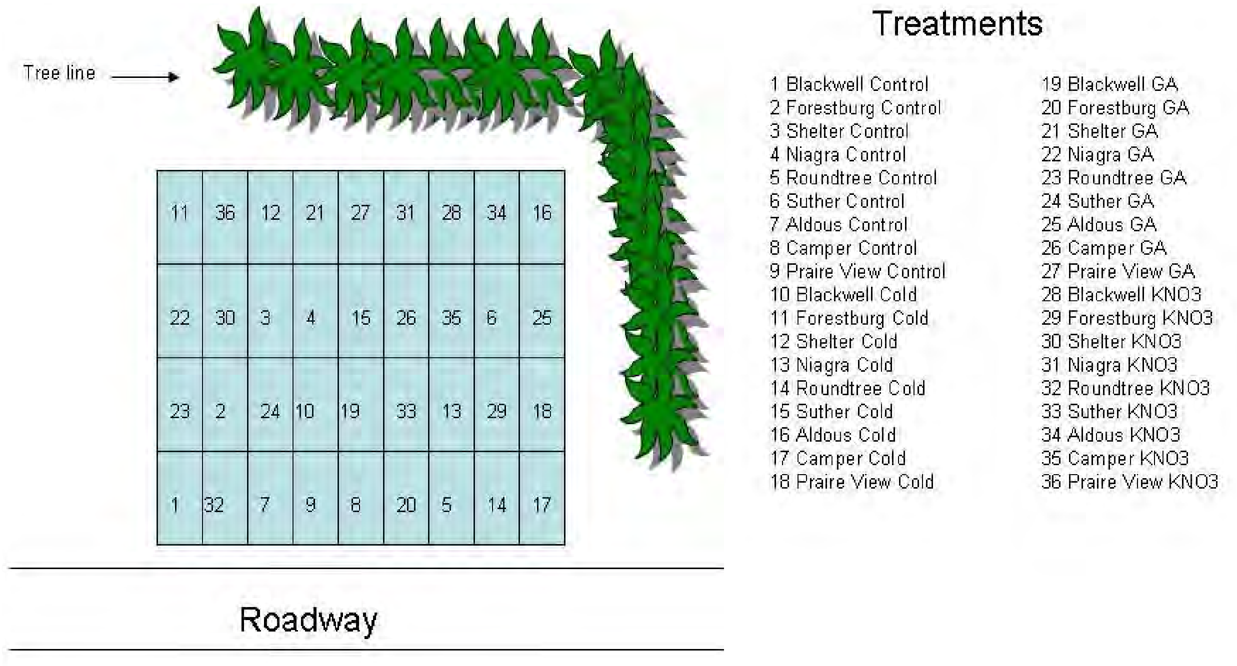

Figure B-2. Schematic of treatment plots for field experiment.

Figure B-3. Preparing the Fort Drum plots for seeding.

Tables B-5 and B-6 describe the results from the field experiments. The dormancy and germination results for each variety of big bluestem, little bluestem, and switchgrass are given in Table B-5 while Table B-6 describes the germination results given different pre-treatments.

The field data suggests that we were able to optimize germination and establishment. Big bluestem and switchgrass as a whole performed significantly better than little bluestem. Analysis indicates both cultivar difference and a significant decline with treatments for little bluestem. This was not the case for either switchgrass or big bluestem. The primary point to be made is that the treatments for pre-germination had a significant negative impact to the overall performance of little bluestem, where as both big bluestem and switchgrass showed improved germination overall with treatment. Possible influences were observed in the field primarily due to site aspect and sandy soils. It was observed that wind scour negatively impacted certain plots.

Table B-5. Percent germination results across multiple varieties of big bluestem, little bluestem, and switchgrass, respectively.

Variety	**% PLS**	**% Dormant**	**June**	**September**
Niagra	95	8	69.7	77.8
Roundtree	74	8	69.7	77.8
Suther	92	65	55.3	71.1
	LSD @ 0.05 = 4.95			
Aldous	94	40	78	65.5
Camper	95	9	53.7	34.7
Prairie View	96	91	49.3	28.9
	LSD @ 0.05 = 5.13			
Blackwell	88	73	66	71.8
Forestburg	95	54	76.1	65.3
Shelter	94	0	57.2	76.2
	LSD @ 0.05 = 5.79			

Table B-6. Summarized percent germination results across multiple varieties of big bluestem, little bluestem, and switchgrass, differing pre-germination treatment, and seasonality.

Treatment	**Big Bluestem (Niagra, Roundtree, Suther)**		**Little Bluestem (Aldous, Camper, Prairie View)**		**Switchgrass (Blackwell, Forestburg, Shelter)**	
	June	**September**	**June**	**September**	**June**	**September**
Control	60.8	70.7	69.7	40.4	68.8	64.8
Cold (20 ℃)	64.5	67.3	50.9	45.3	54.6	76.5
GA	68.8	92.6	56.5	51.2	61.1	53.4
KNO_3	57.7	69.7	64.2	35.2	81.1	89.5
LSD @ 0.05 =	5.72		5.93		5.79	

APPENDIX C:

OBSERVATIONS AND RECOMMENDATIONS FOR NATIVE SEED PRE-TREATMENT PRACTICES

1.) Seed obtained from commercial sources can generally be assumed to have a high percentage of viable or pure live seed. However, germination and dormancy can vary greatly between sources and/or varieties (Appendix B: Table B-1). Therefore, pre- seeding germination and dormancy testing can be useful for ensuring seeding establishment.

2.) In part because of after-ripening and dormancy effects on germination and therefore stand establishment, pre-seeding germination testing during different seasons (months) should be considered (Appendix B: Table B-5).

3.) Pre-germination treatment that uses cold, gibberellic acid, or potassium nitrate can enhance germination (Appendix B: Tables B-3, B-4, and B-6; Figure B-1).

4.) If pre-treatment with gibberellic acid is employed, low concentrations (100 – 500 ppm) are more effective in stimulating germination (Appendix B: Tables B-3 and B-4; Figure B-1).

5.) If pre-treatment application of potassium nitrate is used, germination is enhanced by a wide range of concentrations (e.g., 500 - 2000 ppm) (Appendix B: Tables B-3, B-4, and B-5; Figure B-1).

6.) A combination of pre-germination treatment and seasonal planting that is optimal for the seed variety can enhance germination and subsequent seed establishment (Appendix B: Table B-6).

Appendix D:

ACRONYMS AND ABBREVIATIONS

Term	Spellout
AR	Army Regulation
CAA	Clean Air Act
CECW	Directorate of Civil Works, U. S. Army Corps of Engineers
CEMP	Directorate of Military Programs, U. S. Army Corps of Engineers
CERL	Construction Engineering Research Laboratory
CFR	Code of the Federal Regulations
CWA	Clean Water Act
CONUS	Continental United States
DA	Department of the Army
DPW	Directorate of Public Works
DoD	Department of Defense
EPA	Environmental Protection Agency; also USEPA
ERDC	Engineer Research and Development Center
ESA	Endangered Species Act
GA	gibberellic acid
HQUSACE	Headquarters, U.S. Army Corps of Engineers
KNO_3	potassium nitrate
LSD	least square denominator (of means)
NH_4NO_3	ammonium nitrate
OCONUS	outside Continental United States
NEPA	National Environmental Policy Act
PDF	portable document file
POC	point of contact
ppm	parts per million
PWTB	Public Works Technical Bulletin
URL	universal resource locator
USACE	U.S. Army Corps of Engineers
WBDG	Whole Building Design Guide
WWW	World Wide Web