Treatments to Overcome Dormancy of Eastern Gamagrass Seed

by Janet M. Grabowski and Joel L. Douglas[1]

Eastern gamagrass is a warm season perennial grass, native to the eastern and central portions of the United States and the West Indies (Hitchcock, 1951). It has been recognized as a highly palatable forage species (Polk and Adcock, 1964; Rechenthin, 1951); however, its utilization in forage systems has largely been hampered by seed production and establishment problems (Ahring and Frank, 1968; Anderson, 1990).

The seed unit of eastern gamagrass consists of a caryopsis surrounded by a hard, indurate, cupulate fruit case (Anderson, 1985). Many years ago, researchers such as Crocker (1916) recognized that hard seed or fruit coats could prevent germination by restricting the absorption of water or oxygen, or by physically restricting growth of the embryo. Seed coverings of many species will also contain chemical inhibitors that prevent germination from occurring (Hartmann and Kester, 1975). Anderson (1985) found that germination inhibitors were not present in the fruit coverings of eastern gamagrass and that these coverings did not restrict germination by preventing the passage of respired carbon dioxide out of the fruit. Since the coverings do not act as a barrier to movement of carbon dioxide, they likely would not impose any restrictions on the absorption of oxygen. Removal of the cupule promoted germination (Anderson, 1990) but, unfortunately, there is no effective way for seed producers to accomplish this for larger seed lots without causing a significant amount of damage to the seed. Anderson (1985) suggested that the cupule mainly affected germination by imposing limits on the environmental conditions under which germination could occur. The restrictive effect of the cupule can be overcome by a period of cold, moist stratification (Ahring and Frank, 1968; Anderson, 1985). Anderson (1990) found that there were genetic differences between populations that affected their response to stratification. Ahring and Frank (1968) tested various stratification intervals ranging from one to nine weeks and obtained best germination for the two seed lots tested with 6 weeks of stratification at 5 to 10°C.

This stratification requirement presents several agronomic problems for potential growers. First of all, the seed producer must be capable of providing the stratification treatment. Current recommendations are to hydrate the seed by soaking overnight in a 0.5 percent solution of Thiram 42S fungicide to control seed pathogens, and stratifying for 6 to 12 weeks at 1 to 4°C (Row, 1998). If stratified seed is subsequently exposed to environmental conditions that are not conducive to germination, then the seed may enter secondary dormancy

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(Hartmann and Kester, 1975; Simpson, 1990) and will not germinate until the following year. This problem is most severe when adequate soil moisture is not available after planting, and in many cases, would require irrigation to ensure establishment (Row, 1998). Also, many growers are not accustomed to handling and planting stratified seed. It should be refrigerated before planting and protected from high temperatures during the planting operation. For these reasons, an alternative seed treatment that could simplify the production and planting operation would be desirable.

Hot water treatments have been used to modify hard seed coats and promote germination (Hartmann and Kester, 1975). Keith (1981) successfully treated a hard-seeded cotton (*Gossypium hirsutum* L.) breeding line by soaking the seed in a hot water bath for 60 seconds at 85°C. Seed is usually planted immediately following hot water treatment, but certain types of seed have been allowed to dry and were then stored without greatly affecting germination percentages (Hartmann and Kester, 1975). If eastern gamagrass seed responded to hot water treatment, it might be less likely to encounter the potential secondary dormancy problems associated with stratification and would therefore become more attractive to potential growers.

Various types of chemical stimulants have been shown to promote germination (Hartmann and Kester, 1975). If an appropriate stimulant or combination of stimulants could be found for use on eastern gamagrass seed, planting operations could be simplified and establishment rates improved. Soaking seed in a potassium nitrate (KNO_3) solution is one method commonly used to improve germination of freshly harvested seed (Hartmann and Kester, 1975). Ahring and Frank (1968) found that soaking eastern gamagrass seed in sodium hypochlorite or KNO_3 solutions had no effect on germination. Soaking in a solution of ethylene chlorohydrin slightly increased germination, but the effect was not significant and germination percentages were much lower than stratified seed. Row (1998) found that soaking in KNO_3 or Thiram did not improve germination over soaking seed in water alone. Exogenous applications of gibberellins and cytokinins have also been shown to stimulate germination of various types of seed (Hartmann and Kester, 1975). It has been reported that eastern gamagrass seed is deficient in gibberellins and cytokinins. Treating seed with gibberellic acid (GA) improved early, but not total germination of eastern gamagrass seed (Anderson,1985).

METHODS

Seed from two accessions of eastern gamagrass were tested for the efficacy of hot water soaking, 9058543, which was originally collected in Pushmataha County, Oklahoma, and 9062708, from Williamsburg County, South Carolina. Seed were hand collected from plants growing at the Jamie L. Whitten Plant Materials Center (PMC) in Coffeeville, Mississippi during the months of July and August 1997. Seed harvested from eastern gamagrass often contains a fairly high and often variable percentage of unfilled seed, either without a caryopsis or

with a shriveled, non-viable caryopsis (Ahring and Frank, 1968; Douglas et al., 1997). To determine seed fill, 30 seed of each lot were opened and examined for the presence of a healthy caryopsis. It was found that 9058543 contained an average of 87 percent filled seed and 9062708 averaged 80 percent filled seed. Seed fill was fairly high because the seed was collected by hand, not combine harvested.

To determine appropriate hot water temperatures for testing, a non-replicated preliminary study was conducted using a seed lot from a previous experiment that contained a mixture of accessions cleaned to 90 percent fill. Temperatures used were 70, 80, 90, and 100°C and soaking duration ranged from 60 to 240 seconds in increments of 30 seconds using the methods outlined below. Seed from both the 90 and 100°C treatments exhibited zero percent germination. Because of this result, the 100°C temperature was dropped from further testing and 90°C was retained as the upper testing limit. Treatment intervals for final testing were not altered from those used in the preliminary test.

Seed treatments used in this study were the hot water treatments outlined above, plus stratification, and an untreated control. Twenty-five seed were used for each treatment. Samples were hot water treated by placing the seed in a basket constructed of hardware cloth (6 mm square openings) lined with aluminum window screen; an attached wire handle facilitated placement in and removal from the water bath. A cover was made from similar window screen to prevent seed from floating out of the basket during treatment. The basket containing the seed was submerged in a one liter beaker containing distilled water placed on a multiple setting hot plate, previously calibrated to provide the appropriate temperatures. Care was taken to ensure that temperatures did not vary by more than 2°C from the target temperature during the treatment period. After hot water treatment, seed samples were placed in a greenhouse and allowed to dry before planting. A quantity of seed was prepared for stratification by soaking overnight in tap water. It was then enclosed in a self-closing plastic bag with a minimal amount of free water, and put in a cooler maintained at 42°F with no humidity control.

The experiment was conducted twice. For the first treatment run, seed of both accessions were stratified on 24 February 1998. Seed of 9058543 was hot water treated on 25 March 1998 and all seed treatments for that accession were planted the following day. Accession 9062708 was hot water treated on 26 March 1998 and all treatments were also planted the following day. For the second run, seed was stratified on 24 April 1998, 9062708 was hot water treated on 26 May 1998, and 9058543 was hot water treated on 27 May 1998 with all treatments planted the day after hot water treatment as before. Germination containers used were 17.8 cm x 13.3 cm x 5.9 cm plastic bedding plant liners and seeds were planted 0.6 to 1.3 cm deep in a commercial potting medium. The test was arranged as a randomized complete block with three replications in a split plot design with accessions as the main plot and seed treatments as the

split plot. Germination containers were placed in a germinator maintained at 20/30°C night/day regime, with an eight-hour day period when the internal lights were on. There are no Association of Official Seed Analysts recommendations for eastern gamagrass germination testing; however, this temperature regime was recommended by seed laboratory personnel experienced in testing this species (J. Franklin, personal communication). All containers were irrigated thoroughly following planting and watered throughout the testing period as necessary. Germination counts were made every seven days over a five week period and a total germination percentage was determined for each treatment. This data was subjected to an analysis of variance (ANOVA) using MSTAT-C (Michigan State Univ., 1988) and appropriate mean separations were performed at the five percent level of probability ($P<0.05$) using a least significant difference test.

A non-replicated field trial using seed of both accessions was planted on 28 May 1998 at the PMC on an Oaklimiter silt loam soil. Seed treatments used were stratification (seed placed in stratification on 24 April 1998), untreated seed, and the highest ranking of the hot water treatments from the first run of the germinator experiment. Hot water treatments used were 70°C for 60, 90, 120, 150,180, 210, 240 sec, and 80°C for 60 sec. Seed sample size was 25 seed as in the germinator test. Hot water treated seed was dried in the greenhouse for one to two days before planting. Seed samples were planted by forming a shallow row with a hoe, hand sowing the seed, and covering to a depth of about 2.5 cm. Germination counts were made every three or four days until 9 July 1998.

Accession 9062680 (collected in Montgomery County, Tennessee) has been selected by PMC personnel as a superior accession. Seed of this accession was not available in early 1998 when the original testing was performed, so it could not be included in those trials. Seed was collected in July and August of 1998 and was subsequently tested in the germinator to determine its response to hot water treatment. For this study, seed quality was improved by separating out the heavier seed using a South Dakota Seed Blower (Seedburo Equipment Co., Chicago, Ill.); fill was determined to be 87 percent using similar methods to those of the previous test. Limited seed quantities were available which restricted the number of seed treatments that could be tested. Treatments used were stratification, control, and hot water soaking at 70°C for 240 sec, which ranked as the top hot water treatment for both accessions tested previously. This study was arranged as a randomized complete block with three replications. The seed was stratified on 8 August 1998, and was hot water treated in the morning of 10 November 1998 and all treatments were planted later that day. Methods used were the same as those for the previous germinator trials, except that germination counts were made for six weeks rather than five, because germination rates were slightly slower in this test. Data was subjected to ANOVA using MSTAT-C (Michigan State Univ., 1988) and mean separation was performed using Tukey's honestly significant difference test (HSD) at $P<0.05$.

All three accessions were tested for the effect of soaking in KNO_3 or GA solutions on germination rate and percentage of hot water treated and stratified seed. Stratification treatments consisted of soaking seed in a 0.2% KNO_3 solution, 1000 mg L^{-1} GA solution, or distilled water for 24 hours before commencement of the cold period. ProGibb, a commercial formulation of gibberellic acid was used as the GA source. After soaking, seeds were rinsed with distilled water before being placed in the cooler on 9 March 1999. Seed samples were hot water treated at 70°C for 240 sec and then soaked in the same treatments as used for stratification. Seeds were then rinsed with distilled water and kept moist until planting in the field or germinator. An untreated control was also included. Seed samples for the field test were hot water treated on 3 May 1999 and all treatments planted the following day. Stratified seed was placed in an insulated container to limit heat exposure while being transported to and before planting in the field. Seed for the germinator test were hot water treated on 5 May 1999 and planted 6 May 1999. Sample sizes used were 25 seed for the field test and ten seed for the germinator test. The tests were arranged as a two factor randomized complete block with six replications for the field test and four for the germinator test. The field test was planted at the PMC on an Oaklimiter silt loam soil using similar methods to those for the preliminary field test above. After planting, the study area was treated with 1.68 kg ha^{-1} of atrazine for weed control. Seed samples for the germinator test were planted using the methods outlined for the initial hot water tests. Germination counts were made weekly for 42 days, when the germinator study was terminated; counting seedlings in the field test became difficult after this time period due to weed growth, but the study was left in place in order to do a final survival count in the fall. Data from these tests were subjected to an analysis of variance (ANOVA) using MSTAT-C (Michigan State Univ., 1988) and appropriate mean separations were performed at the five percent level of probability ($P<0.05$) using a least significant difference test.

RESULTS

Hot Water Treatment: The germination percentages for each accession and each trial run were first analyzed separately to determine trends in responses to the treatments. The accessions responded in a similar manner to the seed treatments in each run of the experiment, so the data for each accession was averaged across the two runs. This data is presented in Tables 1 and 2. Germination rates were fairly low as is typical of eastern gamagrass. It appears that all 90°C treatments and the 80°C treatments soaked for 150 sec or longer may have been lethal or otherwise prevented from germinating. The true cause for this lack of germination was not determined, because it was apparent that such treatments would be unacceptable which made further examination irrelevant.

Table 1. Total germination percentages of eastern gamagrass accession 9058543 exposed to various seed treatments averaged over two trial runs.

Seed Treatment	Soaking Time (Sec)	Water Temperature (°C)		
		70	80	90
		----------%----------		
Hot Water Soak	60	11	19	0
	90	7	6	0
	120	13	1	0
	150	9	0	0
	180	12	0	0
	210	17	0	0
	240	22	0	0
Untreated		11		
Stratified		57		

Table 2. Total germination percentages of eastern gamagrass accession 9062708 exposed to various seed treatments averaged over two trial runs.

Seed Treatment	Soaking Time (Sec)	Water Temperature (°C)		
		70	80	90
		----------%----------		
Hot Water Soak	60	27	21	0
	90	29	5	0
	120	28	1	0
	150	25	0	0
	180	28	0	0
	210	30	0	0
	240	30	0	0
Untreated		24		
Stratified		42		

Those treatments that yielded zero germination were dropped from the final ANOVA. The resulting data analysis showed that there was a significant interaction effect between accession and seed treatment (Figure 1). There are several factors that could have contributed to this interaction. First, germination percentages for all seed treatments, except the stratification treatment and the 80°C treatments, were much higher for 9062708. Secondly, germination of stratified 9058543 was much greater than that of any of the other treatments for this accession, but for 9062708, several of the hot water treatments had germination percentages more closely similar in magnitude to those of the stratification treatment. In fact, the germination percentage of 9058543 stratified seed was significantly higher than that of same treatment for the other accession. Anderson (1990) noted variability in stratification response between genotypes of

eastern gamagrass from different locations. From these results, it seems likely that of the two accessions, 9062708 is not as dependent on stratification for optimum germination. Also, the two accessions did not respond in a similar manner to the hot water treatments. When each accession is looked at separately, the 70°C 240 sec treatment provided the best germination of any of the hot water treatments. However, the 80°C 60 sec treatment would be ranked as the second best hot water treatment for 9058543, whereas this treatment would have ranked as the eighth best hot water treatment for 9062708, below even the control treatment. Germination percentages of the 80°C 90 and 120 sec treatments were very low, which indicates that there may have been damage to the seed.

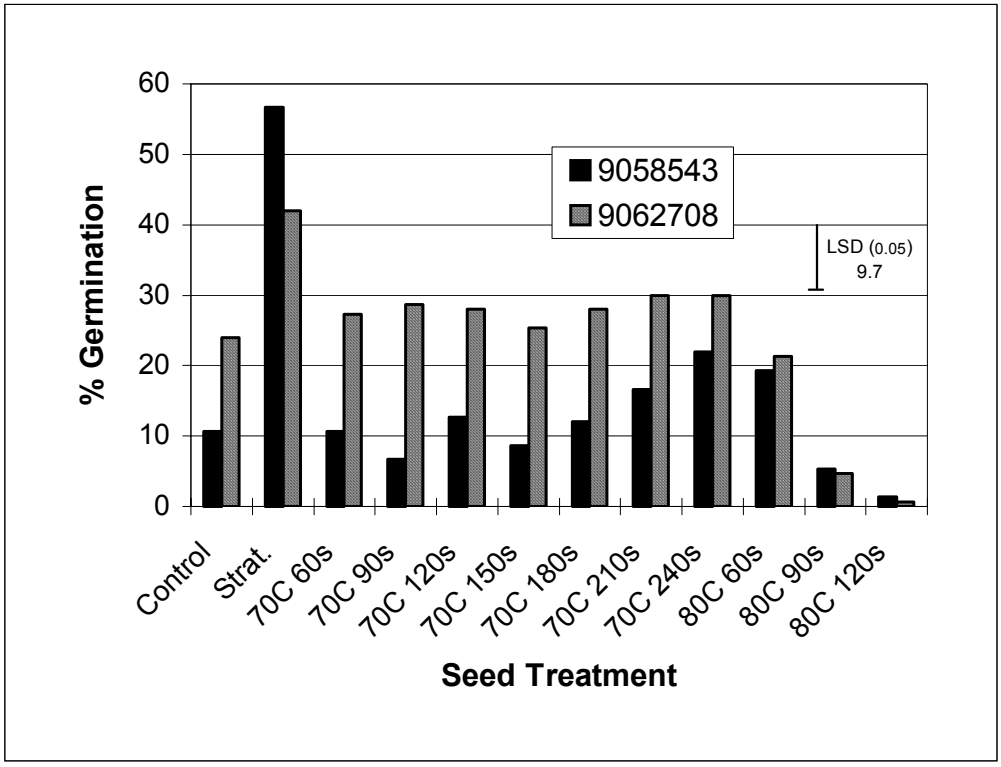

Figure 1. Interaction effect of selected seed treatments on total germination percentages of eastern gamagrass seed.

Figure 2 illustrates differences in germination rate for selected treatments by graphing the germination percentage mean over both accessions at each evaluation interval. Stratified seed germinated much more quickly than the other treatments. Anderson (1985) found that stratified seed exposed to appropriate temperatures germinated very rapidly, with several treatments showing relatively high germination percentages within five days. In this experiment, none of the treatments germinated by the first count at seven days. Anderson used filter

paper as the germination medium and could have detected germination much more quickly than in this study where seed were planted in a potting medium. Also, Anderson was working with populations of eastern gamagrass that originated from southern Illinois, which is father north than the collection site of the accessions used in this test. He notes that eastern gamagrass has naturally occurring races with various ploidy levels. Although Anderson did not specify the ploidy level of the seed used, most northerly accessions tend to be diploid whereas the southern accessions used in this study were tetraploid (C. Dewald, personal communication). Tetraploid seed has been shown to have a larger, heavier fruit case than diploid seed (Douglas, 1999) and this larger fruit case probably imposes more restrictions on the embryo, which may have slowed the germination rate for these accessions. The germination rate of the hot water treated seed was somewhat similar to that of the control, although final germination percentages were higher than those of the control. This response has profound agronomic implications. Even if final germination percentage of the hot water treated seed were equal to that of stratified seed, the fact that it germinates more slowly subjects the seedling to increased competition from other plant species, which could prevent establishment.

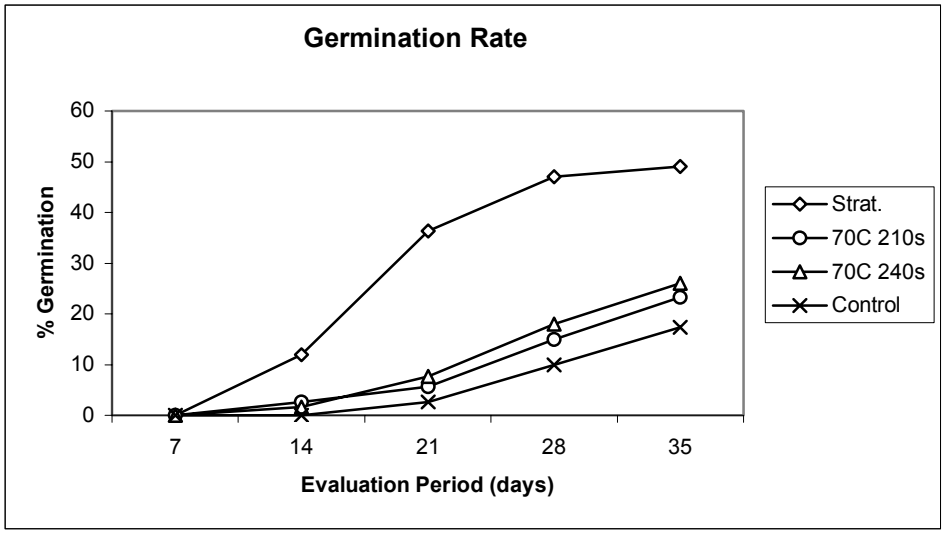

Figure 2. Mean germination percentages of stratified (Strat.), two selected hot water treatments, and untreated seed (Control).

Data for the preliminary field planting is presented in Table 3. No herbicides were used, so locating the seedlings for germination counts was somewhat difficult and this probably affected the counts made on several evaluation dates. The data presented is the maximum count recorded for each treatment, and may not be the true total germination percentage. Field response of the hot water treated seed was disappointing; however, weather conditions during the treatment period were unusually hot and dry. There was almost no germination recorded for any of the hot water treated seed of accession 9062708, the same accession that showed a more favorable response in the germinator. Those hot

water treatments with the highest germination percentages for 9058543 in the field were not those that performed best for this accession in the germinator. Keith (1981) found a similar disparity in the hard-seeded cotton line between those hot water treatments with the best germination percentages in the laboratory as opposed to those that germinated best in the field. Final recommendations for treating this cotton seed line were based on those treatments that performed best in the field. Due to the unusual environmental conditions experienced during this study, such conclusions would not be appropriate in this case. It is interesting to note the high germination percentages recorded for stratified seed. Several researchers have documented that secondary dormancy can be induced in stratified eastern gamagrass seed by drought (Row, 1998); however, germination percentages for these accessions were higher in the hot dry conditions in the field than they were for the more ideal conditions in the germinator. This could possibly be an instance of the seed responding favorably to high temperatures. Anderson (1985) noted that stratified seed germinated much better at a higher temperature (32°C) than at a lower temperature (25°C). Possibly 20°C/30°C is not the appropriate temperature regime to produce optimum germination percentages of eastern gamagrass seed and seed testing methods should be altered.

Table 3. Total germination percentages for a preliminary field test of two eastern gamagrass accessions exposed to various seed treatment regimes.

Seed Treatment	Germination	
	9058543	9062708
	----------%----------	
Untreated	0	0
Stratified	68	84
70°C 60 sec	0	0
70°C 90 sec	4	0
70°C 120 sec	16	0
70°C 150 sec	0	0
70°C 180 sec	24	4
70°C 210 sec	0	0
70°C 240 sec	0	0
80°C 60 sec	0	0

Table 4 shows the response of accession 9062680 to the three seed treatments used. This accession showed a somewhat favorable response to the 70°C 240 sec hot water treatment, but seed germination for this treatment was still significantly lower than that of the stratified seed. From this data, it appears that the response of this accession to the hot water treatment is probably similar to that shown for 9058543.

Table 4. Germination of eastern gamagrass accession 9062680 exposed to three seed treatment regimes.

Seed Treatment	Germination
	----------%----------
Untreated	4b*
Stratified	23a
70°C 240 sec	15ab

* Treatment means followed by different letters are significantly different by Tukey's HSD at $P<0.05$.

Chemical Seed Stimulants: Germination percentages for both locations of testing are presented in Table 5. There was an interaction effect between accession and seed treatment in both tests, because accessions responded differently to the various seed treatments. Germination of hot water treated seed was much higher in the germinator than in the field, which concurs with results from preliminary field testing (Table 3). Non-conducive environmental conditions were cited as a possible cause for the lack of germination in the preliminary test, but conditions were highly favorable during the germination period in this study (data not presented). It appears that hot water soaking does not provide sufficient seed coat modification to permit field establishment of eastern gamagrass seed. Germination of stratified 9062680 seed was lower than the other two accessions and the difference was significant for both accessions in the field test and one accession in the germinator test. Soaking in KNO_3 or GA slightly improved germination of hot water treated seed in the germinator; however, neither of these chemicals consistently improved germination of stratified seed. Row (1998) reported that a 24-hour exposure to KNO_3 reduced germination of some seed lots, but no such reduction was noted in this study. Response to KNO_3 appeared to be greater in the field than in the germinator test.

Table 5. The effect of chemical stimulants on germination of stratified and hot water treated seed of three eastern gamagrass accessions.

Accession	Seed Treatment	Germination	
		Germinator	Field
		----------%----------	
9058543	Stratified	63	49
	GA + Stratified	60	57
	KNO_3 + Stratified	58	62
	Hot Water	8	2
	Hot Water + GA	23	3
	Hot Water + KNO_3	13	4
	Untreated	3	1
9062708	Stratified	70	49
	GA + Stratified	58	45
	KNO_3 + Stratified	43	49
	Hot Water	20	2
	Hot Water + GA	23	0
	Hot Water + KNO_3	13	1
	Untreated	5	2
9062680	Stratified	51	38
	GA + Stratified	27	47
	KNO_3 + Stratified	21	36
	Hot Water	8	3
	Hot Water + GA	21	5
	Hot Water + KNO_3	21	1
	Untreated	8	1
LSD (0.05)		19	10

Figure 3 illustrates germination rates for the three stratification treatments and the control in the germinator test. Germination rate data from the field test is not presented because interference from weeds prevented accurate counts during later evaluation dates. Early germination was improved by GA exposure; however, the difference was not significant in the germinator. In the field, early germination was significantly greater for two of the three accessions. This finding is in agreement with Anderson (1985), who reported that GA increased early but not total germination percentages of seed with intact fruit cases. The GA treatment caused abnormal elongation and chlorosis of the seedlings, which made the shoots highly susceptible to being broken by wind or physical contact. Although Anderson (1985) also used a 1000 mg L^{-1} concentration of GA, abnormal growth was not reported in that study. Anderson (1985) did not specify the length of the GA treatment period or what formulation of GA was used. In this study, GA was added to the pre-stratification soak, which would appear to be a commercially acceptable treatment method. Reduced concentrations may be

required to effectively treat seed with the GA formulation and treatment duration used in this study. KNO_3 had no effect on germination rate.

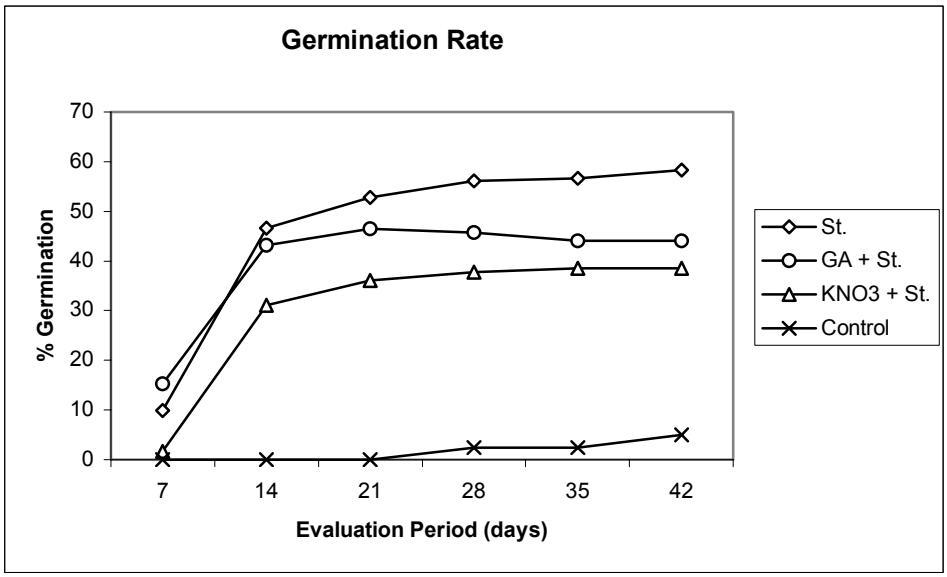

Figure 3. Mean germination percentages of stratified (St.) seed with or without pretreatment with chemical stimulants (GA or KNO_3) and untreated seed (Control) in the germinator.

CONCLUSIONS

Although eastern gamagrass seed responded to hot water soaking in the germinator, the effect on field-sown seed was minimal. Hot water treatments were not able to overcome the fruit coat imposed dormancy as effectively as cold stratification. Germination rate of hot water treated seed was much slower than that of stratified seed, which could affect its establishment potential. Treating seed with GA increased early emergence of stratified seed; however, appropriate rates and treatment methods need to be determined before commercial use could be recommended. Treatment with KNO_3 did not appear to adversely affect germination. Various combinations of these two chemicals will be tested in the future, along with other stimulants such as cytokinins. Seed of different eastern gamagrass genotypes appeared to respond differently to all seed treatments tested.

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