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Eastern Gamagrass Establishment: II. Effect of Hot Water Treatment on Seed Germination

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ABSTRACT

Seed dormancy in eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is imposed by a hard, woody fruit covering surrounding the caryopsis that must be modified before germination can occur. A test was conducted in the germinator using two accessions of eastern gamagrass (9058543 and 9062708) to compare germination rate and percentage for seed exposed to hot water soaking to those of seed treated with the standard commercial practice of cold stratification and an untreated control. Hot water treatments used were 70, 80, and 90°C and treatment duration ranged from 60 to 240 seconds in 30-second increments. Germination rate was faster and total percentage was significantly higher for stratified seed of both accessions than any of the hot water treated seed or the control. Percent germination of seed soaked at 70°C for 240 seconds was higher than germination percentages of the other hot water treatments and was significantly greater than germination of the control for both accessions. The two accessions responded differently to both the hot water soak, with 9062708 showing a greater response, and stratification, with 9058543 showing a greater response. The moderately favorable response to hot water treatment exhibited in the germinator was not apparent in a field test; however, environmental conditions were very hot and dry during the testing period and probably affected germination. Another accession, 9062680, was also tested in the germinator using only the stratification, 70°C 240 second hot water, and control treatments. It also responded favorably to the hot water treatment, but germination percentages were lower than those stratified seed. Additional testing is required to determine if hot water soaking is a feasible treatment for eastern gamagrass seed.

INTRODUCTION

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 (Ahring and Frank, 1968); however, its utilization in forage systems has largely been hampered by seed production and seed establishment problems (Ahring and Frank, 1968; Anderson, 1990).

The seed unit of eastern gamagrass consists of a caryopsis surrounded by a hard, indurate fruit case often referred to as a cupule (Anderson, 1985). Throughout this paper, the

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term "seed" will often be used when referring to the entire seed unit in order to simplify the discussion. Many years ago, researchers such as Crocker (1916) recognized that hard seed or fruit coats can 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 without damaging the seed. Anderson (1985) suggested that the cupule mainly affected germination by imposing limits on the environmental conditions under which germination can occur. The restrictive effect of the cupule can be overcome by a period of cold, moist stratification (Ahring and Frank, 1968; Anderson, 1985). However, 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. Stratified seed germinated better at a higher temperature (32°C) than at a lower temperature (25°C) (Anderson, 1985).

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 (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 instances, irrigation capabilities would be required 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. Generally, seed is 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. The results presented here are from an initial study conducted to determine if hot water treatment proved to be a viable method to overcome dormancy imposed by the fruit covering of eastern gamagrass seed.

MATERIALS AND METHODS

Seed from two accessions of eastern gamagrass were used in this test; 9058543, which was originally collected in Pushmataha County, Oklahoma, and 9062708, from Williamsburg County, South Carolina. Seed was 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. The seed was placed in a cloth bag and stored at room temperature without any cleaning or processing. 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 the 9058543 seed lot contained an average of 87 percent filled seed and 9062708 an average of 80 percent filled seed. The seed fill was fairly high because the seed was hand collected, 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 containing 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. Seed was hot water treated by placing it in a basket constructed of hardware cloth (1/8 inch square openings) lined with aluminum window screen; an attached wire handle facilitated placement in and removal from the water. A cover was made from similar window screen to prevent seed from floating out of the basket during treatment. The seed was submerged in a one liter beaker containing distilled water placed on a multiple setting hot plate calibrated to provide the appropriate temperatures. A thermometer was submerged to read at approximately the level of the seed in the treatment basket to monitor the temperature of the bath. Care was taken to ensure that treatment temperatures did not vary by more than 2°C from the target temperature. Treatment periods were measured with a timer. After treatment, the seed was placed in a greenhouse and allowed to dry before planting. Seed was stratified by soaking the seed overnight in tap water, placing the seed in self closing plastic bags with a minimal amount of free water, and placing these bags 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 was stratified on February 24, 1998. Seed of 9058543 was hot water treated on March 25, 1998 and all seed treatments for that accession were planted the following day. Accession 9062708 was hot water treated on March 26, 1998 and all treatments were also planted the following day. For the second run, seed was stratified on April 24, 1998, 9062708 was hot water treated on May 26, 1998, and 9058543 was hot water treated on May 27, 1998 with all treatments planted the day after hot water treatment as before. Germination containers used were 7 inch x 5-1/4 inch x 2-5/16 inch plastic bedding plant liners and the seed was planted 1/4 to 1/2 inch 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. The tight confines of the germinator required that the trays be moved to the greenhouse for irrigation and they were then allowed to drain thoroughly for several hours before being returned to the germinator. 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) and appropriate mean separations were performed at the five percent level of probability ($P \leq 0.05$).

A non-replicated field trial using seed of both accessions was planted on May 28, 1998. Seed treatments used were stratification (seed placed in stratification on April 24, 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 seconds, and 80°C for 60 seconds. Hot water treated seed was dried for 1 to 2 days before planting. Seed was planted by forming a shallow row with a hoe, hand sowing the seed, and covering to a depth of about one inch. Germination counts were made every three or four days until July 9, 1998.

Accession 9062680 (collected in Montgomery County, Tennessee) was previously 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 it was subsequently tested to determine the response of this accession to hot water treatment. For this study, quality of the seed was improved by separating out the heavier seed using a South Dakota Seed Blower (Seedburo Equipment Co., Chicago, Ill.) and 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 seconds, 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 August 8, 1998, and was hot water treated in the morning of November 10, 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 and mean separation was performed using Tukey's honestly significant difference test (HSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

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 with a duration of 150 seconds or longer may have been lethal. Simpson (1990) reported that high temperatures are capable of inducing secondary dormancy in seed of several grass species, but this phenomenon occurred when the seed was fully hydrated and ready to germinate. In this test, the seed was stored dry until it was placed in the water bath and would not have been primed for germination; therefore, it is unlikely that secondary dormancy was induced. The true cause for this lack of germination was not determined, because it was apparent that these treatments would not be acceptable 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	Water Temperature (°C)		
	(Seconds)	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 (Seconds)	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 accession 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. It seems likely that 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 second treatment provided the best germination of any of the hot water treatments for both accessions; however, the 80°C 60 second treatment would be ranked as the second best hot water treatment for 9058543, but 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 second treatments were very low, which indicates that there may have been damage to the seed.

Figures 3 and 4 illustrate the rate of seed germination during the study period for the control, stratified, 70°C 210 second, and 70°C 240 second treatments. They show that the stratified seed of both accessions germinated much more quickly than the other treatments. Anderson (1985) found that stratified seed exposed to the appropriate germination temperatures germinated very rapidly, with several of his treatments showing relatively high germination percentages within 5 days. In this experiment, none of the treatments germinated by the first count at 7 days. Anderson used filter paper as his germination medium so he could have detected germination much more quickly than in this study where the seed was 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 in his test, 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 cupule than diploid seed (Douglas, 1999) and this larger cupule is probably more restrictive, which may have slowed the germination rate for these accessions. The germination rate of the hot water treated seed was more 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 the stratified seed, the fact that it germinates more slowly makes the seed more susceptible to competition from other plant species which could prevent establishment.

Data for the preliminary field planting is presented in Table 3. No herbicides were applied to the planting site, so locating the seedlings for germination counts was somewhat difficult and probably affected the counts for 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, the 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 accession 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. He based his final recommendations on those treatments that performed best in the field; however, due to the unusual environmental conditions experienced during this study, such conclusions would not be appropriate in this case. What is interesting is the high germination percentages for the stratified seed. It has been noted by several researchers 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 the high temperatures. Anderson (1985) noted that stratified seed germinated much better at higher temperatures. Possibly a 20°C/30°C is not the appropriate temperature regime to produce optimum germination percentages of eastern gamagrass seed and the 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 exhibited a somewhat favorable response to the 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 when 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.

CONCLUSIONS

Although eastern gamagrass seed did respond to hot water soaking, these 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. Seed of different eastern gamagrass genotypes appeared to respond differently to the hot water treatments and to the stratification treatment. If hot water soaking is to be used commercially, further work would be required to refine treatment methods and additional seed treatments may be necessary to increase the germination rate of hot water treated seed. Future studies will explore these aspects of the use of hot water treatments.

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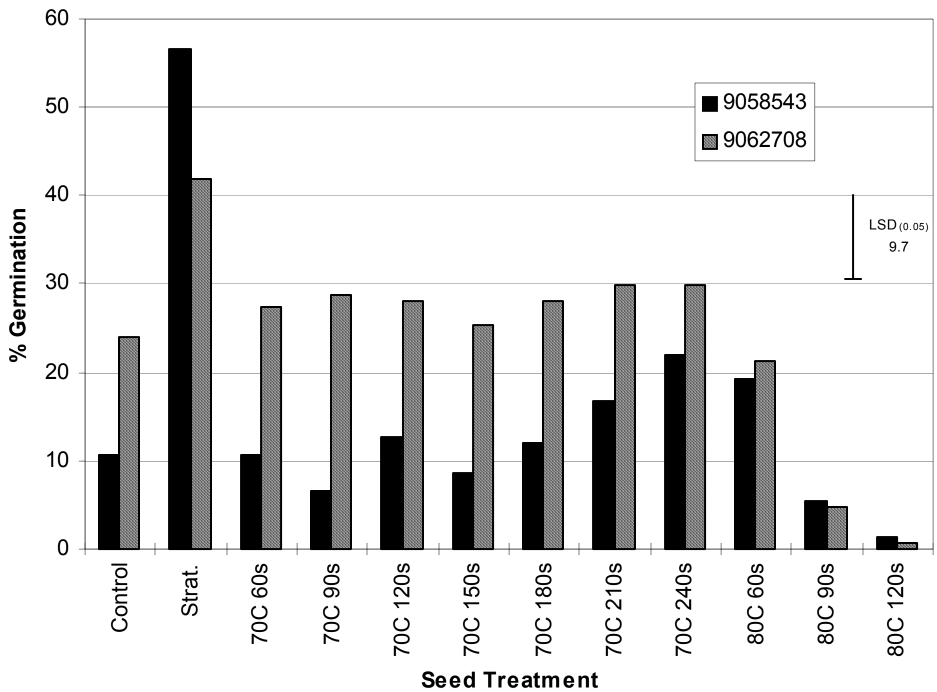

Figure 1. Interaction effect of selected seed treatments on total germination percentages of eastern gamagrass seed.

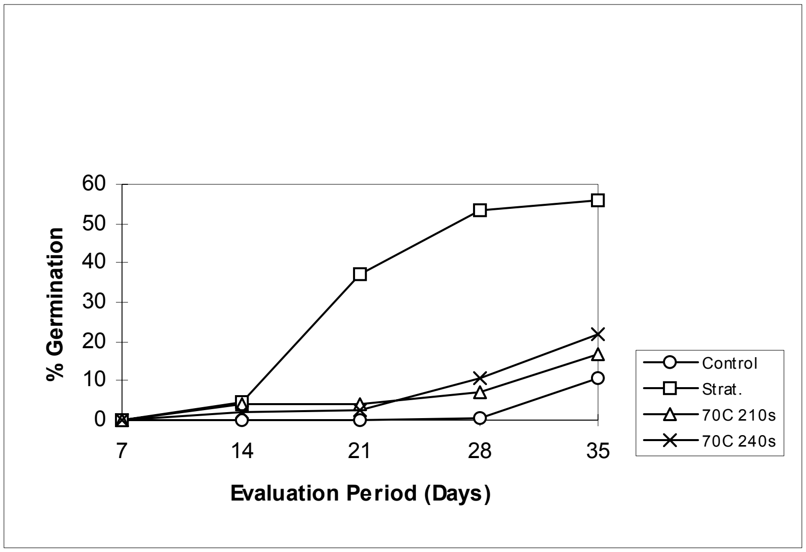

Figure 2. Germination rate for eastern gamagrass accession 9058543 for three selected seed treatments and control.

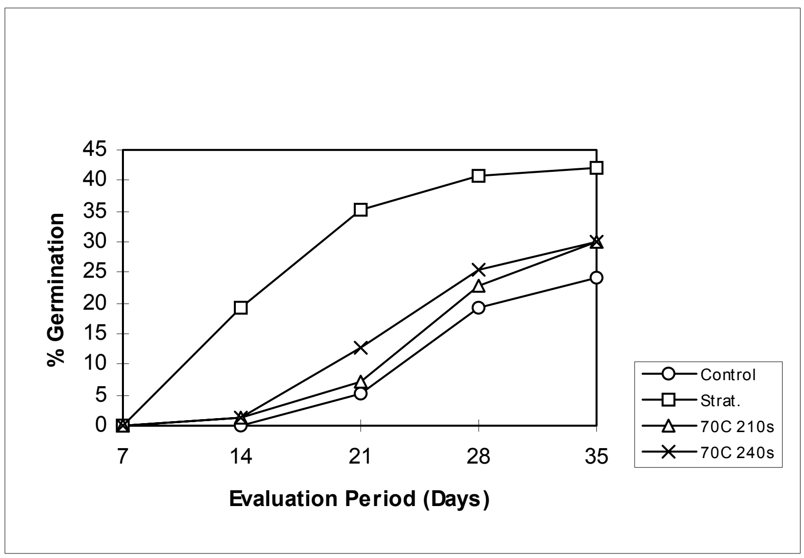

Figure 3. Germination rate for eastern gamagrass accession 9062708 for three selected seed treatments and control.

NOTES