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Seed dormancy, germination and fungal infestation of eastern gamagrass seed

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ABSTRACT

Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is a US native perennial warm-season grass. Commercial seed lots commonly have low percentage germination with high levels of dormant seed. This study was conducted to develop technologies to break seed dormancy and enhance germination and seedling survival of eastern gamagrass in laboratory germination tests. Germination tests for all seed treatments were conducted at alternating temperatures of 20/30 °C with corresponding dark/light cycles consisting of 16 and 8 h, respectively. A method of physically removing the caryopsis from the cupule and then scarifying the caryopsis was previously described in the literature and termed scarified caryopsis (SC). Our research program developed a one-step method of simply grinding off the top of the seed, termed cupulated caryopsis top removal (CCTR). The CCTR treatment breaks the integrity of both the cupule and pericarp. Intact cupules and non-scarified caryopses of eastern gamagrass seed lots had high percentages of dormant seed, whereas the CCTR and SC treatments evaluated in this study reduced or even eliminated dormancy. Additionally, stratification for 8 or 12 weeks respectively broke dormancy of intact cupules of the two seed lots tested. Stratification followed by drying or drying + storage for four weeks caused dormancy reversion; however, reversion did not occur in the CCTR treatment. Seed contamination was evaluated in four seed lots. *Chaetomium*, *Pithomyces* and *Rhizopus* were detected in all seed lots. The fungicide seed treatments captan and thiram significantly reduced germination of CCTR treatments in comparison with the non-fungicide treated control. The combination of pyraclostrobin and fluxapyroxad seed treatments had the best control of fungal seed contaminants and final percentage germination in laboratory germination tests.

1. Introduction

Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is a native perennial warm-season grass with inherently poor seed quality due to low germination potential and high levels of dormant seed. Previous research has shown that seed dormancy and low germination rates of eastern gamagrass were related to tissues surrounding the embryo (Tian et al., 2002). Seed tissues may contain inhibitors or act as physical barriers limiting water uptake, gas exchange and/or embryo expansion. These tissues may also prevent leaching of potential inhibitors from the embryo (Adkins et al., 2002; Baskin and Baskin, 2004; Springer et al., 2001). Dormancy was not associated with the embryo as isolated embryos were shown to germinate within two days (Huarte et al., 2007). Additionally, Anderson (1985) reported that no inhibitors existed in the cupule. Thus, physiological dormancy of eastern gamagrass was related to the seed coat and physical restriction by covering layers. Mechanical seed treatments that break the integrity of the cupule and pericarp may reduce seed dormancy and improve germination of eastern gamagrass seed.

Cold moist stratification (pre-chilling) is the most cost-effective and therefore the most commonly recommended seed treatment for breaking dormancy of perennial warm-season grasses (Haynes et al., 1997; Olszewski and Folin, 2009; Rogis et al., 2004; Watkinson and Pill, 1998). This is due in part to the weakening of the embryo covering tissues, which lowers the force required for radical protrusion. However, several limitations of stratification have been

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Table 1
Fungicide seed treatments and seed treatment additives with application rates.

Compound	Trade name and manufacturer	Application rate
Captan	Captan 400C, Bayer CropScience	2.45 mg a.i./g seed
Thiram	Thiram 42-S, Bayer CropScience	2.5 mg a.i./g seed
Ipconazole	Rancona 3.8 FS, Chemtura AgroSolution	1 mg a.i./g seed
Pyraclostrobin	Stamina, BASF	0.1 mg a.i./g seed
Fluxapyroxad	Xemium, BASF	0.1 mg .i./g seed
Colorant	Pro-ized Red Colorant, Bayer CropScience	13 μl/g seed
Binder	Precise Seed Finisher 1009, Bayer CropScience	13 μl/g seed

reported such as handling difficulties and increased shipping cost as well as loss of seed viability during the stratification process (Finneseth, 2010; Henson and Fenchel, 2007). If seed moisture content is too low before planting and soil moisture is low after sowing, stratified seed may return to the dormant state. This process is referred to as dormancy reversion, which has been studied in switchgrass (Shen et al., 2001) and eastern gamagrass (Finneseth, 2010). Extended stratification and sufficient after-ripening can prevent dormancy reversion of switchgrass (Shen et al., 2001). The mechanisms responsible for dormancy reversion of eastern gamagrass have not been identified. Germination tests of dried stratified seed are needed to study dormancy reversion in eastern gamagrass seed. Mechanical seed treatments following stratification and drying have the potential to reduce or eliminate dormancy reversion.

Fungicide seed treatments or seed dressings have been widely used to protect seed from fungal infections and improve germination, seedling growth and survival. Severe fungal contamination of eastern gamagrass seed during germination tests has been reported (Tian et al., 2002). Captan and thiram (Table 1) are both labeled for use on seed of several forage grasses (Bayer CropScience LP, 2011, 2007). However, only the efficacy of thiram has been reported on eastern gamagrass (Tian et al., 2002). In their study, thiram seed treatments had negative effects on germination especially for seed with cupules removed (Tian et al., 2002). Ipconazole and pyraclostrobin are also labeled for several forage crops as seed treatments to control seed rot, damping-off and seedling blight caused by fungal pathogens (*Zygomycetes*, *Ascomycetes*, *Basidiomycetes* and *Deutoromycetes*) (Table 1) (BASF Corp., 2015; Chemtura AgroSolution, 2012). Additionally, a recently released fungicide, fluxapyroxad (Table 1) has been shown to be effective for control of a wide range of fungal pathogens in many crops, particularly when combined with pyraclostrobin (Cavell et al., 2012; Walker et al., 2011). Due to their efficacy as seed treatments and control of several plant pathogens, these fungicides were selected to evaluate their influence on eastern gamagrass seed germination and survival.

The objectives of this study were to (1) evaluate germination of eastern gamagrass as influenced by various mechanical and physical seed treatments, including breaking the integrity of the cupule and pericarp, seed stratification, seed drying and storage following stratification, (2) investigate effects of fungicides on seed germination and control of fungal pathogens and (3) characterize seed-borne microorganisms from selected seed lots.

2. Materials and methods

2.1. Seed materials

Four seed lots of two eastern gamagrass cultivars were used in this study. Pete is a diploid variety and Meadowcrest is a tetraploid (Huang et al., 2016). One seed lot of the cultivar Pete designated Pete 1 that was primed and treated with thiram was obtained from Gamagrass Seed Company (Falls City, NE, USA) in 2007. The Pete 1 seed lot, labeled in 2007, was washed (approximately 10 min) with tap water using a mesh strainer until the outflow water was visually clear, and air-dried to remove the residual fungicide treatment. One seed lot of the cultivar Meadowcrest designated Meadowcrest 1 was harvested in 2013, and was provided by USDA/NRCS Big Flats Plant Materials Center (PMC), Big Flats, NY, USA. Two additional seed lots Pete 2 and Meadowcrest 2 were hand harvested from seed production fields at the Big Flats PMC in 2013. These two hand harvested seed lots were air-dried at room temperature for approximately two weeks. A South Dakota seed blower (Model D-34, E. L. Erickson Products, Brookings, SD, USA) was used to clean and increase seed quality of Pete 2 and Meadowcrest 2 at airflow velocities of 10.4 (gate setting 5) and 12.3 m/s (gate setting 8), respectively. All four seed lots were stored at 5 °C in a cold seed storage room located at the Seed Science and Technology program, in Geneva, NY prior to study.

2.2. Seed lot viability

Caryopses were obtained by removing cupules with hand-held clippers from all seed lots and were subsequently used to estimate seed lot viability. Four replicates of 50 caryopses of each seed lot were placed in plastic boxes (9 cm × 9 cm × 3.5 cm) between two layers of blue blotters moistened with distilled water and preconditioned overnight at 25 °C. The caryopses were bisected longitudinally with a razor blade to expose the embryo and other tissues. Both halves of each caryopsis remained attached after cutting and were stained in a 0.1% tetrazolium (TZ) solution for 1.5 h at 32 °C. Stained caryopses were evaluated according to guidelines described for Tripsacum in the Tetrazolium Testing Handbook (AOSA/SCST, 2010). A microscope and an illuminator were used for examination when necessary.

2.3. Seed germination

For each treatment, four replicates of 25 seeds were uniformly placed in plastic germination boxes (9 cm × 9 cm × 3.5 cm) on two layers of blue blotter paper (Anchor Paper Co., Saint Paul, MN, USA) moistened with approximately 20 mL distilled water. Germination tests were performed in a growth chamber (Model I-36LL, Percival Scientific Inc., Perry, IA, USA) at alternating temperatures of 20/30 °C (16/8 h). Lights were on during the 8 h at 30 °C. Light intensity of 65 μmoles/m^2/s was provided by two 17 W fluorescent lamps horizontally mounted in pairs. Germination counts were made weekly and converted to percentage germination. Seed were considered germinated if either the root or shoot structure protruded from the cupule or exceeded approximately 2 mm of the caryopsis. Germinated, dead and contaminated seed were removed at each count to minimize cross infection to the non-germinated seed. Distilled water was added to the blotters as needed. At the end of the test period, the percentage germination was recorded and non-germinated seed were subjected to TZ test to determine the percentage dormant seed. The percentage viable seed was the sum of the percentage of germinated and dormant seed. The time to 50% germination (T50) for mechanical seed treatments was calculated using the following equation (Coolbear et al., 1984):

$$T50 = t_i + \frac{((N/2) - n_i)(t_i - t_j)}{n_i - n_j}$$

where N is the final number of seed germinated, n_i and n_j are total number of seed germinated by adjacent counts at time t_i and t_j when $n_i < (N/2) < n_j$.