Effects of Osmopriming on Bermudagrass Germination and Establishment

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Summary. Bermudagrass (*Cynodon dactylon*) is one of the most frequently-used turfgrasses for golf, sport, and recreational turf throughout tropical, subtropical, and transition zone regions of the world. Recently, a number of seeded bermudagrass cultivars have been released into the turfgrass market and are quickly becoming a popular option for turfgrass managers. A major limitation with these seeded bermudagrasses is poor or slow germination. This can lead to weed-infestation, which can ultimately result in a weak turfgrass stand. One possible means to overcome slow germination is the use of a pre-germination treatment such as osmopriming. The following study was designed to investigate the effects of two osmotic priming agents, polyethylene glycol (PEG) and potassium nitrate (KNO_3) on the germination and establishment of a seeded bermudagrass. The cultivar, Jackpot, was exposed to seven different osmotic potential treatments within each solution, ranging from -0.20 to -0.5 MPa. Establishment after a 48 h exposure to these treatments was monitored for 6 weeks. Throughout the experiment, no conclusive evidence was obtained that priming of bermudagrass seed with varying osmotic solutions of PEG (avg. mol. wt. 10000) or KNO_3 was an effective means to promote faster germination and establishment. No statistical difference could be detected between KNO_3 and PEG and KNO_3 actually provided inferior results to the control. Further research is needed to determine if other priming methods would be more effective to enhance bermudagrass germination.

Bermudagrass (*Cynodon dactylon* L. Pers.) is a widely used turf and forage grass in the southern United States. While the majority of bermudagrasses are propagated by sod, plugs, or sprigging, recently developed seeded cultivars may be used for seeding or over-seeding lawns, golf courses and other turf areas. Seeding might be a more cost effective method of establishing new or over-seeded areas because plant material costs would be lower and easily available equipment could be used. To enhance the seeding process, methods to speed the germination and emergence of seed would be beneficial.

Osmopriming is a well-established practice used to enhance germination of field and row crops, but relativity few studies have applied this technique to turfgrasses. Mauromicale and Cavallaro (1996) demonstrated that osmotic solutions of KNO_3 and polyethylene glycol (PEG) enhanced the germination of a cool-season grass, tall fescue (*Festuca arundinacea* Schreb) and a warm-season grass, crabgrass (*Dactylis glomerata* L.). In addition, research on bahiagrass (*Paspalum notatum* Flügge) indicated a positive germination response to priming using PEG (Gates and Mullahey, 1997). Bush et al. (2000), working on the warm-season grasses, carpetgrass (*Axanopus affinis* Chase) and centipedegrass [*Eremochloa ophiuroides* Munro. (Kanz)], found that priming seed with KNO_3 had a very positive effect on days to germination and percentage germination in both of these species. Collectively, these studies suggest that priming may have applications with bermudagrass to enhance the germination and establishment of seeded cultivars.

Little specific work has been conducted on priming bermudagrass seed. In one study, Young et al. (1976) observed enhanced germination of common bermudagrass using solutions of KNO_3, as well as solutions containing KNO_3 and the plant growth regulator kinetin. In a study using 'Guymon' bermudagrass, hulled and unhulled seed were exposed to solutions of PEG (avg mol. wt. 8,000) and K_3PO_4. Hulled seed responded more favorably to the PEG solution while the un-hulled responded more to potassium phosphate (K_3PO_4) (Brede and Brede, 1986). In another study in which common bermudagrass seed was planted through a hydro-seeder (Kay et al., 1977), solutions of a kinitin, gibberellin (GA_3) and potassium nitrate (KNO_3) were used to osmoprime the seed. This study found that soaking seeds for 24 h in a combination of kinitin, gibberellin (GA_3) and potassium nitrate (KNO_3) yielded the most effective results.

Osmopriming and/or pre-germination of bermudagrass seed may allow for faster establishment, which will enhance the use of seeded bermudagrass cultivars. Unfortunately, there has been no research on many of the newer seeded bermudagrass cultivars. As such, the objective of this study was to determine if osmotic priming is an effective means to enhance the germination and establishment of bermudagrass.

Materials and methods

Studies were conducted in the greenhouse facilities of the University of Arkansas, Rosen Research Center (Fayetteville, Ark.). The greenhouse facility is atmospherically controlled with temperature and cooling equipment. Temperature in the greenhouse over the 36-day evaluation period varied from 25.0 – 26.7ºC in the daytime and 21.1 – 23.0ºC at night, while relative humidity ranged from 60-80% during the study. An overhead mist irrigation system was used to establish and maintain the crop. The bermudagrass cultivar, Jackpot, was used in the study. The seed was hulled and coated with a fungicide by Simplot-Jackpot Seed Division, Post Falls, ID.

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Potassium nitrate (KNO_3) and polyethylene glycol (avg. mol. wt. 10000) were selected as the germination solution for pre-soaking the 'Jackpot' seed. Each chemical was prepared in solutions corresponding to osmotic potentials of 0.20, 0.25, 0.30, 0.35, 0.40, and 0.50 MPa. The amount of PEG and KNO_3 needed for these osmotic potential solutions was calculated using the van't Hoff equation which calculates osmotic potential based on the modality of the solution. The van't Hoff equation reads as follows:

$$\prod = miRT$$

$\prod$ = osmotic potential
m = molality of the solution (moles solute / 1000 g H_2O)
i = ionization constant (= 1 for most solutions in H_2O)
R = gas constant (0.00831 L Mpa mol^{-1} K^{-1})
T = temperature ºK = degrees C + 273

For this application, the equation was re-adjusted to solve for molality. As previously identified, a range of solution osmotic potentials was desired. The temperature used for all calculations was 25ºC, or 298 °K.

A 500 mg (0.017 oz) sample of seed was used for each priming unit and represented approximately 2,170 seeds. These seeds were placed in an Erlenmyer flask containing 5.0 mL (0.169 oz.) of the various osmotic potential solutions and soaked for 48 h at 25°C (77°F). The seeds were removed from the flask, washed thoroughly in deionized water, placed in plastic weigh dishes and air dried for 96 h. For germination studies, a 100-seed sub-sample of the 500 mg treated seed sample was collected based on weight [23 mg (0.008 oz)]. An untreated, 100-seed sample was also weighed as the control. Each 100-seed sample was planted in a single cell of a planting flat that contained twenty-four cells [6 cm (2.36 in.) diam.] filled with a standard potting soil (Scotsman Standard No. 1). Vermiculite was lightly spread over the top of the seeded cells to preserve moisture during establishment. Four replications of each priming treatment were prepared and seeds were planted in a completely randomized design. The trays were randomly moved approximately every 5 to 9 days on the bench to minimize variability in the greenhouse.

Digital image analysis was used to monitor growth and establishment rates of the seed samples (Richardson et al., 2001). Digital images were obtained using an Olympus C3030Z (Olympus Optical Co., London, UK) digital camera mounted on a tripod. The tripod was set so that the camera was positioned 38 cm (15 in.) above each cell. For this study, a frame was used to isolate the area inside of the pot where the seeds were planted. The frame was constructed of red mat board in which a 6.0 cm (2.36 in.) circle was cut from the center of the board. The frame was placed over each cell so that the cell and seedlings were exposed to the camera while the remainder of the field was the red frame. The collected images were saved in the JPEG (joint photographic experts group, .jpg) format, with a color depth of 16.7 million colors, and an image size of 480 x 640 pixels. Camera settings included a shutter speed of 1/400 s, an aperture of F4.0, and a focal length of 32mm.

Digital images were downloaded to a personal computer and analyzed using SigmaScan Pro imaging software (v. 5.0, SPSS, Inc., Chicago, Ill, 60611). The color threshold feature in the SigmaScan software allows the user to search a digital image for a specific color or a range of color tones. The first step in the process is to determine the total number of pixels of information that are in the image, which for this study was 307,200 (480 x 640). The next step was to determine the number of pixels that fell in the red range (hue range from 210 to 255 and a saturation range from 25 to 100). Finally, a scan was made to determine the area of green in the image (hue range from 57 to 107 and a saturation range from 0 to 100). After developing an overlay of the red and green areas of the image, the measurement tools in the software package were used to count the total number of selected red and green pixels. From these values, the percentage turfgrass growth was determined by the following equations:

Number of pixels in cell = (total pixels in the image) - (red pixels)
% area of turf = (green pixels) / (number of pixels in the cell) x 100

Digital images were taken weekly for 6 weeks and batch-analyzed in SigmaScan software using a macro developed by Dr. Doug Karcher of the University of Arkansas. Data for turfgrass cover were analyzed as a completely randomized design using analysis of variance (PROC ANOVA) techniques in the SAS statistical analysis software program (SAS, Inc., Cary, NC). Means were separated according to Fishers protected least squares difference.

Results and discussion

The analysis of variance indicated a significant main effect of priming solution on the germination of the bermudagrass seed and no significant main effect of the osmotic potential of the solutions. There was no interaction between the type of solution and the osmotic potential of the solution and all data are therefore presented as the means of the main effects.

All seed treatments had some level of germination within 8 days after planting (DAP). At 10 DAP, there was no statistical difference in turfgrass cover based on either the osmotic potential of the solution or the type of solution (Table 1). However beginning at 16 DAP and continuing through 36 DAP, there was a statistical difference between the control seeds and those treated with KNO_3. On those dates, the control seeds actually out-performed the seeds treated with KNO_3. There was no statistical difference between the control and PEG 10000 or the PEG 10000 and KNO_3 on DAP 16 and DAP 21. Through the remaining evaluation dates, the control and PEG 10000 treatments had significantly greater cover than the KNO_3 treatment. There was no positive or negative effect of osmotic potential at any evaluation date.

Over the duration of the experiment, there was no conclusive evidence that priming of bermudagrass seed with varying osmotic solutions of PEG 10000 or KNO_3 was an effective means to promote faster germination and establishment. When comparing the two priming solutions, no statistical difference could be detected and KNO_3 actually provided inferior results to the control.

Although priming has been effectively used in numerous other field crops (Frett and Pill, 1995; Parera and Cantliffe, 1994) its applicability is apparently dependent on the species and possibly even the cultivar being studied. Some grasses such as fescue (*Festuca*) species (Frett and Pill, 1995), Kentucky bluegrass (*Poa pratensis*) (Phaneendranath and Funk, 1978), carpetgrass and centipedegrass (Bush, et al., 2000) and bahiagrass (Gates and Mullahey, 1997) have responded positively to various priming techniques, including those tested in this study. In addition, previous studies with other cultivars of bermudagrass have also yielded positive results (Young et al. 1977; Brede and Brede, 1986). A replicate study was also conducted as part of this project using a hulled, untreated bermudagrass seed with the same techniques and osmotic solutions. The second study yielded even more unfavorable results than that of the

first due to poor overall germination of the seed (data not shown).

Considering the success of some previous researchers, further research in the area of osmopriming bermudagrass seed should be examined even though the current techniques were ineffective. Future studies might consider solutions that not only contain an osmotic agent, but also a plant hormone such as gibberrelic acid, as this approach has been successful in other studies on grasses.

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Table 1. Effects of various osmotic priming solutions and osmotic potentials on the establishment of 'Jackpot' seeded bermudagrass.

Solution	DAP[z] 10	DAP 16	DAP 21	DAP 27	DAP 36	AVG.
			% cover			
Control	2.25	11.20	17.43	25.13	33.19	17.34
PEG 10000	2.29	10.90	16.58	23.26	32.54	17.11
KNO_3	1.95	9.06	14.34	19.31	26.28	14.19
LSD (0.05)	ns[y]	2.05	2.92	3.84	5.53	2.72
Osmotic potential (-MPa)						
			% cover			
Control	2.25	11.20	17.43	25.13	33.19	17.34
0.20	2.43	10.17	16.78	22.99	30.90	16.65
0.25	2.06	10.03	15.70	21.20	28.98	16.48
0.30	1.92	9.49	14.71	19.75	28.71	15.86
0.35	2.06	10.97	16.11	22.76	30.50	15.94
0.40	2.25	10.09	15.28	21.49	30.20	14.92
0.50	2.04	9.14	14.18	19.53	27.17	14.41
LSD (0.05)	ns	ns	ns	ns	ns	ns

[z] DAP = days after planting

[y] ns = not significant at the 0.05 level of probability, according to analysis of variance