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Comparative Effects of Hydro-, Hormonal-, Osmotic-, and Redox-Priming on Seed Germination of Creeping Bentgrass under Optimal and Suboptimal Temperatures

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Abstract. **Reseeding of creeping bentgrass (*Agrostis stolonifera* L.) under unfavorable temperature (≈10 °C) is a common practice on golf putting greens and fairways. Seed priming to enhance germination and early emergence increases seeding success. Seed priming comparing abscisic acid (ABA), gibberellic acid (GA), glycinebetaine (GB), hydrogen peroxide (H_2O_2), and polyethylene glycol (PEG) has not been investigated in turfgrass. Our objective was to compare these chemical primers at three concentrations with water- and unprimed-seed at two competing germinating temperatures (10 vs. 25 °C). Two seed lots of 'T-1' creeping bentgrass were compared. Curve fitting of daily germination was used to compute days to 50% germination (D_{50}) and maximum germination percentage (G_{max}). Cold (10 °C) significantly inhibited emergence (higher D_{50}) more than G_{max}. The effects of primers and their rates varied with the seed lot and temperature. Enhancement of seed germination measured as early emergence (lower D_{50}) and/or higher G_{max} were only detected at 10 °C. Osmotic primers (GB and PEG) were most effective in promoting germination relative to unprimed seed followed by hormone primers (ABA and GA) with redox primers (H_2O_2) least effective. Glycinebetaine primed seed was the only primer effective at all concentrations, with the 100 mM concentration the only concentration to enhance germination by increasing both G_{max} and early emergence (lower D_{50}) compared with unprimed seed.**

Overseeding and reestablishment of damaged golf greens and fairways planted to creeping bentgrass (*Agrostis stolonifera* L.) is a common practice following injuries. Reseeding is a necessary and costly investment to promote recovery and to maintain adequate density and uniformity for play (Green et al., 2018). Adverse conditions such as cold soil temperatures (≈10 °C) typical of early spring plantings in temperate areas of the northeastern United States can delay seed germination and diminish establishment vigor of cool-season grasses compared with more favorable soil temperatures of 20 to 30 °C (He et al., 2013; Liu et al., 2001; McGinnies, 1960; Wright et al., 1978). Delayed growth from slow to emerge turfgrass plants can reduce competition and therefore contribute to lower contributions of planted species in the turf stand (Larsen et al., 2004). In addition, a delay in the germination may result in high susceptibility to stresses, such as drought (Young and Evans, 1982) and weeds (Murphy et al., 2005).

Seed priming is a preplant method of promoting partial germination before planting as seed. During seed priming (i.e., controlled soaking treatment), the emergence of the radicle is initiated, but the process is interrupted (inhibited) by drying of the seeds. In a recent review of priming agents in agricultural crops (Jisha et al. (2013), numerous primers, including water (hydropriming), hormonal, osmotic, matric, and redox primers, have been shown to enhance final germination percentages, reduce the time to germination, improve synchronization of germination, and increase the stress tolerance of seedlings following priming compared with unprimed seed. To that end, priming enhancement of germination may be achieved by either inducing physiological changes in the seed before germination as a pre-soaking or pre-conditioning treatment and/or after emergence as an exogenous application to the seedling plant. In priming of turfgrass seed, hydropriming with distilled water, osmotic-priming using GB (Pill and Necker, 2001; Zhang and Rue, 2012; Zhang et al., 2014) and PEG (Danneberger et al., 1992; Wang et al., 2014), matrix priming (Yamamoto et al., 1997b), and redox priming using H_2O_2 (Wang et al., 2014) have been investigated with mixed results. To our knowledge, seed priming using hormones such as ABA and GA have not been investigated in turfgrass. Moreover, comparative studies of hydro-, hormone-, osmotic-, and redox-primers and their efficacy as primers has not been reported in turfgrass.

Previous research in agricultural crops has demonstrated the success of seed priming in eliciting beneficial response is specific to the priming agent, the rate or concentration of the primer, and the stress (Iqbal and Ashraf, 2005). In turfgrass seed priming studies conducted by Zhang et al. (2014), most of the six turfgrass species evaluated showed limited response to GB concentrations (5, 10, and 50 mM) in both their effect on daily germination and final germination percentages. The authors reported creeping bentgrass seed primed with the 5-mM concentration promoted higher daily germination rates (i.e., measure of the speed of germination) than unprimed seed while no effect was observed on final germination percentage. Alternatively, Zhang and Rue (2012) in a similar comparison of the same cool-season turfgrass species reported no difference in daily germination rates relative to unprimed seed with GB concentrations ranging from 50 to 200 mM. However, all rates of GB enhanced final germination percentage compared with unprimed seed. Zhang et al. (2014) concluded priming to ameliorate salinity, drought, and temperature stress is species and concentration (rate) specific.

In agronomic crops, the evaluation of priming agents and the effects of temperature have observed a greater enhancement of germination under suboptimal germination temperatures (<15 °C) (Bodsworth and Bewley, 1981; Hardegree, 1994; Heydecker et al., 1975; Knypl and Khan, 1981). Similarly in turfgrass priming studies the response to priming was more readily apparent under cool temperatures for both slow germinating kentucky bluegrass (*Poa pratensis* L.) (Yamamoto et al., 1997a) and quick to emerge perennial ryegrass (*Lolium perenne* L.) (Danneberger et al., 1992). In general, researchers have observed greater differences in germination characteristics due to priming under less favorable germinating temperatures (Danneberger et al., 1992; Pill, 1994; Yamamoto et al., 1997a).

Priming-induced tolerance has been investigated for various stresses; however, the efficiency of various priming agents such as hormones, hydropriming, osmotic, and redox priming has not been comparatively investigated. Creeping bentgrass is an economically important turf species to the golf industry, and the efficacy of the vast array of primers to stimulate early emergence at suboptimal germinating temperatures has not been investigated. Our specific objective was to compare various primers and their efficacy to enhance germination characteristics of creeping bentgrass under suboptimal germination temperatures.

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Materials and Methods

Seed priming and priming concentrations. A popular (commercially) available cultivar T-1 creeping bentgrass seed, with parent germplasm originating from New England golf courses, was selected for germination tests. The following primers were evaluated including seed primed with water (double deionized), hormonal primers (ABA and GA), osmotic primers (GB and PEG 6000), and redox primer hydrogen peroxide (H_2O_2). Three concentrations of each primer (except water-primed) were evaluated at low, medium, and high concentrations corresponding to ABA (0.05, 0.1, and 0.2 μM; Gao et al., 2002), GA (100, 200, and 300 mg·L^{-1}), GB (50, 100, and 150 mM; Zhang and Rue, 2012), H_2O_2 (0.1, 1, and 100 mM; Yadav et al., 2011), and PEG (100, 200, and 300 g·L^{-1}; Murray, 1990). Concentrations were chosen based on previous research from selected studies allowing for a baseline to determine optimal concentrations for each primer. Seeds were surface sterilized using 95% ethanol for 2 min and 2% sodium hypochlorite solution for 10 min, triple rinsed with distilled water and then air dried at room temperature for 24 h. All priming treatments contained 1g of seed to 1.5 mL of priming solution. Priming solutions with seed were placed in microtubes with tubes agitated constantly for 24 h to allow for optimal absorption. Seed was then air dried for at least 24 h. Twenty-five seeds were placed on filter paper saturated with deionized water using 8-cm diameter petri plates for each primer and concentration for a total of three petri plates as subsamples per primer concentration.

Temperature treatments. Two growth chambers were used with one chamber set to 25 °C as optimal and a second chamber set to 10 °C as the low temperature treatment for tests for creeping bentgrass under stressful conditions. Unprimed seed, water-primed seed along with all five primers and their three concentrations (17 treatments × 3 subsamples = 51 petri plates) were randomized within each of the two growth chambers at the two set temperatures. The process and randomization was repeated for a total of three replications which were blocked as timing variables. Temperature was analyzed as main plot with unprimed seeds, water-primed seeds, and primers and their corresponding concentrations as subplots. Relative humidity within each chamber fluctuated between 50% and 80%. Petri plates with seeds were placed into the growth chambers under a 10-h photoperiod with low light levels (≈70 PAR). Distilled water was applied to petri dishes as necessary to prevent filter paper and seeds from drying. The experiment was repeated as Expts. 1 and 2 using a different seed lot because the germination characteristics can vary with the seed lot of the same cultivar (Liu et al., 2001).

Data collection and analysis. Seed germination was defined as 2 mm emerged radical visible under 2× magnification. Counts were made daily for 22 d at 10 °C and 10 d at 25 °C until no further germination was observed. Daily germination data were analyzed by curve fitting using a four-parameter sigmoid model (Sigma Plot; SPSS, Chicago) where Y (i.e., germination percentage) and the independent variable X (i.e., days) were fitted to determine days to 50% germination (D_{50}, days) for each replicate and each combination of priming treatment (primer and priming concentration) and temperature (10 and 25 °C). Maximum germination percentage (G_{max}, %) was simultaneously determined by sigmoidal curve fitting. Curve fitting of the individual subsamples (petri plates) were averaged and then analyzed by analysis of variance (ANOVA) using MINITAB (State College, PA). Seed primers (i.e., unprimed, water primed, ABA, GA, GB, PEG, and Redox primers) and priming rates (i.e., linear and quadratic components for ABA, GA, GB, PEG, and Redox primers) main effect sum of squares (SS) were partitioned into a set of orthogonal single degree of freedom (df) contrast to test for differences between group means comparing various primers (6 df in all) and associated priming rates or concentrations (10 df in all). Hence, all 16 df for main effect of primers and associated concentrations were partitioned. The six df for primers were partitioned as 1) Control (water primed + no water priming) vs. all other primers; 2) Water-priming vs. no priming; 3) Hormone (ABA + GA) vs. osmopriming (GB + PEG); 4) ABA vs. GA, 5) GB vs. PEG; and 6) H_2O_2 vs. ABA + GA + GB + PEG. The 10 df for priming rates as linear ($_L$) and quadratic ($_Q$) components are reported as ABA_L, ABA_Q, GA_L, GA_Q, GB_L, GB_Q, H_2O_{2L}, H_2O_{2Q}, and as PEG_L and PEG_Q, respectively. The 16 df orthogonal contrasts for main effects due to primers (6 df) and associated concentrations (10 df) were crossed with temperature (10 vs. 25 °C) to partition the 16 df and associated SS for interaction to detect significant ($P \leq 0.10$) departures from seed priming main effects caused by interaction with temperature. Priming + priming rate and temperature effects were crossed with Expt. (1 and 2, i.e., different seed lots) to determine interaction with seed lots. Fisher's protected least significant difference (LSD) values at the 0.05 level are reported in the text for various comparisons between treatment means. No departures from the assumptions of the ANOVA were detected in homogeneity of variance or departures from normality.

Results and Discussion

Significant interactions were observed for measured responses between experimental runs 1 and 2 (different seed lots of 'T-1' creeping bentgrass) and associated treatment main effects of seed priming and priming rate and their interaction with temperature (10 vs. 25 °C). The results for days to 50% germination (D_{50}) and maximum germination percentage (G_{max}) are therefore reported by individual experimental runs (Expts. 1 and 2). Response to priming can vary, which is often attributed to variations in seed lot (Larsen and Bibby, 2004; Liu et al., 2001; Snapp et al., 2008).

Expt. 1: Temperature main effects on D_{50}. Temperature (10 vs. 25 °C) main effect accounted for ≈79% of the total variation in seed priming treatment (priming + priming rate) and their interaction with temperature. Low germinating temperature (10 °C) significantly reduced D_{50} by a factor of 3.4 (11.0 d to 50% germination) compared with 25 °C (3.2 d to 50% germination; Tables 1 and 2). As such, 'T-1' creeping bentgrass germinated faster (i.e., lower D_{50}) by achieving 50% germination in 3.2 d at 25 °C. He et al. (2013) reported initial emergence of 4 to 5 d in kentucky bluegrass (*Poa pratensis* L.) when measured under constant temperatures between 20 to 30 °C, whereas at 10 °C initial germination time ranged from 11 to 14 d. When seeding in spring, cold soil temperature is often one of the major limiting factors affecting seed germination, which can significantly delay turfgrass establishment and cover during spring periods and postpone play on damaged areas under repair. Liu et al. (2001) found rough bluegrasses (*Poa trivialis* L.) germination at 10 °C (daytime) temperature to be significantly delayed by several days compared with 25 °C. Similarly, Hardegree (1994) reported a 2-fold higher D_{50} at 10 °C (8.7 d) than at 25 °C (4.1 d) for sheep fescue (*Festuca ovina* L.) and reported most cool-season grasses required between 4 to 11 d longer to achieve 50% germination at 10 °C. Delay of seed germination by only a few days due to cold temperatures in spring has been shown to be sufficient to affect seedling survival (Young and Evans, 1982).

Expt. 1: Priming main effects on D_{50}. Priming orthogonal contrast main effects (6 df) were significant ($P \leq 0.05$, Table 1). Partitioning of the priming main effects into pre-planned single df contrasts indicated the contrast comparing redox (H_2O_2) priming vs. hormone priming (ABA + GA) and osmotic priming (GB + PEG) accounted for this statistical priming effect ($P \leq 0.01$). Redox priming averaged 8.2 d to 50% germination (i.e., D_{50}) and delayed germination compared with the group average for hormone and osmotic priming of 6.9 d (Table 2). Similarly, ABA delayed germination compared with GA by ≈1.1 d ($P \leq 0.10$). Hormone primer GA (D_{50} = 6.6 d) and osmotic primer GB (D_{50} = 6.3 d) were statistically equal to the control D_{50} average of 6.4 d (water primed and unprimed average) (Tables 1 and 2). All other priming treatments such as ABA and H_2O_2 significantly delayed germination to 50% compared with the control. No significant interaction between priming treatment and temperature was observed (Table 1). Use of priming technology to accelerate seed germination rate under less favorable periods serves as an option to enhance seeding success (Jisha et al., 2013). However, such enhancement in germination rate by various priming treatments over the water primed or unprimed control were not observed in Expt.

Table 1. Expt. 1 analysis of variance for days to 50% germination (D_{50}) and maximum germination (G_{max}) derived from curve fitting of 'T-1' creeping bentgrass germination as affected by two temperatures and 17 seed-priming treatments.

Source of variation	df	D_{50}	G_{max}
Temperature (T): 10 vs. 25 °C	1	***	***
Treatment (TRT): Priming and rate	16	***	NS
Priming (P) orthogonal contrasts	6	*	NS
Control (water primed + no) vs. all	1	NS	NS
Water-priming vs. unprimed	1	NS	NS
Hormone[z] vs. osmopriming[y]	1	NS	NS
ABA vs. GA	1	†	NS
GB vs. PEG	1	NS	NS
H_2O_2 vs. ABA + GA + GB + PEG	1	**	NS
Priming rate (R) orthogonal contrasts[x]	10	***	*
ABA_L	1	*	NS
ABA_Q	1	†	***
GA_L	1	NS	*
GA_Q	1	NS	NS
GB_L	1	NS	NS
GB_Q	1	NS	NS
H_2O_{2L}	1	***	NS
H_2O_{2Q}	1	*	NS
PEG_L	1	*	NS
PEG_Q	1	NS	NS
TRT × T	16	NS	*
P × T orthogonal contrasts	6	NS	†
Control (water primed + no) vs. all × T	1	NS	*
Water-priming vs. unprimed × T	1	NS	NS
Hormone[z] vs. osmopriming[y] × T	1	NS	NS
ABA vs. GA × T	1	NS	NS
GB vs. PEG × T	1	NS	*
H_2O_2 vs. ABA + GA + GB + PEG × T	1	NS	NS
R × T orthogonal contrasts	10	NS	†
ABA_L × T	1	NS	NS
ABA_Q × T	1	NS	NS
GA_L × T	1	NS	NS
GA_Q × T	1	NS	*
GB_L × T	1	NS	***
GB_Q × T	1	NS	NS
H_2O_{2L} × T	1	NS	NS
H_2O_{2Q} × T	1	NS	NS
PEG_L × T	1	**	†
PEG_Q × T	1	NS	NS

[z]Hormone treatments represent GA (gibberellic acid) and ABA (abscisic acid).
[y]Osmopriming treatments represent GB (glycinebetaine) and PEG (polyethylene glycol).
[x]Rates for priming treatments were partitioned as linear (L) and quadratic (Q) orthogonal contrasts.
†, *, **, ***Significant at $P \leq 0.10$, 0.05, 0.01, or 0.001, respectively.

1 as indicated by the nonsignificant contrast: Control (water primed + no) vs. all (Table 1). This is consistent with other reports in turfgrass (Pill and Necker, 2001; Zhang et al., 2014; Zhang and Rue, 2012).

Expt. 1: Priming rate main effects on D_{50}. The effects of rate (linear and quadratic components) of priming were significant for ABA, H_2O_2, and PEG (Table 1). Linear and quadratic single df contrasts and the effects of rate were statistically unimportant for GA and GB and therefore their priming effects were independent of rate. Similarly, Zhang and Rue (2012) observed no rate effect when priming seed using GB at five rate levels ranging from 50 to 200 mM. Osmotic primer PEG promoted faster germination (i.e., lower D_{50}) at higher rates (200 and 300 g·L^{-1}), whereas redox primer H_2O_2 promoted lower D_{50} with lower rates (0.1 and 1 mM) (Table 2). The hormone primer ABA promoted statistically lower D_{50} at the midpoint rate (0.1 μM). These statistically lower D_{50} for ABA, H_2O_2, and PEG germination rates did not, however, promote lower D_{50} compared with the water primed or unprimed controls (Table 2). However, some priming rates for ABA (0.2 μM, D_{50} = 9.2 d), H_2O_2 (100 mM, D_{50} = 11.3 d), and PEG (100 g·L^{-1}, D_{50} = 8.8 d) were statistically higher and therefore may potentially reduce the competitive advantage of creeping bentgrass during establishment compared with the water primed and unprimed control (D_{50} = 6.4 and 6.3 d, respectively) (Table 2). Delayed establishment indicated by higher D_{50} may lead to greater environmental stress during establishment.

Expt. 1: Priming rate × temperature interaction effects on D_{50}. The priming rate main effect for the osmotic primer PEG was dependent on temperature main effect (Table 1). The PEG_L × temperature interaction was the only significant ($P \leq 0.01$) single df contrast observed (Table 1). Germination D_{50} at 25 °C for PEG was 2.4, 2.6, and 3.0 d for 100, 200, and 300 g·L^{-1}, respectively. Unlike the main effect for PEG, no statistical difference was observed between the different PEG concentrations at 25 °C. This is a major departure from the main effects for PEG discussed earlier. Effects of rate were only observed for PEG at 10 °C where higher PEG rates (200 and 300 g·L^{-1}) exhibited lower D_{50} corresponding to 9.9 and 10.1 d to 50% germination, respectively, which were statistically lower than the D_{50} of 15.3 d observed at 100 g·L^{-1} ($LSD_{0.05}$ = 2.9 d). The water primed and unprimed control averaged 9.8 d to 50% germination at 10 °C, and therefore the low rate of PEG promoted a significantly higher D_{50} (slower emergence rate) than the control. Hardegree (1994) also reported the effects of matric (water potential) priming on D_{50} were relatively greater at 10 °C than observed at favorable temperatures of 25 °C. Other researchers have also observed the promotive effects from osmopriming were more beneficial when seeds were germinating under less favorable conditions such as low temperature (Danneberger et al., 1992).

Expt. 1: Temperature main effects on G_{max}. Maximum germination percentages (G_{max}) were inversely related to germination rate (D_{50}), r value = –0.55 ($P \leq 0.001$, n = 102). Higher germinating temperatures (25 °C) promoted significantly higher germination percentages (71.4%) than seeds germinating at 10 °C (62.0%) (Table 2). Fewer days to 50% germination (lower D_{50}) and in

Table 2. Expt. 1 fitted main effect means for days to 50% germination (D_{50}) and maximum germination (G_{max}) as affected by two temperatures and 17 seed-priming treatments on 'T-1' creeping bentgrass germination.

Treatments	D_{50}	G_{max} (%)
Temperature (°C)		
10	11.0 a[z]	62.0 b
25	3.2 b	71.4 a
Controls		
No water priming	6.3	65.0
Water priming	6.4	64.9
Priming and rate		
ABA (abscisic acid)		
0.05 µM	7.2 ab	63.4 b
0.1 µM	6.6 b	76.3 a
0.2 µM	9.2 a	58.4 b
GA (gibberellic acid)		
100 mg·L^{-1}	7.0	70.0 ab
200 mg·L^{-1}	6.1	71.1 a
300 mg·L^{-1}	6.7	60.9 b
GB (glycinebetaine)		
50 mM	7.0	65.5
100 mM	5.9	69.7
150 mM	6.1	69.1
H_2O_2 (hydrogen peroxide)		
0.1 mM	6.6 b	66.2
1 mM	6.7 b	70.8
100 mM	11.3 a	63.4
PEG (polyethylene glycol)		
100 g·L^{-1}	8.8 a	65.5
200 g·L^{-1}	6.2 b	68.0
300 g·L^{-1}	6.5 b	65.9
Priming		
Control	6.4	65.0
Chemicals		
ABA	7.7 ab	66.0
GA	6.6 bc	67.4
GB	6.3 c	68.1
H_2O_2	8.2 a	66.8
PEG	7.2 abc	66.5

[z]Numbers followed by the same letter(s) within a main effect group are not significantly different at $P \leq 0.05$ level. Statistical effects are shown only for significant main effects as indicated in Table 1.

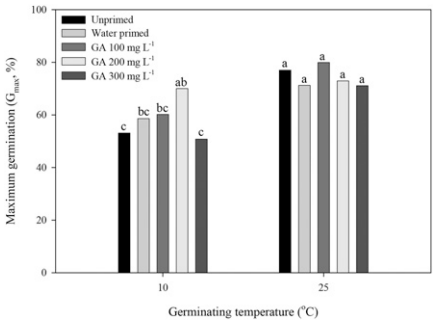

Fig. 1. Expt. 1 interactive effect for maximum germination percentage (G_{max}) comparing seed priming control (water primed and unprimed) with gibberellic acid (GA) at three rates and two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

turn faster germination rates were generally associated with higher G_{max}. These two measured variables, however, explained only 30% of the total variation as covariables, suggesting that lower D_{50} does not necessarily equate to higher germination percentages (G_{max}). For example, no statistical difference was observed in G_{max} due to the effects of priming treatments and associated single df contrasts (Tables 1 and 2) even though statistical differences in D_{50} were observed among priming treatments in Expt. 1. The effect due to temperature explained only 37% of the total treatment variation in G_{max}, which was less than half of the 79% accounted for by temperature main effects on D_{50}. The speed of germination measured as D_{50} was influenced more by the effects of temperature compared with temperature effects on maximum germination percentage measured as G_{max}. Zhang et al. (2014) also reported that daily germination percentages, the measure of the speed of germination, were more sensitive to environmental stress (drought, salinity, and temperature) than final germination percentages.

Expt. 1: Priming rate main effects on G_{max}. Overall main effect of priming rate on G_{max} (10 df) was significant ($P \leq 0.05$, Table 1) with two single df orthogonal contrasts identified 1) ABA (ABA_Q, $P \leq 0.001$) and 2) GA (GA_L, $P \leq 0.05$), which accounted for the statistical significance in priming rate. Priming seed using ABA was most effective in increasing G_{max} to 76.3% using the midpoint rate of 0.1 µM, which corresponded to the same ABA rate most effective in achieving faster germination rates (lower D_{50}) (Table 2). Hormone primer GA at 100 and 200 mg·L^{-1} was generally associated with higher G_{max} (> 70%) compared with GA priming at 300 mg·L^{-1} (G_{max} = 60.9%) (Table 2).

Expt. 1: Priming × Temperature interaction effects on G_{max}. No significant statistical main effect due to priming treatment and associated contrasts were observed for G_{max}. However, significant interactions were detected between temperature and priming main effects (Table 1). Two single df orthogonal (interaction) contrasts were detected for the priming × temperature interaction 1) Control (water primed + no) vs. all × temperature and 2) GB vs. PEG × temperature. Differences between the control (water primed + unprimed) vs. all other priming treatments (hormone + osmopriming + redox) varied with the germinating temperature (10 vs. 25 °C). Close inspection of the interaction revealed that no difference in G_{max} between the control (74.1%) vs. priming (71.1%, hormone + osmopriming + redox) was observed at 25 °C, which is consistent with the priming main effects. However, at 10 °C seed priming (hormone + osmopriming + redox) afforded significantly better germination percentages (62.8%) than the control (55.9%) ($LSD_{0.05}$ = 6.8%). The effects comparing the two competing osmopriming treatments (GB vs. PEG) varied with temperature. Polyethylene glycol exhibited significantly lower G_{max} at 10 °C (59.8%) compared with 25 °C (73.1%) ($LSD_{0.05}$ = 6.7%), which is consistent with the temperature main effect. Alternatively, the G_{max} for GB at the colder germinating temperature of 10 °C (67.0%) that was statistically equivalent to G_{max} at the more favorable germinating temperature of 25 °C (69.2%), which is a major departure from temperature main effects (Tables 1 and 2). Moreover, osmopriming with GB primed seed at 10 °C promoted significantly higher G_{max} (67.0%) than observed with PEG (59.8%). The interactive effects of seed priming treatments (hormone + osmopriming + redox) with temperature revealed some enhancement of G_{max} over the water primed and unprimed control at low germinating temperatures. These results at low germinating temperatures are consistent with previous studies in cool-season turfgrass in which germination is generally more variable at unfavorable (10 °C) temperatures than at warm temperatures (25 °C) (Liu et al., 2001). These same authors reported similar G_{max} averages of ≈56.0% to our 62.0% (Table 2) for the same germinating temperature of 10 °C.

Expt. 1: Priming rate × temperature interaction effects on G_{max}. Two significant single df contrasts were identified for priming rate × temperature interaction including 1) GA_Q × temperature ($P \leq 0.05$) (Fig. 1) and 2) GB_L × temperature ($P \leq 0.001$) (Fig. 2). Seed germinating at 10 °C primed with GA at 200 mg·L^{-1} promoted G_{max} that was statistically equivalent to G_{max} germinating at 25 °C (Fig. 1). Creeping bentgrass seed primed with GA using 200 mg·L^{-1} concentration exhibited greater G_{max} than unprimed seed at the cold germinating temperature. Although seed priming with GA showed a statistical benefit over unprimed seed under unfavorable temperatures, no differences between GA treatments and the controls were observed at favorable germinating temperatures of 25 °C (Fig. 1). GB-treated seed primed using the lowest rate (50 mM) also provided higher G_{max} than the unprimed control when germinating under unfavorable temperatures (10 °C) (Fig. 2). Alternatively, the same 50 mM concentration of GB when germinating under more favorable temperatures (25 °C) significantly inhibited G_{max} compared with the unprimed control (Fig. 2). The effects of GB and associated positive or negative rate effects on G_{max} were therefore highly dependent on the germinating temperature (Table 1). Farooq et al. (2008) reported seeds of maize (*Zea mays* L.) presoaked in GB at 50, 100, and 150 mg·L^{-1} enhanced the chilling tolerance during germination with the best results occurring with 100 mg·L^{-1}. Zhang et al. (2014) found that the effectiveness of priming with GB during seed germination of several turfgrass species was rate (concentration) dependent.

Expt. 2: Temperature main effects on D_{50}. Similar to Expt. 1, in Expt. 2 the effect of temperature (10 vs. 25 °C) accounted for the majority (98.5%) of the total treatment variation in germination rate (D_{50}). Low temperature (10 °C) reduced D_{50} by 5.6 d (8.1 d to 50% germination) compared with 25 °C (2.5 d to 50% germination; Tables 3 and 4). On average, Expt. 2 (5.3 d to 50% germination) was 34% faster (lower D_{50}) than Expt. 1 (7.1 d to 50% germination).

Expt. 2: Priming main effects on D_{50}. Priming effects (6 df) were highly significant ($P \leq 0.001$) with three single df orthogonal contrasts accounting for the variation including 1) Water-priming vs. no priming ($P \leq 0.05$), 2) ABA vs. GA ($P \leq 0.001$), and 3) H_2O_2 vs. ABA + GA + GB + PEG ($P \leq 0.001$). Overall, the following order from slower to faster germination rate (lower D_{50}) was observed: H_2O_2 (5.6 d), ABA (5.4 d), PEG (5.3 d), GB (5.1 d), and GA (5.0 d) (Table 4). Consistent with Expt. 1, redox (H_2O_2) priming significantly delayed germination compared with all other seed priming treatments (ABA + GA + GB + PEG); group average of 5.6 vs. 5.2 d to 50% germination, respectively. Also, hormone primer ABA significantly delayed germination (D_{50} = 5.4 d) compared with priming seed with the hormone GA (D_{50} = 5.0 d) (Table 4). Interestingly, unlike the results reported in Expt. 1, seed primed with water (D_{50} = 5.1 d) exhibited faster germination rate than unprimed seed (D_{50} = 5.6 d). According to priming main effects, GA and GB priming treatments were the only treatments with statistically lower D_{50} and faster in germination rate than the controls (D_{50} = 5.4 d, Table 4). The benefits of priming to achieve faster germinations with GA and GB primed seeds compared with the controls (average of unprimed + water primed) were not observed in Expt. 1.

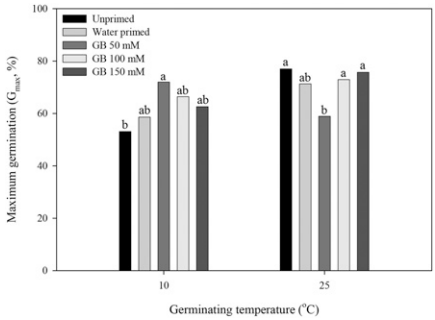

Fig. 2. Expt. 1 interactive effect for maximum germination percentage (G_{max}) comparing seed priming control (water primed and unprimed) with glycinebetaine (GB) at three rates and two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

Expt. 2: Priming rate main effects on D_{50}. The effects of rate (linear and quadratic components) of priming were significant for GA and H_2O_2 (Table 3). Similar to Expt. 1, redox primer H_2O_2 promoted lower D_{50} (faster germination rates) with lower rates of H_2O_2 (0.1 and 1 mM) compared with the highest priming rate of 100 mM (Table 4). Hormone primer GA optimal concentration for germination rate was the midpoint value of 200 mg·L^{-1}. Therefore, the quadratic component was the principal source of variation for D_{50} with GA; the GA_Q was highly significant ($P \leq 0.001$, Table 3). Seed primed with GA at 200 mg·L^{-1} promoted the lowest D_{50} (4.7 d) observed among all priming main effects (Table 4), which was statistically lower than seed primed with water (5.2 d, $LSD_{0.05}$ = 0.4). Plants are more susceptible to environmental stress in the seedling stages especially under conditions such as low temperature (Pill and Finch-Savage, 1988). Faster establishment indicated by lower D_{50} with creeping bentgrass seed primed with GA

Table 3. Expt. 2 analysis of variance for days to 50% germination (D_{50}) and maximum germination (G_{max}) derived from curve fitting of 'T-1' creeping bentgrass germination as affected by two temperatures and 17 seed-priming treatments.

Source of variation	df	D_{50}	G_{max}
Temperature (T): 10 vs. 25 °C	1	***	***
Treatment (TRT): Priming and rate	16	***	NS
Priming (P) orthogonal contrasts	6	***	*
Control (primed + no) vs. all	1	NS	†
Water-priming vs. unprimed	1	*	NS
Hormone[z] vs. osmopriming[y]	1	NS	NS
ABA vs. GA	1	***	NS
GB vs. PEG	1	NS	NS
H_2O_2 vs. ABA + GA + GB + PEG	1	***	*
Priming rate (R) orthogonal contrasts[x]	10	***	NS
ABA_L	1	NS	NS
ABA_Q	1	NS	NS
GA_L	1	†	NS
GA_Q	1	***	NS
GB_L	1	NS	NS
GB_Q	1	NS	NS
H_2O_{2L}	1	***	NS
H_2O_{2Q}	1	NS	NS
PEG_L	1	NS	NS
PEG_Q	1	NS	NS
TRT × T	16	***	**
P × T orthogonal contrasts	6	*	**
Control (primed + no) vs. all × T	1	NS	NS
Water-priming vs. unprimed × T	1	**	NS
Hormone[z] vs. osmopriming[y]	1	NS	NS
ABA vs. GA × T	1	†	*
GB vs. PEG × T	1	NS	NS
H_2O_2 vs. ABA + GA + GB + PEG × T	1	NS	**
R × T orthogonal contrasts	10	**	*
ABA_L × T	1	NS	†
ABA_Q × T	1	NS	NS
GA_L × T	1	**	NS
GA_Q × T	1	**	**
GB_L × T	1	*	NS
GB_Q × T	1	NS	†
H_2O_{2L} × T	1	*	*
H_2O_{2Q} × T	1	†	NS
PEG_L × T	1	NS	NS
PEG_Q × T	1	NS	NS

[z]Hormone treatments represent GA (gibberellic acid) and ABA (abscisic acid).
[y]Osmopriming treatments represent GB (glycinebetaine) and PEG (polyethylene glycol).
[x]Rates for chemical priming treatments were partitioned as linear (L) and quadratic (Q) orthogonal contrasts.
†, *, **, ***Significant at $P \leq 0.10$, 0.05, 0.01, or 0.001, respectively.

Table 4. Expt. 2 fitted main effect means for days to 50% germination (D_{50}) and maximum germination (G_{max}) as affected by two temperatures and 17 seed-priming treatments on 'T-1' creeping bentgrass germination.

Treatments	D_{50}	G_{max} (%)
Temperature (°C)		
10	8.1 a[z]	58.1 b
25	2.5 b	76.5 a
Controls		
No water priming	5.6 a	72.1
Water priming	5.1 b	70.8
Priming and rate		
ABA (abscisic acid)		
0.05 µM	5.3	68.1
0.1 µM	5.6	64.8
0.2 µM	5.4	60.2
GA (gibberellic acid)		
100 mg·L^{-1}	5.4 a	67.7
200 mg·L^{-1}	4.7 b	68.0
300 mg·L^{-1}	5.1 a	69.2
GB (glycinebetaine)		
50 mM	5.3	74.1
100 mM	5.2	68.5
150 mM	5.0	70.2
H_2O_2 (hydrogen peroxide)		
0.1 mM	5.2 b	60.1
1 mM	5.5 b	62.5
100 mM	6.0 a	64.0
PEG (polyethylene glycol)		
100 g·L^{-1}	5.3	69.6
200 g·L^{-1}	5.2	66.8
300 g·L^{-1}	5.3	67.4
Priming		
Control	5.4	71.5
Chemicals		
ABA	5.4 ab	64.4 bc
GA	5.0 d	68.3 ab
GB	5.1 cd	70.9 a
H_2O_2	5.6 a	62.2 c
PEG	5.3 bc	67.9 ab

[z]Numbers followed by the same letter(s) within a main effect group are not significantly different at $P \leq 0.05$ level. Statistical effects are shown only for significant main effects as indicated in Table 3.

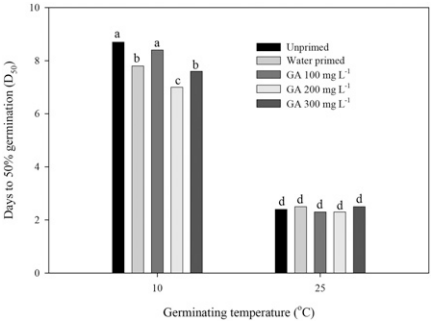

Fig. 3. Expt. 2 interactive effect for days to 50% germination (D_{50}) comparing three rates of gibberellic acid (GA) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

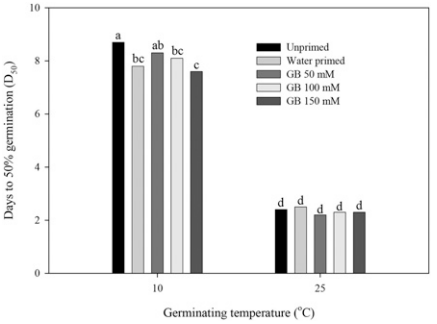

Fig. 4. Expt. 2 interactive effect for days to 50% germination (D_{50}) comparing three rates of glycinebetaine (GB) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

at 200 mg·L^{-1} may lead to less environmental stress during establishment.

Expt. 2: Priming × temperature interaction effects on D_{50}. In Expt. 1 no interaction between seed priming treatments with temperature was observed for D_{50} (Table 1). Two significant single df orthogonal contrasts for interaction were observed, however, during Expt. 2 including 1) water-priming vs. no priming × temperature ($P \leq 0.01$) and 2) ABA vs. GA × temperature ($P \leq 0.1$) (Table 3). Although main effect means for priming treatments indicated that priming with water significantly increase the speed of germination by reducing D_{50} compared with the unprimed control, the effect was temperature specific. For example, at 25 °C no difference between water-primed and unprimed seed was found for D_{50} (2.4 vs. 2.5 d, respectively. At 10 °C, however, a statistically significant difference was observed between the water-primed vs. unprimed seed, which averaged 7.8 vs. 8.7 d to 50% germination, respectively ($LSD_{0.05}$ = 0.5 d). In addition, the effect of seed priming on D_{50} using hormone ABA and GA was temperature dependent with GA promoting faster germination (D_{50} = 7.7 d) than ABA (D_{50} = 8.3 d) ($LSD_{0.05}$ = 0.3 d) under less favorable germinating temperatures (10 °C), whereas no difference was observed at favorable germinating temperatures of 25 °C (GA D_{50} = 2.4 d vs. ABA D_{50} = 2.6 d).

Expt. 2: Priming rate × temperature interaction effects on D_{50}. Three significant single df contrasts were detected for priming rate × temperature interaction (Table 3) including 1) GA_L × temperature and GA_Q × temperature (Fig. 3), 2) GB_L × temperature (Fig. 4), and 3) H_2O_{2L} × temperature and H_2O_{2Q} × temperature (Fig. 5). Seed primed with GA using the 200 mg·L^{-1} concentration was most effective in promoting rapid germination under cold germinating temperatures (10 °C) followed by 300 and 100 mg·L^{-1} (Fig. 3). This GA effect is consistent with the main effect for priming rate. Moreover, seed primed with GA at 200 mg·L^{-1} concentration was the only treatment to promote faster germination than the unprimed and water-primed controls under cold germinating temperatures. Alternatively, no rate effect due to priming with GA was observed under more favorable germinating temperatures of 25 °C. Seed priming with GB at the highest concentration of 150 mM was also effective is reducing D_{50} (Fig. 4). In addition, GB priming of creeping bentgrass seed at 150 mM concentration promoted faster germination than the unprimed control. No effect was observed according to priming rate main effects. Similarly, no difference between any GB concentrations was observed at the more favorable germinating temperatures (25 °C) (Fig. 4). Redox primer H_2O_2 exhibited no effect on germination rate measured as D_{50} at 10 °C (Fig. 5). At 25 °C the highest concentration (100 mM) of H_2O_2 significantly reduced germination vigor by increasing days to 50% germination (Fig. 5) and delaying days to germination to 50% by ≈1 d compared with all other concentrations evaluated. Seed priming with H_2O_2 has been reported to enhance the chilling tolerance of some species (Yadav et al., 2011). The level of change due to H_2O_2 is dependent on the stress, the concentration, and the length of exposure (Miller et al., 2009). In our study the highest concentration of H_2O_2 (100 mM) was inhibitory to creeping bentgrass seed germination vigor according to Expts. 1 and 2 (Tables 2 and 4). This was especially the case under more favorable temperatures for seed germination (Fig. 5). High levels of H_2O_2 may be toxic to plant cells and damaging to photosynthesis when applied externally (Bowler and Fluhr, 2000; Sairam et al., 2002). In wheat (*Triticum aestivum* L.), seeds primed with 80 mM H_2O_2 promoted optimal seedling growth under drought, whereas 120 mM inhibited seedling growth during

germination relative to the water-primed seed (He et al., 2009). Priming efficacy can be highly rate dependent.

Expt. 2: Temperature main effects on G_{max}. Results due to the effects of temperature on G_{max} were consistent with Expt. 1. Colder germinating temperatures (10 °C) inhibited creeping bentgrass maximum germination percentage and reduced G_{max} by ≈30% compared with 25 °C (Tables 3 and 4). During Expt. 1, only a 15% reduction due to the effects of colder temperatures was observed. Germination percentages were higher under 25 °C and lower at 10 °C in Expt. 2 relative to Expt. 1. The main effect of temperature accounted for ≈66% of the total variation in G_{max} during Expt. 2 to only 37% observed in Expt. 1, which is most likely due to the effects of the different seed lots.

Expt. 2: Priming main effects on G_{max}. In Expt. 1, no difference due to priming main effects (6 df) or single df contrasts were observed (Table 1) while in Expt. 2 significant main effects due to priming treatments and orthogonal contrasts were detected (Table 3). GB primed seed promoted the highest G_{max} (70.9%) with H_2O_2 (62.2%) the lowest G_{max} (Table 4). According to single df contrasts, H_2O_2 reduced maximum germination percentage compared with all other priming treatments (ABA + GA + GB + PEG). Hormone primer ABA (G_{max} = 64.4%) and redox primer H_2O_2 (G_{max} = 62.2%) inhibited G_{max} compared with the water-primed and unprimed seed (G_{max} = 71.5%, $LSD_{0.05}$ = 6.3%). No seed priming treatment was better than the water-primed and unprimed control in enhancing G_{max}. Interactions were detected between the effects of temperature with priming main effects and associated single df contrasts (Table 3). Therefore, these results due to priming were dependent on temperature.

Expt. 2: Priming rate main effects on G_{max}. Unlike Expt. 1, no significant main effect due to the rate of priming was observed in Expt. 2 (Table 3). Any differences in Expt. 2 that were observed due to the effect of priming rate were the result of priming rate interaction with temperature, discussed next.

Expt. 2: Priming × temperature interaction effects on G_{max}. Priming × temperature interactive effects were significant for priming main effects (6 df, $P \leq 0.01$, Table 3) including two of the following single df contrasts 1) ABA vs. GA × temperature ($P \leq 0.05$) and 2) H_2O_2 vs. ABA + GA + GB + PEG × temperature ($P \leq 0.01$). According to main effects no difference in G_{max} was observed between hormone primers ABA and GA (Tables 3 and 4). Hormone primer GA, however, afforded higher G_{max} (61.3%) than ABA (50.5%) ($LSD_{0.05}$ = 7.9%) under unfavorable germinating temperatures (10 °C), whereas at 25 °C, no difference between the two primers was observed in G_{max} (GA = 75.3%, ABA = 78.2%). Maximum germination percentage was lower following priming with H_2O_2 at 25 °C (Fig. 6) compared with all other primers (ABA + GA + GB + PEG), which is consistent with priming main effects (Tables 3 and 4). Alternatively at 10 °C, no difference between H_2O_2 and the combined mean of all other primers was detected (Fig. 6). Additionally, no effect of temperature on G_{max} was observed following priming with H_2O_2 (Fig. 6), which is a major departure from temperature main effects (Table 4). Optimal germinating temperature of 25 °C is expected to increase G_{max} compared with less favorable germinating temperature of 10 °C (Liu et al., 2001). However, the effect of H_2O_2 was especially inhibitory to germination compared with all other priming treatments under optimal germinating temperatures, which is readily apparent in Fig. 6. Furthermore, when compared with the average G_{max} of water-primed and unprimed seed at 25 °C (G_{max} = 83.4%), H_2O_2 significantly reduced G_{max} by 30% to 64.9% ($LSD_{0.05}$ = 8.9%).

Expt. 2: Priming rate × temperature interaction effects on G_{max}. No priming rate main effect was observed, however, significant priming rate main effect by temperature interactions were detected for the following single df contrasts 1) ABA_L × temperature ($P \leq 0.1$), 2) GA_Q × temperature ($P \leq 0.01$), 3) GB_Q × temperature ($P \leq 0.1$), and 4) H_2O_{2L} × temperature ($P \leq 0.05$). Hormone primer ABA and associated rates (0.05, 0.1, and 0.2 μM) had no effect on G_{max} at 10 °C, which ranged from 48.5 to 52.2%. At favorable germinating temperatures of 25 °C the same concentrations of ABA (0.05, 0.1, and 0.2 μM) were significantly linear; G_{max} decreased with increasing concentrations of ABA from 85.3, 81.1% to 68.2%, respectively ($LSD_{0.05}$ = 13.7%). The G_{max} for water-primed seed (84.8%) and unprimed seed (81.8%) were significantly higher than ABA seed primed using the 0.2 μM concentration at 25 °C. Therefore, hormone primer ABA significantly inhibited creeping bentgrass seed germination at the higher concentration under favorable germinating temperatures. The rate effect of hormone primer GA and osmotic primer GB on G_{max} were quadratic and dependent on the germinating temperature. At 10 °C germinating temperature, G_{max} of creeping bentgrass seed primed with GA at 200 mg·L^{-1} concentration was superior to all other GA rates including water-primed and unprimed seed (Fig. 7). At 25 °C the same GA concentration of 200 mg·L^{-1} was statistically no different from other GA priming concentrations but significantly inhibited G_{max} compared with water-primed and unprimed seed (Fig. 7). Differences between seed priming rates using the osmotic primer GB were only observed at 10 °C. Maximum germination percentage using GB at 10 °C germinating temperature was highest at the lowest concentration of 50 mM, which increased G_{max} compared with the 100 mM concentration and was superior to the water-primed and unprimed seed (Fig. 8). Redox primer H_2O_2 at the highest concentration (100 mM) increased G_{max} compared with the 0.1 mM concentration, whereas no differences among H_2O_2 concentrations were observed under favorable germinating temperatures of 25 °C (Fig. 9). Most concentrations or rates of H_2O_2 significantly inhibited germination at 25 °C compared with the unprimed and water-primed seed (Fig. 9). Interestingly, G_{max} in response to increase concentrations of H_2O_2 were linear (H_2O_{2L}, Table 3) with a positive trend at

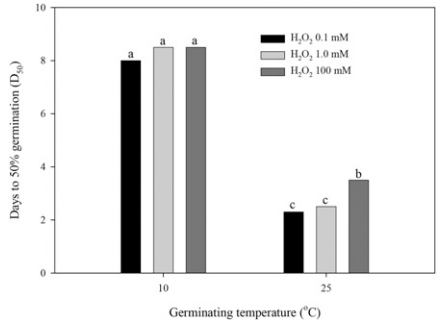

Fig. 5. Expt. 2 interactive effect for days to 50% germination (D_{50}) comparing three rates of redox primer (H_2O_2) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

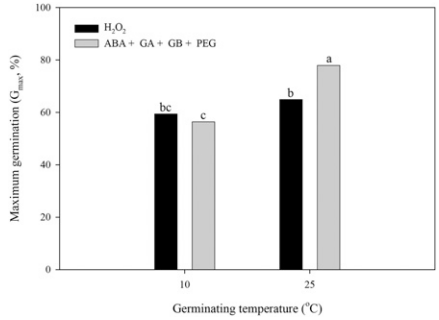

Fig. 6. Expt. 2 interactive effect for maximum germination percentage (G_{max}) comparing redox seed primer (H_2O_2) with all other priming treatments (abscisic acid, ABA; gibberellic acid, GA; glycinebetaine, GB; and polyethylene glycol, PEG) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

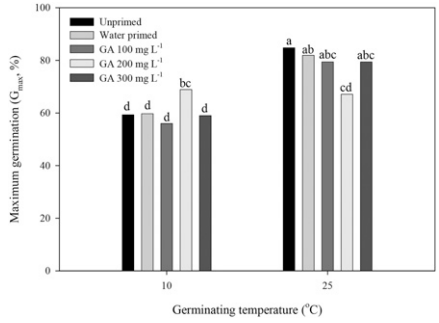

Fig. 7. Expt. 2 interactive effect for maximum germination percentage (G_{max}) comparing three rates of gibberellic acid (GA) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

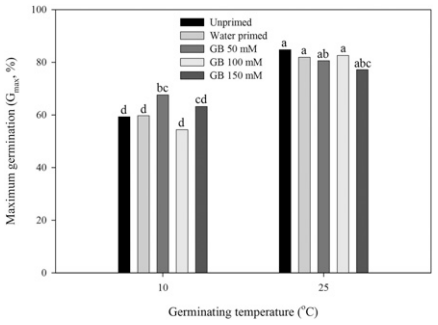

Fig. 8. Expt. 2 interactive effect for maximum germination percentage (G_{max}) comparing three rates of glycinebetaine (GB) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

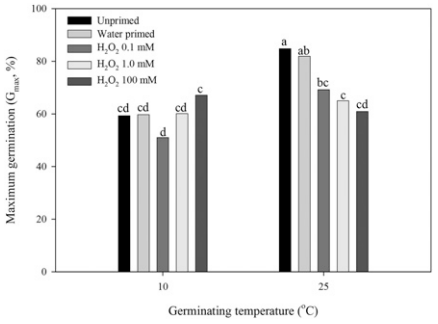

Fig. 9. Expt. 2 interactive effect for maximum germination percentage (G_{max}) comparing three rates of redox primer (H_2O_2) at two germinating temperatures. Means followed by the same letter(s) are not statistically different ($P \leq 0.05$).

10 °C and negative trend in response to H_2O_2 at 25 °C (Fig. 9). Results from seed priming with GB (Fig. 2) were similar to priming with H_2O_2 (Fig. 9) with G_{max} exhibiting positive and negative trends in response to increasing concentrations of GB at unfavorable and favorable germinating temperatures, respectively.

The interactive effect between temperature and priming rate for GB (Fig. 2) and H_2O_2 (Fig. 9) are problematic from a practical perspective because of their competing effects that increase germination at 10 °C while inhibiting germination at 25 °C. Cool-season grasses at planting may be exposed to a range of soil temperatures during establishment. Seed priming treatments and their rates or concentrations to be effective should not retard maximum germination percentage or delay the speed of germination by increasing D_{50} relative to unprimed seed at either unfavorable (10 °C) or favorable (25 °C) germinating temperatures. Reseeding of cool-season grasses in the spring in northern regions following winter injuries is necessary under suboptimal conditions such as low soil temperature. Germination enhancement (i.e., higher G_{max}) and stimulating early emergence (i.e., lower D_{50}) are usually observed under suboptimal temperatures (Hardegree, 1994; Heydecker et al., 1975). Both of these seed germination characteristics (G_{max} and D_{50}) contribute to the success in the establishment of new turf stands (Newell and Bladau, 1993).

Priming creeping bentgrass seed using redox primer H_2O_2 consistently increased D_{50} in Expts. 1 and 2 when compared with seed primed with hormone and osmotic primers (Tables 2 and 4). Priming creeping bentgrass seed using higher concentrations of H_2O_2 (100 μM) may be especially inhibitory to stimulating faster emergence under warm and more favorable germinating temperatures (Fig. 5) and in achieving adequate germination (Fig. 9). High levels of H_2O_2 may result in toxicity to cellular membranes causing damage to plant cells (Sairam et al., 2002), which in turn may explain the inhibitory effects on germination observed in our study. Similarly, Wang et al. (2014) reported presoaking kentucky bluegrass seed with 0.3% H_2O_2 had negative effects on seed germination and seedling growth. Priming bluegrass seed with H_2O_2 was not recommended by these authors.

Overall Summary of Expts. 1 and 2. Results summarized in Tables 1–4 indicate significant variability in main effects and interaction observed between Expts. 1 and 2 (i.e., different seed lots of 'T-1'). In addition, interactions between primers and associated concentrations with temperature were observed. The significance of the interaction between temperature with treatments (primers and priming rates) accounted for more of the variation in G_{max} than treatment main effects indicated by the partitioning of G_{max} 16 df into single df orthogonal contrasts. For example, partitioning G_{max} revealed 10 significant interactions to only 4 significant main effects when combined across Expt. 1 (Table 1) and Expt. 2 (Table 3). Identifying consistent seed priming treatments that improve germination by increasing G_{max} and/or lowering D_{50} relative to the unprimed control are problematic because of the competing (negative and positive) effects observed through interaction with germinating temperatures. Interactions among turfgrass species, temperature, and priming rate have been reported in previous seed priming studies (Zhang et al., 2014). Inconsistent results as indicated by the numerous interactions observed in our study suggest that seed priming efficacy is dependent on numerous factors in addition to seed lots, such as the priming agent, their rates, and the stress under evaluation (Iqbal and Ashraf, 2005).

Table 5 summarizes the effects relative to the unprimed control for all primers and their concentrations that were observed in Expts. 1 and 2 at the two germinating temperatures (10 and 25 °C). Seed primed with distilled water promoted earlier emergence (lower D_{50}) than unprimed seed at unfavorable germinating temperatures (10 °C) in Expt. 1 with no adverse effects observed (i.e., neither significantly lower G_{max} nor higher D_{50}) during Expts. 1 and 2. Creeping bentgrass seed primed with the hormone ABA at 0.05 μM concentration promoted faster emergence (lower D_{50}) than the unprimed control under cold germinating conditions (Expt. 2) without promoting any adverse effects. In addition, seed primed with ABA at 0.1 μM concentration promoted higher G_{max} than the unprimed control at 10 °C (Expt. 1) without causing any adverse effects on germination relative to unprimed seed. The highest concentration of ABA (0.2 μM) exhibited competing effects on D_{50} and inhibited G_{max} relative to the unprimed seed under favorable germinating temperatures (Expt. 2). These results suggest that seed primed with ABA at 0.05 and 0.01 μM concentrations were most effective for enhancing germination compared with unprimed seed. Hormone primer GA using the 100 mg·L^{-1} concentration exhibited no effect and therefore provided no adverse or beneficial effects on germination relative to unprimed seed. Priming seed using GA at the 200 mg·L^{-1} concentration promoted competing effects on G_{max}, which varied with germinating temperatures. Gibberellic acid at the highest concentration of 300 mg·L^{-1} promoted earlier emergence (lower D_{50}) than the unprimed control at 10 °C (Expt. 2) with no adverse effects observed on germination. As such, priming creeping bentgrass seed with GA using 300 mg·L^{-1} is an effective option for seed priming to achieve faster emergence. Osmotic primer GB enhanced germination relative to unprimed seed at all concentrations (50, 100, 150 mM) tested by promoting either higher G_{max} (Expt. 1) or lower D_{50} (Expt. 2) under unfavorable germinating temperatures of 10 °C. No adverse effects were observed on germination of creeping bentgrass seed using GB. Similarly, osmotic primer PEG at 200 and 300 g·L^{-1} promoted faster emergence by lowering D_{50} (Expt. 2) relative to unprimed seed under cold germinating temperatures with no adverse effects observed. Priming with PEG at 100 g·L^{-1} was associated with competing effects on D_{50} (Expts. 1 and 2) at 10 °C. Redox primer H_2O_2 promoted competing effects and/or adverse effects on G_{max} and D_{50} relative to unprimed seed at all concentrations and germinating temperatures. Similar to the conclusion of Wang et al. (2014), the use of H_2O_2 under our test concentrations provided negative results by inhibiting germination measured as lower G_{max} and/or higher D_{50} relative to unprimed seed.

In summary, of overall seed priming effectiveness relative to unprimed seed (Table 5), the potential for various primers to enhance germination as either higher G_{max} and/or lower D_{50} were as follow: osmotic primers were most effective (GB and PEG, 5 of 6 priming-rate combinations, Table 5) followed by hormone primers (ABA and GA, 3 of 6 priming-rate combinations, Table 5) with redox primers least effective (0 of 3 priming-rate combinations, Table 5). Any enhancement of creeping bentgrass seed germination using hormone and osmotic primers and their appropriate rates were only detected

Table 5. Summary of Expt. 1 and Expt. 2 indicating the statistical effect on days to 50% germination (D_{50}) and maximum germination percentage (G_{max}) following germination at two temperatures. Various seed primers and associated concentrations are compared relative to unprimed creeping bentgrass seed.

	Expt. 1				Expt. 2			
	D_{50}		G_{max}		D_{50}		G_{max}	
Primers and concentrations	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C
Water primed	0[z]	0	0	0	–	0	0	0
ABA (abscisic acid)								
0.05 μM	0	0	0	0	–	0	0	0
0.1 μM	0	0	+	0	0	0	0	0
0.2 μM	+	+	0	0	–	0	0	–
GA (gibberellic acid)								
100 $mg \cdot L^{-1}$	0	0	0	0	0	0	0	0
200 $mg \cdot L^{-1}$	0	0	+	0	–	0	0	–
300 $mg \cdot L^{-1}$	0	0	0	0	–	0	0	0
GB (glycinebetaine)								
50 mM	0	0	+	0	0	0	0	0
100 mM	0	0	+	0	–	0	0	0
150 mM	0	0	0	0	–	0	0	0
H_2O_2 (hydrogen peroxide)								
0.1 mM	0	0	0	0	–	0	0	–
1 mM	0	0	+	0	0	0	0	–
100 mM	+	+	0	0	0	+	0	–
PEG (polyethylene glycol)								
100 $g \cdot L^{-1}$	+	0	0	0	–	0	0	0
200 $g \cdot L^{-1}$	0	0	0	0	–	0	0	0
300 $g \cdot L^{-1}$	0	0	0	0	–	0	0	0

[z]Coding indicates statistical difference ($P \leq 0.05$ level) relative to unprimed (control) seed where "0" indicates no difference from the control; "+" indicates significantly higher from the control; "–" indicates significantly lower from the control.

under unfavorable germinating temperatures of 10 °C. Germination enhancement with hormone and osmotic primers were principally the result of faster emergence due to lower D_{50}. These results suggest that hormone and osmotic primers can benefit germination under cold (unfavorable) conditions typical of early spring establishment in northern regions. However, hormone and osmotic primers may not necessarily enhance germination under warmer or near optimal germinating temperatures of 25 °C. Creeping bentgrass seed primed with water also offered faster emergence (lower D_{50}) than unprimed seed under low temperature with no enhancement of maximum germination percentage (i.e., higher G_{max}). Seed primed with GB using the 100 mM concentration was the only primer and concentration to enhance germination under low temperature by increasing both G_{max} and early emergence (i.e., lower D_{50}) compared with unprimed seed. Investigations into the interactive effects of different hormone-osmotic-redox combinations and associated rates under different germinating temperatures and field conditions are needed.

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