

Optimizing Pre-germination Techniques for Kentucky Bluegrass and Perennial Ryegrass¹

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Abstract

Many intensively trafficked areas such as athletic fields and golf courses require constant overseeding to maintain suitable turfgrass cover. Rapid seed germination and development are critical to managing these high wear areas. The objectives of this research were to determine the effect of water aeration, seed soaking duration, and water temperature on mean germination time (MGT) and final germination percentage (FGP) of Kentucky bluegrass (*Poa pratensis* L., KBG) and perennial ryegrass (*Lolium perenne* L., PRG). Two separate controlled environment studies were conducted. PRG soaked in aerated water from 8 to 48 h had a 20% decrease in MGT compared to an untreated control, while treated KBG decreased MGT by only 10% compared to an untreated control. Soaking duration and water temperature had significant effects on KBG. KBG MGT was optimized at 20 C (68 F) water temperature with a soaking duration of 24 h. MGT of PRG was optimized when soaked for 8 h while water was aerated. There was no significant difference in FGP for any of the treatments tested.

Index words: turfgrass, aeration, seed soaking.

Species used in this study: Kentucky bluegrass (*Poa pratensis* L.); perennial ryegrass (*Lolium perenne* L.).

Significance to the Horticulture Industry

Sports turf areas are often intensively trafficked and require constant overseeding to maintain uniform cover. In these high wear situations, the speed of seed germination and development are important to the success and usability of these areas. When establishing worn turf areas, conditions are often not optimal and are likely to be time sensitive. Managers are usually facing hard deadlines for playable events. Therefore, knowing the optimal technique for germinating turf seed quickly would be an extremely useful tool for any turf manager. Many anecdotal recipes exist in trade magazines, but there is little scientific literature on the subject. This article helps to fill some of the gaps in the literature and gives turf managers the ability to choose techniques that fit their particular situation to optimize the speed of germination for highly trafficked turf areas.

Introduction

Turfgrass establishment is a challenging process that oftentimes must occur during suboptimal growing conditions in a very short time period. Turfgrass cover is reduced frequently due to high traffic, excessive divoting, sub-optimal weather conditions, or pest damage. Poor turfgrass cover can increase weed encroachment, reduce ball roll and footing, increase soil erosion, and reduce the aesthetics of turfgrass swards (Brede 1992). Recovery of damaged areas is essential to maintain the functionality of turfgrass

surfaces. Seeding is the most common establishment method used for turfgrass areas (Emmons and Rossi 2015). Given limited time for re-establishment, reducing seed germination time is extremely important for turfgrass managers.

Kentucky bluegrass (*Poa pratensis* L., KBG) and perennial ryegrass (*Lolium perenne* L., PRG) are two of the most widely used turfgrass species. PRG germinates relatively quickly and develops rapidly compared to KBG (Proctor et al. 2015). Rapid germination and subsequent seedling development are critical to managing high traffic areas. Priming and pre-germinating seed are two methods that can be utilized to speed the rate of turfgrass establishment. Priming seed is a procedure where water is added at sufficient levels to initiate the germination process without causing radical protrusion (Bush et al. 2000). Conversely, pre-germinating seed is an irreversible action where radical protrusion is imminent (Jamil et al. 2016). Seed priming and pre-germination have been shown to increase the rate of seed germination of many agronomic and vegetable crops (Farooq et al. 2006, Shim et al. 2008, Welbaum et al. 1998). Primed seed has advantages such as ease of spreading and temporary storage, but pre-germinating seed is generally preferred where time is critical since it is more likely to produce turfgrass cover faster (Stier 2001).

Currently “recipes” exist in various professional publications for pre-germinating seed to decrease germination time and increase the rate of seedling development (Brede 1992, Trulio 1994, Stier 2001). However, most of these are based on anecdotal observations and have not been evaluated using replicated research. Many variables such as turfgrass species, soaking duration, water temperature, and the moisture content of the seed at sowing are suggested to be important factors in the success of pre-germination (Zapiola and Mallory-Smith 2010, Stier 2001). Few studies have surveyed these effects on KBG or PRG. Previous studies have examined effects of pre-soaking, aerating and temperature on crops such as

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corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.) and *Solanaceous* species (Argerich and Bradford 1989, Barakat et al. 2013, Finch-Savage et al. 2004, Murungu et al. 2003). Previous studies have shown there is a clear relationship between temperature and turfgrass seed germination and are often described using a thermal time model (Larsen et al. 2004). Thermal accumulations vary depending on species. Kentucky bluegrass has been shown to have a higher thermal requirement than perennial ryegrass (Larsen et al. 2004). Air temperatures between 15.5 and 24 C (60-75 F) are optimal for the growth of most cool season grasses (Emmons and Rossi 2015). Still, water soaking temperature prior to germination has not been widely explored, nor has the duration of soaking seeds at various temperatures. Water temperature could be critical depending on the time of year and water source.

Aeration has been shown to have a positive effect on large monocot seeds. Germination times for aerated seeds significantly decreased compared to non-aerated seeds (Finch-Savage and Leubner-Metzger 2006). However, it is unclear how long a seed can be soaked without additional aeration and still germinate. Non-aerated creeping bentgrass seed remained viable after 17 weeks of soaking at 20 C (68 F) (88% germination) and 4 C (39 F) (46% germination) (Zapiola and Mallory-Smith 2010). This research indicates that some turfgrass seeds can survive long soaking durations with minimal impact on survival. However, the objectives of the Zapiola and Mallory-Smith study were not related to pre-germinating seed for the purpose of increasing the establishment rate of turfgrasses, nor were the seeds immediately germinated. Furthermore, previous research investigating methods of pre-germinating seed have focused more on the storage and viability of seed than the rate of germination and establishment. Studies have also limited levels of soaking duration and have not evaluated water temperature or aeration as a variable. Nor do these studies include some of the most widely used cool-season turfgrass species (Bush et al. 2000, Dudeck and Peacock 1986).

Pre-germinating seed is labor intensive and requires some specialized equipment. There is a need to understand which methods optimize germination speed, especially for the most commonly used turfgrass species. The objectives of this research were to determine the effect of water aeration, soaking duration, and water temperature on the MGT and FPG of KBG and PRG.

Materials and Methods

Two studies were conducted in controlled environment growth chambers (Environmental Growth Chambers, Chargin Falls, OH) located in the Agricultural Biotechnology Laboratory Building, University of Connecticut, Storrs, CT. Seeds of 'Wild Horse' KBG (Pennington Seed Co., Lebanon, OR - Lot #Z1-10-1182) and 'Soprano' PRG (Pennington Seed Co., Lebanon, OR - Lot #MG10SOP771) were used. Seeds for each experiment were used from the same lot to maintain consistency across both studies. Seed bags were stored at 20 C (68 F) until needed.

Study 1: The Effect of Soaking Duration and Aeration on MGT and FPG. This study was arranged as a 2 by 4 factorial split-plot design with an untreated control. The first factor, water aeration (yes and no), was the main plot with soaking duration of 8, 24, 48 or 72 hours (h) as the sub-plot. The study was performed independently with KBG and PRG with three runs being conducted for each species. Seeds were first soaked in plastic containers (15 L) containing deionized (DI) water (8 L). Each plastic container used along with the DI water was placed in an environmental growth chamber maintained at 20 C (68 F) 24 h prior to the start of the soaking period. This was done in order to allow the water to equilibrate to 20 C (68 F). A 15.24 cm (6 in) air stone disk (Ecoplus, model number 728417, Vancouver, WA) was placed at the bottom of each container containing an aerated treatment to produce a uniform column of bubbles. An air compressor, manifold, and vinyl tubing were used to provide constant air flow throughout each soaking duration time period for the four aerated treatments. The four non-aerated treatments contained only DI water. After equilibration, seeds (900 g (2 lb) per container) of each species were then added to the appropriate container starting with the 72 h soaking duration and then continuing sequentially to 48, 24 and 8 h so the seeds could be removed from all the containers at the same time to allow for simultaneous testing of all treatments. Each container was then considered an experimental unit.

Once the allotted soaking time had passed, 90 seeds from each experimental unit were individually placed on moistened, 43 kg (95 lb) unbleached brown blotter paper (Stults Scientific Engineering Corporation, Springfield, IL) and placed in 15 by 23cm (6 by 9 in) clean, plastic germination boxes (Pioneer Packaging, Dixon, KY). Three replicates were taken from each experimental unit for evaluation. Non-soaked seeds were used as a control for each species. Each germination box was sealed with aluminum tape to prevent the blotter paper from drying. The germination boxes were transferred to another growth chamber and completely randomized. Turfgrass seeds can show conditional dormancy to varying day/light conditions (Larsen, et al. 2004). Therefore, the growth chamber temperature was maintained at 25 C (77 F) for an 8 h photoperiod and then reduced to 15 C (34 F) for 16 h of darkness in order to simulate day/night. The germination boxes were rotated daily on each shelf to minimize any spatial difference in light intensity. Light intensity was maintained at 14 to 21 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (1000 to 1550 lux).

Study 2: The Effect of Soaking Duration and Water Temperature on MGT and FPG. This study was arranged as a 3 by 3 factorial, nested with an untreated control. The main factors were water temperature (4, 20, and 30 C; 39, 68, and 86 F) and soaking duration (8, 24, and 48 h). Soaking duration was nested within each water temperature. The study was performed independently with KBG and PRG seed. The soaking procedure was similar to Study 1 with the exception of the soaking temperatures. Plastic containers (15 L; 4 gal) were filled with 8L (2 gal) of DI water and allowed to acclimate for 24 h in an environmental growth chamber prior to the start of soaking at

Table 1. Study 1: The effects of soaking duration averaged over aeration and aeration averaged over soaking duration on mean germination time (MGT) and final germination percentage (FGP) of Kentucky bluegrass (KBG) and perennial ryegrass (PRG).

Main Effects	Kentucky bluegrass		Perennial ryegrass	
	MGT days	FPG ^Z %	MGT days	FPG %
Duration (DU)				
Control	7.7a ^Y	93	4.2	79
8 h	7.0b	89	3.2	85
24 h	6.7d	91	3.1	84
48 h	7.0cb	92	3.2	83
72 h	6.8cd	89	3.0	76
Aeration (AE)				
Control	7.7a	93a	4.2a	79
No	6.6c	93a	3.4b	81
Yes	7.1b	88b	2.8c	83
Source of variation ^X	<i>P</i> > <i>F</i>			
DU	*	NS	NS	NS
AE	***	**	**	NS
DU by AE	**	NS	NS	NS
Orthogonal Contrast				
Control vs. All durations	ND ^W	ND	ND	NS

^ZFPG was calculated based on two replicates.

^YMeans in a column for each main effect followed by the same letter(s) are not significantly different according to Fisher's Protected LSD test (*P*=0.05).

^X*, **, ***, NS, Significant at the *P* ≤ 0.05, 0.01, 0.001 probability levels, or not significant, respectively.

^WNot determined; ND.

either 4, 20, or 30 C (39, 68, or 86 F). KBG or PRG seed (900g) was added to each container starting with the 48 h soaking duration and then continuing sequentially to 24 and 8 h so all seeds could be removed from all the containers at the same time to allow for simultaneous testing of all soaking durations and species within soaking temperature. After soaking times were complete, all the seeds were removed and arranged in germination boxes as in Study 1.

Treatments were arranged in a completely randomized design within each soaking temperature. Treatments were replicated three times within each soaking temperature in the growth chamber and three separate runs for each temperature (4, 20, or 30 C; 39, 68, or 86 F) were completed. The treatments were then subjected to the same light and environmental conditions as the treatments in Study 1 for seed germination.

Data collection and analysis. Radicle protrusion counts were conducted daily for each treatment for a total of 10 or 28 d for studies 1 and 2, respectively, and a final germination count was taken on day 28 of both studies. A radicle was considered protruded if the tip could be seen without the aid of a hand lens emerging from the seed. When the radicle protruded the seed was deemed to have germinated and was noted in the count. FGP was calculated using the number of seeds showing radical protrusion at 28 days divided by the total number of seeds tested. Mean Germination Time (MGT) was calculated using a weighted average to determine how long the seed took to germinate

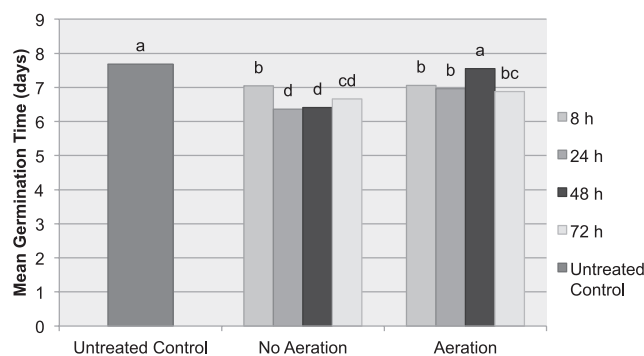


Fig. 1. Study 1: The interaction of soaking duration and aeration on mean germination time for Kentucky bluegrass compared to a control without soaking or aeration. Bars with different lettering are significantly different according to Fisher's Protected LSD (*P*=0.05).

using the following equation (Salehzade et al. 2009):

$$\text{MGT} = \frac{\sum Dn}{\sum n} \quad (1)$$

where:

n = The number of seeds, which were germinated on day *D*

D = The number of days counted from the beginning of germination

Data were analyzed using the MIXED procedure in SAS/STAT 14.1 software (SAS Institute Inc., 2015, Cary, NC) along with relevant orthogonal contrasts with the 0.05, 0.01, and 0.001 significance levels reported. Mean separation tests were performed using Fisher's protected least significant difference test at *P* = 0.05.

Results and Discussion

Kentucky bluegrass. In the aeration and soaking duration study (Study 1), overall soaking duration and aeration main effects were significant for KBG (Table 1). Aeration increased MGT and decreased FGP compared to non-aerated soaked treatments of KBG. However, because there was a significant interaction between soak duration and water aeration the main effects need to be interpreted with caution. Soak duration effects were not consistent across aeration levels (Fig. 1). Non-aerated seeds generally had shorter MGT than aerated seeds, except at the 8 and 72 h soaking durations, which were no different than aerated seeds. Within non-aerated treatments, soaking durations ≥ 24 h reduced MGT by 0.6 days (d) compared 8 h. However, no differences in MGT between soaking durations of 24 to 72 h were observed. Conversely, among aerated seeds, no effect of soaking duration was observed, except at 48 h which increased MGT 0.6 d compared to the average MGT of the other aerated seeds. MGT seen with aerated seed soaked for 48 h was not significantly different than the untreated control, whereas all other treatments reduced MGT compared to the non-soaked control. Additionally, aerated seed had significantly lower FGP than non-aerated seed (Table 1). Brede and Brede (1989) speculated that aerating a partially germinated seed can be physically harsh on a delicate seed. It may be possible that the aerated seeds were physically damaged and therefore showed lower

Table 2. Study 2: The effects of soaking duration and water temperature on mean germination time (MGT) and final germination percentage (FGP) of Kentucky bluegrass (KBG), and perennial ryegrass (PRG).

Main Effects	Kentucky bluegrass		Perennial ryegrass	
	MGT days	FPG ^Z %	MGT days	FPG %
Duration (DU)				
Control	8.8a ^Y	93	4.7	90
8 h	7.2b	92	4.2	90
24 h	7.0c	92	4.1	88
48 h	6.7d	93	4.1	88
Temperature (TP)				
Control	8.8a	93	4.7	90
4 C	7.5b	90	4.3	90
20 C	6.7c	92	4.1	86
30 C	6.7c	94	4.1	90
Source of variation ^X	<i>P</i> > <i>F</i>			
DU	**	NS	NS	NS
TP	***	NS	NS	NS
DU by TP	**	NS	NS	NS
Orthogonal Contrast				
Control vs. All durations	ND ^W	NS	***	NS

^ZFPG was calculated based on two replicates.

^YMeans in a column for each main effect followed by the same letter(s) are not significantly different according to Fisher's Protected LSD test (*P*=0.05).

^X*, **, ***, NS, Significant at the *P* ≤ 0.05, 0.01, 0.001 probability levels, or not significant, respectively.

^WNot determined; ND.

germination rates. Based on these results, aerating KBG seed showed no additional benefit to MGT when compared to the soaked seed. In addition, soaking for more than 24 h showed no added benefit to MGT.

In the soak duration and temperature optimization study (Study 2), water temperature and soaking duration main effects, and the interaction had a significant effect on MGT (Table 2). All water temperatures and soaking duration treatments decreased MGT compared with the control. Seed soaked at 4 C (39 F) had greater MGT than 20 C (68 F) or 30 C (86 F) regardless of duration, except at the longest time interval (48 h), which was comparable to the higher water temperatures. However, there was also a significant interaction between water temperatures and soaking durations, indicating that soaking duration did not have the same effect across all water temperatures (Fig. 2). At 4 C (39 F), MGT decreased as soaking duration increased. However, no differences between soaking durations were observed at 20 C (68 F) and 30 C (86 F). The effect of temperature on breaking seed dormancy has been well documented (Bradford 2002). KBG seeds soaked at 4 C (39 F) were likely slower to imbibe water and thus retarded germination compared to the higher temperatures. However, there were no detrimental effects of soaking at 4 C (39 F), as the MGT at all three soaking durations were significantly lower than the untreated control (Fig. 2). There were no significant soak duration or water temperature effects on FGP for KBG (Table 2).

Based on the results of Study 1 for KBG, aeration is not needed to optimize MGT. Results for soaking duration

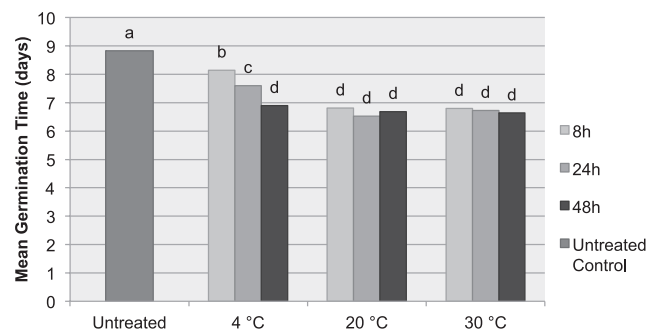


Fig. 2. Study 2: The interaction of soaking duration and water temperature compared to an untreated control without soaking on mean germination time for Kentucky bluegrass. Bars with different lettering are significantly different according to Fisher's Protected LSD (*P*=0.05).

were not consistent across the two studies. Study 1 indicated an optimal soak duration of 24h at 20 C (68 F) (Fig. 1) for KBG. However, while the MGT was slightly lower at 24 h compared to 8 h at 20 C (68 F) in Study 2, this difference was not statistically significant (Fig. 2). The reason for this discrepancy is not exactly clear, but it is likely due to higher variability in Study 2. Based on these results, optimal soak duration for KBG is highly dependent on water temperature. Therefore, the soak duration should be adjusted based on water temperature (i.e. the lower the water temperature, the longer the optimal soak duration: 4 C (39 F) /48 h, 20 C (68 F) /24 h, and 30 C (86 F) /8 h).

Perennial ryegrass. No significant differences for soaking duration were observed for PRG in the study examining the effect of aeration and soaking duration (Study 1) on MGT (Table 1). However, aeration significantly reduced MGT compared to no aeration (Table 1). Averaged over soaking temperature, aeration decreased MGT from 3.4 days with no aeration to 2.8 days with aeration (Table 1). There was a benefit to using aeration regardless of soaking duration with PRG that was not seen with KBG. Even though the benefit was small (~14 h shorter MGT), it may be crucial in situations where timing is vital.

In the water temperature and soaking duration study (Study 2), there was no significant soak duration or water temperature main effect or interaction (Table 2). However, when an orthogonal contrast comparison was performed, comparing all the treated seeds (all seeds that had been soaked) to the control, the soaked seeds had lower MGT compared to the untreated control (Table 2) (Fig. 3). This indicates that the optimal soak duration may be less than 8 h. Unlike KBG, FGP for PRG was not significantly different for any of the treatments for either of the two experiments (Tables 1 and 2). When pre-germinating perennial ryegrass, turfgrass managers should soak the seed for 8 h, possibly less.

Aeration of PRG provided a slight benefit, while there was no benefit to aerating KBG. This is consistent with Dudeck and Peacock (1984), who showed varying sensitivity to aeration among species. Because seeds were soaked for three days or less, sufficient oxygen needed for

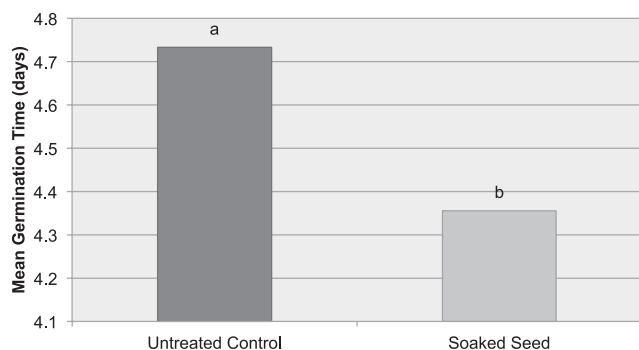


Fig. 3. Study 2: The effect of untreated perennial ryegrass seeds compared to soaked perennial ryegrass seeds averaged across all treatments on mean germination time. Bars with different lettering are significantly different according to orthogonal contrast ($P=0.001$).

metabolic processes involved with germination could have been present with no additional aeration needed. The results may be different for longer soaking durations.

MGT of PGR was optimized when seed was aerated and soaked for 8 h. Soaking duration and water temperature were not critical factors for PRG within the parameters measured. The optimal soaking duration for PRG could be less than 8 h due to its rapid germination time. Additional studies are needed to confirm this hypothesis. Additionally, the data show there was no detrimental effect on PRG or KBG MGT when soaking at longer durations or at varying temperatures, indicating that PRG and KBG could be soaked together if seeding with a mix. The benefits of pre-germination are likely to become most evident when growing conditions are adverse or when mixing a slow germinating species such as KBG with a fast germinating species such as PRG. PRG often outcompetes KBG in mixed stands, causing poor survival of KBG. Pre-germination would be useful when the allotted time between athletic events is minimal.

As seen in Hardegree and Van Vactor (2000), we expect that the MGT and viability of seeds could be different in laboratory and field settings. However, these experiments provide vital information to turfgrass managers about soaking duration, soaking temperature, and aeration for two of the most commonly used turfgrass species. Based on the range of temperature and soaking duration optima observed for these turfgrass species, more research is needed to determine optimal pre-germination conditions for other commonly used turfgrasses.

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