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Seed Germination of Southern Seaoats (*Uniola paniculata*) as Influenced by Stratification, Temperature, and Light[1]

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Abstract

Seeds of southern seaoats (*Uniola paniculata* L.) were removed from storage at 4C (39F) and stratified (moist-prechilled) for 0, 15, or 30 days at 4C (39F). Following stratification, seeds were germinated at 25C (77F) or 30C (86F) or at 8/16 hr thermoperiods of 30/20C (86/68F) or 35/25C (95/77F) with daily photoperiods at each temperature of 0 (total darkness), 2, 4, 8, 12, or 24 hr. Germination was recorded every 3 days for 30 days. Light had no effect on germination. Regardless of photoperiod the influence of light was nonsignificant ($P = 0.45$). On the other hand, temperature and stratification were significant ($P = 0.0001$) and there was a significant interaction ($P = 0.001$) between the two parameters. Averaged across all treatments, the highest total germination was realized at 35/25C (95/77F) (60%) followed by 30/20C (86/68F) (48%), 30C (86F) (37%), and 25C (77F) (31%). Stratification was not a requirement for germination but stratification for 15 days increased the rate of germination but not total germination. However, stratification for 30 days decreased germination due to seed decay caused by fungal growth despite seed treatment with 1.3% sodium hypochlorite prior to stratification. Seed decay during germination was observed and treatments to reduce decay should be investigated since viability tests with 2,3,5-triphenyltetrazolium chloride (TTC or TZ) indicated that initial seed viability was >95%.

Index words: sexual propagation, sand dune species, beach and dune restoration, Poaceae.

Significance to the Nursery Industry

Results demonstrate that seed germination of *U. paniculata* is relatively easy to accomplish. Seeds do not require stratification (moist-prechilling) for germination but stratification for 15 days will increase the rate of germination. Longer durations of stratification may be beneficial but seed decay is a problem. Light has no effect on germination whereas temperature plays a major role. Of the various temperature-stratification treatments investigated in this study, the highest germination (70%) was realized for seeds stratified for 15 days followed by germination at an 8/16 hr thermoperiod of 35/25C (95/77F).

Introduction

Southern seaoats (*Uniola paniculata* L.) is a perennial dune grass that ranges from southern Virginia to eastern Mexico (9). It is one of the primary components in the dune-strand ecosystem (9). Ecologically, *U. paniculata* is extremely important in formation and maintenance of sand dunes, and is an integral part of the food web for the animals, birds, and

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insects that characterize this habitat (9). The species could have considerable ecological and economic potential because of its dune building and sand holding abilities (10). Currently, seedling transplants are in high demand for restoring beaches and sand dunes damaged or destroyed by tropical storms and erosion (2).

Seed production of *U. paniculata* is generally low although the potential exists for much greater production. The species produces six to eight fertile florets per spikelet, but few of these ever set seed (9). It appears as though viable embryos are produced and subsequently abort (9). Seeds are susceptible to attack by the fungi *Alternaria* Nees sp. and *Helminothosporium* Lk. sp. that occur commonly in many grass species (9). Although these fungi are able to attack and destroy viable ovules directly, it is more likely they are secondary invaders attacking the aborted ovules following insect feeding or bacterial infection (9). High humidity and summer rain common in this habitat can increase the incidence of fungal infection on the seeds (2). Consequently, plants produce on average less than two viable seeds per spikelet (9). Seed germination is not difficult to accomplish but research is needed to optimize germination such as studying the influence of various environmental factors (e.g., light and temperature) on germination. Such work could improve current production of seedling transplants.

Little information has been published on the effects of light on seed germination of *U. paniculata*. Although Westra and Loomis (10) reported that light does not influence germination, this does not appear to have been thoroughly investigated. On the other hand, several studies have been published dealing with the effects of temperature on both seed germination and seedling growth (3, 7, 8, 10). It has been reported that alternating 17 ± 1/7 ± 1 hr temperatures of 32/16C (90/61F) (8) and 35/18C (95/64F) (6, 7) yield maximum germination and seedling growth. Seneca (7) reported that both higher constant temperatures [30C (85F) and 35C (95F)] and alternating 17 ± 1/7 ± 1 hr thermoperiods [35/19C (95/65F) and 30/19C (85/65F)] increased germination compared to cooler constant [24C (75F) and 19C (65F)] temperatures and alternating [24/19C (75/65F)] 17 ± 1/7 ± 1 hr thermoperiods. Colosi (3) reported optimal temperatures for germination exceeded 35C (95F).

Stratification (moist-prechilling) is not necessary for germination of *U. paniculata* but research has indicated it will stimulate greater germination in comparison to nonstratified seeds (7, 8). Seneca (8) studied the effects of stratification on several Atlantic and Gulf coast populations of *U. paniculata*. Results indicated Atlantic coast Florida populations were unaffected by stratification, whereas stratification stimulated germination of populations from Virginia and North Carolina. The response of Gulf coast populations was intermediate between the aforementioned populations. When stratification increased germination, the duration necessary to maximize germination varied from 15 to 30 days depending on the geographic locality from which the seeds were collected (8). In contrast, Hester and Mendelssohn (6) reported that stratification did not increase total germination of seeds from four Louisiana populations of *U. paniculata* but increased the rate of germination. Therefore, to further define optimum environmental conditions for seed germination of the species, the following research was conducted to study the influence of stratification, temperature, and light on seed germination of *U. paniculata*.

Materials and Methods

Spikelets of *U. paniculata* were collected from a population of plants growing on Oak Island (Brunswick County), NC, on October 13, 1998. The plants were growing on sand dunes facing the Atlantic Ocean. As the spikelets were collected they were placed in plastic bags and transported to Raleigh, NC. The spikelets were then removed from the plastic bags and placed on trays for drying at 21C (70F) for 5 weeks followed by seed extraction and storage at a moisture content of 9% in a sealed glass bottle at 4C (39F). Seed moisture content was determined by calculating the mean moisture content of six 50-seed samples following drying at 105C (221F) for 24 hr.

In January 1999, seeds were removed from storage and graded under a dissecting scope, which allowed removal of abnormal or damaged seeds, and any debris. Graded seeds [approximately 6000 pure seeds per 28 g (1 oz)] were then stratified (moist-prechilled) for 0, 15, or 30 days at 4C (39F) in the following manner.

Dry sand was sieved through a 16-mesh [0.06-in (1.59-mm)] screen and the fine separate retained. Seeds were surface disinfested by submerging them in a 1.3% NaOCl solution for 15 min followed by several rinses with tap water. Fifty cleaned/graded seeds were mixed with 20 ml (0.68 fl oz) moist sand [dry sand:water (10:1 by vol)] and were placed in 476 ml (1 pt) nonvented, polyethylene freezer bags. After the designated stratification interval, 96 randomly selected bags were removed from stratification. Seeds were separated from sand by flushing with tap water in a colander and sown in covered 9-cm (3.5-in) glass petri dishes (50 seeds per dish). Each dish contained two prewashed (rinsed) germination blotters (Filtration Sciences Corp., Mt. Holly Springs, PA) uniformly moistened with tap water. All dishes were placed in black sateen cloth bags and seeds were allowed to imbibe over night at 21C (70F). The following day, dishes were randomized within four growth chambers [C-chambers (4)] at the Southeastern Plant Environment Laboratory (NC State Univ., Phytotron), Raleigh, NC. The chambers were maintained at 25C (77F) or 30C (86F), or at 8/16 hr thermoperiods of 30/20C (86/68F), or 35/25C (95/77F). Chamber temperatures varied within ± 0.5C (0.9F) of the set point.

Within each temperature regime, seeds were subjected daily to the following photoperiods: 0 (total darkness), 2, 4, 8, 12, or 24 hr. Regardless of stratification or temperature, photoperiod treatments were administered the same time each day. All photoperiod treatments for the alternating temperatures of 30/20C (86/68F) or 35/25C (95/77F) began with the transition to the high-temperature portion of the cycle, with the exception of total darkness and 24 hr.

Growth chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400–700 nm) of 30–40 □mol·m^{-2}·s^{-1} (2.1–2.8 klx) as measured at the level of the dishes and outside the dishes with a cosine-corrected LI-COR LI-185 quantum/radiometer/photometer (LI-COR, Lincoln, NE). All photoperiod treatments except total darkness and 24 hr were regulated by removal and placement of the petri dishes in black sateen cloth bags. For the 24 hr photoperiod treatment, the dishes remained continuously unbagged in open chamber conditions. Regardless of the photoperiod, temperatures within the petri dishes, as measured by a thermocouple, never exceeded ambient temperature by more than ± 1C (2F) of the set point. The constant darkness treatment was maintained by keeping the petri dishes

in the black sateen cloth bags throughout the experiment, and all watering and germination counts were performed in a darkroom utilizing a fluorescent lamp equipped with a green acetate filter (Rosco Laboratories, Port Chester, NY). Germination blotters were kept moist with tap water throughout the duration of the experiment. Seeds showing signs of decay were removed immediately from the dishes.

For each temperature, all photoperiod-stratification treatments were replicated four times with a replication consisting of a petri dish containing 50 seeds. Germination counts were recorded every 3 days for 30 days. A seed was considered germinated when radicle emergence was ≥1 mm (0.04 in).

The experimental design was a split-split plot with temperatures as the main plots, stratification treatments as the subplots, and photoperiods as the sub-sub plots. Data for total percentage germination (total germination at the end of the 30-day germination period) and time course of germination (percentage germination recorded every 3 days for 30 days) were subjected to analysis of variance procedures and means were separated by Fisher's protected least significant difference (LSD) at $P < 0.05$.

Results and Discussion

Light had no effect on germination; it neither inhibited nor stimulated germination. Regardless of photoperiod, the influence of light was nonsignificant ($P = 0.45$). Although a previous report (10) mentioned that light had no influence on germination, the authors were unable to find any indication that the influence of light had been subjected to rigorous investigation, which prompted this aspect of the present study. On the other hand, the influence of stratification and temperature were significant ($P = 0.0001$) and there was a significant interaction ($P = 0.001$) between the two parameters. Although the interaction between temperature and stratification was statistically significant, the error mean square of the main effects of temperature (10,984) and stratification (13,567) dwarfed that of the interaction term (294). The significance of the interaction was likely due in part to the large sample size of the experiment (n = 288).

Stratification was not necessary for germination as percentage germination was the same for nonstratified seeds and seeds stratified for 15 days (Fig. 1). Stratification for 15 days increased the initial rate of germination compared to nonstratified seeds; by day 21 germination of nonstratified seeds and seeds stratified for 15 days was identical. Although statistical analysis of the data revealed stratification to have a significant effect on germination ($P = 0.0001$), this occurred only because there was a decrease in germination with 30 days stratification. Total germination of 51%, 50%, and 30% resulted following stratification for 0, 15, and 30 days, respectively. The decrease in germination following 30 days stratification resulted from extensive seed decay during germination despite treatment of the seeds with 1.3% NaOCl for 15 min prior to stratification. Seed decay appeared to be caused by various fungal and bacterial pathogens although no attempt was made to identify these organisms.

When seeds were placed in the petri dishes for germination following stratification for 30 days there were no noticeable signs of decay. However, after 2 days it became apparent that decay would be a problem as evidenced by mycelium growth on the seeds. Apparently, stratification for 30 days stimulated growth of various seed pathogens. Perhaps, if a more effective means of seed treatment had been employed prior to stratification for 30 days and even 15 days, greater germination may have been realized. In fact, the authors also included a stratification treatment of 45 days. However, because of the extensive seed decay resulting from 30 days stratification coupled with reduced germination, the authors decided not to germinate those seeds that were stratified for 45 days. To further study the influence of stratification durations >15 days will first require some means to eliminate/control bacterial and fungal growth during stratification.

Even though several authors have reported on seed germination of *U. paniculata* (2, 3, 6, 7,8), none of these reports indicated that seed decay is a problem during stratification and germination. Either this has not been a problem previously or has been ignored.

The only benefit that the authors were able to observe following stratification was the increase in the rate of germination following 15 days stratification (Fig. 1). Whether strati-

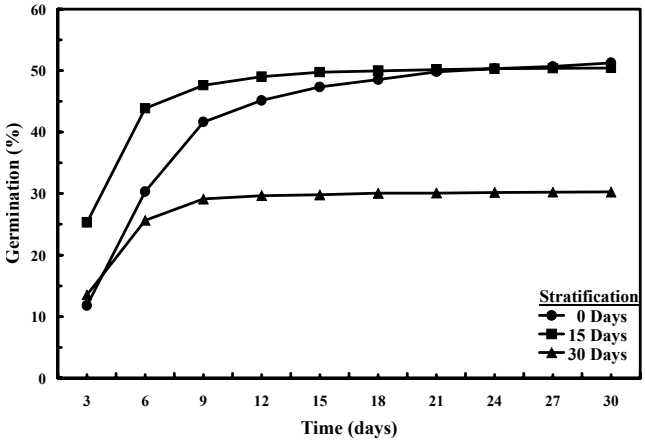

Fig. 1. Influence of stratification on seed germination of *U. paniculata* combined over all temperatures. $LSD_{0.05}$ = 0.8 for comparisons among stratification treatments for a given day.

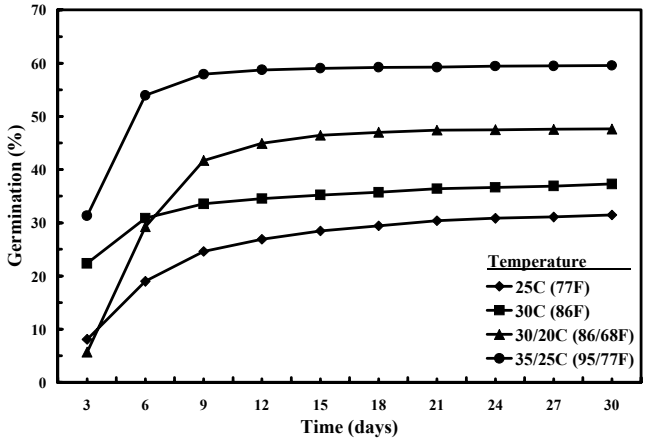

Fig. 2. Influence of temperature on seed germination of *U. paniculata* combined over all photoperiod and stratification treatments. $LSD_{0.05}$ = 0.9 for comparisons among temperatures for a given day.

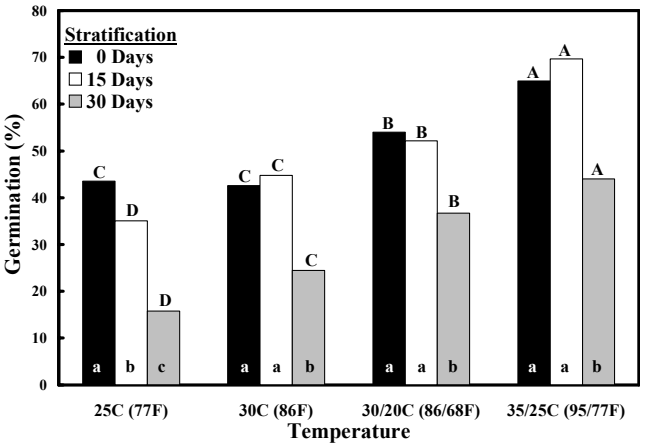

Fig. 3. Influence of stratification and temperature on seed germination of *U. paniculata* combined over all photoperiods. Lowercase letters within the vertical bars denote mean separation among stratification treatments for a particular temperature at $P < 0.05$. Uppercase letters above vertical bars denote mean separation among temperatures for a particular stratification treatment at $P < 0.05$. $LSD_{0.05} = 5$ for all comparisons.

fication would be useful in terms of commercial production of *U. paniculata* is debatable particularly with problems of seed decay following stratification for 30 days. Adkins et al. (1) reported that stratification broadened the range of temperatures over which seed germination of *Abies fraseri* (Pursh) Poir. (Fraser fir) occurred and a similar phenomenon might exist for *U. paniculata*. Such a response could have practical significance since it would permit optimum germination over a range of temperatures. However, to test this would require a means of suppressing fungal and bacterial growth during seed stratification of *U. paniculata* and stratifying seeds for durations exceeding 30 days.

Temperature was also highly significant ($P = 0.0001$) when averaged across all photoperiod and stratification treatments. The highest total germination was realized at 35/25C (95/77F) (60%) followed by 30/20C (86/68F) (48%), 30C (86F) (37%), and 25C (77F) (31%) (Fig. 2). These findings tend to agree in part with those of Seneca (7). All temperature treatments were significantly different from each other.

As mentioned previously, there was a significant ($P = 0.001$) interaction between temperature and stratification. When seeds were not stratified, germination at 35/25C (95/77F) and 30/20C (86/68F) were significantly different and both thermoperiods resulted in higher total germination than either 30C (86F) or 25C (77F), which were not significantly different (Fig. 3). For 15 and 30 days stratification, germination at all temperatures was significantly different.

At 25C (77F), each stratification duration was significantly different (Fig. 3). However, at 30C (86F), 30/20C (86/68F), and 35/25C (95/77F), stratification for 0 or 15 days was not significantly different, but both resulted in significantly greater germination than 30 days stratification. These differences were likely due to increased fungal growth and seed decay at 25C (77F) and the longer stratification treatment of 30 days.

Of the various stratification-temperature treatments investigated in this study, the highest total germination (70%) was realized for seeds stratified for 15 days followed by germination at an 8/16 hr thermoperiod of 35/25C (99/77F) (Fig. 3). However, the potential may exist for greater germination since initial viability tests utilizing 2,3,5-triphenyltetrazolium chloride (TTC or TZ) (5) indicated that viability was >95%. To achieve germination comparable to results of the TTC/TZ tests, if possible, will require use of different environmental conditions for germination, particularly temperature, coupled with suppression of seed pathogens.

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