ABSTRACT

BURGESS, TYLER LYNNE. Seed germination studies of southern seaoats (*Uniola paniculata*). (Under the direction of Frank A. Blazich).

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 [NaOCl (chlorine bleach)] prior to stratification. Since viability tests with 2,3,5-triphenyltetrazolium chloride indicated that initial seed viability was >95%, treatments to reduce decay were further investigated. Seeds were treated with the following selected surface disinfestants and/or fungicides: nontreated (control), 1.3% sodium hypochlorite, 2.6% sodium hypochlorite, RTU®-PCNB (pentachloronitrobenzene), RTU® (thiram +

thiabendazole), combinations of 1.3% sodium hypochlorite and RTU®, 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB. Following treatment, seeds were germinated at an 8/16 hr thermoperiod of 35/20C (95/68F). The seed treatments and germination thermoperiod utilized were based on three trials that investigated the influence of selected surface disinfestants, fungicides, and temperature on seed germination of the species. Germination was recorded every 3 days for 30 days. Seed treatment was highly significant (P=0.0001) for both total percentage germination and total percentage of decayed seeds. Germination of nontreated seeds was 45% and four treatments resulted in germination >80% [RTU®-PCNB (81%), 2.6% sodium hypochlorite and RTU® (83%), 1.3% sodium hypochlorite and RTU® (87%), and 1.3% sodium hypochlorite and RTU®-PCNB (89%)]. A subsequent experiment investigated the effects of the aforementioned treatments with the exception of 1.3% sodium hypochlorite and RTU®, both used alone, on subsequent seedling growth of the species. Following treatment, seeds were sown in containers filled with a peat-based medium and the containers placed in a growth chamber maintained at an 8/16 hr thermoperiod of 35/20C (95/68F) with long day conditions. Emergence data were recorded every 3 days for 45 days and seedlings were fertilized daily after emergence and once the first leaf was visible. After 45 days, the study was terminated and additional data recorded to include plant height (height of main stem), leaf number, length and width of the two longest leaves, and top and root dry weights. Surface disinfestant and/or fungicide treatments were highly significant (P=0.0004). Percentage emergence of the nontreated seeds was 35% and five of the seven treatments resulted in emergence $\geq$75% [2.6% sodium hypochlorite (75%), 1.3%

sodium hypochlorite and RTU® (75%), 1.3% sodium hypochlorite and RTU®-PCNB (76%), 2.6% sodium hypochlorite and RTU®-PCNB (81%), and 2.6% sodium hypochlorite and RTU® (83%)] with negligible effects on subsequent seedling growth. There were significant treatment differences regarding some of the variables used to evaluate seedling growth. These differences in most cases were due to seedlings from nontreated seeds having lower values for each measured variable than values from treated seeds. Results demonstrate the need and practicality of seed treatment during production of seedling transplants of *U. paniculata*.

Seed Germination Studies of Southern Seaoats (*Uniola paniculata*)

by

Tyler Lynne Burgess

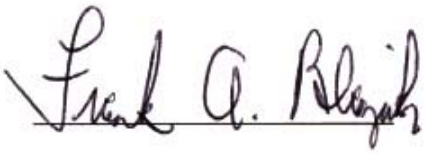
A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the requirements for the
Degree of Master of Science

Department of Horticultural Science

Raleigh

2002

Approved by:



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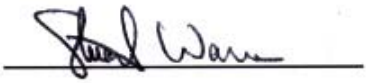

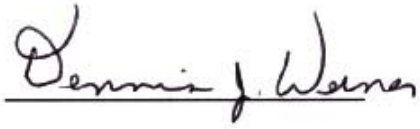

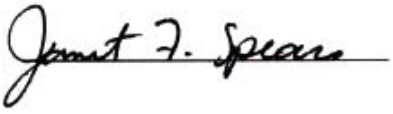

DEDICATION

This thesis is dedicated to my entire family for supporting me throughout my life.

To my parents for teaching me the value of a work ethic and giving me the wings to fly.

To my grandmother, Marni Rankin who has been my greatest cheerleader throughout.

To my grandfather, Robert Zech whose lifelong commitment to education has been truly inspiring to me.

BIOGRAPHY

Tyler Lynne Burgess was born May 21, 1976 to William and Zalinda A. Burgess in Seattle, Washington. She discovered her two greatest loves, gymnastics and horticulture, early in life. She began taking gymnastics classes at the age of 5 and excelled throughout high school and college. During her gymnastics career she won several state, regional, and national championships. From 1990 to 1993 she attended Interlake High School in Bellevue, Washington where she began her studies in horticulture.

In August 1993, she enrolled at Michigan State University, East Lansing, on a full athletic scholarship as a member of the Women's Gymnastics Team and pursued a BS degree in Horticulture. After 2 years she transferred to the University of Washington, Seattle, where she earned a BS in Urban Forestry in June 1997.

In August 1997, Tyler was admitted to the Graduate School at North Carolina State University to pursue a MS in Horticultural Science.

During her undergraduate studies she found a passion for endangered and ecologically significant plant species which was the inspiration for this project.

ACKNOWLEDGMENTS

I would like to recognize my Advisory Committee members, Drs. Frank A. Blazich, Janet F. Spears, Stuart L. Warren, and Dennis J. Werner whose guidance helped me develop both personally and professionally throughout this degree program. Special thanks go to David L. Nash who provided unlimited support and guidance throughout this project.

In addition, I would like to thank Juan R. Acedo and Brenda T. Penny for technical assistance, Dr. Betsy Randall-Schadel (NCDA Seed Lab) for her time and energy devoted to this project, and Dr. William H. Swallow and Joy M. Smith for invaluable assistance with statistical analyses.

I would also like to recognize Rachel McLaughlin and my fellow graduate students whose support and encouragement were invaluable. I would like to give special acknowledgement to Anthony Lebude, Jean-Jaques DuBois, Todd Lasseigne, Jason Griffin, and Amy Noelle Wright who went out of their way to help me in many ways.

Finally, I would like to express gratitude to the people in the Neurology & Psychiatry Clinical Development and Regional Monitoring Departments at GlaxoSmithKline who invited me into their families and made my job with this organization something to look forward to.

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GENERAL INTRODUCTION

Southern seaoats (*Uniola paniculata* L.) is a perennial dune grass that ranges from southern Virginia to eastern Mexico (7). 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 insects that characterize this habitat (7).

An important element of the physical environment in the maritime dune habitat is the effect of wind as a daily presence, in the form of storms, and as a bearer of salt spray and sand (7). Zonal distribution of plant species in the dune environment is explained by the tolerance of wind (7) and windborne salt (4). Although salt spray has little or no direct effect on *U. paniculata*, it is important in controlling competing species (7).

The growth characteristics of individual species inhabiting the dune environment influence the growth and configuration of sand dunes (10). *Uniola paniculata* has an extensive root system due to the necessity for uptake of water and mineral nutrients over a large area, which creates a strong foothold in the shifting unstable sand. It also provides the firmest colonizer or sand stabilizer by intercepting windborne sand with its aerial structures (7). Another species, American beachgrass (*Ammophila breviligulata* Fernald) is similar to *U. paniculata* in terms of establishment and sand accumulation producing a gently sloping dune but is short-lived (10). When grown alone *U. paniculata* produces a steep dune front (10), however, it colonizes a dune at a much slower rate than *A. breviligulata* (11). When planted together in North Carolina, there has been a gradual replacement of *A. breviligulata* by *U. paniculata* (11). In addition, *A. breviligulata* is less drought tolerant and subject to more insect and disease damage than *U. paniculata* (9,

11). Temperature appears to be a major factor limiting the northern range of *U. paniculata* and the southern range of *A. breviligulata* (6), although the original southern limit of *A. breviligulata* near northern North Carolina has been obscured by extensive planting (11).

Severe storm along the Atlantic and Gulf Coasts during the 1980s and 1990s renewed interest in the use of vegetation for beach restoration and dune stabilization. Thus, there is currently high demand for transplants of various dune species for restoring beaches damaged by tropical storms and erosion (1). The demand has attracted considerable interest in commercial production of some dune species, particularly *U. paniculata*, because of its dune building and sand holding abilities (8). Commercial production of transplants of *U. paniculata* has the potential to be extremely lucrative if efficient propagation and cultural protocols can be developed. Although some research has been conducted in those areas additional research is needed.

Uniola paniculata can be propagated by both sexual (seed) and asexual (vegetative) means. Direct seeding for plant establishment is ineffective due in part to seed dormancy and sand movement (8). Plants that are direct seeded are much smaller relative to nursery or greenhouse-grown plants due to the need to expend so much energy in the first and second growing season for root system establishment in sand compared to nursery or greenhouse substrate (9, 11). Vegetative reproduction for dune propagation is reportedly expensive (8), although it is the primary means of reproduction in the dune habitat (1). This means of reproduction only creates larger stands of *U. paniculata* and colonization of new areas that are spatially distant is accomplished primarily by seed (7).

Survival of transplants from one dune stand to another dune has shown to be low so nursery or greenhouse production of planting stock is essential for large-scale production of high quality transplants (11). At present, it appears that greenhouse propagation by sexual means has the greatest potential for mass production of seedling transplants. However, seed production and germination are not without inherent problems.

Seed production of *U. paniculata* is generally low although the potential exists for much greater production. In its natural habitat the species typically produces six to eight fertile florets per spikelet, but few of these ever set seed, which mature by October in North Carolina (7). It appears as though viable embryos are produced and subsequently abort (7). Seeds are susceptible to attack by the fungi *Alternaria* sp. (Nees) and *Helminothosporium* sp. (Lk.) that occur commonly in many grass species (7). Although these fungi are able to attack and destroy viable ovules directly, it is more likely that they are secondary invaders attacking the aborted ovules following insect attack or bacterial infection (7). High humidity and summer rain in the maritime environment increase the incidence of fungal attack (1). Consequently, plants produce on average, two viable seeds per spikelet (7). Spikelets on exposed sand are subject to destruction by birds and small mammals and few seeds are available for germination the following spring (7).

Research by the author has indicated that seed decay during germination of *U. paniculata* is a serious problem and warrants attention. Surprisingly, this has not been mentioned in previous reports dealing with seed germination of the species (1, 2, 3, 5, 6) although all of these investigations report use of sodium hypochlorite [NaOCl (chlorine

bleach)] for surface disinfestation of seeds ranging from 1.3% to 1.6% for durations of 15 to 30 min.

Seed germination of *U. paniculata* is not difficult to accomplish and most of the research conducted on germination of the species has dealt with the effects of temperature and stratification (moist-prechilling). Light may also have an influence on germination and although there are reports that it is not necessary for germination, this does not appear to have been thoroughly tested (8). On the other hand, several studies have appeared dealing with the effects of temperature on both seed germination and seedling growth. Alternating temperatures of 32/16C (6) and 35/18C (3, 5) appear to yield the best germination and seedling growth. Colosi (2) reported that all optimal temperatures for germination exceeded 35C.

Stratification is not necessary for germination but research has indicated it will stimulate greater germination in comparison to nonstratified seeds (5, 6). Seneca (6) 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 stimulated germination the duration necessary to maximize germination varied from 15 to 30 days depending on the geographic locality from which the seeds were collected (6). Hester and Mendelssohn (3) reported that stratification did not increase total germination of seeds from four Louisiana populations of *U. paniculata* but increased the rate of germination.

If large-scale greenhouse production of seedling transplants of *U. paniculata* for beach restoration and dune stabilization is to become a reality, additional research is needed regarding both seed germination and subsequent seedling growth. The first phase of such a production scheme will involve seed germination, which prompted the following research with three objectives: (A) determine the influence of light, temperature, and stratification on seed germination of *U. paniculata* (B) screen selected surface disinfestants or fungicides for the capacity to control seed decay and in turn promote improved germination and seedling emergence, and (C) determine whether those surface disinfestants or fungicides that control seed decay influence subsequent seedling growth.

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Chapter 1

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

(In the format appropriate for submission to the

Journal of Environmental Horticulture)

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

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[1]Received for publication_____. This research was funded in part by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. Assistance of Juan R. Acedo, Brenda T. Penny, William H. Swallow, and the staff of the Southeastern Plant Environment Laboratory (Phytotron) is gratefully acknowledged. From a thesis submitted by T.L.B. in partial fulfillment of the requirements for the MS degree.

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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 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 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 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 (8) and 35/18C (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)] 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), North Carolina 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, North Carolina. 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 h.

In January 1999, seeds were removed from storage and graded under a dissecting scope, which allowed removal of abnormal, damaged, or under-sized 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 [10 dry sand:1 water (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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (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 nonstratified seeds also germinated (Fig. 1). Stratification for 15 days increased the rate of germination

compared to nonstratified seeds although 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 d 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 stratification 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 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 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 were 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-triphenyltetrazoleum 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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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.

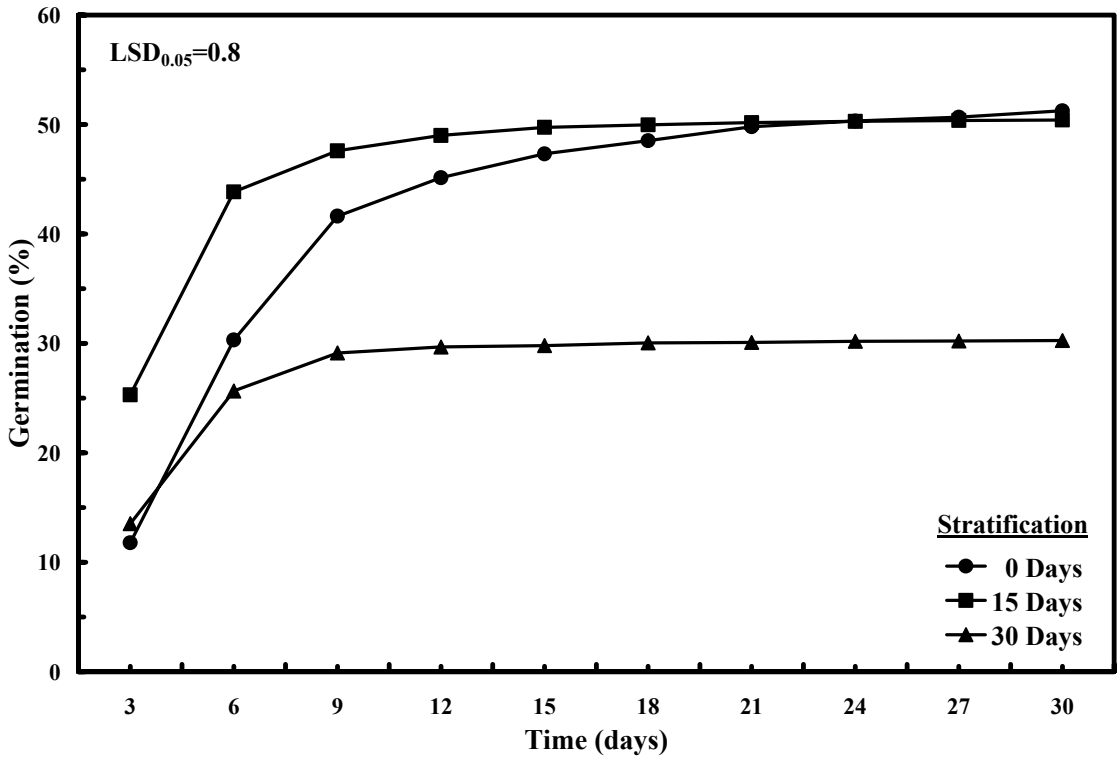

LSD0.05=0.8
Germination (%)
Time (days)
Stratification
0 Days
15 Days
30 Days
0
10
20
30
40
50
60
3
6
9
12
15
18
21
24
27
30

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.

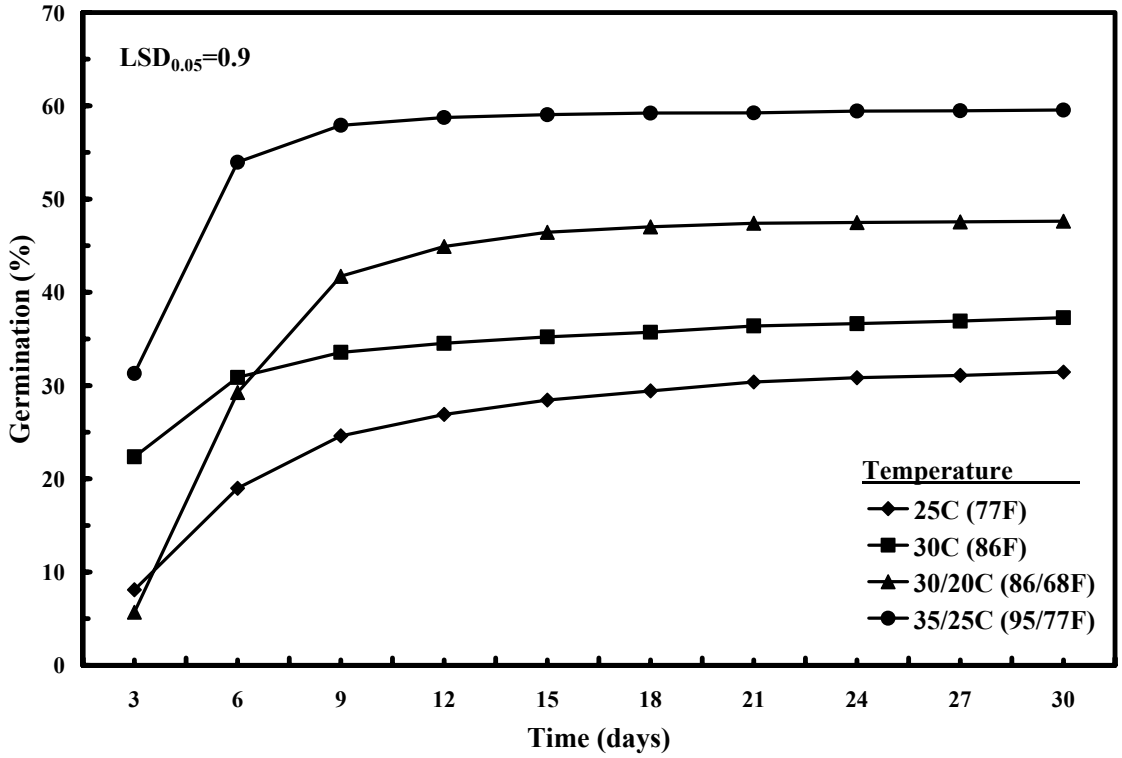
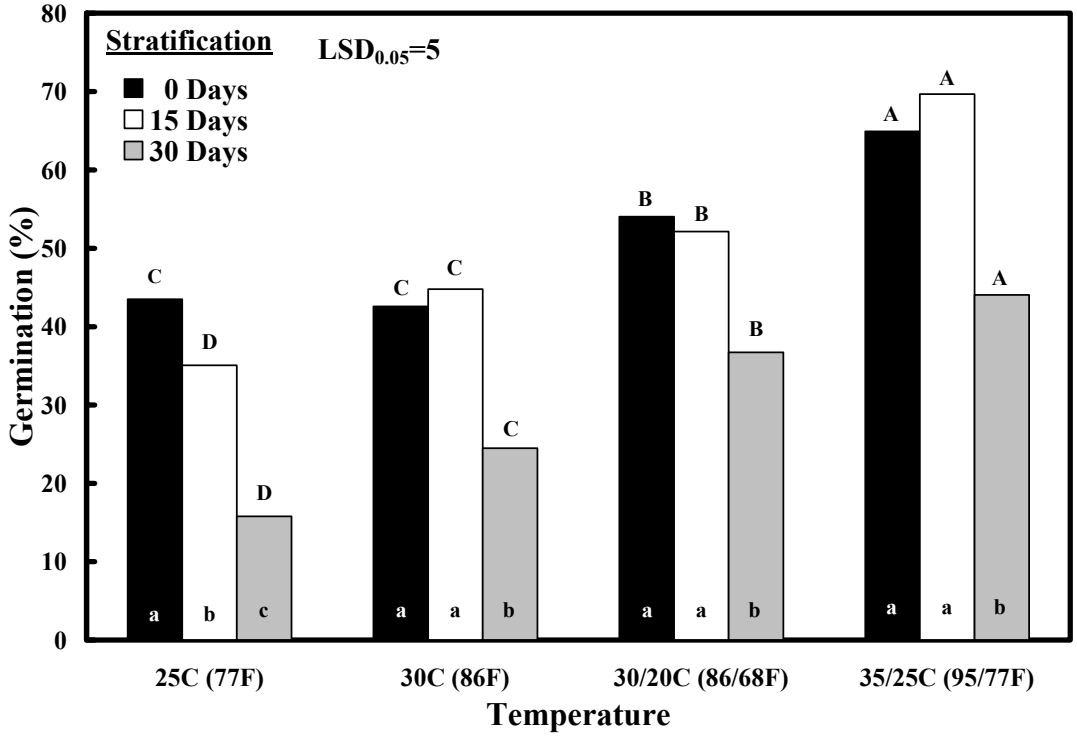

LSD0.05=0.9
Germination (%)
Time (days)
Temperature
25C (77F)
30C (86F)
30/20C (86/68F)
35/25C (95/77F)
0
10
20
30
40
50
60
70
3
6
9
12
15
18
21
24
27
30

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.


Stratification
LSD0.05=5
0 Days
15 Days
30 Days
Germination (%)
80
70
60
50
40
30
20
10
0
C
D
D
C
C
C
B
B
B
A
A
A
a
b
c
a
a
b
a
a
b
a
a
b
25C (77F)
30C (86F)
30/20C (86/68F)
35/25C (95/77F)
Temperature

Chapter 2

Influence of Selected Surface Disinfestants, Fungicides, and Temperature on Seed Germination of Southern Seaoats (*Uniola paniculata*)

(In the format appropriate for submission to the

Journal of Environmental Horticulture)

Influence of Selected Surface Disinfestants, Fungicides, and Temperature on Seed Germination of Southern Seaoats (*Uniola paniculata*)[1]

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[1]Received for publication_____. This research was funded in part by the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. Assistance of Juan R. Acedo, Brenda T. Penny, William H. Swallow, and the staff of the Southeastern Plant Environment Laboratory (Phytotron) is gratefully acknowledged. From a thesis submitted by T.L.B. in partial fulfillment of the requirements for the MS degree.

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Abstract

Seeds of southern seaoats (*Uniola paniculata* L.) were removed from storage at 4C (39F) and treated with the following selected surface disinfestants and/or fungicides: nontreated (control), 1.3% sodium hypochlorite [NaOCl (chlorine bleach)], 2.6% sodium hypochlorite, RTU®-PCNB (pentachloronitrobenzene), RTU® (thiram + thiabendazole), combinations of 1.3% sodium hypochlorite and RTU®, 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB. Following treatment, seeds were germinated at an 8/16 hr thermoperiod of 35/20C (95/68F). The seed treatments and germination thermoperiod utilized were based on three trials that investigated the influence of selected surface disinfestants, fungicides, and temperature on seed germination of the species. Germination was recorded every 3 days for 30 days. Seed treatment was highly significant (P=0.0001) for both total percentage germination and total percentage of decayed seeds. Germination of nontreated seeds was 45% and four treatments resulted in germination >80% [RTU®-PCNB (81%), 2.6% sodium hypochlorite and RTU® (83%), 1.3% sodium hypochlorite and RTU® (87%), and 1.3% sodium hypochlorite and RTU®-PCNB (89%)]. A subsequent experiment investigated the effects of the aforementioned treatments with the exception of 1.3% sodium hypochlorite and RTU®, both used alone, on subsequent seedling growth of the species. Following treatment, seeds were sown in containers filled with a peat-based medium and the containers placed in a growth chamber maintained at an 8/16 hr thermoperiod of 35/20C (95/68F) with long day conditions. Emergence data were recorded every 3 days for 45 days. After 45 days, the study was terminated and

additional data recorded to include plant height (height of main stem), leaf number, length and width of the two longest leaves, and top and root dry weights. Surface disinfestant and/or fungicide treatments were highly significant (P=0.0004). Percentage emergence of the nontreated seeds was 35% and five of the seven treatments resulted in emergence ≥75% [2.6% sodium hypochlorite (75%), 1.3% sodium hypochlorite and RTU® (75%), 1.3% sodium hypochlorite and RTU®-PCNB (76.2%), 2.6% sodium hypochlorite and RTU®-PCNB (81.0), and 2.6% sodium hypochlorite and RTU® (83.3%)] with negligible effects on subsequent seedling growth. There were significant treatment differences regarding some of the variables used to evaluate seedling growth. These differences in most cases were due to seedlings from nontreated seeds having lower values for each measured variable than values for the same variables from treated seeds. Results of both experiments demonstrate the need and practicality of seed treatment during production of seedling transplants of *U. paniculata*.

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

Significance to the Nursery Industry

Seedling transplants of *Uniola paniculata* (southern seaoats) are in great demand for beach and sand dune restoration and stabilization. However, seed decay is a problem that reduces germination and seedling emergence during production of transplants. Results herein demonstrate the importance of seed treatment of the species and identify surface disinfestant and/or fungicide treatments that will inhibit decay and permit emergence ≥75% without adverse effects on subsequent seedling growth.

Introduction

There is currently much interest in production of seedling transplants of *Uniola paniculata* (southern seaoats), a perennial dune grass that ranges from southern Virginia to eastern Mexico (12). This interest has arisen because of the high demand for transplants of the species for restoring beaches and stabilizing sand dunes destroyed or damaged by tropical storms and erosion (1).

Seed germination of *U. paniculata* is not difficult, as the seeds do not posses any rigid internal dormancy at maturity. Seeds can be germinated without pretreatment but stratification (moist-prechilling) in some cases will increase total germination and the rate of germination (2, 6, 10, 11). However, when conducting germination studies with *U. paniculata*, the authors have observed that seed decay caused by various fungal and possible bacterial pathogens can be a problem (2). Studies regarding germination of this species have not reported any serious problems of seed decay, but most investigations describe treatment of seeds with sodium hypochlorite [NaOCl (chlorine bleach)] at rates ranging from 1.3% for 15 min (6, 10, 11) to 1.6% for 30 min (1) before initiation of experiments. The authors have used 1.3% sodium hypochlorite for 15 min as a surface disinfestant with mixed results and have concluded that more effective treatments are needed.

Temperature is also an important factor affecting seed germination of *U. paniculata*. Burgess et al. (2) investigated germination of the species at 25C (77F) or 30C (86F) or at 8/16 hr thermoperiods of 30/20C (86/68F) or 35/25C (95/77F) and observed the highest total germination at 35/25C (95/77F). However, Seneca (10) noted

optimum seed germination at 35/18C (95/65F). Therefore, the following research was undertaken to study the effects of selected surface disinfestants, fungicides, and temperature on seed germination and subsequent seedling growth of *U. paniculata*.

Materials and Methods

Screening trials. Prior to conducting the two experiments described herein, three studies were conducted (trials) to screen selected surface disinfestants and/or fungicides for the capacity to reduce seed decay during seed germination of *U. paniculata* at various germination temperatures (Table 1). Results indicated that some seed treatments were more effective than others and that a germination temperature of 35/20C (95/68F) appeared to be optimum (Table 2). Based on these data the following two experiments were conducted. The first experiment (Expt. 1) examined the influence of those surface disinfestants and/or fungicides found during the three trials to be most effective in controlling seed decay. Seeds were germinated at 35/20C (95/68F). The second experiment (Expt. 2) examined subsequent seedling growth of *U. paniculata*, as influenced by treatment with a particular surface disinfestant and/or fungicide. The surface disinfestants and/or fungicides utilized in Expt. 2 were selected based on results of Expt. 1. With the exception of the Clearys 3336 (thiophanate methyl) plus Captan 400 (captan) and hydrogen peroxide (30%) treatments, the methodologies employed in the aforementioned trials for seed treatment and germination were identical to those employed in Expts. 1 and 2 which were conducted as discussed below. The Clearys 3336 plus Captan 400 treatment was administered by soaking the seeds in 11 g Clearys 3336 (2 tablespoons) plus 20 g Captan 400 (2.5 tablespoons) / 3.8 liter (1 gal.) of water. The

seeds were soaked for 15 min. followed by drying at 21C (70F) for 30 min. Seeds treated with 30% hydrogen peroxide were soaked in a solution for 30 min., rinsed with tap water, and dried at 21C (70F) for 30 min.

For each screening trial, germinated and decayed seeds were recorded every 3 days for 30 days and were removed from the dishes. A seed was considered germinated when radicle emergence was $\geq$1 mm (0.04 in). The experimental design was a split-plot with temperatures as the main plots and surface disinfestant and/or fungicide treatments as the subplots. Data were subjected to analysis of variance (ANOVA) procedures and means separated by Fisher's protected least significant difference (LSD) at $P<0.05$.

Harvest of spikelets, seed extraction, and storage. Spikelets of *U. paniculata* were collected from a population of plants growing on Oak Island, (Brunswick County) North Carolina on October 12, 1999. 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, North Carolina. 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). Moisture content of the seeds was determined by calculating the mean moisture content of six 50-seed samples following drying at 105C (221F) for 24 h.

Influence of selected surface disinfestants and/or fungicides on seed germination (Expt. 1). On September 8, 2000, seeds were removed from storage and graded under a dissecting scope, which allowed removal of abnormal, damaged, or under-sized seeds and any debris. Graded seeds [approximately 6000 pure seeds per 28g (1 oz)] were subjected

to the following treatments: nontreated (control), 1.3% sodium hypochlorite [NaOCl (chlorine bleach)], 2.6% sodium hypochlorite, RTU®-PCNB (pentachloronitrobenzene), RTU® (thiram + thiabendazole), combinations of 1.3% sodium hypochlorite and RTU®, 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB. Seeds treated with sodium hypochlorite were soaked in a solution of the chemical for 15 min, rinsed with tap water, and allowed to dry at 21C (70F) for 30 min. The fungicide treatments were prepared as slurries by mixing 1 ml of the flowable formulations of these materials with 1 ml of distilled water. One half milliliter of each slurry was then used to treat seeds designated for fungicide treatment. After slurry treatment, the seeds were dried at 21C (70F) for 30 min. The seeds treated with a combination of sodium hypochlorite and a particular fungicide were first soaked in either 1.3% or 2.6% sodium hypochlorite for 15 min then rinsed with tap water and allowed to dry for 30 min followed by the slurry treatment and drying.

Following seed treatment, seeds were placed 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 placed in an unlit growth chamber [C-chamber (4)] at the Southeastern Plant Environment Laboratory (NC State Univ., Phytotron), Raleigh, NC. The chamber was maintained at an 8/16 hr thermoperiod of 35/20C (95/68F). Chamber temperature varied within ± 0.5C (0.9F) of the set point. Temperatures within the petri dishes, as measured by a thermocouple,

never exceeded ambient temperature by more than ± 1C (2F) of the set point. Germination blotters were kept moist with tap water throughout the duration of the experiment. Germinated and decayed seed counts were recorded every 3 days for 30 days and were removed from the dishes. A seed was considered germinated when radicle emergence was ≥1 mm (0.04 in).

The experimental design was completely randomized with nine treatments replicated four times with each replication consisting of a petri dish containing 50 seeds. Data were subjected to analysis of variance (ANOVA) procedures and means separated by Fisher's protected least significant difference (LSD) at $P<0.05$.

Influence of selected surface disinfestants and/or fungicides on subsequent seedling growth (Expt. 2). On January 3, 2001, seeds were removed from storage and treated following the procedures described in Expt. 1 with the following surface disinfestants and/or fungicides: nontreated (control), 2.6% sodium hypochlorite, RTU®-PCNB, combinations of 1.3% sodium hypochlorite and RTU®, 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB.

Following treatment, two seeds were sown per tube in Ray Leach SuperCells [cell diameter = 3.8 cm (1.5 in), height = 21 cm (8.3 in), volume = 164 cm^3 (10 in^3) (Stuewe and Sons, Corvallis, OR)] filled with ferti·lome® which consists of Canadian sphagnum peat (80%), perlite, and wood charcoal (Voluntary Purchasing Groups, Inc., Bonham, TX).

The tubes were then placed in a growth chamber [C-chamber (4)] at the Southeastern Plant Environment Laboratory (NC State Univ., Phytotron), Raleigh, NC. The chamber was maintained at an 8/16 hr thermoperiod of 35/20C (95/68F) with an 8-hr photoperiod during the high temperature portion of the cycle. Chamber temperature varied within ± 0.5C (0.9F) of the set point. During the 35C (95F) portion of the cycle the chamber used a combination of cool-white fluorescent and incandescent lamps that provided a photosynthetic photon flux (PPF, 400-700 nm) of 411 μmol/m^2/sec (31.1 klx) plus photomorphogenic radiation (PR, 700-850 nm) of 7.4 W/m^2. Incandescent lamps providing a PPF of 40 μmol/m^2/sec (2.0 klx) plus PR of 5.7 W/m^2 were used to interrupt the 16-hr dark period between 11:00 PM and 2:00 AM daily.

Seedlings were thinned to one seedling after emergence (leaving the larger of the two seedlings) and fertilized daily with half-strength Phytotron nutrient solution (4), which was applied after seedling emergence and once the first leaf was visible. To ensure that each tube designated for nontreated seedlings had one seedling, additional nontreated seeds were germinated in a covered 9-cm (3.5 in) glass petri dish inside the growth chamber. On day 15, no seedlings had emerged in 14 tubes sown with nontreated seeds. One germinated seed was planted in each of these tubes in order to provide an adequate number of nontreated seedlings for comparison.

Emergence data were recorded every 3 days for 45 days. After 45 days, the study was terminated and additional data recorded to include plant height (height of main stem), leaf number, length and width of the two longest leaves, and top and root dry weights. Leaf width measurements were taken at the midpoint of each leaf and dry

weights were recorded after drying for 48 hr at 70C (158F). Percentage emergence and average days to emergence for nontreated seeds was calculated excluding the 14 seeds planted on day 15. The experimental design was a randomized complete block with six blocks, seven treatments per block, and seven tubes per treatment. Data were subjected to ANOVA procedures and means separated by Fisher's protected LSD at $P<0.05$. Pairwise comparisons of percentage emergence and average days to emergence were calculated using the "PDIFF" option on LSMEANS under PROC GLM of SAS (SAS Inst., Inc., Cary, NC).

Results and Discussion

Influence of selected surface disinfestants and/or fungicides on seed germination (Expt. 1). Seed treatment with sodium hypochlorite and a particular fungicide either alone or in combination was absolutely essential to achieve germination >50% (Fig. 1). Seed treatment was highly significant (P=0.0001) for both total germination percentage and total percentage of decayed seeds. Generally, treatment of seeds with sodium hypochlorite in combination with a particular fungicide resulted in greater germination than seed treatment with only a fungicide or sodium hypochlorite. For example, the highest total germination was realized for seeds treated with a combination of 1.3% sodium hypochlorite and RTU®-PCNB (89%) followed by 1.3% sodium hypochlorite and RTU® (87%), 2.6% sodium hypochlorite and RTU® (83%), and RTU®-PCNB (81%).

As would be expected, the greatest percentage of decayed seeds was noted for the nontreated seeds (38%) which was significantly different as compared to all the other

treatments (Fig. 1). Interestingly, although 1.3% and 2.6% sodium hypochlorite alone were relatively effective in reducing seed decay this did not necessarily correspond to increased germination (<75%). Some treatments had significantly greater germination with the same amount of seed decay as the sodium hypochlorite treatments (>80%).

Although four of the seed treatments resulted in germination >80% with the highest germination of 89% for seeds treated with 1.3% sodium hypochlorite in combination with RTU®-PCNB, there may be room for improvement (Fig. 1). Prior to conducting this research viability tests with 2,3,5-triphenyltetrazolium chloride (TZ or TTC) (5) estimated that seed viability was >95%. Thus, germination approaching 100% may be feasible. This may, however, be difficult to attain because of what appears to be a multitude of fungal pathogens such as *Alternaria* Nees sp., *Eppicocum* Lk. sp., *Fusarium* Lk. sp., and *Helminthosporium* Lk. sp., which were tentatively identified from a sample of seeds taken from the seed lot used to conduct the present research. In addition to various fungal pathogens, bacteria may also contribute to seed decay. The presence of various seed pathogens, particularly fungi, may to be related to the extremely humid and moist maritime environment in which *U. paniculata* is endemic (12). Despite the possibility of germination greater than that reported herein (Fig. 1), germination of 80% would appear to be commercially acceptable.

Seed decay of *U. paniculata* during germination, which prompted this investigation, may have influenced previous seed germination studies of this species (1, 3, 6, 10, 11). However, these reports provide no indication that seed decay was a problem, which in turn may have influenced the results of the aforementioned studies.

Seeds treated with RTU®PCNB germinated readily, however, abnormal radicle development was observed among these seeds. The radicle appeared to twist and lacked root hairs on all seeds that germinated after being treated with RTU®PCNB either alone or in combination with sodium hypochlorite. This observation concerned the authors and raised the possibility of potential adverse effects from RTU®PCNB treatment influencing subsequent seedling growth, which provided in part the basis for Expt. 2.

Influence of selected surface disinfestants and/or fungicides on subsequent seedling growth (Expt. 2). Surface disinfestant and/or fungicide treatments were highly significant (P=0.0004) and were essential to achieve emergence >60% with five of the seven treatments resulting in emergence >75% (Table 3). Emergence for nontreated seeds was only 35% and emergence for all surface disinfestant and/or fungicide treatments were significantly greater than the nontreated seeds and ranged from 64% to 83%. Results of the various seed treatments generally confirm what was observed in Expt. 1 (Fig. 1) regarding the need for seed treatment of *U. paniculata* and the report by Burgess et al. (2) that decay is a problem during seed germination of the species.

There were no apparent adverse effects of the various seed treatments on subsequent seedling growth (Table 3). Among these treatments there were no significant differences in stem height, length of the second longest leaf, width of the longest leaf, top dry weight, and root dry weight. However, as mentioned previously there were significant differences in percentage emergence. There were also significant differences regarding average days to emergence, number of leaves, length of the longest leaf, and width of the second longest leaf. In most cases, the significant differences were due to

the nontreated seeds having lower values for each measured variable than values for the same variables of the treated seeds.

Although percentage emergence was lowest for the nontreated seeds (35%, Table 3) these seeds emerged essentially in the same length of time as the treated seeds with two exceptions. Average days to seed emergence for seeds treated with 2.6% sodium hypochlorite and RTU® and 1.3% sodium hypochlorite and RTU®-PCNB were longer (14.7 and 17.3 days, respectively) in comparison to nontreated seeds (12.0 days). Although these differences were significant, the authors question whether a difference of 2.7 to 5.3 days would have any practical significance regarding commercial production of seedling transplants.

The authors noted in Expt. 1 that seeds treated with RTU®-PCNB either alone or in combination with sodium hypochlorite germinated readily although abnormal radicle development was observed. This raised the possibility that RTU®-PCNB would have adverse effects on subsequent seedling growth. Despite RTU®-PCNB resulting in reduced emergence in comparison to some of the other treatments, it had no deleterious affects on the other recorded variables used to evaluate seedling growth.

In Expt. 1 the various surface disinfestant and/or fungicide treatments were evaluated in terms of germination by defining germination as radicle emergence $\geq$ 1 mm (0.04 in.). However, in Expt. 2 germination was not recorded because of the nature of the study and percentage emergence was recorded. Thus, it is difficult if not impossible to compare directly percentage germination data in Fig. 1 with percentage emergence data in Table 3. However, if one compares values for percentage emergence of the seven

treatments used in Expt. 2 that were also included in Expt. 1 but evaluated in terms of percentage germination the values are generally less in Expt. 2. For example, germination of the nontreated seeds in Expt. 1 was 45% in comparison to seedling emergence of 35% for the nontreated seeds in Expt. 2. Also in Expt. 1 there were four treatments that resulted in germination >80% [RTU®-PCNB (81%), 1.3% sodium hypochlorite and RTU® (87%), 2.6% sodium hypochlorite and RTU® (83%), and 1.3% sodium hypochlorite and RTU®-PCNB (89%)] but in Expt. 2 those same treatments resulted in percentage emergence of 64%, 75%, 83%, and 76% respectively. Since the manner in which Expt. 2 was conducted simulates more closely greenhouse production practices, values for percentage emergence may be a more realistic indicator of how the various fungicides and/or surface disinfestants will perform under actual seedling production conditions.

Although there have been no previous reports on the use of various surface disinfestants and/or fungicides to reduce seed decay during germination of *U. paniculata*, beneficial effects of fungicide seed treatments have been noted for many forage grasses. Michail and Carr (9) reported significant improvement of establishment of species of ryegrass (*Lolium* L. sp.), fescue (*Festuca* L. sp.), cocksfoot (*Dactylis* L. sp.), and timothy (*Phleum* L. sp.) by fungicidal seed treatments. Seed treatment of cultivars of ryegrass with the fungicides Benlate (benomyl) and Captan provided excellent protection against *Fusarium* sp. even on the most susceptible cultivars (7, 8). Lewis and Clements (8) also reported that the combination of Subdue (metalaxyl) and Mycozol (thiabendazole) provided results similar to that of Benlate plus Captan.

In summary, five of the seven surface disinfestant and/or fungicide treatments utilized in this experiment resulted in percentage emergence of 75% to 83% with negligible effects on subsequent seedling growth of *U. paniculata*. This demonstrates that a variety of treatments/materials may be used to combat seed decay during production of seedling transplants of the species. Choice of a particular treatment will undoubtedly need to include consideration of various factors for example, effectiveness, cost, and whether or not the material(s) selected for use are registered for use on *U. paniculata*. Although the surface disinfestant and/or fungicide treatments tested provided control for seed decay the potential for higher germination and emergence percentages may exist because, as mentioned previously, viability tests with 2,3,5-triphenyltetrazolium chloride (TZ or TTC) (5) indicated that initial seed viability was >95%.

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Table 1. Trade names, common names, and chemical names and active ingredients for each surface disinfestant and fungicide screened in the initial trials and used subsequently in Expts. 1 and 2.

Trade name	Common name	Chemical name/active ingredients
Captan 400	captan	N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (37.4%)
		related derivatives (0.85%)
Clearys 3336 + Captan 400	thiophanate methyl	dimethyl 4,4'-o-phenylenebis(3-thioallophanate) (50%)
	captan	N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (37.4%)
		related derivatives (0.85%)
RTU®	thiram	tetramethylthiuram disulfide (12.6%)
	thiabendazole	2-(4-thiozolyl)-benzimidazole (0.34%)
RTU®-PCNB	pentachloronitrobenzene	pentachloronitrobenzene (24%)
RTU®-Vitavax®-Extra	carboxin	5,6-dihydro-2-methly-N-phenyl-1,4-oxathiin-3-carboxamide (16.7%)
	imazalil	1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)-ethyl]-1H-imidazole (1.2%)
	thiabendazole	2-(4-thiazolyl)-benzimidazole (1.5%)
RTU®-Vitavax®-Thiram	carboxin	5,6-dihydro-2-methly-N-phenyl-1,4-oxathiin-3-carboxamide (10%)
	thiram	tetramethylthiuram disulfide (10%)
Hydrogen peroxide (30%)	hydrogen peroxide	hydrogen peroxide (30%)
Sodium hypochlorite (1.3%)	chlorine bleach	sodium hypochlorite (1.3%)
Sodium hypochlorite (2.6%)	chlorine bleach	sodium hypochlorite (2.6%)

Table 2. Results of three trials that investigated the influence of selected surface disinfestants, fungicides, and temperature on seed germination of *U. paniculata*. Each value for percentage germination represents mean germination percentage at 30 days of four petri dishes each containing 50 seeds.

	Trial 1 Germination temperature				Trial 2 Germination temperature					Trial 3 Germination temperature		
Surface disinfestant or fungicide (trade name)	25C	30C	30/20C	35/25C	30/20C	30/25C	35/25C	35/30C	35C	30/20C	35/20C	35/25C
	Germination (%)				Germination (%)					Germination (%)		
Nontreated	3.6 c B[z]	6.5 d B	8.9 e B	23.5 d A	12.1 d C	13.1 c C	36.2 d A	20.8 c BC	25.4 b B	21.0 d B	44.7 c A	25.9 d B
Captan 400	31.3 a B	28.5 ab B	53.1 a A	49.4 ab A	54.9 bc A	30.5 b C	54.1 bc A	38.1 b BC	45.5 a AB			
Clearys 3336 + Captan 400					49.8 ab A	30.6 b B	53.0 c A	36.4 b B	37.6 a B	38.9 c C	69.2 b A	57.5 bc B
RTU®	22.0 b C	32.0 a B	47.3 ab A	50.5 ab A	55.2 bc A	41.0 a B	61.5 bc A	41.3 ab B	35.5 a B	50.5 b B	74.5 ab A	65.4 bc A
RTU®-PCNB	23.3 ab C	28.4 ab C	43.3 bc B	52.2 ab A	75.3 a A	44.5 a B	72.6 a A	49.2 ab B	24.0 b C	71.0 a A	74.4 ab A	77.6 a A
RTU®-Vitavax®-Extra	13.5 b A	13.4 cd A	21.7 d A	19.3 d A								
RTU®-Vitavax®-Thiram	32.1 a A	22.6 bc B	38.9 bc A	34.3 c A								
Hydrogen peroxide (30%)	20.1 b B	17.1 c B	36.7 c A	41.7 bc A						50.3 b B	73.8 ab A	54.3 c B
Sodium hypochlorite (1.3%)					61.0 b A	38.3 ab B	63.8 ab A	46.3 ab B	43.9 a B	49.7 b B	72.7 ab A	64.9 b A
Sodium hypochlorite (2.6%)										49.2 b C	80.4 a A	62.5 bc B

[z]Mean separation within columns (lowercase letters) and rows (uppercase letters) for a trial by Fisher's protected LSD, $P<0.05$

Table 3. Influence of selected surface disinfestants and/or fungicides on subsequent seedling growth of *U. paniculata* (Expt. 2).

Values for each parameter are means of six blocks each consisting of seven seedlings per treatment.

Surface disinfestant or fungicide	Emergence (%)	Avg. days to emergence	Plant ht. (mm)	No. of leaves	Length of longest leaf (mm)	Length of second longest leaf (mm)	Width of longest leaf (mm)[z]	Width of second longest leaf (mm)[z]	Top dry wt. (mg)	Root dry wt. (mg)
Nontreated	34.5 c[y]	12.0 a[y]	33.1[w]	3.4 b[x]	89.1 b[x]	59.1[w]	1.9[w]	1.6 c[x]	16[w]	14[w]
RTU®PCNB	64.3 b	14.2 ab	37.1	3.5 ab	102.9 ab	68.3	2.2	1.8 ab	18	16
2.6% sodium hypochlorite	75.0 ab	13.5 ab	41.0	3.8 a	112.0 a	73.6	2.2	1.9 a	22	20
1.3% sodium hypochlorite + RTU®	75.0 ab	14.2 ab	38.3	3.7 a	110.7 a	70.2	2.4	1.9 a	20	19
2.6% sodium hypochlorite + RTU®	83.3 a	14.7 b	39.8	3.7 a	114.4 a	74.3	2.2	1.9 ab	22	18
1.3% sodium hypochlorite + RTU®-PCNB	76.2 ab	17.3 c	36.5	3.4 b	103.2 ab	63.5	2.1	1.7 bc	18	16
2.6% sodium hypochlorite + RTU®-PCNB	81.0 a	13.5 ab	38.1	3.6 ab	108.4 a	66.6	2.1	1.8 abc	20	17

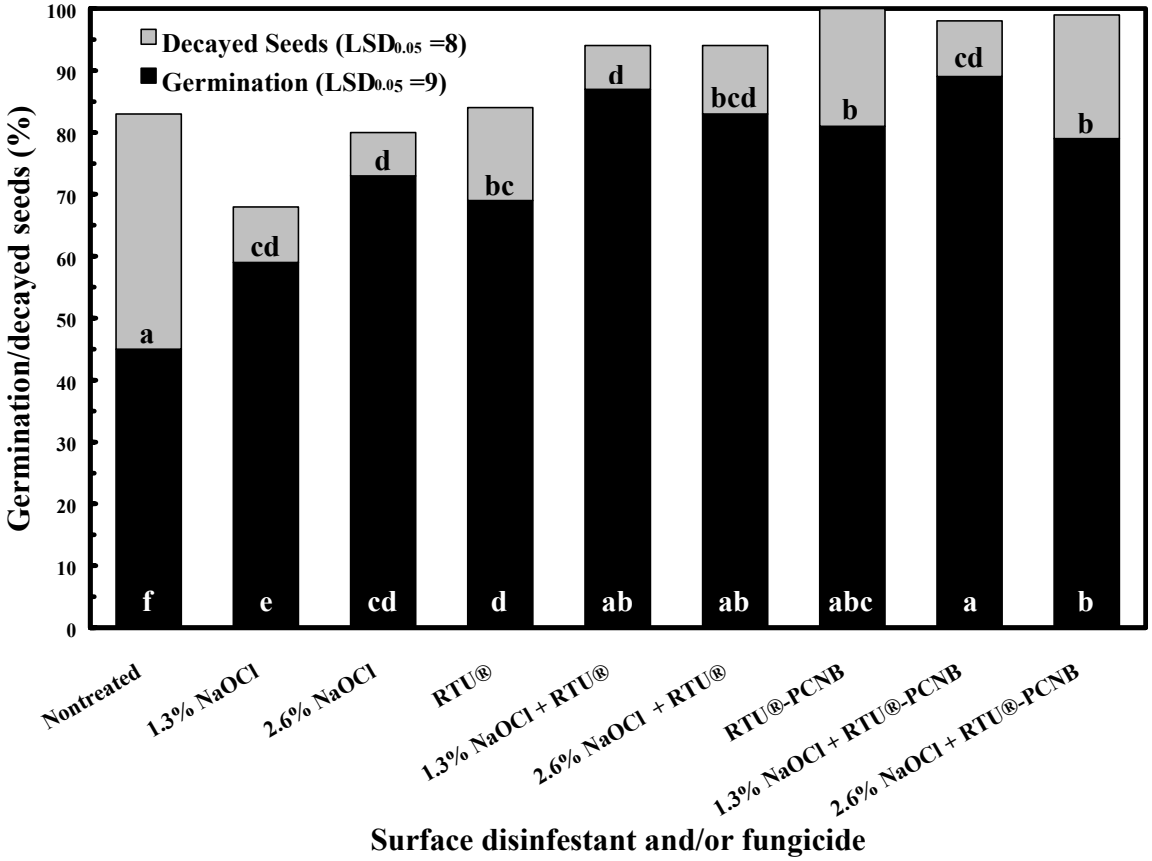
[z]Measured at the midpoint.

[y]Pairwise comparisons made using the "PDIFF" option on LSMEANS under PROC GLM of SAS (SAS Inst., Inc., Cary, NC)

[x]Mean separation within columns by Fisher's protected LSD, $P<0.05$.

[w]Not statistically significant

Fig. 1. Influence of temperature and selected surface disinfestants and/or fungicides on seed germination and seed decay of *U. paniculata* (Expt. 1). Lowercase letters within the black vertical bars denote mean separation among surface disinfestant and/or fungicides for total germination by Fisher's protected LSD at $P<0.05$. Lowercase letters within the gray vertical bars denote mean separation among surface disinfestant and/or fungicides for total decayed seeds by Fisher's protected LSD at $P<0.05$.


Decayed Seeds (LSD0.05 =8)
Germination (LSD0.05 =9)
Germination/decayed seeds (%)
100
90
80
70
60
50
40
30
20
10
0
a
cd
d
bc
d
bcd
b
cd
b
f
e
cd
d
ab
ab
abc
a
b
Nontreated
1.3% NaOCl
2.6% NaOCl
RTU®
1.3% NaOCl + RTU®
2.6% NaOCl + RTU®
RTU®-PCNB
1.3% NaOCl + RTU®-PCNB
2.6% NaOCl + RTU®-PCNB
Surface disinfestant and/or fungicide