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Optimization of Select Native Seed Propagation©

Shane H. Huff, Richard L. Harkess, and Brian S. Baldwin
Mississippi State University, Mississippi State, Mississippi 39762
Email: shh23@pss.msstate.edu

Gary R. Bachman
Coastal Research and Extension Center, Mississippi State University, Coastal Research and Extension Center, Biloxi, Mississippi 39532

Eugene K. Blythe
Coastal Research and Extension Center, Mississippi State University, South Mississippi Branch Experiment Station, Poplarville, Mississippi 39470

Ecological restoration has become a grave concern due to long-term, anthropogenic impacts. The Coastal Roots program, a U.S. Gulf States project, provides educational opportunities working with local schools to inform and instruct students and teachers regarding successful propagation of various native species for ecological restoration. Two plant species native to Crosby Arboretum, Picayune, Mississippi, and surrounding natural areas have been chosen due to the lack of commercial availability, propagation knowledge, wildlife significance, and threatened or endangered status. *Vernonia angustifolia* Michx. (tall ironweed) and *Coreopsis nudata* Nutt. (pink coreopsis) seed were collected and acquired. Seed were placed in coin envelopes and stored under refrigeration at 5 °C until germination tests were conducted. Seed were germinated on Whatman® filter paper (#1, Whatman International Ltd., Maidstone, England) hydrated with 5 mL Captan® Fungicide 50WP (2.37 g a.i./L H_2O) (Southern Agricultural Insecticides, Boone, North Carolina) solution in Petri dishes to determine optimal temperature regime. Seed were exposed to five alternating temperature regimes set at 5 °C increments to simulate day and night temperatures: 10/5, 15/10, 20/15, 25/20, and 30/25 °C. To check for seed viability after germination, the remaining ungerminated seed were pricked and soaked overnight in 0.1% tetrazolium chloride (TZ). Germination tests from 2010 and 2011 indicated pink coreopsis germinated best under warmer temperature regimes (30 day / 25 °C night to 25 day / 20 °C night) resulting in 41.45 and 7.65% overall germination, respectively. Tall ironweed germinated best under the mid- to upper-temperature range (20 day / 15 °C night to 25 day / 20 °C night) resulting in 36.15% and 9.35% germination, respectively.

INTRODUCTION

There is an ever increasing need for ecological restoration due to many natural and man-made disasters such as hurricanes, toxic oil spills, and mining. At least 83% of the Earth's land surface has been altered with estimations around 60 percent of our ecosystems being classified as degraded from such practices as overgrazing and logging (Groom et al., 2006). Many definitions have been given to define ecological restoration, but for the purposes of this research the working definition chosen is "*the returning of a system that has been altered, degraded, or destroyed to a state that closely mimics pre-disturbance conditions*" (Anderson, 2007).

As land use intensifies from a more natural to a more artificial landscape, there is a great reduction in the populations from all species of flora and fauna. This reduction is directly associated with the presence of humans and their impacts to ecosystems from practices such as unsustainable agriculture, leaching of toxic chemicals, urbanization, logging, and mining (Groom et al., 2006). As a result of anthropogenic impacts and natural disasters, several programs have been developed to help with restoration efforts. One such program is the Coastal Roots School Seedling Nursery Program developed by Louisiana State University (LSU) in 2000 (Coleman and Bush, 2002). This program has now been adopted by the Coastal Research and Extension Center (CREC) of Mississippi State University (MSU). The Coastal Roots program works with schools that range from elementary to high schools. By constructing a small nursery on the schools' premises, science students are able to propagate, grow, and care for native plants to later be used in restoration plantings within a predesignated restoration site. In the classroom, the students learn about the issues and importance of ecological restoration, nursery maintenance, plant growth, wetlands, as well as other restoration and conservation issues. Within Mississippi, Woolmarket Elementary School in Biloxi was chosen to be the first school for the nursery program with their associated restoration site located within the Crosby Arboretum, Picayune, Mississippi (Coker et al., 2008). From this, there is a great need for information on how to propagate many of our native species. Popular species are well known in the nursery industry and easily propagated; however, they represent a very small portion of the plant species which historically contribute to a given ecosystem's biodiversity. Many other species important to biodiversity have very little known about their propagation and need more research to be conducted. Reintroducing composites greatly enhances plant diversity within a degraded ecosystem (Coffey and Kirkman, 2006). Promoting biodiversity in ecological restoration is crucial to creating a true healthy and functional system. To address these issues the project objectives are as follows: (1) to identify two native plants of significance for ecological restoration from various habitats located in southern Mississippi; specifically, plants with potential for production in a small, school-based nursery system; (2) determine the viability of seed propagation of these two native plants applicable in a small, school-based nursery system; and (3) determine the optimal temperature regime producing the greatest germination percentage for each species.

MATERIALS AND METHODS

Working in association with Crosby Arboretum, two plants native to their properties were chosen for seed germination research due to their threatened or endangered status, lack of commercial availability, ornamental value, and significance to wildlife populations.

Seed germination studies with *Vernonia angustifolia* Michx. (tall ironweed) and *Coreopsis nudata* Nutt. (pink coreopsis) seed were conducted separately for two consecutive years. Due to the lack of seed source on-site, tall ironweed and pink coreopsis were acquired elsewhere. Tall ironweed, Florida ecotype, was purchased from Ernst Conservation Seeds, Meadville, Pennsylvania, in January 2010, and from the Florida Wildflowers Growers Cooperative, Crescent City, Florida, in January 2011 because Ernst Conservation Seeds had none available. Pink coreopsis was collected from Apalachicola National Forest, Liberty County, Florida, in May 2010 and May 2011. Once collected, the seed were cleaned and prepped accordingly. Tall ironweed was debearded and then aspirated. Pink coreopsis was sieved to remove as much trash and debris as possible. Seed were counted by an electronic seed counter and placed in coin envelopes. Each year, individual coin envelopes contained 50 seed and were stored under refrigeration (5 °C) until May 2010, and January 2011.

Petri dishes (100 × 15 mm) were lined with Whatman® filter paper (#1, Whatman International Ltd., Maidstone, England) and hydrated with a 5 mL Captan® Fungicide 50WP (2.37 g a.i./L H_2O) (Southern Agricultural Insecticides, Boone, North Carolina) solution. Seed from each plant species were placed within the Petri dishes and then placed inside five germination incubators. The incubators were set at varying temperature regimes under a long-day photoperiod (16 h). Temperatures were alternated to simulate environmental conditions set at 5 °C increments to distinguish day and night ranges: 10/5, 15/10, 20/15, 25/20, and 30/25 °C. Eight replications of 50 seed per species per temperature regime were used for both species. Lighting was correlated to day temperatures.

Seed were placed under alternating temperature regimes for 28 days with germination counts conducted every 2 days. Determination of the optimal germination temperature was then made for each species based from the regime with the highest germination percentage within the 28-day period. Any remaining ungerminated seed from each species were placed within the determined optimal temperature for an additional 10 days with germination counts conducted every 2 days to determine quiescence. After the completion of the 10-day germination period, viability tests were conducted on the remaining ungerminated seed. Seed were pricked by a hypodermic needle within close proximity to their embryos and then soaked in a 0.1% 2,3,5-triphenyl-2H-tetrazolium chloride (TZ) solution for 12 h. The remaining viable seed were determined from the presence of a pink to red stained embryo which classified them as being dormant (Baskin and Baskin, 2001; Peters, 2000). Total viability was calculated by combining the germinated and remaining viable or dormant seed. The data collected include: germination counts conducted every 2 days, overall percentage of germination, and an overall percentage of seed viability. The data were analyzed as a randomized complete design, using generalized linear model of SAS version 9.2 (SAS Institute Inc., Cary, North Carolina) with mean separation according to the least significant difference test, $\alpha = 0.05$.

RESULTS

In 2010, pink coreopsis and tall ironweed resulted with overall germination percentages of 41.45% and 36.15%, respectively. Significant differences were found with pink coreopsis seed in relation to germination under separate temperature regimes for the 28-day period. The 28-day germination period indicated pink coreopsis germinated best under warmer temperature regimes (30/25 °C); whereas, tall ironweed showed the highest germination percentages at moderate temperatures (20/15 °C). At the completion of the study, viability percentages for pink coreopsis and tall ironweed were 60.2% and 65.95%; respectively.

Germination percentages varied between consecutive years among species. In 2011, pink coreopsis and tall ironweed resulted in 7.65% and 9.35%, respectively, which was substantially lower than the year prior. Pink coreopsis and tall ironweed had higher germination percentages under 25/20 °C. Viability percentages in 2011 for pink coreopsis and tall ironweed were 32% and 9.60%; respectively.

An indication of a cold, moist stratification (10/5 °C) was found to enhance the germination percentage of tall ironweed. This was especially observed from the results of 2010; whereas, it was not as evident the following year (Figs. 1 and 2). The seed source differed in 2011 and initial observations upon arrival lead to the belief that much of the seed had desiccated and were not viable before undergoing experimentation.

In 2010, germination counts across the temperature regimes indicated warmer temperatures (30/25 °C) increased the germination of pink coreopsis; however, in 2011, pink coreopsis resulted in higher germination only after seed were exposed

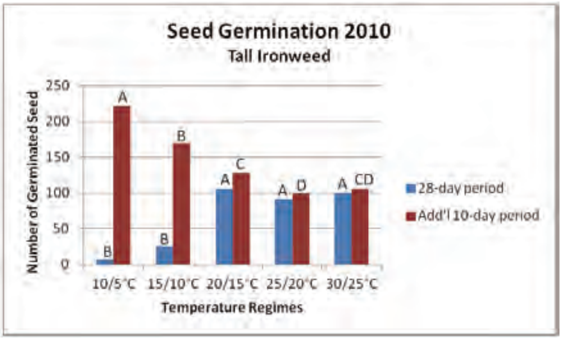

Figure 1. Germination counts across temperature regimes to determine optimal temperature (20 day / 15 °C night) combined with the additional 10-day period to determine quiescence under the optimal regime for tall ironweed (*Vernonia angustifolia* Michx.) in 2010.

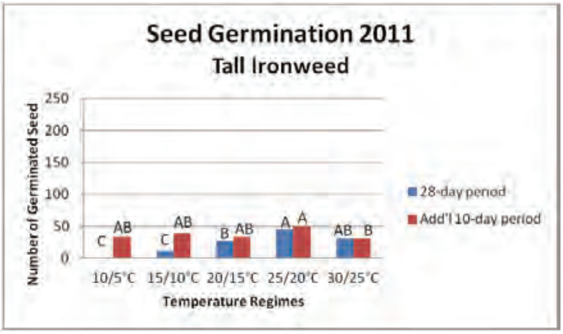

Figure 2. Germination counts across temperature regimes to determine optimal temperature (25 day / 20 °C night) combined with the additional 10-day period to determine quiescence under the optimal regime for tall ironweed (*Vernonia angustifolia* Michx.) in 2011.

to cooler temperatures of 10/5 °C and 15/10 °C and then placed under the optimal temperature that was determined to be 25/20 °C (Figs. 3 and 4). This suggests pink coreopsis may enter some form of dormancy and benefits from a cool, moist stratification process which is similar to the results of *Coreopsis floridana* and *C. leavenworthii* (Rukuni, 2008). However, indications have been reported that an after-ripening period is beneficial toward the germination of some *Coreopsis* species (Kabat, 2004; Norcini and Aldrich, 2007; Rukuni, 2008). Further research should be conducted to address after-ripening effects on pink coreopsis seed.

DISCUSSION

In conclusion, biodiversity needs to be promoted through research for ecological restoration to be most beneficial (Groom et al., 2006). With programs arising to

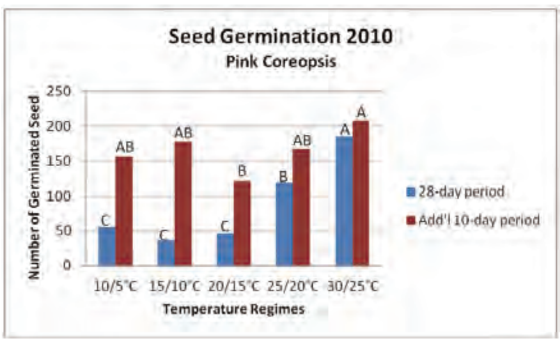

Figure 3. Germination counts across temperature regimes to determine optimal temperature (30 day / 25 °C night) combined with the additional 10-day period to determine quiescence under the optimal regime for pink coreopsis (*Coreopsis nudata* Nutt.) in 2010.

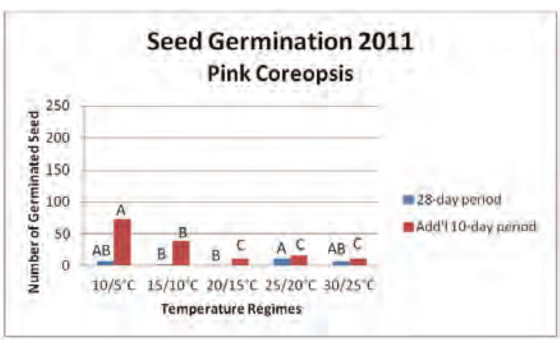

Figure 4. Germination counts across temperature regimes to determine optimal temperature (25 °C day / 20 °C night) combined with the additional 10-day period to determine quiescence under the optimal regime for pink coreopsis (*Coreopsis nudata* Nutt.) in 2011.

help aid against harmful impacts on the environment through restoration efforts, additional native plants continue to need research to be conducted. Germination of these selected native species from this research was influenced by temperature; therefore, optimal temperature regimes and germination percentages were able to be determined for both species. This determination greatly enhances the knowledge of seed propagation for these native plant species and gives some indication on the applicability of using these species within restoration efforts and the commercial native plant industry. Based on the results from 2010, additions to the Coastal Roots program's plant palette could include pink coreopsis and tall ironweed which would also benefit restoration efforts by promoting biodiversity. However, the source of the seed may prove to be difficult and limiting at times. The results from 2011 for both species vary in germination and viability percentages; thus, additional research within a nursery setting needs to be conducted for each of these two species before making a definite decision.

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