

"A botanist explores the longleaf pine woods of Virginia in the early 1800's"

Cover illustration by Gary Pendleton for Meadowview Biological Research Station, see article on page 154.

# The Longleaf Alliance

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## Collection, germination, and propagation of Virginia longleaf pine

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ABSTRACT: A stand of longleaf pine along the Blackwater River in Suffolk City, Virginia was visited November 3-14, 1997. Two thousand six hundred fourteen seeds were obtained from 121 cones with an average yield of 22 seeds per cone. One thousand seven hundred forty eight seeds were used in an experiment to determine whether hydrogen peroxide treated seeds would germinate at a significantly greater rate than controls. Seeds were sown on sand/peat beds in a greenhouse during mid-December and covered to a depth of 0.6 cm with soil. Greenhouse temperatures were maintained above freezing at night and daytime temperatures averaged 24°C. Germination commenced within one week of planting and by the end of January there was no significant difference in germination (mean 52%) between the controls and hydrogen peroxide treated seeds.

#### INTRODUCTION

Longleaf pine reaches its northern limit in Virginia and is listed as an S1 or extremely rare species (Ludwig 1997). There is currently no commercial supplier of native Virginia longleaf stock and we were interested in determining the viability of wild harvested stock for restoration purposes. Barnett and McGilvray (1997) recommend a hydrogen peroxide treatment to minimize germinant losses due to pathogenic fungi. We therefore wanted to determine whether hydrogen peroxide treatment would significantly increase the germination rate of wild harvested seed compared to controls and enhance our restoration efforts.

## MATERIALS AND METHODS

Several visits were made to Union Camp Corporation block 100 in the City of Suffolk, Virginia from November 3-14, 1997 to collect longleaf pine cones. The site is located on the east bank of the Blackwater River and contains one of the few native stands of longleaf pine in Virginia. An extension pole was used to snip branch tips containing cones. Cones were air-dried in a greenhouse on a sand bed and seeds manually extracted. Branches were used to prepare herbarium specimens. Seeds were then counted, the average number of seeds per cone calculated, and seeds were then placed in a plastic container and stored in a refrigerator. Seeds were weighed on December 10, 1997 and average weight per seed was calculated.

Seeds were then divided into lots for hydrogen peroxide (713 seeds) and control (1035 seeds) treatments. The hydrogen peroxide treatment consisted of submerging seeds for 1 hour in a 1% hydrogen peroxide solution (3% Food Lion hydrogen peroxide diluted to 1/3 concentration) and then rinsing seeds 4 times with tap water.

A 50/50 mixture of pre-moistened sand and peat was then prepared and placed in beds in the greenhouse. Beds were 9 cm. deep and were plastic lined with several holes for drainage. Seeds were then placed within a grid and covered with 0.6 cm. of soil. Three beds were used for control seeds and two beds for the hydrogen peroxide treated seeds. Bed position in the greenhouse was determined using a random number table. Air temperature in the greenhouse at night was maintained above freezing by heating and daily temperatures were monitored.

Germination data was then collected on January 13 and 31, 1998. A Chi-Square goodness of fit test was used to determine whether there was a significant difference in overall germination rate among treatments.

#### RESULTS

On average, 22 seeds per cone were collected from this Virginia population of longleaf pine (2614 seeds/121 cones). The average weight per seed was 0.06g.

The first germinations were noted on the third day after sowing and average germination reached 49% after 31 days. After 49 days germination had reached an average of 52% (Table 1) and the experiment was terminated in order to transplant the seedlings to pots. No significant difference in overall germination rate was found (Table 1). Four seedlings of the longleaf x loblolly pine hybrid Pinus x sondereggeri Chapman were also recorded within our data set (0.4% of seedlings are hybrids).

#### DISCUSSION

Two factors stand out in our work with Virginia longleaf pine, the low number of seeds per cone and a relatively low germination rate. These results are partly explained by the methods used in this experiment.

Our cone collection was done in early November and by then the cones were fully open. Longleaf pine cone collection is normally done throughout October in the southern part of its range with an optimum collection in mid-October (Barbour 1998). We thought that an early November harvest would be necessary in Virginia to allow for the change in latitude from southern harvest dates. Our attempts to collect seed in 1998 have also been foiled by early maturing cones. An earlier cone harvest may therefore be necessary, potentially indicating a Virginia longleaf genotype, which matures ahead of its southern counterparts.

A number of cones appeared to have produced few if any seeds and the germination rate of the collected seed is somewhat below reported figures (Barnett and Jones 1993). Other than discarding undeveloped seeds we made no attempt to discriminate seed quality. Our lack of selection may have resulted in inclusion of poor quality seed, which would have been removed by conventional separation techniques. This poor quality seed may have depressed the germination average below that of other workers.

Longleaf pine in Virginia frequently occurs as scattered individuals over a several acre tract. This paucity of individuals may be affecting fitness through self-pollination and subsequent inbreeding depression. Tree species, particularly pines, are known to be highly outcrossed (Ledig 1986). Inbreeding depression from self-pollination affects several fitness parameters including seed number, viability, and quality. Seedlings resulting from self-pollination essentially do not reach maturity (Savolainen 1994). Restoration strategies with longleaf pine in Virginia may need to address tree density for successful cross-pollination in extant stands. Failure to address this important issue may result in lack of seedling recruitment.

Allen (1961) found that 18% of his putative Virginia longleaf pine seedlings from Nansemond County (now City of Suffolk) were in fact the Sonderegger pine hybrid. Although this is substantially more than our observed hybridization rate it does suggest an ongoing level of genetic exchange between longleaf and loblolly pine in Virginia. While we were collecting seeds for this study and during our census we did observe a number of mature pines, which appear to be the Sonderegger hybrid. An investigation of the amount of gene flow between these two pine species and its impact on fitness may be worthy of investigation.

Although hydrogen peroxide treatment did not provide an advantage in our germination experiment higher strengths may be more effective. We originally interpreted Barnett and McGilvray's (1997) 30% hydrogen peroxide solution to refer to a store bought dilution. In fact, they were reporting the use of industrial strength peroxide (100%) diluted to 30%. Stronger hydrogen peroxide solutions may therefore be beneficial as a prophylactic if there is a concern over potential pathogens. Our emphasis was on finding a technique easily available to a grower which was both low cost and safe. We found no benefit in using a 1% hydrogen peroxide treatment on longleaf pine seeds.

## **ACKNOWLEDGMENTS**

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Table 1. Germination data of control and hydrogen peroxide treated Virginia longleaf pine seed.

	# sown	# germ.	% germ.	X <sup>2</sup>	treatment
	460	236	0.51	0.03	H <sub>2</sub> O <sub>2</sub>
	253	130	0.51	0.03	H <sub>2</sub> O <sub>2</sub>
	460	239	0.52	0.00	control
	322	163	0.51	0.14	control
	253	142	0.56	0.75	control
Total	1748	910		0.95*	
Avg.	(*************************************	•	0.52		

<sup>\*</sup>not significant, d.f. = 1, P<0.05