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RESEARCH REPORT 04 – 1

THE GERMINATION OF LONGLEAF SEED IN ARTIFICIAL MEDIA BY FAMILY, SOWER AND SEED TREATMENT WITH FUNGICIDE AND RHIZOBACTERIA

by

Bill Carey, Scott Enebak and Juliana Couto

INTRODUCTION

Recent evaluations of fungicide treatments for longleaf pine seed have been successful. Several studies by Nursery Cooperative Personnel have reported significant improvements in seedling production for treating the seed of certain seed lots with fungicides or combinations of fungicides. In addition, we found that small differences in sowing depth greatly affected both germination and seedling development (Carey *et al.* 2003). In the current study we reevaluated one of the more promising seed treatments and combined it with a bacterial seed treatment (PGPR). Effects were evaluated as cavity fill in artificial media for longleaf pine seed from three half-sib families that differed in contamination with the pitch canker fungus, *Fusarium circinatum (*Carey *et al.* 2000).

METHODOLOGY

The three half-sib longleaf families used in this study are reported in previous fungicide studies (Carey *et al.* 2001, Carey *et al.* 2003, and Carey and Folegatti 2003). The seed were from cones collected at the Bladen Lakes seed orchard on Oct 9, 2001, extracted at Auburn, and kept at 34° F in plastic bags until July 21, 2003 when treated. Treatments were a factorial combination of three levels of a fungicide treatment (TOPS® 90) and a bacterial seed treatment (Kodiac®) at two levels (0 and 1.5 g of the rhizobacteria product / lb seed). The TOPS® 90 (38% Captan, 13% PCNB, 8.5% Carboxin and 13.5% thiophanate-methyl) was applied at either 0, 11.7 or 23.5 oz product/100 lbs of seed. Both the fungicide and bacteria treatments were as a surface treatment to surface moistened seed and were distributed evenly by mixing in a plastic bag. Seeds from two longleaf clones (137 and 135) received all 6 fungicides by bacterial treatments and the third

clone (119) received only the bacterial treatment. Approximately 500 longleaf seed with wings of each half-sib family received each treatment. After treatment, each half-sib family by treatment was divided into four subsets of approximately 80 seed, then returned to storage (34-36° F) until sown the next morning.

Four sowers each single-sowed two 40-cell container racks with each treatment by seedlot combination with sower identity recorded. Before sowing, containers were pre-filled with a peat/pearlite media (Premeier® Pro-Mix) and seed were placed on top of the Pro-Mix and later covered with coarse sand. Sown containers were randomly distributed on two greenhouse tables and kept moist through germination. Germination was recorded weekly from Week 2 to Week 9 and seedlings were harvested and root and shoot weights determined for each sown container rack. All data were analyzed for the effects of family, fungicide, bacteria, sower, and for family by fungicide interaction using SAS GLM. Separate (more balanced) analyses were made for the effect of fungicide on those clones receiving all treatments and for the effects of clones and bacteria, on seed not treated with fungicide. Differences between means were determined using SAS Duncan's.

RESULTS

Due to significant interactions between the effects of fungicide rate and longleaf clone the results of analyses for fungicide by clone are presented in Table 1 along with data for sower and bacteria. The standard rate (11.7 oz product) of fungicide treatment improved germination of both clones and the interaction of rate with clone can be seen (in Table 1) to result from reduced germination at the high rate for Clone 137 but not Clone 135. The effects of sower were always significant with sower No. 3 the lowest, sower No. 2 the highest, and these sowers always significantly differed from each other. Bacterial treatment had no effect on any seedling parameter analyzed when restricted to clones receiving fungicide treatments.

Data for the analysis restricted to seed not treated with fungicide are presented in Table 2. Sower effected germination (as described previously), and Clone 119 did not germinate as well as clones 135 and 137 which did not differ. Only total germination (numbers of live plus dead seedlings 63 days after sowing) differed significantly for bacterial treatment and, as in the non-significant differences for live seedlings, treatment with bacteria significantly reduced seed performance.

DISCUSSION

The results of both the bacteria and the fungicide seed treatments were as in previous studies. That is, fungicides increased production (Carey *et al.* 2003) and bacteria had little effect on the germination, seedling densities, or size (Enebak *et al.* 2002). The standard rate (11.7 oz) of the fungicide increased germination about 30% in both treated half-sib families. The bacterial treatment, although seldom significant, reduced total germination and live fill.

The relative germination among the three tested clones differed from that in previous studies. Percent germination of non-treated seed from this collection sown in 2002 and in 2003 is presented in Table 3. The germination by clone in 2002 (see Carey and Folegatti 2003) was typical for these sources in other studies. That is, clone 135 was best and 137 worst. Although germination declines in storage under our conditions (that is undried seed at 34°F), we assumed

Table 1. Longleaf seedlings per 40 sown cells by sower and by fungicide and bacterial seed treatments of seed from two seed orchard clones.

Clone	Variable	Level	Number live at 14 days	Number live at 63 days	Total sum germination
135	Sower	1	13.9 c	15.1 b	18.2 b
		2	22.1 a	21.5 a	24.3 a
		3	12.3 c	12.6 b	15.1 b
		4	17.9 b	19.5 a	23.0 a
		lsd	*3.5*	*4.0*	*4.0*
137	Sower	1	15.5 ab	14.9 ab	17.5 ab
		2	19.8 a	19.1 a	21.8 a
		3	11.5 b	11.2 b	14.2 b
		4	17.8 a	16.4 ab	19.5 ab
		lsd	*5.4*	*2.2*	*5.9*
135	Fungicide	0	15.4	14.1 b	17.0 b
		1x	17.2	18.4 a	21.6 a
		2x	16.8	19.3 a	22.0 a
		lsd	*3.0*	*3.5*	*3.5*
137	Fungicide	0	16.6 ab	14.1 b	17.5 ab
		1x	19.5 a	19.2 a	21.7 a
		2x	12.4 b	12.7 b	15.6 b
		lsd	*4.7*	*4.6*	*5.1*
135	Bacteria	No	17.0	17.5	20.5
		Yes	15.9	16.7	19.7
		lsd	*2.5*	*2.9*	*2.9*
137	Bacteria	No	16.8	15.9	18.6
		Yes	15.5	14.7	17.9
		Lsd	*3.9*	*3.7*	*4.2*

the three clones would rank, roughly, as in previous tests. Barnett (1993), reported that moisture content effected viability of longleaf seed stored at 34°F. He found viability declined little in one year for seed stored at 14% moisture but was reduced about 60% (from 80% to 47%) for seed with 18% moisture. Because moisture content of seed was not regulated before storage it is possible that changes in viability among clones was due to differences in moisture content at time of storage.

It is difficult to imagine cause(s) for the differences in germination attributable to sower. What reason could there be for the large difference (see Table 2) between Sower No.3 and the others. Having stopped the practice of sticking longleaf seed into the media in favor of just placing seed on the surface (Carey *et al.* 2003), there seems little left for the sower to do to effect germination.

Table 2. Longleaf seedlings per 40 sown cells by clone, by sower and by seed treatment with bacteria for seed that received no fungicidal treatment.

Variable	Level	Number live at 14 days	Number live at 63 days	Total Germination
Clone	119	5.1 b†	6.9 b	8.5 b
	135	15.4 a	14.1 a	17.0 a
	137	16.6 a	14.1 a	17.5 a
	lsd	*3.5*	*3.9*	*3.5*
Bacteria	No	13.7	12.7	15.8 a
	Yes	11.0	10.7	12.9 b
	lsd	*2.8*	*3.2*	*2.9*
Sower	1	11.7 b	11.4 a	13.7 b
	2	17.3 a	15.9 a	19.1 a
	3	7.1 c	6.7 b	9.2 c
	4	13.3 ab	12.7 a	15.4 ab
	lsd	*4.0*	*4.5*	*4.1*

† Means for a variable and within a column followed by different letters differ at 0.05 by Duncan's.

However, some minor things influenced by the depth of sow were observed. For instance, although all container cells were filled to the top (by hand), and refilled once after the media settled; additional settling occurred in cells as the racks were moved and manipulated. Sowers were not closely monitored during sowing (so technique cannot be analyzed) but the most successful sower (No. 2) carefully filled any not full cells before placing seed on top to insure a uniform and small amount of sand would be placed on the seed. Other sowers added media as they thought fit. Another potential factor is that the worst sower (No. 3), who started sowing last, had fewer of the most uniformly pre-filled racks to use because these had been preferentially selected by other sowers to reduce their labor. These differences between sowers indicate that careful supervision of carefully instructed sowers can significantly increase longleaf germination, production, and profit.

Table 3. Percent germination 15 days after sowing for not treated seed from three longleaf pine clones collected in 2002 and stored without drying at 34° F till sown.

Clone	Germination in 2002	Germination in 2003
137	34.6	41.5
135	64.8	38.5
119	34.6	12.7

MANAGEMENT IMPLICATIONS

The fungicide treatments increased production (averaged for the two families) by 22%. Although total germination was lower than last year's, probably due to storage conditions, this is still a very cost effective treatment for longleaf seed. Once again, the importance of TLC and proper technique (not clearly explained here) in sowing longleaf seed and minimum sowing depth is demonstrated.

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