Auburn University Southern Forest Nursery Management Cooperative

RESEARCH REPORT 04 - 5

THE GERMINATION OF LONGLEAF SEED IN ARTIFICIAL MEDIA BY FAMILY, SEED TREATMENT AND ORCHARD.

by

Bill Carey, Scott Enebak and Thais Dreza

INTRODUCTION

The seed efficiency of longleaf pine is rarely as good as that of loblolly or slash pines and for some seedlots it has been very bad indeed. Problems are sometimes linked to infestation of the seed by *Fusarium circinatum (syn. F. subglutinans)* and poor container production has been correlated with differences in infestation of seed related to differences in vegetative symptoms among seed orchard clones (Carey et al 2001). Some fungicidal seed treatments have been effective but the best of these have either not been registered or have lost registration. This study evaluated the effects of three seed treatments (two fungicides and a bacterial treatment), on the germination and survival of longleaf seed sown in artificial media. As in several recent trials, four longleaf clones which have differed in previous trials for pitch canker incidence within the seed orchard and for container fill were used.

METHODOLOGY

Longleaf pine cones were collected at two seed orchards within the North Carolina Division of Forestry Bladen Lakes State Forest on October 7-8, 2003. These were transported to and allowed to open and shed seed naturally in the greenhouse. Shed seed were collected through mid-December, placed in plastic bags and kept in the refrigerator at 36□F until treated for the study. Cones were collected from four clones and from two seed orchards. From these collections, five seed sources were tested consisting of two clones from the old orchard (137 and 135) and three clones (135, 131 and 119) from the new or Expansion orchard.

Seed treatments were applied March 22, 2004. The control was a 10 minute soak in distilled water. For the control and all other treatments, 60 gm of seed from each of the five seed sources was treated in a separate batch that was stirred occasionally during treatment then surface dried on paper towels, placed in ziplock bags, and put back in the refrigerator over night. A seed soak in Topsin-M® was applied as a 2.5% suspension (25 gm of 70 WP thiophanate methyl/L). Sixty grams of seed from each source were stirred occasionally for 10 minutes and then surface dried and stored as for the control. A new fungicide treatment obtained from Gustafson (as a replacement for the Tops 90® previously evaluated) is designated as the "4-Way" treatment, contains Captan, PCNB, Carboxin and thiophanate methyl. This was applied as a seed dressing by putting 0.89 gm of product in a mechanical tumbler with each 60 gm replicate of seed (a rate = 23.5 oz/100 lb seed) and tumbling for 10 minutes with occasional stirring. In the tumbler, seed were sprayed with just enough distilled water to make the treatment spread evenly then seed were surface dried, bagged, and returned to the refrigerator. A biological seed treatment of *Bacillus subtilis*, (GB34) Yield-Shield was the fourth treatment. This bacterial strain has been shown to be an antibiotic producer with some Induced Systemic Resistance effects. Yield-Shield has been approved by EPA and for use in organic production. The Yield Shield was applied as a seed dressing (at 1.5 gm/lb rate) at 0.2 gm/ 60 gm replicate in the seed tumbler exactly as the 4-way treatment.

A day after treatment (March 23), every seed source by treatment was divided into five equal replicates and one replicate of each assigned to each of five sowers. All seeds were placed flat on the surface of each of 80 containers cells (two 40 cell racks of 80 cm^3 cells) containing peat/pearlite media (Premeier® Pro-Mix). After sowing, all containers were covered with a thin layer of sand and then randomly distributed on two greenhouse tables. Containers were kept moist through germination, then watered as needed and re-randomized within the greenhouse about once each month through the study period.

The number of emerged seedlings was recorded weekly for the first four weeks and at 100 days after sowing. Among emerged seedlings, disease symptoms were recorded as to either originating from the seed where attached to the needles or within the root system. That is as "top-down" (seed associated) or "bottom-up" damping-off. Numbers for live seedlings and numbers for symptom origins were assessed by seed sources, seed treatments and sowers and for the interaction of treatments and sources were analyzed using SAS ANOVA and differences between means were compared using SAS Duncan's.

RESULTS

Numbers of seedlings varied ($Pr > F < 0.01$) among seed sources and seed treatments and for the interaction of those variables from day 14 on (see Table 1), but did not differ by sower. Numbers of seedlings differed for the two orchard sources of clone 135 (Table 1) and by seed treatment within that clone between the two orchard sources (Table 2).

Table 1. Longleaf seedling per rack (40 single-sown-cells) at 28 and 100 days after sowing by seed treatment and by seed source (clone by ramet location) and Pr> F for the interaction.

Treatment	Fill 28 (# per 40 sown seed)	Fill 100
None	17.0 a	17.0 a

Yield-Shield (PGPR)	17.7 a	17.8 a
Four-Way	16.8 ab	16.7 ab
Thianophanate	15.8 b	15.7 b
lsd (a 0.05)	*1.1*	*1.1*
Seed Source (orchard clone by ramet location)		
137 / Old Orchard	9.7 e	9.6 e
131 / Expansion	11.8 d	11.9 d
119 / Expansion	21.2 b	21.4 b
135 / Old Orchard	17.8 c	17.6 c
135 / Expansion	23.6 a	23.4 a
lsd (a 0.05)	1.2	1.2
Pr>F for Treatment*Seed Source	< 0.01	< 0.01

Due to the interaction of treatment and seed source, the effects of treatment are presented by seed source in Table 2. Compared to non-treated seed, one seed source (clone 119 expansion orchard) was improved by the 4-Way fungicidal treatment and one seed source was improved by the biological treatments (clone 135 old orchard). One seed source (clone 131 expansion orchard) was negatively impacted by both fungicidal treatments and two sources (clone 137 old orchard and clone 135 expansion orchard) were not affected by treatments.

Table 2. Longleaf seedling per 40 single-sown-seed at 28 and at 100 days after sowing by seed treatment for each seed source (orchard clone by ramet location).

Clone	Orchard	Day	Control	Yield-Shield	4-Way	Topsin M	*lsd*
137	Old Orchard	28	10.0	8.0	10.8	10.1	*2.6*
137	Old Orchard	100	9.7	8.0	10.7	10.1	*2.6*
131	Expansion	28	15.3 a[†]	14.6 a	9.0 b	8.3 b	*2.1*
131	Expansion	100	15.5 a	14.7 a	8.9 b	8.5 b	*2.3*
119	Expansion	28	19.3 b	19.8 b	23.9 a	21.6 ab	*3.1*
119	Expansion	100	19.8 b	20.5 b	23.7 a	21.8 ab	*2.6*
135	Old Orchard	28	17.0 b	21.3 a	16.7 b	16.5 b	*2.3*
135	Old Orchard	100	16.7 b	21.2 a	16.6 b	16.0 b	*2.3*
135	Expansion	28	23.3	25.0	23.7	22.3	*3.1*
135	Expansion	100	23.3	24.6	23.6	22.3	*3.3*

[†] Within a row, means followed by the same letter do not differ at $a = 0.05$.

The numbers of non-healthy seedlings (top-damping-off or root-damping-off) per container rack were analyzed as percentages of the number of emerged seedlings by 40 cell rack. Data for both types of damping-off produced similar inferences, but since top-damping off is much more precisely characterized by its symptom, we will consider its analysis here. Top-damping-off differed significantly at 28 and 100 days after sowing for the effects of treatment and for seed source without interaction for those effects. At both sample dates, the effect of source was due only to more damping-off for clone 131 than the other sources. Also at both dates, both fungicides reduced top-damping-off compared to not treated seed. There was less damping-off among seedlings receiving the 4-Way treatment but the difference between fungicides was significant only for the 28 day data.

DISCUSSION

Work with the four clones used here, though previously only from the old-orchard at the Bladen Lakes State Forest, has indicated that survey estimates for pitch canker symptoms rank consistently (Carey et al 2000) between years and that surveyed severity of orchard symptoms correlates with seed efficiency in containers (Carey et al 2001). For example, clone 135 has always been among the least infected at the orchard and has had the best seed efficiency at the nursery, and clones 137 and 131 are always among the most symptomatic at the orchard and have had the worst seed efficiency. Means for clone 119 are usually intermediate to these other clones but sometimes not significantly different from the better clone (135) depending on variability within the sample. Incidence surveys for the old orchard demonstrate that pitch canker has long been chronic among several clones there. We have not surveyed the new orchard, which has come into seed production only in the last few years, because there have been too few symptoms there to make surveying appear to be worthwhile. It seems reasonable to assume based on apparent (but undocumented) differences in pitch canker incidence at the two orchards that better seed efficiency at the new site would result if pitch canker were a significant factor in that efficiency. Indeed, the better seed efficiency of clone 135 seed from the new orchard appears to support this hypothesis.

Within an orchard, those clones with fewer symptoms of pitch canker consistently produce more seedlings per sown seed (better seed efficiency). This supports two hypotheses: 1) that seed from less symptomatic clones is genetically resistant to *F circinatum,* and 2) that seed from trees with less disease acquire fewer spores by developing further, on average, from those vegetative sites of inoculum production recorded in the survey data. That is, more spores are produced on trees outside the canopy of the mother trees. Differences in seed efficiencies for clone 135 between orchards indicate that a lower general level of inoculum within an orchard (increased distance to infected trees) increased seed efficiency independent of genotype.

Unfortunately, although inferences for the association of pitch canker symptoms with seed efficiency were as in previous studies those for the seed treatment were more variable and do little to help determine an acceptable treatment to improve seed efficiency. It is disappointing that one of the best treatments in previous studies (Tops 90®) is not going to be registered. It is also disappointing that the Topsin M® soak, which in similar concentrations improved germination for clones 137 and 135 in two previous studies (Carey and Folegatti 2003, Carey et al 2004) did not improve those clones' seed efficiency here.

MANAGEMENT IMPLICATIONS

Differences in seed efficiency between seed from clone 135 collected at two orchards differing in the mean level of pitch canker among orchard trees indicate part of the difference is due to distance between where the cones develop and those of spore production. This indicates that removing severely infected trees from an orchard could improve seed efficiency more than just not collecting cones from those trees.

LITERATURE

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