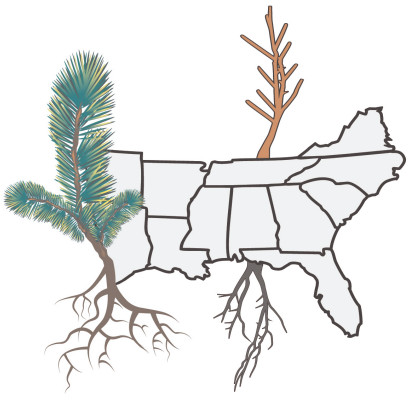

Auburn University
Southern Forest Nursery
Management Cooperative

RESEARCH REPORT 11-04

PITCH CANKER RATINGS OF LONGLEAF PINE CLONES CORRELATE WITH FUSARIUM CIRCINATUM INFESTATION OF SEEDS AND SEEDLING MORTALITY IN CONTAINERS

by
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A note from the Director: *While working on a review paper, I came across some data on longleaf pine that Bill Carey, Steve Oak (USFS – Asheville) and I had worked on in early 2000. Unable to locate the Research Report that should have been published, it dawned on me that, in the aftermath of the April 2005 accident, this report was not completed. Here is that data in a Research Report. -- S.A. Enebak.*

INTRODUCTION

Container production of longleaf pine (Pinus palustris Mill.) has been ongoing since the mid-1970s (Barnett 1975). Container seedlings survive outplanting better than bare root (Goodwin 1976; Boyer 1988), but mortality in containers from pre- and post-emergence damping-off (Wakeley 1954) can cause seedling losses that are both more expensive and extensive than in bare root nurseries (Pawuk and Barnett 1974). Mortality in container nurseries was often 50%, and in some seedlots germination of only 35% might be compounded by 55% post-emergent mortality (Pawuk 1978). Several virulent isolates of Fusarium from dead longleaf seed and seedlings were identified as F. moniliforme (Pawuk 1978) [= F. moniliforme var. subglutinans; = F. subglutinans f. sp. pini = F. circinatum Nirenberg and O'Donell (Nirenberg and O'Donell 1998)]. Fungicide seed treatments helped reduce seedling losses (Pawuk and Barnett 1974) and refinements in watering and fertility management improved production (McRae and Starkey 1996) such that losses of longleaf pine seedlings like those in early production now occur only sporadically (Runion and Bruck 1988; Carey and Kelley 1994a). However, increases in container longleaf pine production, up to 30 million seedlings in 1996 (McRae and Starkey 1996), combined with poor longleaf seed crops through the 1990s caused some marginal longleaf seedlots to be sown resulting in costly losses for nursery managers (Anonymous 1998).

Although *F. circinatum* is considered a wound pathogen, research has shown that there is a wide range of susceptibility to pitch canker as differences in both disease incidence and severity has been reported from natural stands, plantations and seed orchards (Kuhlman et al. 1982; Dwinell et al. 1985; Rockwood et al. 1988). It seems reasonable that a greater inoculum source within a tree would result in a higher percent of infested seed from that tree. However, there have been no studies to quantify the relationships among seed source, seed infestation and subsequent longleaf seedling mortality. These trials tested the hypothesis that pitch canker in the seed orchard correlates with infestation of the seed from more severely infected clones, and subsequent seedling mortality in the nursery.

MATERIALS AND METHODS

Assessing pitch canker among seed orchard clones: The three oldest orchard blocks at the North Carolina Division of Forest Resources' longleaf pine seed orchard near Elizabethtown, NC were surveyed for symptoms of pitch canker during the second week of October in 1997, in 1998 and in 1999. Infections near branch tips often kill just a few inches of the terminal where dead needles persist creating a symptom called a flag. The rating system combined estimates for differences in flagging, and for large stem cankers with their impacts on crown loss and general vigour. A single surveyor estimated disease for all trees without knowing any clone's identity. Trees were subjectively rated from 1 to 4.5 in intervals of 0.5 (i.e. eight steps). Symptomless trees were rated 1, trees with few to moderate to abundant flagging were rated 0.5–2.5, and trees losing canopy area because of extensive flagging or cankers low on larger branches 3–3.5, trees with obviously compromised vigour were rated 4–4.5. Pitch canker ratings were averaged by clone, year and seed orchard block and analyzed for differences in disease incidence and severity using an anova, SAS Version 6.02, Cary, NC. Clones not represented in either all orchard blocks or by less than six ramets per clone were omitted during the analysis.

Cone collection and seed processing: Longleaf pine cones were collected October 12, 1999 from five clones across the range of pitch canker disease ratings among the seed orchard trees. Cones were picked by ramet, dropped to the ground and collected by clone in large paper bags for transport to Auburn, AL. At Auburn, the cones were transferred to open plastic bins by clone and allowed to open naturally at a minimum temperature of 20°C in the greenhouse. Seed from the cones was taken to the International Forest Company (IFCo.) facility at Odenville, AL on November 14, 1999 where it was partially de-winged, cleaned using a Seedboro aspirator and dried over a period of approximately 48 h to between 6 and 7% moisture on a forced air dryer calibrated for longleaf seed. Processed seed was sealed in plastic bags, returned to Auburn and kept at 5°C until sown in nursery trials or used to assess *Fusarium* infestation.

Assessing seed infestation by Fusarium circinatum: Sub-samples from each longleaf clone were assayed for *F. circinatum* at the USDA Forest Service's Asheville, NC laboratory using the blotter paper technique developed by Anderson (1986) for loblolly and slash pine, but modified for the larger longleaf pine seed. A sheet of blue blotter paper (Anchor Seed Germ Paper, St. Paul, MN, USA) was placed in the bottom of a sterile 130 x 136 mm box and soaked with 18 ml of a *Fusarium* selective medium (Nelson et al. 1983). Sixteen seeds were evenly spaced on the blotter paper and their seed coats cracked using a sterile plastic plate slightly smaller than the inside dimension of the box. Two drops of selective medium were then placed directly on each

seed, the box covered, then incubated at 20°C for 7 days. Isolates from fungal colonies with growth characteristics of *F. circinatum* were transferred to carnation leaf agar and incubated in the dark at 28°C for 3 days prior to identification (Nelson et al. 1983). Two boxes of 16 seeds each were considered a replicate sample and a minimum of three replicate samples were evaluated for each of the five-longleaf clones.

Container nursery study: On April 16, 2000, four pre-sow treatments were applied to seeds of each of the five- longleaf clones (see Table 1). Treatments were: a 9 min soak in distilled water (non-treated control), or a 9 min soak in 2.5% active ingredient (ai) benomyl (50 g/l Benlate® SP), or in 2.5% ai thiophanate-methyl (35.7 g/l Topsin M®), or a 3 min stirring in 10% ai mancozeb (140 g/l Manzate® 200 DF). Mancozeb treatments were in 100 ml of 'slurry' and other treatments were in 500 ml suspensions with each clone by treatment processed in a fresh treatment preparation. Five hundred seeds of the five clones' received each of the four treatments, which were then subdivided into six sub-lots of ≥80 seeds each, placed in plastic bags and stored overnight at 4°C.

On April 17, 2000 seed were sown at the IFCo. container nursery in containers (plastic racks of 40 conical 85 cm3 planting cells each 4 cm wide by 9 cm deep) that had been machine-filled with soil-less media. The medium was comprised of peat, vermiculite and perlite containing slow release macro- and micro-nutrients and a mycorrhizal spore inoculum. One seed lot of each clone (n = 5) by presow treatment (n = 4) was randomly assigned to each of six sowers who were instructed to single sow seeds ≤0.5 cm deep. After sowing, container cells were covered with 0.5 cm of screened pine bark and were randomly distributed among two nursery racks and moved into the growing area where they were maintained by IFCo. on their regular production schedules for water, fertility and pest control (McRae and Starkey 1996).

Numbers of live seedlings were recorded May 15, June 5 and July 15, 2000 (28, 49 and89 days after sowing) and 10 surviving seedlings were harvested from each treatment replicate on October 17, 2000 (6 months after sowing) to determine seedling biomass and root collar diameter. Numbers and sizes of seedlings were analyzed for differences attributable to clone, treatment, sower and clone-by-treatment interaction using anova in SAS, Version 6.02, Cary NC, USA.

RESULTS AND DISCUSSION

The most recent orchard map is from 1987 and lists 208 ramets in the smallest of the three orchard blocks (20 ha). The two larger orchard blocks were surveyed intensively enough to produce the same number of estimates as the smallest block. In all three blocks, 270 ramets comprising 41 clones were rated; 16 clones had ≥3 ramets recorded in each block and 28 clones had ≥6 ramets evaluated in 1997–1999.

Among the 16 clones recorded from each orchard block every year, symptom severity varied among clones ($p < 0.01$) and survey years ($p < 0.01$) but not between orchard blocks ($p > 0.78$). The subjective nature of the disease ranking in the orchard may affect the variability (s^2) among ratings within years making comparisons among years questionable. However, with that qualification, the mean rating for 1998 of 1.67 was less (lsd = 0.22) than the 1997 or the 1999 ratings (2.13 and 2.29, respectively).

One important objective of these trials was to determine what effect disease in the orchard had on subsequent seed infestation and seedling mortality. Terminal destruction by pitch canker (flagging), affects the terminal shoots where flowers are produced, and so could affect the number of mature cones 2 years later. Although factors such as weather and pests affect cone production there is sufficient internal variability of cone production in longleaf to obscure correlations with external factors. For this reason, cones were only produced in sufficient numbers in 1999 to allow assessment of clone x seed infestation. To do this, cones were collected from five of those 16 clones with ramets within all three-orchard blocks. Disease severity ratings for these five clones are presented in Table 1 with statistics from the entire 16 clones analysis. Clones 131 and 137 were rated as the most severely infected clones by pitch canker among those 16 clones. Clones 119 and 135 were among the least affected by pitch canker and were rated, respectively, 14th and 16th in severity. Clone 118 rated as intermediate in disease and was 8th among the 16 clones.

The percentage of longleaf seed from which *F. circinatum* was isolated is presented by seed orchard clone in Table 2. The percent of seed with *F. circinatum* correlated with the 1999 orchard ratings for pitch canker. Although this relationship was linear (Fig. 1a) it seems appropriate, for a partially subjective disease rating survey, to simply conclude that seed from more severely diseased trees contain more *F. circinatum*.

The number of live seedlings per 80 cavities sown for the first and last survey dates, seedling mortality and biomass are presented by clone for non-treated seeds in Table 3.

The number of live seedlings was greatest on May 15 and counts from that date are the best measure of total germination. Seedling shoot weights decreased as the percentage survival increased (Table 3) apparently as a function of more space for surviving seedlings (Mexal 1980). While a clones' seed infection rate with *F. circinatum* did not correlate with the initial germination rate ($p = 0.13$) it did correlate with both the final germination ($p = 0.02$) and with the post-germination mortality ($p = 0.02$). Overall, as seed orchard disease rating increased, the percent of seeds with *F. circinatum* infection increased (Fig. 1a) and as seed infection with *F. circinatum* increased, the seedling mortality in the container nursery increased (Fig. 1b).

The effect of seed treatments on germination and seedling numbers are presented in Table 4. With respect to benomyl, the results were similar to those of Barnett et al. (1999) as both germination and seedling survival, especially of the poorer seedlots (clones 131 and 137), were improved by benomyl treatment. There was a significant interaction between treatment and family such that those families that had the greatest percent germination when not treated (clones 135 and 119) were negatively affected by treatment, but the other clones were improved by treatment with benomyl. However, the label for benomyl has been withdrawn and the chemical is no longer available in the US. The other two seed treatments with thiophanate methyl or mancozeb did not show significant effects as applied in this study.

Intra-specific differences in resistance to pitch canker have been demonstrated for loblolly and slash pines (Kelley and Williams 1982; Kuhlman et al. 1982; Dwinell et al. 1985; Rockwood et al. 1988) and the heritability of aspects of resistance as shown for loblolly as differential canker development among inoculated seedlings (Rockwood et al. 1988; Carey and Kelley 1994b). However, where wounds are required for infection, difference in resistance to the internal spread of infections may not correlate strongly with disease incidence, especially among seedlings in a

nursery. This study was the first to show that differences in pitch canker symptoms among clones of mature longleaf pine trees in a seed orchard correlated both with incidence of *F. circinatum* inoculum among their seed and with seedling mortality in the nursery. Unlike the other southern pine species, longleaf seed is not collected and processed by family, but is pooled and processed by area or orchard, therefore, this study indicates that removing seed orchard clones based on the incidence of pitch canker could reduce the infestation of collected seed and the percent of seedlings that become diseased in the nursery.

MANAGEMENT IMPLICATIONS

The incidence and severity of pitch canker was rated among 16 clones of mature longleaf pine (*Pinus palustris*) in a seed orchard and cones were collected from five of those clones across the range of pitch canker disease ratings. The percent of seed with *F. circinatum* correlated with the pitch canker ratings in the orchard for the year of collection ($r = 0.88$, $p = 0.05$) and, when sown without fungicide treatment, with the number of seedlings produced ($r = -0.94$, $p = 0.01$) and with seedling mortality after germination (r ¼ 0.92, p ¼ 0.02). The same orchard clones were more symptomatic of pitch canker through three annual surveys. *Fusarium circinatum* was isolated from a higher percent of seed from more symptomatic clones and a lower percent of their seed produced plantable seedlings. We propose that removing such clones from seed collections should reduce seedling mortality at the nursery and increase seed efficiency.

ACKNOWLEDGEMENTS

Funding for this study was provided through Participating Agreement (R8-97-01) between the USDA Forest Service, Auburn University and the Southern Forest Nursery Management Cooperative. The North Carolina Division of Forestry provided access to the Bladen Lakes Seed Orchard and use of bucket lift for cone collection. International Forest Company provided irrigated nursery space, containers pre-filled with soil-less media and all necessary seedling maintenance through the 2000- growing season when such space was under great demand for production.

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Table 1. Pitch canker survey ratings for five clones of longleaf pine at the Bladen Lakes seed orchard in 1997–1999, Elizabethtown, NC, USA

	Year of Survey							
	1997		1998		1999		Three Year Average	
Clone[1]	Rate[2]	Rank[3]	Rate	Rank	Rate	Rank	Rate	Rank
131	3.5 a[4]	1	2.6 a	1	4.2 a	1	3.4 a	1
137	3.3 a	2	2.4 a	2	3.5 a	2	3.1 a	2
118	1.9 b	10	1.5 b	13	2.4 b	5	1.8 b	8
119	1.4 b	12	1.4 b	10	1.9 bc	12	1.6 bc	14
135	1.2 b	16	1.1 b	16	1.3 c	15	1.2 c	16
lsd[5]	0.85		0.81		0.96		0.51	

[1]Longleaf pine clone designations of the North Carolina Division of Forestry.
[2]Mean pitch canker rating from an 8-step survey scale (1–4.5) where 1 is symptomless and 4.5 is severely affected.
[3]Rank is the order of rated symptom severity (highest = 1 to lowest = 16) among the 16-longleaf clones with ≥6 ramets in each of three orchard blocks.
[4]Mean ratings within a column followed by the same letter are not different at $\alpha = 0.05$ for the five longleaf clones.
[5]Least significant difference at $\alpha = 0.05$.

Table 2. Percent of longleaf seeds from which *F. circinatum* was isolated by clone.

Clone	Percentage (replicates)[1]
131	88 (3)
137	86 (6)
118	82 (3)
119	47 (3)
135	24 (3)

[1]The percentage, out of 96 longleaf seed per replicate, from which *F. circinatum* could be isolated when cracked seed were moistened with selective media.

Table 3. Germination, mortality and growth of container-grown longleaf seedlings, from non-treated seed of five seed orchard clones differing in infestation by *F. circinatum.*

	Germination[1]		Seedling		
Clone	May 15	July 15	Mortality[2]	Root weight (g)[3]	Shoot weight (g)
131	53.2 bc[4]	27.8 d	47 a	1.26 a	4.18 a
137	58.2 ab	34.2 c	41 a	1.21 a	3.24 ab
118	48.2 c	31.7 cd	33 b	0.88 b	2.60 b
119	57.8 ab	39.4 b	32 b	1.29 a	2.86 b
135	65.5 a	56.5 a	13 c	1.21 a	2.55 b
lsd[5]	7.5	4.7	7.3	0.30	1.11

[1]Germination is the number out of the 80 cells sown per replicate that contained a seedling on the dates indicated.
[2]Mortality is the percent of seedlings that died after emergence.
[3]Mean oven dry weights of seedlings 6 months after sowing.
[4]Numbers within a column followed by the same letter are not different at a ¼ 0.05.
[5]Least significant difference at a ¼ 0.05.

Table 4. Seed germination and seedling mortality of container-grown longleaf pine, by seed treatment for five clones differing in infestation by *F. circinatum*, 2000

	Germination[2]		
Treatment[1]	**May 15**	**July 15**	**Seedling mortality[3]**
Benomyl	62 a[4]	50 a	18 a
thiophanate methyl	53 b	37 bc	31 c
Mancozeb	54 b	34 c	37 bc
Control	56 b	38b	33 b
lsd[5]	5	3	4.5

[1]Treatments were a 9 min soak in either, 2.5% ai benomyl, 2.5% ai thiophanate methyl or a 3 min stir in 10% ai mancozeb.
[2]Germination is the number out of 80 cells sown per replicate that contained a seedling on date.
[3]Percent of seedlings that died after emergence.
[4]Numbers within a column followed by the same letter are not different at a ¼ 0.05.
[5]Least significant difference a ¼ 0.05.

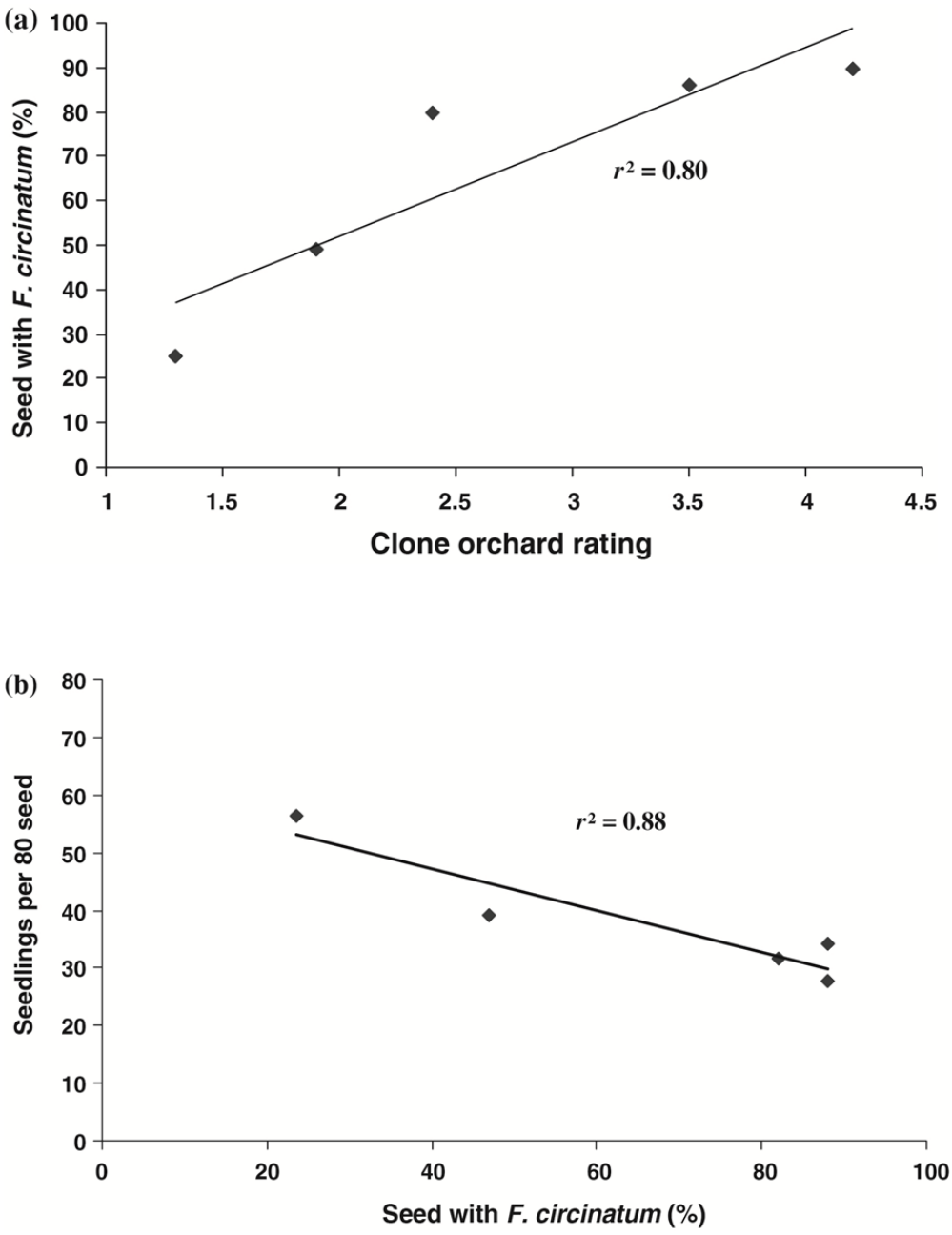

Figure 1. Correlations (a) between orchard ratings for pitch canker by clone and *F. circinatum* on seed and (b) between *F. circinatum* on seed and the number of longleaf seedlings per 80 sown seed in a container nursery.