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Nursery-to-Field Carryover and Post-Outplanting Impact of *Macrophomina phaseolina* on Loblolly Pine on a Cutover Forest Site in North Central Florida

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The charcoal root rot fungus— Macrophomina phaseolina *(Tassi) Goid.— was carried over from nursery seedbeds to a field planting site on asymptomatic, apparently healthy loblolly pine* (Pinus taeda *L.) seedlings. Seedlings on which the pathogen was carried to the field exhibited reduced survival over two growing seasons. The implications of this nursery-to-field carryover are discussed.* Tree Planters' Notes 45(2):68-71; 1994

Charcoal root rot, caused by *Macrophomina phaseolina* (Tassi) Goid., has been and still is considered a problem in many southern forest tree nurseries (Barnard and Gilly 1986, Fraedrich and Smith 1994, Hodges 1962, Rowan 1971, Seymour 1969, Seymour and Cordell 1979, Smith and others 1989). Although once previously reported as a problem in 2-to 7-year-old slash pine *(Pinus elliottii* Engelm.) plantations on reforested sandhills in western Florida (Smalley and Scheer 1963), *M. phaseolina* traditionally has been considered a "forestry problem" primarily in seedling nurseries. Recently, however, Barnard and others (1995) reported *M. phaseolina* to be commonly associated with and a probable contributor to first-year mortality of slash pine seedlings planted on recently converted agricultural croplands.

The concern with any "nursery disease" is the potential for the causal agent itself (i.e., the pathogen) to be carried into the field on healthy-appearing nursery stock, with subsequent disease development after outplanting. This potential has been evaluated for some forest tree pathosystems (Barnard 1984, Barnard and others 1985, Hansen and others 1980, Saunders and others 1992, Smith 1967), but information is lacking with regard to this potential in many others. An outbreak of charcoal root rot at the Florida Division of Forestry's Andrews Nursery in August 1987, provided an opportunity to evaluate nursery-to-field carryover of *Macrophomina phaseolina* and its effect on survival of outplanted loblolly pines (*P. taeda* L.). This paper summarizes the results of this evaluation.

Materials and Methods

A preliminary assessment of the occurrence of *M. phaseolina* on both dead and "healthy" (i.e., asymptomatic, potentially saleable) loblolly pine seedlings was performed on September 30, 1987. Twenty-five side-by-side pairs of live and dead seedlings were removed from disease--impacted seedbeds with a shovel (figure 1) and carried in an ice chest to the laboratory for evaluation. In the laboratory, a single 1-cm root collar segment and four randomly selected 1-cm primary lateral root segments were excised from each sample seedling. These segments were then soaked for 3 minutes in 0.5% sodium hypochlorite, rinsed in sterile deionized water, plated onto acidified (3.3 ml of 50% lactic acid/liter) potato dextrose agar, and incubated at ambient laboratory conditions. Plates were periodically examined over a period of 7 to 10 days for developing colonies of *M. phaseolina.*

In January 1988, seedbeds in which charcoal root rot occurred were divided into two groups: disease-free seedbeds (i.e., seedbeds or areas within seedbeds with no noticeable seedling symptoms or mortality) and disease-impacted seedbeds (seedbeds or areas within seedbeds having abundant seedling mortality). At that time, 50 surviving and apparently healthy seedlings were lifted at random from seedbeds representative of each of the two treatments and evaluated as described above for the presence of *M. phaseolina.* At the same time, 250 surviving and apparently healthy seedlings from each treatment were transported to the field and machine planted at a 6 X 10 ft (about 2 X 3 m) spacing on a cutover pine plantation site in north central Florida in alternating 50-tree row plots (5 replications). Comparative sizes of seedlings between the two treatments were assessed at lifting/ outplanting by measuring root collar diameters to the nearest millimeter on 50 randomly selected seedlings from each of the two treatments.

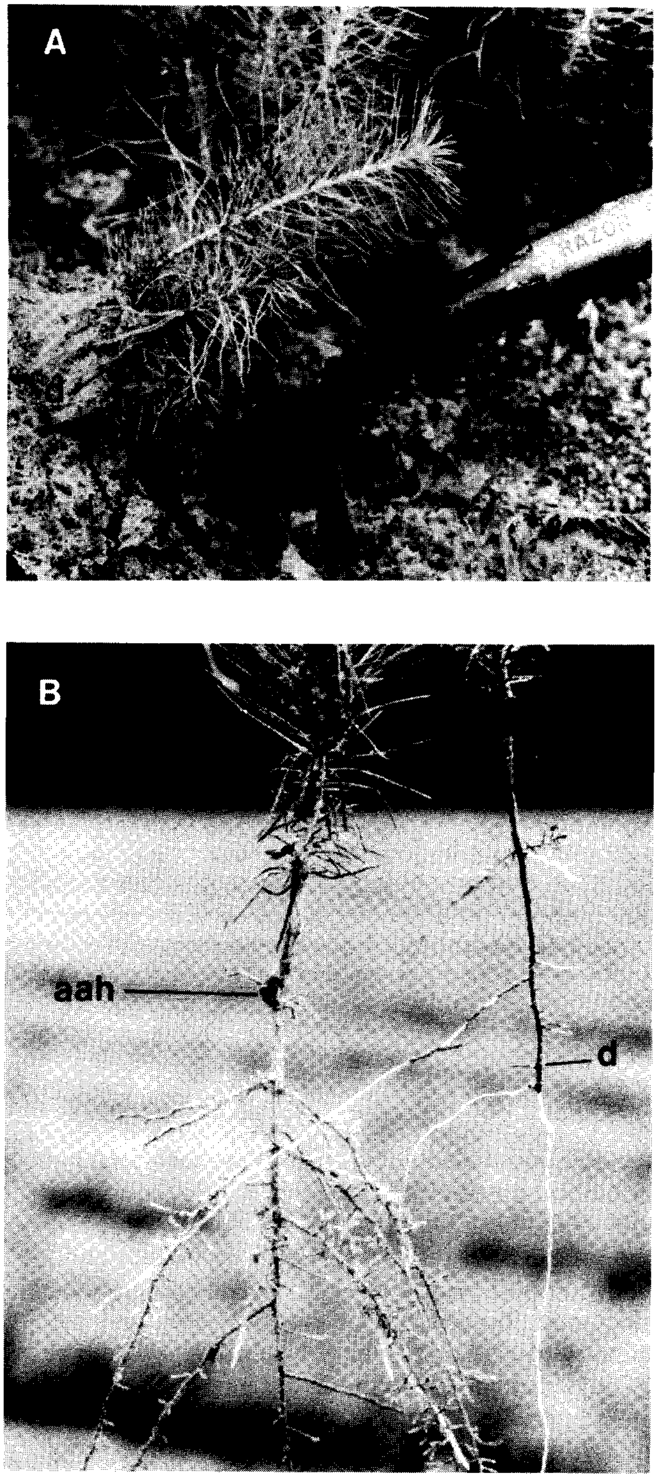

Figure 1A—*Side-by-side sampling of dead* **(d)** *and asymptomatic, apparently healthy* **(aah)** *loblolly pine seedlings in a charcoal root rot-impacted seedbed.* **B**—*Roots of dead* **(d)** *and asymptomatic, apparently healthy* **(aah)** *seedlings.*

The field site was visited in September 1988 and 1989 after one and two growing seasons respectively. On each visit, the percentage of surviving seedlings was determined, and all dead or dying seedlings were carefully dug, transported to the laboratory, and evaluated for the presence of *M. phaseolina.* This evaluation consisted of both visible inspection for the presence of subcortical microsclerotia (Barnard and others 1994, Barnard and Gilly 1986, Smith and others 1989) and culturing for the pathogen as described above. Year-end survival data were subjected to analysis of variance (ANOVA) and differences between treatment means within each measurement year were evaluated for significance at P# 0.05.

Results

In September 1987, *M. phaseolina* was readily recovered from asymptomatic, apparently healthy seedlings adjacent to dead seedlings in disease-impacted seedbeds (table 1). *M. phaseolina* was also recovered in January 1988 from surviving, asymptomatic seedlings taken from disease-affected seedbeds. The percentage of seedlings yielding the pathogen in the January 1988 collection, however, was considerably lower than that in the September 1987 collection. *M. phaseolina* was not recovered from any of the sample seedlings removed from disease-free seedbeds.

At the time of lifting and outplanting in January 1988, the surviving, asymptomatic seedlings from disease--affected seedbeds were slightly larger in stem diameter than seedlings from disease-free seedbeds. Figure 2 shows the distribution of seedling stems within 1-mm diameter classes from each of the two treatments.

Seedlings from disease-free seedbeds exhibited significantly (P # 0.05) better survival than those from disease-affected seedbeds over the course of the 2 years of field monitoring (table 2). In neither of the two treatments, however, was survival particularly poor. The association of *M. phaseolina* with dead and dying seedlings was clearly treatment-related (table 3). The pathogen was detected on approximately 75% of the dead and dying seedlings from disease-affected seedbeds, but only on 5% of those from disease-free seedbeds.

Table 1– *Recovery of* Macrophomina phaseolina *from roots of loblolly pine seedlings removed from charcoal root rot-impacted seedbeds in a Florida forest nursery*

				M. phaseolina isolations			
		No. of trees	No. of roots	Trees		Roots	
Date	Treatment*	sampled	plated	No	%	No.	%
09/30/87	Live	25	125	10	40	10	8
	Dead	25	125	19	76	42	34
01/28/88	3	50	250	0	0	0	0
	33	50	250	9	18	10	4

*Live = healthy seedlings adjacent to dead seedlings; Dead = dead seedlings (see figure 1); 3= healthy seedlings from unaffected portions of seedbeds; 33= "healthy" seedlings removed from portions of seedbeds with abundant seedling mortality.

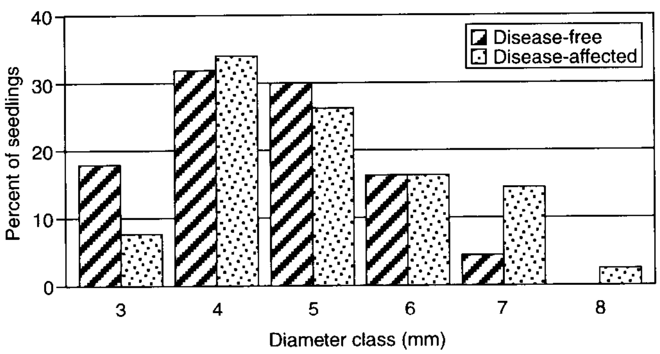

Figure 2—*Frequency distribution of stem diameters for loblolly pine seedlings taken from disease-free and disease-affected nursery seedbeds.*

Table 2 – *Survival of outplanted loblolly pines removed from* Macrophomina phaseolina-*infested seedbeds after 1 and 2 growing seasons in the field*

	% Survival			
	Sept. 2, 1988		Sept. 21, 1989	
Rep. no.	Trt I*	Trt II*	Trt I*	Trt II*
1	97.9	89.8	89.6	87.8
2	98.0	93.9	94.0	85.7
3	100.0	88.5	91.7	82.7
4	100.0	92.2	90.0	88.0
5	100.0	97.9	94.0	89.6
Mean‡	99.2a	92.5b	91.9a	86.8b

*Trt 3 = healthy seedlings from unaffected portions of seedbeds; Trt 33= "healthy" seedlings removed from portions of seedbeds with abundant seedling mortality.

‡Means across computed sampling dates that are followed by different letters differ significantly at P # 0.05.

Discussion

The data reported in this paper demonstrate that *Macrophomina phaseolina* can indeed be carried from the nursery to the field on asymptomatic, apparently healthy seedlings (table 1). In addition, such nursery-to-field carryover can result in reduced survival of outplanted seedlings (table 2). However, these facts must be interpreted within the framework of operational forestry practices, varying weather conditions, differences among field outplanting sites, etc. For example, although differences in survival between seedlings from the two treatments were statistically significant (table 2), it is questionable whether such differences are managerially consequential. It is also probable that in a drier planting year, survival would have been poorer and differences in survival between treatments would have been greater; 1988 was a good planting year with respect to rainfall and soil moisture, and the impact of *M. phaseolina* as a pathogen is typically greater when host plants are under drought or moisture stress (Barnard and Gilly 1986, Barnard and others 1994, Hodges 1962, Palti 1981). At the same time, it can be argued that on sites with abundant indigenous *M. phaseolina*e.g., converted agricultural croplands (Barnard and others 1995), the impact of nursery-to-field carryover of *M. phaseolina* would be inconsequential due to the abundance of inoculum already on site. Typical of cutover forest sites (Barnard, unpublished data), the outplanting site employed in the present study had relatively low levels of *M. phaseolina* (about 0.8 colony-forming units/g of soil compared to about 6 to 13 colony-forming units/g of soil on converted agricultural croplands; Barnard and others 1995) before establishment of the test planting. Finally, consideration must be given to the fact that in

Table 3 — *Numbers of dead loblolly pine seedlings evaluated and percentage recovery of* Macrophomina phaseolina *after 1 and 2 growing seasons in the field (seedlings outplanted from* M. phaseolina-*infested nursery seedbeds)*

Date samples collected	No. dead trees		% Trees positive for *M. phaseolina*	
	Trt I*	Trt II*	Trt I*	Trt II*
09/02/88	2	18	0	66.7
09/21 /89	18	15	5.6	80.0
Overall	20	33	5.0	73.0

*Treatments: defined at lifting from nursery seedbeds (see tables 1 and 2). I= healthy seedlings outplanted from unaffected portions of seedbeds. II = "healthy" seedlings outplanted from portions of seedbeds with abundant seedling mortality.

the present study, every effort was made to purposely obtain seedlings contaminated with *M. phaseolina.* In an operational nursery management situation, such seedlings would likely (and should) be avoided (i.e., skipped during lifting), thus minimizing the "threat" of nursery-to-field carryover of the pathogen.

Acknowledgments

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