Soil Fertilization, Fumigation, and Temperature Affect Severity of Black Root Rot of Slash Pine

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ABSTRACT

Fumigation with methyl bromide apparently eliminated natural soil populations of *Macrophomina phaseolina*, significantly reduced soil populations of *Fusarium oxysporum*, and effectively controlled black root rot of slash pine. The combination of high soil temp, nitrogen (N) fertilization, and the presence of both *M. phaseolina* and *F. oxysporum* produced the most severe root rot symptoms. Nitrogen significantly increased seedling growth, but also increased root rot severity and seedling mortality. Potassium (K) significantly increased seedling top growth at 24 C and seedling mortality at 35 C, but did not increase disease severity or root growth at either temp. The mortality of K-fertilized seedlings apparently was caused by the interaction of K and high soil temp. Phosphorus (P) did not affect seedling growth, root rot severity, or seedling mortality. Fumigation and N, P, and K fertilization also affected the percentage of seedlings from which the two fungal pathogens were isolated. Phytopathology 61:184-187.

Additional key words: Charcoal rot, *Pinus elliottii* var. *elliottii*.

The complex etiology of black root rot of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) is reported to involve *Macrophomina phaseolina* (Tassi) Goid. (*Sclerotium bataticola* Taub.), *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans., *F. solani* (Mart.) Appl & Wr. emend. Snyd. & Hans., and certain parasitic nematodes (5, 6). *Macrophomina phaseolina* and any one of the other pathogens may cause black root rot (5), but charcoal rot is the proper name of this disease when only *M. phaseolina* is present (10). Hodges (5) isolated *M. phaseolina* and *F. oxysporum* from diseased slash pine seedlings in the St. Regis Paper Co. nursery near Lee, Fla., and reported black root rot in this nursery. On the basis of limited inoculations with isolates of *F. oxysporum* and *M. phaseolina* from the same nursery, Seymour (10) concluded that pathogenic strains of *F. oxysporum* caused only damping-off, and that the root rot involved was charcoal rot. Although his inoculation studies did not include the use of naturally infested soil or inoculations with the organisms combined, he reported that *M. phaseolina* was the only root rot pathogen involved and that charcoal rot was the disease present. Regardless of which disease was present, both Hodges (5) and Seymour (10) isolated these fungi from diseased seedlings from this nursery, and both fungi caused damping-off of slash pine.

Although fumigation with methyl bromide is reported to control black root rot (5, 10), this treatment is relatively expensive because the soil in many nurseries must be fumigated every 1 or 2 years for control of the disease. Soil fertility, which has been shown to affect the severity of damping-off of pine seedlings caused by *F. oxysporum* (11), may also affect the severity of black root rot. If certain levels of nitrogen (N), phosphorus (P), and potassium (K) in soil reduce the severity of black root rot, modification of fertilization practices may increase the effective duration of each fumigation and reduce the cost of disease control. The purpose of this study was to determine the effects of N, P, and K fertilization, soil temp, and soil fumigation on the severity of black root rot of slash pine.

MATERIALS AND METHODS.—Ninety-six extruded aluminum pots (15.3 cm diam by 30.5 cm) were filled with soil collected in the St. Regis nursery from areas heavily infested with *M. phaseolina* and *F. oxysporum*. Forty-eight pots of soil were fumigated 72 hr under a plastic cover with 48.8 g methyl bromide/m^2. Soil moisture was near field capacity, and soil temp was 18-20 C during fumigation. Twelve fumigated and 12 nonfumigated pots were randomly selected and placed in each of four constant-temp tanks. Two tanks were set to maintain soil temp at 35 C $\pm$ 3, and two were set at 24 C $\pm$ 3. Eight fertilizer treatments were established in factorial combination, and included N as ammonium nitrate and K as muriate of potash at 0 and 112.1 kg/hectare (ha) and P as superphosphate at 0 and 56.0 kg/ha. An unfertilized check was also included. The fertilizer treatments were randomly placed into 12 fumigated and 12 nonfumigated pots in each temp tank and mixed into the top 10 cm of soil. Three replicates of each treatment were established as a $2 \times 2 \times 8$ split-plot factorial study. A duplicate soil sample, analyzed by the University of Georgia Soil Testing Laboratory, showed that the soil contained 316.1 kg P/ha, 35.9 kg K/ha, 722.9 kg calcium/ha, 31.4 kg magnesium/ha, and 5.2 kg zinc/ha; the pH was 5.7. Slash pine seed were planted in each pot, and seedlings were thinned to 10/pot 1 month later.

After 9 months, we recorded seedling root and top fresh wt, diam of stem at ground line, seedling height, and root rot index based on an arbitrary scale of 0 to 9 (0 indicated the absence of root rot symptoms and 9 indicated the most severe symptoms, in which only a decayed taproot remained). We also recorded seedling mortality and number of seedlings from which *F. oxysporum* and *M. phaseolina* were isolated. Isolations were made from two randomly selected, necrotic (if necrosis were present) root sections from each plant on potato-dextrose agar after surface sterilization for 60 sec with 2.5% sodium hypochlorite. The data were expressed as the average per replication, and percentage data were transformed to arcsin before an analysis of variance

TABLE 1. Effects of fumigation, temp, and fertilization on *Pinus elliottii* var. *elliottii* seedling growth, severity of black root rot, seedling mortality, and isolation frequency of *Fusarium oxysporum* and *Macrophomina phaseolina*. The data are expressed as the average unit of measurement per replication

Treatment	Fertilization	Avg wt (g), roots		Avg wt (g), tops		Avg height, mm		Avg diam, mm		Root rot index		Avg % mortality		Avg % recovery: F. oxysporum		Avg % recovery: M. phaseolina	
		24 C	35 C	24 C	35 C	24 C	35 C	24 C	35 C	24 C	35 C	24 C	35 C	24 C	35 C	24 C	35 C
Fumigated	Check	1.1	1.1	2.7	2.2	218	165	2.6	2.7	0.0	0.0	0.0	0.0	20.0	13.3	0.0	0.0
	N	2.2	1.4	5.3	3.1	215	149	3.9	3.0	0.0	0.1	0.0	0.0	40.0	10.0	0.0	0.0
	P	1.3	1.1	3.2	2.2	230	157	2.8	2.6	0.0	0.0	0.0	0.0	41.9	16.7	0.0	0.0
	K	1.2	1.2	3.3	2.5	242	164	2.7	2.7	0.0	0.0	0.0	3.3	43.3	17.2	0.0	0.0
	N × P	2.0	1.3	5.5	3.3	245	158	3.8	3.1	0.0	0.0	0.0	0.0	50.0	30.0	0.0	0.0
	N × K	2.2	1.1	6.0	2.8	244	147	3.8	2.9	0.0	0.1	0.0	3.3	19.4	17.2	0.0	0.0
	P × K	1.3	1.1	3.6	2.1	247	159	2.8	2.7	0.0	0.0	0.0	0.0	23.3	13.3	0.0	0.0
	N × P × K	2.3	1.2	5.5	2.4	248	141	3.8	2.8	0.0	1.2	0.0	6.7	20.0	14.3	0.0	0.0
	Avg	1.7 a[a]	1.2 a	4.4 a	2.6 b	236 a	155 a	3.3 a	2.8 c	0.0 a	0.2 b	0.0 a	1.7 b	25.8 a	16.5 b	0.0 a	0.0 a
Nonfumigated	Check	1.2	1.0	2.8	2.1	209	156	2.7	2.5	0.2	2.1	0.0	0.0	74.2	93.3	9.7	23.3
	N	1.9	1.1	5.4	2.3	245	155	3.6	2.7	0.1	4.5	0.0	10.0	83.3	92.6	13.3	25.9
	P	1.1	0.9	2.3	2.3	194	163	2.5	2.5	0.1	1.2	0.0	0.0	90.0	93.3	46.7	36.7
	K	1.2	1.1	2.8	2.5	221	161	2.6	2.6	0.5	1.3	0.0	0.0	80.0	90.0	20.0	30.0
	N × P	1.7	1.1	4.8	2.4	209	156	3.5	2.7	0.2	5.1	0.0	6.7	90.0	89.2	23.3	25.0
	N × K	2.0	1.1	5.0	2.2	232	137	3.4	2.8	0.4	5.2	0.0	13.3	90.0	88.5	16.7	19.2
	P × K	1.2	1.2	3.0	2.3	209	153	2.6	2.8	0.1	1.6	0.0	3.3	83.3	79.3	26.7	27.6
	N × P × K	2.2	1.2	5.2	2.5	231	145	3.7	2.7	0.3	3.7	0.0	10.0	90.0	77.8	33.3	7.4
	Avg	1.6 a	1.1 a	3.9 a	2.3 b	219 a	153 a	3.1 b	2.7 d	0.2 b	3.1 c	0.0 a	5.4 b	85.1 c	88.0 c	23.7 b	24.4 b

[a] Average effects of fumigation and temp treatments in each pair of columns are not significantly different if followed by the same letter.

was made. Fertilization treatment data were partitioned into single degrees of freedom for orthogonal comparisons.

RESULTS AND DISCUSSION.—Black root rot and mortality were most severe in N-fertilized, nonfumigated soil at 35 C (Tables 1, 2). Mortality was directly correlated with increasing root rot severity. Seedling growth was also significantly reduced at 35 C, where root rot was most severe (Tables 1, 2). These results agree with reports indicating both black root rot and charcoal rot to be most severe at high temp (1, 5, 7, 8, 12, 13). But these results contradict a report (14) suggesting that cultural methods which stimulate plant growth will control this disease. The addition of N, singly or in combination with P, K, or P and K, significantly increased root rot severity and seedling growth (Tables 1, 2). Seedling diam and top wt were increased by the addition of N at 24 C and 35 C in both fumigated and nonfumigated soil (Tables 1, 2). Root growth was also increased significantly by the addition of N to fumigated and nonfumigated soil at 24 C, but was not significantly increased at 35 C where root rot was most severe (Tables 1, 2). Partitioning of the data also showed that seedling height growth was significantly increased in N-fertilized soil at 24 C where root rot was less severe, and was reduced in N-fertilized soil at 35 C where root rot was most severe (Table 1).

Although N fertilization increased black root rot severity, the frequency of *M. phaseolina* isolation was significantly reduced by N fertilization, and the frequency of *F. oxysporum* isolation was not significantly affected (Tables 1, 2). This lack of increased frequency of *F. oxysporum* isolation is difficult to explain, particularly in view of the report that N fertilization of conifers increases the severity of damping-off caused by *F. oxysporum* and increases the growth of this fungus on artificial media (11). The lack of a correlation between the frequency of isolation of both fungi and black root rot severity may have resulted from a number of causes, such as antagonism by other microorganisms within the decayed roots or rhizosphere (9), natural succession of root-inhabiting fungi (2), or nonselectivity of isolation technique employed.

Partitioning of the data showed that fertilization with K significantly increased seedling top growth at 24 C and seedling mortality at 35 C without promoting root rot severity or root growth at either temp. The mortality associated with K occurred in fumigated soil at 35 C in the absence of *M. phaseolina* (Table 1). Partition analysis also showed that the frequency of *M. phaseolina* isolation was significantly reduced by K fertilization in nonfumigated soil at 35 C. Although *F. oxysporum* was present in fumigated soil at 35 C (Table 1), the mortality associated with K fertilization was not related to the presence of root rot symptoms nor to an increased isolation frequency of *F. oxysporum* (Tables 1, 2). The mortality of K-fertilized seedlings, therefore, appears to have been caused by the interaction of K and high soil temp.

Phosphorus fertilization did not significantly affect root rot severity, seedling mortality, or seedling growth (Tables 1, 2). The highest frequency of *M. phaseolina* isolation was from P-fertilized seedlings, but the frequency of *F. oxysporum* isolation was not significantly affected by P (Tables 1, 2). In conifers, the severity of damping-off caused by *F. oxysporum* is reported to be increased by a P deficiency, but not increased by P fertilization (11). Growth of this fungus on artificial media, however, is promoted by the addition of P (11). Because the soil used in this study contained 316.1 kg available P/ha before fertilization, the effects of a deficiency of P on disease severity and seedling growth were not detected.

In addition to the main effects of the three nutrients described, partition analysis showed that the isolation frequency of *M. phaseolina* was significantly reduced in nonfumigated soil by the interaction of P and K at 24 C, of N and K at 35 C, and of N and P at both temp. The frequency of isolation of *F. oxysporum* was reduced only by the interaction of P and K in fumi-

TABLE 2. Mean square values for treatment effects on *Pinus elliottii* var. *elliottii* seedling growth, root rot severity, seedling mortality, and isolation frequencies of *Fusarium oxysporum* and *Macrophomina phaseolina*

Source of variation	df	Seedling wt		Seedling height	Stem diam	Root rot index	Mortality	Isolation frequency	
		Roots	Tops					*F. oxysporum*	*M. phaseolina*
Fumigation (M)	1	0.4	3.0	22.4	0.6**	59.7**	6.2	49020.3**	
Temp (T)	1	6.3	69.1**[a]	1262.1	4.6**	54.7**	22.1**	633.0	0.9
M × T	1	0.0	0.3	37.9	0.0	42.8**	6.2	1128.9	
Error A	8	0.0	0.6	5.4	0.0	0.9	1.9	225.4	374.6
Fertilizer (F)	7	0.9	6.4**	1.3	1.3**	2.6	2.2	171.0	241.4**
N	1	5.7**	43.5**	0.1	8.8**	17.6**	11.6**	95.4	452.1**
P	1	0.0	0.0	0.3	0.0	0.1	0.0	102.3	473.4**
K	1	0.1	0.3	2.9	0.0	0.0	3.4*	193.0	64.7
N × P	1	0.0	0.0	1.3	0.0	0.0	0.0	0.0	189.8
N × K	1	0.0	0.6	4.3	0.1	0.1	0.3	17.0	29.7
P × K	1	0.1	0.0	0.3	0.0	0.0	0.1	742.0*	419.8**
N × P × K	1	0.1	0.0	0.2	0.0	0.4	0.1	46.2	49.9
M × F	7	0.0	0.2	4.5	0.0	2.1	1.1	141.6	
T × F	7	0.5	3.8	11.7**	0.6**	2.6	2.2	113.5	465.0**
M × T × F	7	0.0	0.3	0.4	0.0	2.2	1.2	224.6	
Error B	56	0.4	0.3	3.2	0.0	0.4	0.8	157.6	47.8

[a] * = Significant differences at the 5% level of confidence; ** = significant differences at the 1% level of confidence.

gated soil at both temp. Although these interactions reduced the isolation frequency of the fungi, root rot severity was not reduced. The interaction of N, P, and K in nonfumigated soil significantly reduced root rot severity, but did not affect the isolation frequency of either fungal pathogen. Because root rot severity was increased by fertilization with N alone or in combination with P or K, the reduction of disease severity by the interaction of N, P, and K may have little practical significance. Seedling growth and mortality were not affected by any of these interactions.

Fumigation with methyl bromide caused a significant increase of seedling stem diam and root and top wt, and caused a significant reduction of root rot severity and the isolation frequency of both fungi (Tables 1, 2). Height growth was greater in fumigated than in nonfumigated soil, greater at 24 than at 35 C, greatest in fumigated soil at 24 C, and least in nonfumigated soil at 35 C, as indicated by a significant interaction of fumigation and temp (Table 2). Root rot was more severe in nonfumigated soil than in fumigated soil, and more severe at 35 C than at 24 C, as shown by the significant interaction of fumigation and temp (Table 2). The significant interaction of temp and fertilization resulted from a greater promotion of seedling growth by N and K at 24 C than at 35 C, and also from higher seedling mortality in N- and K-fertilized soil at 35 than at 24 C (Tables 1, 2). The isolation frequency of *M. phaseolina* in N-fertilized soil was lower at 35 than at 24 C, but the frequency of isolation was higher in P-fertilized soil at 24 than at 35 C, as indicated by the interaction of temp and fertilization (Tables 1, 2).

Fumigation apparently eliminated soil populations of *M. phaseolina* (Tables 1, 2). The fungus was not isolated from 952 root sections removed from 476 seedlings which survived 9 months in fumigated soil. Soil populations of *F. oxysporum* were significantly reduced, but not eliminated, by fumigation (Tables 1, 2). A minor amount of root rot was observed on seedlings grown in fumigated soil at 35 C in the absence of *M. phaseolina* (Table 1). This indicates that strains of *F. oxysporum* that are pathogenic on pine roots exist in the St. Regis nursery. The presence of pathogenic strains of both *F. oxysporum* and *M. phaseolina* in the St. Regis nursery confirms the earlier report of black root rot in this nursery (5).

These data may be summarized as follows: fumigation with methyl bromide at 48.8 g/m^2 will eliminate soil populations of *M. phaseolina*, significantly reduce soil populations of *F. oxysporum*, and effectively control black root rot. Although the amount of N in the nursery soil used in this study was not determined, approx 56 kg N/ha were needed to prevent deficiency symptoms in pine. The addition of 112.1 kg N/ha significantly increased the severity of black root rot. Since the interaction of N, P, and K reduced the severity of black root rot, min amounts of available soil N (56-84 kg/ha) and opt amounts of available soil P (56-112 kg/ha) and K (140-168 kg/ha) should reduce disease severity in nonfumigated areas. Minimal levels of N during the first 2 months after planting should substantially reduce the severity of damping-off caused by *M. phaseolina* and *F. oxysporum*, as the virulence of both pathogens is increased by N fertilization (4, 11). The addition of organic matter to soil and the consequent reduction of available soil N should also reduce disease severity. Frequent irrigation and adequate amounts of seedbed mulch during the hot and dry summer should also reduce root rot severity because the disease is less severe at cool soil temp and in the absence of significant soil water stress (3).

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