Influence of Fertilization on Pitch Canker Development on Three Southern Pine Species

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ABSTRACT

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In greenhouse and field studies, canker length on loblolly, slash, and Virginia pines caused by the pitch canker fungus *Fusarium moniliforme* var. *subglutinans* was increased by applications of a complete fertilizer (containing nitrogen, phosphorus, and potassium). Nitrogen appeared to be the principal nutrient responsible for increased canker severity, whereas phosphorus and potassium had an additive effect when both were applied with nitrogen.

Additional key words: epidemiology, *Pinus elliottii* var. *elliottii*, *P. taeda*, *P. virginiana*

Outbreaks of pitch canker caused by *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. & Reink. (*FMS*) have been associated with poultry manure and applications of chemical fertilizers (2). In a survey of slash pine plantations in Florida, fertilization was one of four factors associated with the incidence of pitch canker (1). In a fertilization study, slash pines receiving annual applications of nitrogen (N), phosphorus (P), and potassium (K) for 6 yr had a higher incidence of pitch canker than trees receiving N alone, P and K, or no fertilizer (2). No attempt was made to determine whether fertilization affected the host, the pathogen, or a possible vector.

In the following studies, fertilized and unfertilized trees were artificially inoculated to determine whether fertilization affects pathogen colonization of pine stems. The interaction of N, P, and K on pathogen colonization of the stem was also evaluated.

MATERIALS AND METHODS

Greenhouse studies. One-hundred 12-mo-old slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seedlings were planted in 6-L metal pots containing peat, sand, and perlite (2:1:1, v/v). Each of 20 seedlings was fertilized eight times at 2-wk intervals with either N, P, K, or N-P-K (two or more elements separated by hyphens indicates that elements were applied together); 20 seedlings served as unfertilized standards. Elements were applied at 0.177, 0.077, and 0.147 g/pot of actual N, P, or K, respectively (equivalent to approximately 56, 25, and 47 kg of actual N, P, or K per hectare, respectively, based on surface area). N as ammonium nitrate and K as potassium chloride were applied in aqueous solutions, and K was applied as finely ground superphosphate.

All seedlings, including unfertilized standards, received two equal applications of calcium, magnesium, sulfur, and micronutrients. All fertilized and unfertilized seedlings were inoculated with *FMS* 4 mo after the fertilization regime was initiated. The inoculum, consisting of a 28.3-mm^2 disk of potato-dextrose agar (PDA) with mycelium and spores of the pathogen, was applied to a wound in the lower stem 7–10 cm above the root collar. Wounds were inflicted to the cambium with a 6-mm-diameter cork borer. The inoculation court was covered with moistened gauze and secured with Parafilm; the gauze and Parafilm were removed after 10 days.

After 21 days, the bark surrounding the inoculation court was removed and canker length was measured. Host tissue from canker margins on 10 randomly selected seedlings in each treatment and standard was placed on PDA to confirm presence of the pathogen. During the study period, early May to late August 1977, seedlings were maintained under natural day-length and were irrigated as needed (usually once each day). Temperatures in the greenhouse varied from approximately 17 to 32 C. Shoot growth and development were present on all seedlings throughout the study.

The same experiment was repeated on 18-mo-old loblolly pine (*P. taeda* L.) seedlings, except that 25 seedlings were used in each treatment and the unfertilized standard. Twenty seedlings per treatment were inoculated with *FMS*, and the remaining seedlings in each treatment served as uninoculated controls. Controls were treated as inoculated seedlings except that sterile PDA was used in place of *FMS*. Host tissue from the canker margins on 10 randomly selected inoculated seedlings and tissue surrounding wounds on three controls in each treatment were placed on PDA to determine presence of *FMS*. The fertilization treatment period extended from mid-September 1977 to mid-January 1978. Throughout the study, seedlings were maintained under a 16-hr photoperiod and watered as needed (usually once every 1–2 days). Ambient temperatures varied from 17 to 30 C during the study period. Shoot growth and development were present throughout the study.

A study utilizing a factorial was undertaken with 80 2-yr-old slash pine and 72 2-yr-old Virginia pine (*P. virginiana* Mill.) seedlings. Seedlings were planted in 6-L metal containers in the medium previously described. Ten slash and nine Virginia pine seedlings each received one of the following fertilizer treatments: N, P, K, N-P, N-K, P-K, N-P-K, or no macronutrients. Each element was applied at the rates and frequencies previously described. All seedlings also received two equal applications of calcium, magnesium, and micronutrients during the treatment period. Four months after initiation of treatments, all seedlings were inoculated with *FMS*; canker lengths were measured 21 days later. Stems surrounding the inoculation point on four slash and four Virginia pines in each treatment were incubated in a moist chamber and observed microscopically for fruiting structures, mycelium or spores of *FMS*. Seedlings were maintained in a greenhouse throughout the course of the study (late April to mid-September 1978).

Field studies. Eight 0.02-ha square plots were established in a 12-yr-old slash pine plantation in the South Carolina Piedmont. Four randomly selected plots were fertilized with N-P-K at the rate of 9.1 kg of actual N as ammonium nitrate, 4.0 kg of actual P as superphosphate, and

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7.6 kg of actual K as potassium chloride per plot. Fertilizers were surface applied on 20 March 1977 as granules. Sufficient distance was left between plots to avoid any fertilizer crossover. All trees were inoculated with *FMS* on 3 May 1977. Inoculum, consisting of a 78-mm^2 PDA disk with mycelium and spores of the pathogen, was placed in a chisel wound inflicted to the cambium on the main stem 1–2 m in height. Canker length on each tree was measured 6 mo after inoculation. Bark surrounding the inoculation site was removed to measure the length of the lesion. A total of 127 fertilized and 155 unfertilized trees was inoculated and evaluated. Host tissue from canker margins on 20 randomly selected fertilized and 20 unfertilized trees was placed on PDA to confirm presence of the pitch canker fungus.

Twenty 0.014-ha rectangular plots were established in a 7-yr-old loblolly pine plantation in the South Carolina Piedmont. Four randomly selected plots each were fertilized with N, P, K, or N-P-K; four plots remained unfertilized. Nutrients were applied at the rate of 7.9, 3.4, and 6.6 kg of actual N, P, and K per plot, respectively, on 20 September 1977 and with 3.2, 1.4, and 2.7 kg of actual N, P, and K, respectively, on 10 May 1978. Nutrient sources were the same as described in the previous field study. Trees on half of each plot were inoculated on 17 May 1978 with 0.75 ml of an aqueous spore suspension containing 1×10^6 spores of *FMS* per milliliter. The inoculum was applied to chisel wounds inflicted to the cambium 1–2 m in height. Trees on the corresponding half of each plot were treated similarly except that sterile water was substituted for inoculum. Four months after inoculation, canker length surrounding each inoculation site was measured as described previously.

A study utilizing a factorial arrangement was established in an 8-yr-old slash pine plantation in the South Carolina Sandhills. Three blocks each contained eight 0.02-ha rectangular plots; treatments within each block were N, P, K, N-P, N-K, P-K, N-P-K, and no fertilizer. Nutrients were applied in split applications: 6.8, 3.0, or 5.6 kg per plot of N, P, or K, respectively, on 9 March 1978 and 4.5, 2.0, or 3.7 kg per plot of N, P, or K, respectively, on 23 May 1978. All trees were inoculated on 31 March 1978 using the same methods as in the loblolly pine field study. Inoculum consisted of 0.75 ml of an aqueous spore suspension containing 1.6×10^7 spores per milliliter. Three trees in each treatment plot were randomly selected as controls, and sterile water was substituted for inoculum. Evaluations were made as previously described in the slash pine study.

In all greenhouse studies, the data were analyzed and Duncan's multiple range test was used to determine significant differences in mean canker lengths among treatments. In the field studies, significant differences were determined by a *t* test in the slash pine study and by Duncan's multiple range test in the loblolly pine and factorial slash pine studies.

RESULTS

Greenhouse studies. Cankers on slash pine seedlings fertilized with N-P-K were significantly longer than those on seedlings fertilized with N, P, or K alone or on unfertilized standards (Table 1). Cankers on seedlings fertilized with N were significantly longer than those fertilized with K. One seedling each in the N-P-K and N treatments was girdled by the canker and died. Isolations from the margins of cankers on all stems that were sampled yielded *FMS*.

Cankers on loblolly seedlings fertilized with N-P-K were significantly longer than those on seedlings fertilized with N, P, or K or on standards; those on seedlings fertilized with N alone were significantly longer than on seedlings receiving no N (Table 1). One seedling in the N-P-K treatment was girdled by the canker and died. All inoculated seedlings sampled yielded *FMS* in culture, whereas none of the uninoculated controls yielded the pathogen.

Slash pine seedlings fertilized with N, either separately or in combination with P, K, or both, had significantly longer cankers than those receiving no N (Table 2). Differences in canker lengths on slash pine seedlings treated with P, K, P-K, or no macronutrients were not significant. One slash pine seedling in the N-P-K treatment was girdled by the canker and died. Cankers on Virginia pine seedlings fertilized with N in combination with P, K, or both were significantly longer than those on seedlings receiving no N (Table 2). Canker lengths did not differ significantly on Virginia pine seedlings among treatments where N was a fertilizer constituent nor on seedlings among treatments where N was not a fertilizer constituent. One Virginia pine seedling each in the N and N-P treatments was girdled and died. Mycelium with conidia of *FMS* formed at the canker margins of all seedlings incubated in moist chambers.

Field studies. The average canker length of 21.3 cm on fertilized slash trees was significantly longer ($P = 0.05$) than the average length of 13.9 cm on unfertilized trees. *FMS* was isolated from 70% of the fertilized trees and 45% of the unfertilized trees that were sampled. The pathogen was isolated from canker margins where callus tissue was absent but not from margins or interiors of cankers where callus was present.

Cankers were significantly longer on loblolly pines fertilized with N-P-K than on trees receiving N, P, K, or no macronutrients (Table 1). Callus tissue surrounded some small cankers, particu-

Table 1. Lengths of cankers on slash and loblolly pine seedlings in the greenhouse and on 7-yr-old loblolly pines in the field that were inoculated with *Fusarium moniliforme* var. *subglutinans* and grown under various fertilizer treatments

	Greenhouse				Field	
	Slash pine		Loblolly pine			
Treatment	Seedlings (no.)	Mean canker length (mm)	Seedlings (no.)	Mean canker length (mm)	Loblolly pine (no.)	Mean canker length (mm)
N-P-K	19	76 a[y]	19	47 a	43	141 a
N	19	56 b	20	34 b	39	93 b
P	20	48 bc	20	19 c	42	56 c
K	20	41 c	20	19 c	42	66 bc
NF[z]	20	48 bc	20	16 c	40	70 bc

[y]Column means followed by the same letters are not significantly different at the 5% level.
[z]Not fertilized.

Table 2. Lengths of cankers on slash and Virginia pine seedlings in the greenhouse and on 8-yr-old slash pines in the field that were inoculated with *Fusarium moniliforme* var. *subglutinans* and grown under various fertilizer treatments

	Greenhouse				Field	
	Slash pine		Virginia pine			
Treatment	Seedlings (no.)	Mean canker length (mm)	Seedlings (no.)	Mean canker length (mm)	Slash pine (no.)	Mean canker length (mm)
N-P-K	9	47 ab[y]	8	53 a	62	76 a
N-P	9	50 a	8	55 a	56	65 abc
N-K	9	41 ab	8	48 a	58	74 a
N	9	39 b	8	45 ab	64	68 ab
P	8	27 c	9	31 c	55	55 c
K	10	23 c	9	33 bc	60	57 bc
P-K	8	18 c	9	31 c	61	63 abc
NF[z]	8	24 c	9	27 c	61	60 bc

[y]Column means followed by the same letters are not significantly different at the 5% level.
[z]Not fertilized.

larly in the plots receiving no N. Cankers did not form on wounded, uninoculated controls, and callus tissue was present around all wounds.

Cankers on trees fertilized with N-P-K or N-K were significantly longer than those on unfertilized standards (Table 2). The remaining treatments were not significantly different from the unfertilized standard. In all treatments, most cankers were very small, and callus tissue was present on the margins at the time of evaluation. Again, *FMS* was isolated only from canker margins with no callus tissue, which represented 30% of all cankered trees sampled in all treatments. Recovery of *FMS* in culture varied little among treatments.

DISCUSSION

Nitrogen appeared to favor pitch canker development on slash, loblolly, and Virginia pine. In all studies, trees fertilized with N-P-K had significantly longer cankers than those receiving no fertilization. Although not statistically significant in every study, trees fertilized with N alone had longer cankers than unfertilized standards. However, in none of the studies was canker length significantly altered by P and K, either alone or in combination. These findings implicate N as the principal element that increases pitch canker severity, whereas P and K had an additive effect when both were applied with N.

These results support the study by Wilkinson et al (2) in which the incidence of pitch canker was greatest in slash pine plots receiving annual applications of N-P-K as compared with trees receiving N, K-P, or no fertilization. In our study and theirs, fertilizers were applied at rates far higher than those traditionally used in forest fertilization. The high rates were used to obtain sufficient nutrients in the soil to ensure a treatment effect. Additional studies with N fertilizers applied at rates typically employed in management of pine in the South are needed for adequate assessment of the impact of fertilization on pitch canker development.

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