Epidemiology of Pitch Canker Disease in a Loblolly Pine Seed Orchard in North Carolina

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ABSTRACT

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Incidence of pitch canker disease among 550 ramets in 28 clones in a 16-yr-old loblolly pine seed orchard varied from 2% of nine resistant clones to 39% of nine intermediate clones and 92% of 10 susceptible clones in 1976. Some branches died back after inoculations in all months of the year, but more dieback resulted from fall and winter inoculations than from those in spring and summer. Seventy-one percent of the dieback from inoculations happened in May–July regardless of when inoculations were made. Since more dieback developed from inoculations of wounds on susceptible than on resistant or intermediate ramets, resistance does not appear to involve penetration. Spores of *Fusarium moniliforme* var. *subglutinans* were present throughout the 1977 growing season on dead branches in the crown, in rainwater falling through infected trees, and in the air; the total indigenous inoculum was sufficient to cause up to 52% of wounded pine seedlings to die back. Nevertheless, symptoms of pitch canker from 1977 natural infections were extremely rare in 1978. In spite of the high incidence of pitch canker in the orchard in spring 1976, cone and seed production was more than twice as large in 1976 as in the preceding 3 yr. Cones initiated in 1975 and 1976 during the pitch canker epidemic matured into record crops in 1976 and 1977. Although resistant ramets produced a plurality of cones most years, intermediate and susceptible ramets also yielded heavy crops in 1976 and 1977.

Additional key words: host phenology, mineral nutrition.

In the mid-1970s, a shoot-dieback disease endemic in southern pine seed orchards for nearly two decades (8) was present in 22 of 25 slash pine (*Pinus elliottii* Engelm. var. *elliottii*) seed orchards surveyed in Florida (16) and was a major problem in at least two loblolly pine (*P. taeda* L.) seed orchards (8). Besides causing shoot-dieback, the causal organism damages pine cones and seeds (14) and affects young seedlings (1).

The disease, which is called pitch canker (9), is caused by *Fusarium moniliforme* var. *subglutinans* (= *F. lateritium* f. sp. *pinii*) (10). Canker-producing species of *Fusarium* usually require a wound to gain entry into their hosts (9,11). Cyclic outbreaks of pitch canker in plantations, which occurs in association with damage caused by the tip moth (*Rhyacionia* sp.) (12), wounding of the host, and vectorings of the pathogen by the deodar weevil (*Pissodes nemorensis*) (3,5), suggest that insect injuries may provide infection sites for *F. moniliforme* var. *subglutinans*. Although application of the systemic insecticide phorate reduced both tip moth incidence and pitch canker, McGraw (13) concluded that tip moths do not transmit the pathogen.

Branch dieback resulting from the disease was reported throughout the year in seed orchards, but the time when infection took place was unknown. In natural stands Hepting and Roth (9) reported successful inoculations with the pitch canker fungus on Virginia pine in April, June, August, and September in western North Carolina. In western North Carolina sporodochia were rarely seen (17), but in Florida sporodochia were found throughout the year in slash pine plantations (4). Airborne spores of *F. moniliforme* var. *subglutinans* were trapped at weekly intervals throughout the year in a slash pine study (2).

The purposes of the present study were to determine the relative susceptibility of various clones of loblolly pine within an established orchard, to determine if phenology of stem elongation or mineral content of foliage contribute to susceptibility, to relate natural infections with insect damage, to determine the coincidence of availability of spores and periods of susceptibility, and to relate disease susceptibility to cone and seed production.

MATERIALS AND METHODS

Disease and seed production surveys. In April 1976, all 16-yr-old loblolly pine ramets were examined in the Piedmont source section of the J. P. Weyerhaueser Orchard at Washington, NC. The crown of each ramet was viewed from a mobile aerial tower. Susceptibility was rated on the basis of the number of branches killed by the pathogen: 1 = none, 2 = 1–3 lateral branches, 3 = terminal, 4 = terminal and 1–3 lateral branches, 5 = 5–10 branches, 6 = >10 branches but less than half the crown, and 7 = more than half the crown. A map of the orchard with the disease rating of each ramet was plotted to determine if the incidence or severity of disease was concentrated in any part of the orchard. Disease incidence by clone was analyzed, and arbitrary limits were established for relative susceptibility: 1.00–1.20 resistant, 1.21–2.85 intermediate, 2.86–7.00 susceptible.

Each year from 1973 to 1978, cone and seed production was tallied by clone to maintain an inventory of seed in stock and to determine the relative productivity of each clone. However, seed from all clones with few cones was extracted en masse. Therefore, the data for cone production were related to disease susceptibility, because they were more precise on a clone basis than were seed production data.

Inoculation. The effect of seasonal inoculation on disease development was studied in two experiments. In the first, 24 ramets with only a few affected branches from one resistant, seven intermediate, and two susceptible clones were inoculated at 2-mo intervals from April 1977 to February 1978. Each 2-mo inoculation was made on tissue of the same age (eg, in April 1977, second flush 1976 tissue; in June 1977, first flush 1977 tissue; and August 1977-February 1978, second flush 1977 tissue). Two pairs of branchlets in the upper crown were selected for treatment. One pair of branchlets was wounded by making a longitudinal 3-cm-long knife slit in each branch. The other pair of branchlets was wounded by making five pinholes with a dissecting needle in a 4-cm^2 area. To each wound 0.03 ml of either water or a water suspension containing 1×10^6 conidia of *F. moniliforme* var. *subglutinans* per milliliter was applied. Conidia were collected from 10-day-old cultures of five isolates of the fungus on potato-dextrose agar. Branchlet dieback was recorded through 1978.

In the second inoculation, 30 ramets were systematically selected by pairs throughout the orchard to provide individuals from clones of the three susceptibility categories. Two branchlets in the upper crown of each ramet were slit wounded each month from June 1978 to May 1979. Inoculated and check treatments were as previously described. Treatments were observed for 7 mo after inoculation for branchlet dieback due to treatment.

Availability of spores. Occurrence of spores of *F. moniliforme* var. *subglutinans* in the orchard in 1977 was determined by four methods.The presence of spores of *F. moniliforme* var. *subglutinans* in the air was determined by exposing two petri dishes with PCNB agar (15) for 30 min at each of nine locations at 2-wk intervals from April to October. After exposure, plates were incubated under fluorescent lights on a 12-hr day at room temperature (approximately 20 C) for 6–8 days; *F. moniliforme* var. *subglutinans* colonies were then counted on the plates. Colonies were occasionally subcultured to confirm the identification.

The availability of sufficient spores to cause infection was determined by exposing 1-yr-old potted loblolly and slash pine seedlings in the orchard for 2-wk periods from 20 April to 4 October and for 1-mo periods starting 18 October, 15 November, and 12 December. Fifty seedlings of each species were wounded with three 5-cm-long slits through the bark and into the wood on the youngest main stem tissue. Ten seedlings of each species were set out in five locations in the Piedmont orchard under trees with disease ratings of 6 or 7. After the 2- or 4-wk exposure, the seedlings were returned to a lathhouse in Research Triangle Park, NC, for observation of symptom development. Stem tissue from all seedlings with dieback was cultured each week on PCNB agar.

Rainwater falling through the crown of heavily and lightly infected and uninfected trees was collected with 17-cm-diameter funnels into plastic bottles on the ground at 12 times from 5 April to 18 October 1977. Three trees in each disease susceptibility group were sampled at two locations. Rainwater samples were refrigerated until processed. One-milliliter samples from these collections were plated on PCNB agar to determine the relative number of spores per milliliter of rainwater.

The relative number of spores on dead branches was determined monthly by collecting the two most recently killed branches from each of 10 trees. Individual branches were rinsed with 50 ml of sterile water. Samples (1 ml) of this rinse water were plated on each of three PCNB plates.

Host phenology. Growth flushing and mineral status of foliage were observed in ramets from resistant, intermediate, and susceptible clones. Ramets were selected systematically throughout the orchard to provide a full range of susceptibility and to minimize movement of the mobile aerial tower. The selected ramets included 18 from seven susceptible clones, 12 from seven intermediate clones, and six from five resistant clones.

Growth flushing was determined for the terminal and four upper lateral branches of each ramet. Growth of each branch was measured at 2-wk intervals from 4 April to 7 September 1977.

Mineral status of foliage was determined for foliar samples collected in April 1977 (1976 needles), July 1977 (1977 first flush), and July 1979 (1979 first flush) from the 36 ramets. Total nitrogen was determined by Kjeldahl procedures; NH_4, NO_3, S, and P were determined by autoanalyzer; and K, Ca, Mg, Mn, Zn, Cu, and B were determined by atomic absorption.

Disease development. Disease development was observed when growth flushes were measured in 1977. Branches that had died recently were examined to determine where infection had occurred. At least five live branches on each of the 36 ramets were examined for insect or other types of wounds that would provide entry for *F. moniliforme* var. *subglutinans*. Samples with possible infections were collected for examination and culturing in the laboratory.

RESULTS

Disease survey. Branch dieback due to pitch canker was common in ramets from Piedmont sources but was sporadic in loblolly pines from other geographic sources and did not occur in slash pine. Pitch canker had been epidemic in the Piedmont sources in the orchard for 1 yr when the 100% survey was made in April 1976. Disease incidence varied greatly among clones, indicating genetic variation in susceptibility (Table 1). The ramets of all clones are randomized throughout the Piedmont source section to enhance cross pollination. Plotting the disease rating of individual ramets on an orchard map further confirmed the occurrence of the disease by clone rather than location. Relative susceptibility was indicated both by the number of ramets infected and by disease severity on ramets within the clones. These differences were used to establish the arbitrary, but distinct, susceptibility categories.

Inoculations. Branch dieback occurred after each of the six inoculation treatments in 1977–1978. However, dieback occurred after only 24% of the inoculations in April, June, and August but

after 72% of those in October, December, and February. Heavy rainfall from November 1977 to February 1978 limited access to 10 of the 24 ramets in February. There was no significant difference according to a chi-square test in dieback between slit wound-inoculated trees (46%) and pinhole wound-inoculated trees (45%). Although branches on ramets from clones of intermediate resistance had 41% dieback and those from susceptible clones had 51% dieback, this difference was not statistically significant in a chi-square test.

The 1978–1979 inoculations confirmed the year-round susceptibility of loblolly pine branches and lower susceptibility in spring and summer than in fall and winter (Fig. 1). Twenty-six percent of the inoculations in April–September caused dieback, whereas 60% of those in October–March caused dieback. Most dieback (71%) occurred in May–July 1979, even though

TABLE 1. Incidence and severity of pitch canker in April 1976 in the Piedmont section of the loblolly pine seed orchard near Washington, NC, arranged by relative susceptibility of the clones

Relative susceptibility	Clones (no.)	Ramets (no.)	Ramets infected (%)	Severity index[a]
Resistant	9	154	2	1.04
Intermediate	9	166	39	2.04
Susceptible	10	230	92	5.34
Total or average	28	550	51	3.14

[a] Based on a rating scale of the number of branches killed by pitch canker per ramet: 1 = none; 2 = 1–3 lateral branches; 4 = terminal and 1–3 lateral branches; 5 = 5–10 branches; 6 = >10 branches, but less than half the crown; and 7 = more than half the crown.

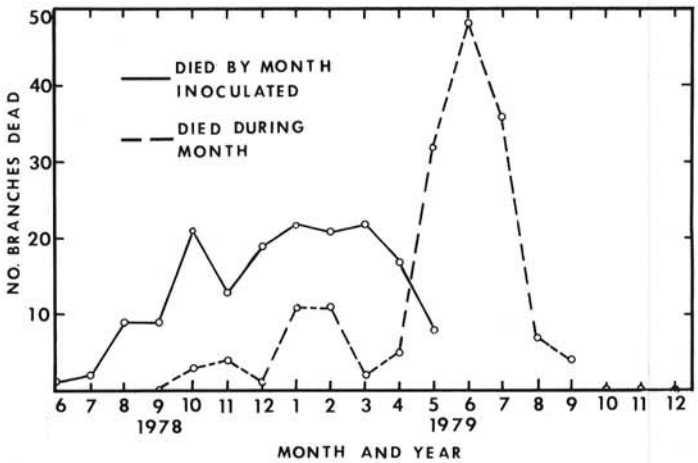

Fig. 1. Branch dieback following monthly inoculations in 1978–1979 of loblolly pine ramets with *Fusarium moniliforme* var. *subglutinans* in a seed orchard. Branch dieback is indicated by both the month of inoculation and by the month dieback occurred.

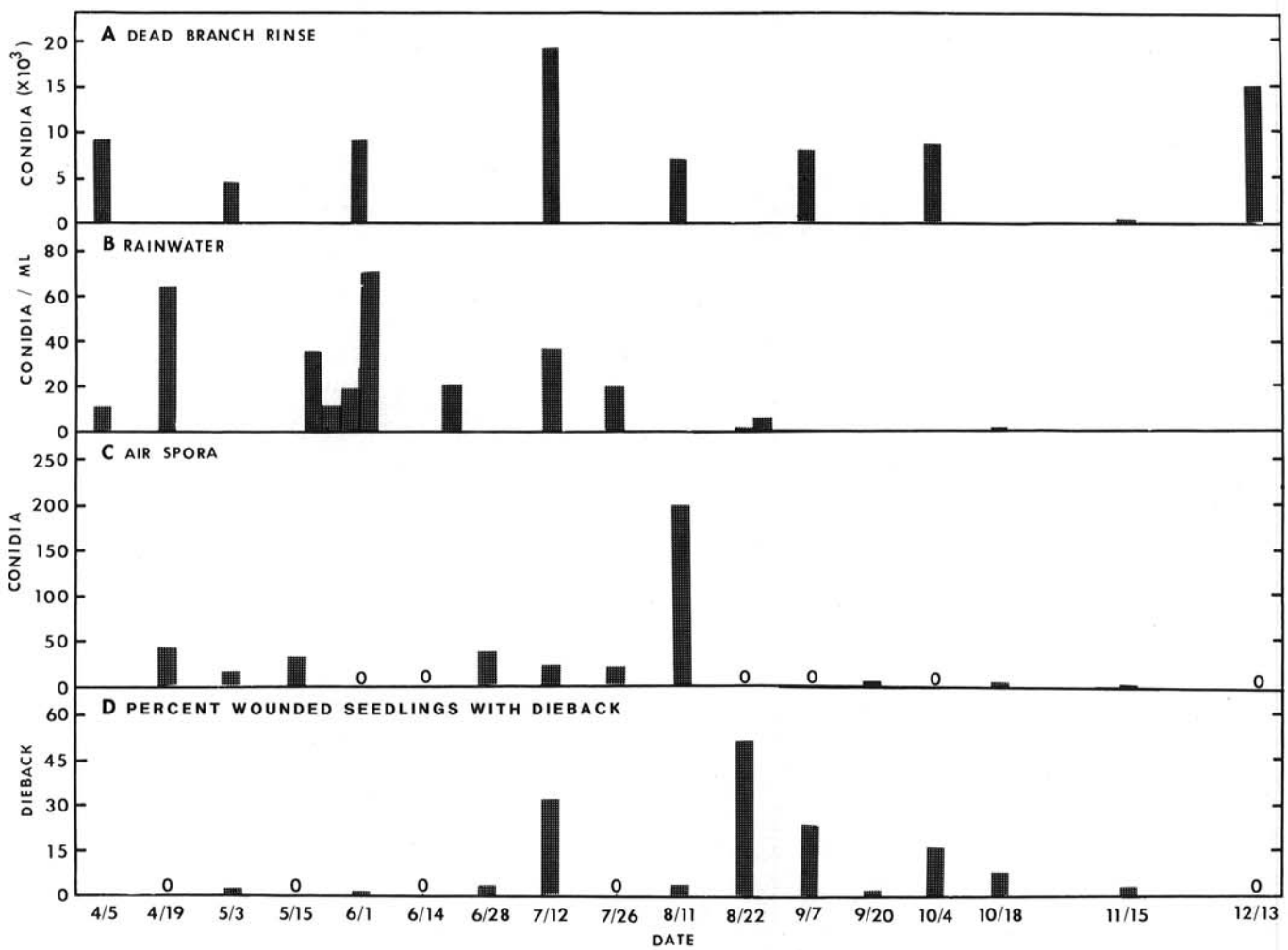

Fig. 2. Availability of conidia of *Fusarium moniliforme* var. *subglutinans* in a loblolly pine seed orchard at various intervals in 1977 as indicated by **A,** rinsing each of 20 dead branches in 50 ml of water (average conidia per branch); **B,** from canopy fall-through rainwater (conidia per milliliter of rainwater); **C,** exposing PCNB plates in air for 30 min (total colonies in 18 plates); and **D,** exposing 100 wounded pine seedlings for 2–4 wk (percent of seedlings with dieback).

inoculations were made throughout the year. Nineteen of the ramets were from susceptible clones. Fifty-eight percent of 228 inoculated branches on susceptible ramets died, whereas only 29% of 108 on intermediate and 4% of 24 branches on resistant ramets died. These differences were significant, $P = 0.01$, according to a chi-square test.

Availability of spores. Dead branches in the upper crowns of infected trees had an abundance of conidia of *F. moniliforme* var. *subglutinans* present in all months except November (Fig. 2A). Rain falling through the foliage of infected trees contained spores at every collection time, although the number per milliliter of water was relatively low (Fig. 2B). Plates exposed at intervals trapped some spores of *F. moniliforme* var. *subglutinans* on 10 of 15 sample dates (Fig. 2C). The greatest number of spores were trapped from the air on 11 August. Wounded pine seedlings placed in the orchard for 2 or 4 wk in 1977 subsequently had dieback due to *F. moniliforme* var. *subglutinans* on 11 of 17 treatment times (Fig. 2D). Fifty-two percent of the seedlings first exposed 22 August developed dieback due to the fungus.

Host development. When growth was first measured on 4 April 1977, the initial growth flush had already extended to 41–57% of its final length (Fig. 3). A second-growth flush had started by 17 May in the susceptible clones and by 31 May in the intermediate and resistant clones. Shoot growth slowed sooner in the resistant trees (after 14 June), but all trees had only very limited growth after 28 June. Branches on trees of intermediate susceptibility had significantly longer growth periods than did those in the other categories according to an analysis of variance. This is indicated by an 11% growth increase between 14 and 28 June.

The amounts of K, NH_4, and B were significantly greater in foliage from trees of intermediate susceptibility than in those from the other two susceptibility categories according to an analysis of variance. The level of Ca was significantly higher in susceptible clones than in resistant and intermediate clones. There were also statistically significant variations in nutrient levels by sample dates. However, all the variations were within the levels considered normal for loblolly pine.

Disease development. Disease development was observed on the 36 ramets on which host development was followed. The initial observations in April 1977 revealed a considerable number of nongirdling infections on 1976 stems near the tips. These initially nongirdling infections caused bud death and wilting and dying of the 1977 shoots. The amount of stem infected and the proximity to the buds determined how long the buds expanded before wilting and dying. Every 2 wk from 4 April until 23 August, one hundred 1977 shoots in the upper crown of each of the 36 trees were tallied as live or freshly killed. The percentage of freshly killed shoots in the susceptible trees increased until 15 May. Most 1976 infections had girdled the stem by 30 June, but scattered dieback from these infections occurred until 23 August 1977. Variation in susceptibility was apparent. Susceptible trees had a maximum dieback of 27%. Trees from clones with intermediate resistance had a maximum of 3% dieback. Trees from resistant clones were free of disease in 1977.

Early symptoms providing information about infection courts were sought on the 1977 growth. Wounds on 1976 needles caused by needle midges (*Contarinia* sp.) yielded *F. moniliforme* var. *subglutinans* in December 1976, but the fungus was not recovered from 75 needle midge wounds in 1977. Dead needle bundles had been associated with infections on the 1976 stem tissue, but only 3% of 75 dead needle bundles from 1977 stem tissue yielded the pathogen in July and August 1977. From September to December 1977, 47 branches with dead buds, resinosis, and/or dead needles were collected and only 30% yielded *F. moniliforme* var. *subglutinans*. These were the only branches on the 36 study trees that developed symptoms. In the spring of 1978, the disease was essentially absent from the orchard.

Cone and seed production. From 1973 to 1975, cone and seed production in the Piedmont source section of the orchard was stable (Table 2). From 1976 to 1978, production increased dramatically to record levels for the orchard. The resistant ramets made up only 28% of the orchard, but produced a plurality of the cones in 4 of 6 yr. Resistant ramets had the highest production per ramet in each of the 6 yr, but production differences were significant only in 1976 and 1977 due to the large variation in production by individual clones.

DISCUSSION

Variations in disease susceptibility among clones in the Piedmont source section of the loblolly pine orchard were detected in both the survey and the inoculation studies. The basis for this variation appears to be internal, since the 1978–1979 inoculations were more successful on susceptible than on resistant and intermediate trees. If resistance was due to the absence of infection courts, the inoculation experiment would have bypassed this defense. The inoculation data support the suggestion of others (6,7) that seedling inoculations could be used to test for resistance to this pathogen.

We expected pitch canker disease to have a drastic effect on cone and seed production. At the Weyerhaueser orchard, most susceptible trees lost half or more of the upper portions of their crown to dieback in 1975–1976. Since most cones are produced in upper portions of the crown, a large decline in cone production might have been expected in years when the disease was epidemic. In fact, cone production was at record levels on both resistant and susceptible clones during the height of the epidemic. Floral buds are present on the first growth flush, then after pollination the cones take 18 mo to mature. Thus, the record production in 1976 and 1977 started in 1975 and 1976 when pitch canker was epidemic.

Several factors must be considered to be interesting in these observations. First, variation in cone production among clones was tremendous. A few clones had heavy cone production every year, a

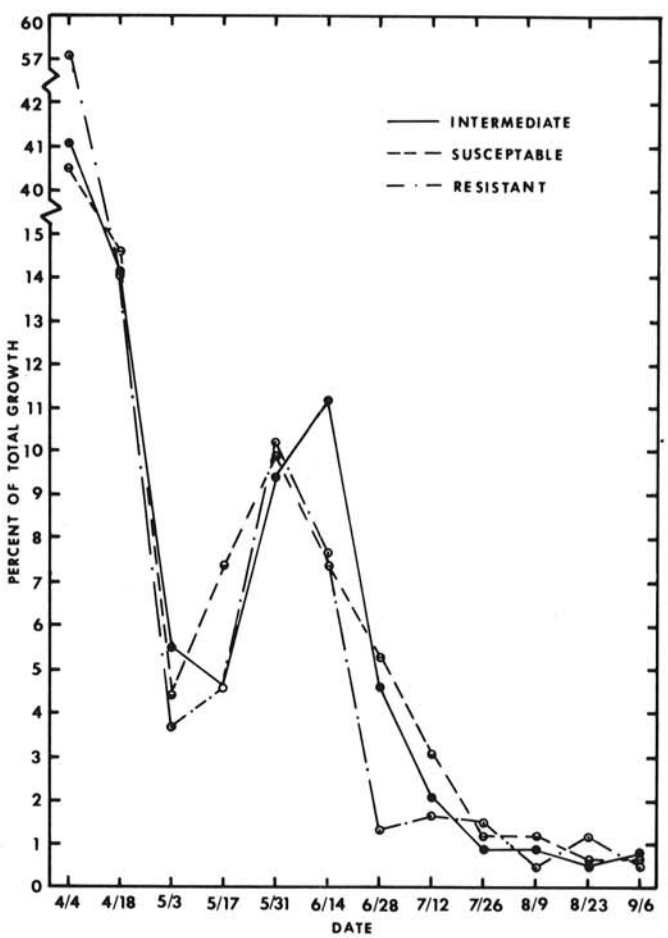

Fig. 3. Relative growth rate in 1977 of the terminal and four lateral branches of ramets in the three pitch-canker disease categories expressed as the percentage of total growth for 1977.

TABLE 2. Total and relative cone and seed production by the 550 ramets in the Piedmont source of loblolly pine at Washington, NC, from 1973 to 1978, presented by the susceptibility to pitch canker disease

Production measure	Clone type	Ramets (%)	1973	1974	1975	1976	1977	1978	Avg
Seed (kg)	All	100	127	123	137	331	628	609	
Cones (kl)[a]	All	100	8.7	5.3	6.0	12.9	24.5	27.0	
Cone production (%)	Resistant	28	45	35	58	52	63	37	48
	Intermediate	30	19	18	11	25	17	24	19
	Susceptible	42	38	47	30	23	20	40	33
Avg cones/ramet (liters)	Resistant	28	18	11	20	36[b]	92[c]	57	39
	Intermediate	30	7	4	2	14	21	34	14
	Susceptible	42	10	10	6	13	19	45	17

[a]Total cone yield (kl = kiloliters) per year.
[b]Significantly more cones per ramet than intermediate and susceptible clones, $P = 0.10$.
[c]Significantly more cones per ramet than intermediate and susceptible clones, $P = 0.003$.

few had infrequent, light production. The most productive clone had 16.4 kl in 6 yr, the least productive had 0.06. Second, the disease-resistant clones produced more cones per tree every year than did clones in the other disease categories, although the difference was statistically significant in only 2 yr. Therefore, even though the resistant clones were only 28% of the orchard population, they consistently produced most of the crop. Third, clones of intermediate disease susceptibility that had only an average of one to three diseased branches had low cone production (Table 2), further suggesting that the inherent cone-producing capacity of the clones rather than the disease intensity affected cone production. Finally, environmental factors conducive to the disease and to cone production occurred at the same time. The disease has a pruning effect and causes lateral buds to develop profusely. This effect could have enhanced cone production, but the resistant and intermediate sources also increased production. Pine seed orchard managers should note that even during a severe disease outbreak, record cone and seed production occurred. In contrast, a Mississippi orchard reported a dramatic reduction in cone production during a severe pitch canker outbreak (8). However, cone and seed production at the Mississippi orchard were presented for only 2 yr, and production by various disease susceptibility categories was not given.

Results of inoculations in various seasons show that infections can develop throughout the year, but that those occurring in fall and winter most frequently cause mortality. The long period of susceptibility, together with the erratic disease occurrence, suggest that chemical control is not practical.

Since most infections occurred on the youngest tissue, we thought susceptibility might be related to shoot phenology. No important relationships were found between disease susceptibility and shoot growth or mineral content of foliage.

Inoculum of *F. moniliforme* var. *subglutinans* was present throughout the 1977 growing season (Fig. 2). Reports from Florida indicate that 98% of the *F. moniliforme* var. *subglutinans* inoculum in slash pine stands is pathogenic to slash pine (2). The inoculum endemically present at Washington, NC, was sufficient to cause dieback of up to 52% of the wounded seedlings exposed in the orchard. New infections were rarely seen in 1977 and by 1978 the disease had essentially disappeared from the orchard. Unfortunately we were unable to determine the factors that were either ideal for disease development in 1975–1976 or were limiting in 1977–1978.

Large scars created by cone harvesting have been suggested as likely infection courts for entry for the pathogen. Infections on the 1976 shoots were usually on the second, noncone-bearing flush. These infections were on the youngest stem tissue near the buds, whereas cones generally occurred in the middle of the first flush. Thus, special precautions during cone harvest are not needed to limit this disease.

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