Effects of Carbofuran and Thiabendazole on Incidence of Pitch Canker of Loblolly Pine

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ABSTRACT

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In eastern North Carolina, the percentage of loblolly pine (*Pinus taeda*) seedling terminals damaged by pine tip moths (*Rhyacionia* spp.) was positively correlated with the percentage of terminals infected with the pitch canker fungus, *Fusarium subglutinans*. The insecticide carbofuran reduced terminal shoot infection by *F. subglutinans* and terminal shoot damage by pine tip moths, and seedlings were significantly taller than when no carbofuran was used. Multiple applications of the fungicide thiabendazole significantly lowered the percentage of infected terminals and resulted in significantly taller seedlings compared to no thiabendazole but did not affect damage by pine tip moths.

Pitch canker, caused by *Fusarium subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun, & Marasas (19), was recognized as an important problem on loblolly pine (*Pinus taeda* L.) in North Carolina in 1983 (13). The fungus can infect and damage virtually all vegetative and reproductive parts of most southern pine species throughout their lives (2,3,7,17). On loblolly pine in eastern North Carolina, *F. subglutinans* usually causes a dieback of terminal and lateral shoots in 4- to 8-yr-old plantation trees. Losses are attributed to reduction in growth, loss of form and, occasionally, mortality.

F. subglutinans requires a wound to gain ingress into a host (3), and Hepting and Roth (12) were the first to report that insects may serve as wounding agents. Matthews (15) was the first to report an association between pine tip moths (*Rhyacionia* spp.) and pitch canker incidence on slash pine (*P. elliottii* Engelm. var. *elliottii*). Several other insects have been suspected of playing a role in the epidemiology of the disease (5,10,28), but only the deodar weevil (*Pissodes nemorensis* Germar) (6) and engraver beetles (*Ips mexicanus* (Hopkins) and *I. paraconfusus* Lanier) (11) are proven vectors of the fungus.

While conducting studies in a plantation of severely infected 7-yr-old loblolly pines in eastern North Carolina, we observed that terminal and lateral shoots exhibiting typical pitch canker symptoms also exhibited obvious signs of tip moth infestation. *F. subglutinans* was isolated from an average of 91% of loblolly pine shoots exhibiting pine tip moth damage and from about 50% of larvae and 70% of pupae aseptically excised from these pine shoots (22). Because of the limited movement of larvae and because the motile stage (adults) does not damage pine tissues, pine tip moths are probably not vectors of *F. subglutinans* (15,16). However, they do play a major role in pitch canker on loblolly pine in eastern North Carolina by providing infection courts for the pathogen via larval feeding (16,22).

Pine tip moths are a prevalent and damaging pest of loblolly pine throughout the southeastern United States, with incidences in plantations approaching 100% (4). Carbofuran, a systemic insecticide, has been used to control several species of pine tip moths (8,27) and is applied in a controlled-release granular formulation to loblolly pine plantations in the southeastern United States when high tip moth infestation is anticipated. The insecticide has reduced pine tip moth infestations on loblolly pine seedlings (20).

Thiabendazole, a systemic and resid-

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ual fungicide, has been effective in reducing the incidence of diseases caused by *Fusarium* spp. (21) and other fungi (26) on various tree hosts. Thiabendazole has also been effective in reducing the impact of *F. subglutinans* on inoculated and naturally infected loblolly pine seedlings (23).

This study was undertaken to determine the effects of carbofuran and thiabendazole on the incidence of pitch canker and damage by pine tip moths on loblolly pine seedlings in eastern North Carolina.

MATERIALS AND METHODS

In March 1984, an area of about 3 ha in Beaufort County, NC, was planted to a single half-sib loblolly pine family with 1-yr-old nursery-grown seedlings. Plots of 36 seedlings in six bedded rows (2.5 m apart) with six seedlings along a bed (2 m apart) were established with two border rows and four buffer trees between plots. Because initial seedling survival was poor, the area was interplanted in April 1984.

Carbofuran (Furadan 10G) was applied just beneath the soil surface adjacent to each seedling at 0, 500, or 1,000 mg a.i. per tree the day after interplanting. Thiabendazole (Mertect 340F) was applied at 0, 14.2, 21.3, or 28.4 g a.i./L within the first week after interplanting. Thiabendazole was applied to the foliage until runoff (about 1 L of suspension per plot). Trees that did not receive thiabendazole were sprayed to runoff with sterile distilled water. Treatments were applied in all combinations of the carbofuran and thiabendazole concentrations in a randomized complete block design with three replicates.

The study was repeated in a modified form in March 1985 on a similar-size site in Washington County, NC. The 1985 study used the same treatments and experimental design as in 1984, except that no buffer trees were included between plots, a different half-sib loblolly pine family was used, and thiabendazole was applied every 4 wk from the first week after planting in March until November. In 1985, carbofuran was applied just beneath the soil surface adjacent to each seedling 3 days after planting.

In both years, the study sites received an aerial application of hexazinone (Velpar 4L) at 0.37 kg a.i./ha in May to control grasses and herbaceous vegetation. The sites were located about 100 and 200 m (1984 and 1985 studies, respectively) downwind from 8-yr-old loblolly pine plantations with high pitch canker incidence.

After the seedlings had been exposed to natural inocula of *F. subglutinans* for 9 mo, the number of dead trees was recorded for each plot, and the percentage mortality was determined. Tree height was recorded for surviving trees, and the terminal shoot of each was evaluated for signs of tip moth damage. After the tip moth evaluations were completed, the terminal shoot of live trees was removed. In 1984, all live trees in each plot were sampled in this way. In 1985, only the central four trees from each row were sampled for each plot; the remaining trees were considered buffer trees.

A 4- to 5-cm section was cut from each terminal and surface-disinfested for 10 min in a continuously stirred solution of 1.05% NaOCl, 1 ml of lactic acid per liter, and 1 ml of Tween 20 per liter. The sections were then rinsed three times in sterile distilled water and placed on Nash and Snyder selective medium (18). Petri plates containing the shoot sections were incubated under ambient laboratory conditions (approximately 20 C) for 10 days, at which time the presence or absence of *F. subglutinans* was determined with a compound microscope (19).

Arbitrarily selected colonies of *F. subglutinans* from 5 and 10% (1984 and 1985, respectively) of the terminals were tested for pathogenicity in the greenhouse as follows: a droplet (5 μl) of spore suspension (10^6 spores per milliliter) was placed into a wound on the terminal shoot of a 6-mo-old loblolly pine seedling from a half-sib family known to be susceptible to pitch canker. Terminals that yielded *F. subglutinans* in culture were assumed to be infected by the fungus.

In both years, study sites were visited monthly. To verify the presence of *F. subglutinans* inocula, during each visit 20 petri plates of Nash and Snyder *Fusarium*-selective medium (18) were exposed horizontally, 0.2–1.0 m above the ground, for 3 hr. Subsets of colonies of *F. subglutinans* from the trap plates were tested for pathogenicity as described previously.

Mean-to-variance plots for all data demonstrated no heterogeneity of variance, and thus the data were not transformed before analysis. Data were analyzed with the general linear models procedure (25) and the correlation procedure (24) of the Statistical Analysis Systems. Except where otherwise stated, values were considered to be significant if they differed at the $P < 0.05$ level.

RESULTS

Terminal infections. Use of carbofuran reduced the percentage of loblolly pine terminals infected with *F. subglutinans* (Table 1). In 1984, only the highest concentration of carbofuran reduced the percentage of infected terminals, and there was a significant linear response of disease incidence to carbofuran concentration. In 1985, both concentrations of carbofuran tested reduced the percentage of infected terminals, and there were significant linear and quadratic responses of disease incidence to concentration of the insecticide.

All concentrations of thiabendazole reduced the percentage of terminals infected with *F. subglutinans* in 1985 (Table 1). There were significant linear and quadratic responses of disease incidence to thiabendazole concentration. There was no significant interaction of thiabendazole with carbofuran with respect to infection by *F. subglutinans*.

In both years, the percentage of terminals infected by *F. subglutinans* was negatively correlated with increasing concentrations of carbofuran ($r = -0.464$ and -0.569 for 1984 and 1985, respectively) and thiabendazole ($r = -0.319$ and -0.636). The percentage of infected terminals was positively correlated with the percentage of terminals damaged by *Rhyacionia* spp. ($r = 0.588$ and 0.440 for 1984 and 1985, respectively).

Tip moth damage. In both years, significantly lower percentages of loblolly pine terminals were damaged by pine tip moths when either concentration of carbofuran was applied compared to no carbofuran treatment (Table 1). There were significant linear and quadratic responses of tip moth incidence to carbofuran concentration in both years. Thiabendazole had no effect on tip moth damage in either year, and there were no interactions of carbofuran with thiabendazole affecting tip moth damage in either year.

In both years, the percentage of terminals damaged by pine tip moths was positively correlated with the percentage of terminals infected by *F. subglutinans*, as previously described. The percentage of terminals damaged by pine tip moths was negatively correlated with carbofuran concentration ($r = -0.853$ and -0.623 for 1984 and 1985, respectively).

Seedling height. Both concentrations of carbofuran resulted in significantly taller seedlings in both years (Table 1). There was a significant linear response of loblolly pine seedling height to carbofuran in 1984. In 1985, linear and quadratic responses of seedling height to carbofuran were significant. The highest concentration of thiabendazole resulted in taller seedlings in 1985 (Table 1), and there was a significant linear response of height to thiabendazole.

Seedling height was negatively correlated with the percentage of terminals damaged by pine tip moths in 1984 ($r = -0.565$) and 1985 ($r = -0.304$; $P = 0.0597$). Seedling height was also negatively correlated with the percentage of terminals infected with *F. subglutinans* in 1985 ($r = -0.533$). Height was positively correlated with carbofuran concentration in both years ($r = 0.480$ and 0.667 for 1984 and 1985, respectively) and with thiabendazole concentration in 1985 ($r = 0.333$). There was no significant interaction of thiabendazole with carbofuran affecting loblolly pine seedling height in either year of study.

Mortality. No seedling mortality in

either year of study could be reliably attributed to *F. subglutinans*. The various treatments did not differ significantly in the percentage of mortality at the end of the experiment in either year.

Pathogenicity tests. Viable inocula of *F. subglutinans* were collected on each visit to the study sites. The average number of *F. subglutinans* colonies formed per petri plate for each visit ranged from 0.5 to 2.8 in 1984 and from 0.2 to 1.6 in 1985. Pathogenic isolates of the fungus were collected during each visit, but only 40% of the aerially trapped isolates tested were virulent (resulted in tissue necrosis) when inoculated onto susceptible loblolly pine seedlings. However, most (85–90%) isolates of *F. subglutinans* recovered from terminal shoots produced symptoms (either terminal necrosis or discolored lesions around the point of inoculation) when inoculated onto susceptible loblolly pine seedlings.

DISCUSSION

The fact that carbofuran lowered the incidence of pine tip moth damage and the incidence of infection by *F. subglutinans* suggests a direct relationship between the moths and *F. subglutinans* and supports the hypothesis that pine tip moths play a role in the epidemiology of the pitch canker disease complex in eastern North Carolina. In this and other studies (G. B. Runion and S. C. Cade, *unpublished*), the percentages of tip moth-infested loblolly pine terminals infected by *F. subglutinans* were consistently high. However, *F. subglutinans* infection persisted even when tip moth damage was reduced with carbofuran, which indicates that the use of this insecticide shifted the primary wounding agent associated with *F. subglutinans* infection from pine tip moths to some other biotic or abiotic agent. The quadratic effects observed for carbofuran suggest that doubling the concentration of the insecticide resulted in limited additional reductions in disease and insect incidence and a limited increase in loblolly pine seedling growth above that obtained with 500 mg.

Only repeated foliar applications of the fungicide thiabendazole lowered the percentage of terminals infected with *F. subglutinans*. The reduction in infection incidence provided by thiabendazole beyond that provided by carbofuran suggests that the fungicide protected the seedlings from fungal infections associated with wounding agents other than pine tip moths. The nature of these other wounding agents and their roles in the infection processes of pitch canker on loblolly pine need to be elucidated.

Although thiabendazole reduced the incidence of terminal shoots infected by *F. subglutinans*, the reductions in disease incidence observed in this study are not considered sufficient to warrant its use. Application of thiabendazole on a 4-wk spray schedule would not be economical in most forestry situations regardless of its efficacy. However, the two higher concentrations of thiabendazole tested in this study reduced disease incidence for up to 12 wk (23), and effects on pitch canker incidence similar to those in this study might be obtained with a less frequent spray schedule. The quadratic effect of the fungicide on disease incidence suggests that the 21.3-g concentration was the most effective, and this hypothesis is supported by previous work with this compound (23).

Recording pitch canker symptoms on the terminal shoots collected in the fall in this study would have underestimated disease incidence because symptom expression begins in the fall and continues through the winter and spring (9). Some of the isolates of *F. subglutinans* recovered from pine terminals could have come from inocula that escaped disinfestation within tip moth larval galleries or within other wounds rather than from infected tissue. However, because most of the recovered isolates tested were pathogenic on loblolly pine seedlings, isolation of the fungus from surface-disinfested shoots should give a more accurate and reliable indication of infection than visual assessment of symptom expression in the fall.

The effects of the two pesticides in increasing loblolly pine seedling height probably resulted from protection from the insect and/or the fungus. The significant negative correlations observed for seedling height with percentage of terminals damaged by pine tip moths and with percentage of terminals infected with *F. subglutinans* support this conclusion. The increased growth might also be due in part to the ability of one or both of the systemic pesticides to act as growth regulators in loblolly pine tissues (1,14).

Carbofuran reduced the incidence of pine tip moth damage and reduced infection by *F. subglutinans* associated with tip moth damage on loblolly pine seedlings during the first year after outplanting in eastern North Carolina. Limited additional reductions in disease incidence were achieved with repeated foliar applications of thiabendazole. These data may have application for

Table 1. Percentage of loblolly pine terminal shoots infected with *Fusarium subglutinans*, percentage of terminal shoots damaged by *Rhyacionia* spp., and average height of loblolly pine seedlings in 1984 and 1985[w]

Chemical	Chemical concentration[x]	Infected terminals[y] (%)		Damaged terminals[y] (%)		Seedling height[y] (cm)	
		1984	1985	1984	1985	1984	1985
Carbofuran	0	48.7	74.7	48.8	32.2	52.9	54.5
	500	36.9	51.2*	14.4*	11.7*	59.3*	64.1*
	1,000	30.3*	51.1*	8.8*	10.3*	61.3*	66.3*
Source	df	Pr > *F*[z]	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Main Effect	2	0.0220	0.0001	0.0001	0.0001	0.0022	0.0001
Linear	1	0.0064	0.0001	0.0001	0.0001	0.0009	0.0001
Quadratic	1	0.8730	0.0057	0.0001	0.0086	0.2885	0.0162
Thiabendazole	0	49.6	77.4	34.7	20.1	55.0	57.6
	14.2	32.0	59.5*	21.3	18.2	57.2	62.1
	21.3	35.6	45.6*	24.7	20.3	61.2	61.1
	28.4	37.2	52.6*	20.1	17.8	57.1	64.5*
Source	df	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Main effect	3	0.1540	0.0001	0.0929	0.7749	0.2100	0.0358
Linear	1	0.2941	0.0001			0.3548	0.0111
Quadratic	1	0.0862	0.0020				
Interaction	6	0.0968	0.8107	0.6955	0.3847	0.1819	0.2322

[w] Carbofuran data are based on 350–400 and 250–300 terminals for 1984 and 1985, respectively; thiabendazole data are based on 250–300 and 180–220 terminals for 1984 and 1985, respectively.

[x] In mg a.i. per tree for carbofuran and g a.i./L for thiabendazole.

[y] Asterisks (*) indicate treatments that differed from the zero concentration of the respective chemical at $P \leq 0.045$ according to contrasts under the general linear model procedure. The 0.045 level was used to assess significance because of nonindependence of contrasts.

[z] Significance levels are the probability of a greater *F* value. Significance values for higher-order terms omitted from the table were not significant for either year.

growers of Virginia pine (*P. virginiana* Mill.) for Christmas trees, because the use of pesticides would be more economical for this more valuable tree crop.

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