Laminar Necrosis, Growth Inhibition, and Death of Tobacco Plants Caused by Toxic Extracts of Phytophthora cryptogea

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ABSTRACT

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Extracts of oat cultures and of mycelium of *Phytophthora cryptogea* grown on glucose-glutamate liquid medium in shake cultures were toxic to tobacco plants. The severity of growth inhibition and foliar necrosis increased with time of exposure of the roots to extracts. Long (60 min) exposures of the roots to the extracts often resulted in death of tobacco plants. Burley and flue-cured tobacco types, either resistant or susceptible to the black shank disease, were affected by the extracts. Even though the black shank pathogen, *P. parasitica* var. *nicotianae*, reduced growth and killed plants transplanted into infested soil, extracts of oat cultures of this fungus were not toxic. Growth inhibition of tobacco seedlings caused by these two fungi appears to be caused by different mechanisms.

Isolates of the Pythiaceae have the ability to reduce the growth of a variety of crop plants without the development of typical root and stem necrosis associated with these fungi (1, 5, 6, 8). One of the most active of these fungi, an isolate of *Phytophthora cryptogea* Pethyb. & Laff., caused a severe reduction in growth (1, 5, 8) and necrotic foliar symptoms (1) in commercially grown cultivars of tobacco. Soilborne populations of the fungus did not increase in the presence of stunted plants (8) as they do in host-pathogen combinations in which parasitism is known to occur (3, 4).

Tobacco plants were reduced in growth when planted into soil amended with nonviable, lyophilized powdered mycelium as well as in soil infested with the living fungus (1). These results suggested that a toxin was involved in the reduction in growth of tobacco. The present study further investigates the stunting phenomenon and attempts to distinguish the disease caused by a known parasite of tobacco, *P. parasitica* var. *nicotianae*, from the disease caused by *P. cryptogea*, a documented non-parasite of tobacco (9,10).

MATERIALS AND METHODS

Phytophthora cryptogea isolate 147 isolated from bean in Kentucky (2) and *P. parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker race 0, isolate 42 (6) isolated from burley tobacco were used in this study.

Phytophthora cryptogea was grown on glucose-glutamate medium, and the mycelium was separated from the filtrate as previously described (1). The mycelium was lyophilized, weighed, sealed in plastic bags, and stored in a freezer. Mycelial extracts of *P. cryptogea* were prepared by blending lyophilized mycelium at the rate of 1 g lyophilized mycelium to 50 ml of deionized water in a Waring Blendor for 30 sec on high speed. The mixture was filtered through four layers of cheesecloth, and the filtrate was acidified to pH 2.5 - 3.0 with 1N HCl and centrifuged in 200-ml plastic bottles for 20 min at 8,000 *g*. The clear supernatant liquid was decanted and stored in the freezer.

In other experiments, *P. cryptogea* and *P. parasitica* var. *nicotianae* were cultured on oats (6). Infested soil was prepared by blending oat cultures with a minimum of water and mixing the slurry with steam-pasteurized soil at the rate of two and one-half petri dish cultures/kg as previously described (6).

Extracts of oat cultures were prepared from 2-wk-old cultures of the fungi by blending one petri dish culture with 200 ml of deionized water in a Waring Blendor on high speed for 30 sec. A clear extract was prepared as described above.

The extracts were sterilized either by autoclaving or by filtering through a 0.45-μm Millipore filter. The toxic property of the extracts was found to be stable to autoclaving or storage at acid pH, but not at pH 8. All extracts were adjusted to pH 4.0 before treatments.

Tobacco plants were grown in black 50-ml tubes filled with sand (1). Plants with roots washed free of sand were graded for uniformity in weight within experiments. Initially, plants were 6-8 wk old and weighed 1.5 - 3.0 g.

Tobacco seedlings with roots immersed in 5 ml of extract in 10-ml vials (Fig. 4) were incubated at 27 C in a growth chamber with overhead fluorescent lights emitting an average of 15,300 lux at the plant surface. The

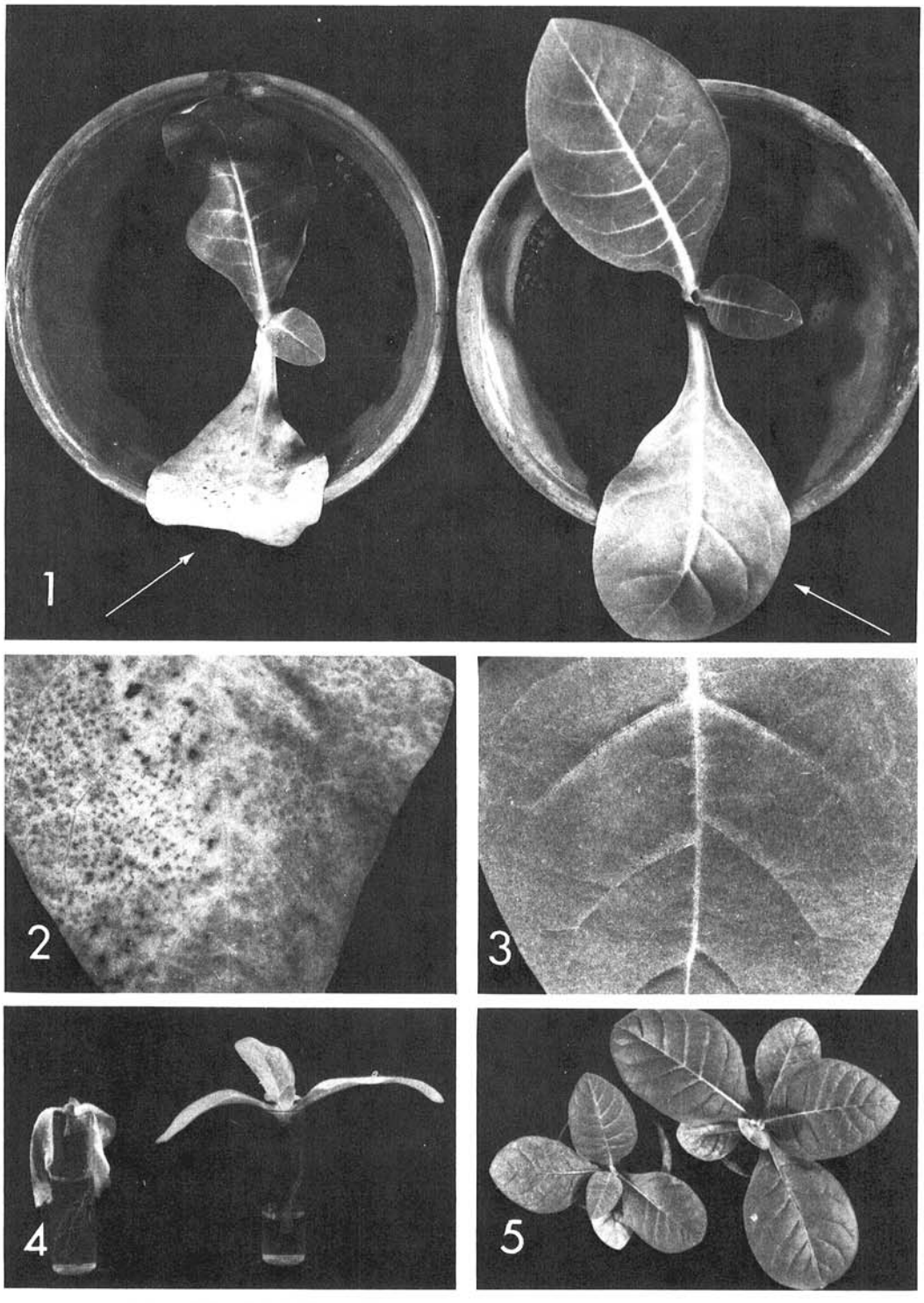

Fig. 1-5. Effect of extracts of mycelium of *Phytophthora cryptogea* on Burley 21 tobacco plants. **1)** Plants 3 days after 60-min root exposure to mycelial extract. Left: plant treated with active extract. Right: plant treated with extract inactivated by autoclaving at pH 8. **2)** Enlargement of leaf from Fig. 1, left (arrow), showing chlorosis, necrosis, and laminar collapse. Leaf was fully expanded at the time of treatment. **3)** Enlargement of leaf from Fig. 1, right (arrow). Leaf was fully expanded at the time of treatment. **4)** Effect of 24-hr exposure to diluted (1:25) active (left) and inactivated (right) extracts on intact tobacco plants. **5)** Effect of 20-min of exposure to active (left) and inactivated (right) extracts on growth of plants. Plants were treated, transplanted into soil, and grown 24 days.

plants were incubated in the chamber for 10 to 60 min. The roots were rinsed with deionized water, and the plants were transplanted into 400 g of steam-pasteurized soil (7) and incubated in the greenhouse (1).

RESULTS

Growth of Burley 21 transplants was reduced by exposures of roots to the mycelial extract (Table 1). Plants whose roots were immersed in the extract for 60 min usually developed necrosis of the veins and collapse of the laminae within a few days (Fig. 1-3), and some of them died. Plants treated with diluted extract for 24 hr were killed (Fig. 4). Surviving plants later appeared normal except for reduction in size (Table 1, Fig. 5). Plants treated for shorter periods had less necrosis and were not as severely reduced in growth. Plants treated for the shortest time (10 min) did not develop laminar symptoms, but they were slightly reduced in growth.

Four *Nicotiana tabacum* cultivars were reduced in growth by the mycelial extract (Table 2). Some of the more severely stunted plants developed mild veinal necrosis and laminar collapse soon after they were treated, but subsequent growth was free of the symptoms. Tobacco plants treated with mycelial extracts autoclaved at pH 8.0 displayed no necrosis or stunting.

Seedlings transplanted into soil infested with *P. cryptogea* were severely reduced in growth (Table 3). In a few cases, minor veinal necrosis and laminar collapse occurred, but plants did not have stem lesions or obvious root necrosis. Tobacco transplanted into soil infested with *P. parasitica* var. *nicotianae* developed typical black shank symptoms, lower stem lesions, and root necrosis,

TABLE 1. Effect of an extract of mycelium of *Phytophthora cryptogea* produced on glucose-glutamate medium on growth and survival of tobacco (cultivar Burley 21)

		Experiment 1[a]		Experiment 2[b]		Experiment 3[a]	
Solution	Uptake time (min)	Shoot weight (g)	Plants killed/ plants treated (no.)	Shoot weight (g)	Plants killed/ plants treated (no.)	Shoot weight (g)	Plants killed/ plants treated (no.)
Mycelial extract[e]	10	9.26 ± 0.81[c]	0/10	11.52 ± 0.72	0/9	5.74 ± 0.69	0/7
	20	6.85 ± 0.93[d]	1/10	11.52 ± 0.45	0/9	4.57 ± 0.45	0/7
	40	2.02 ± 0.43	0/10	7.93 ± 1.24	0/9	1.43 ± 0.52[d]	1/7
	60	3.59 ± 0.88[d]	3/10	5.93 ± 0.90	0/9	1.23 ± 0.19[d]	4/7
Inactivated mycelial extract control[e,f]	60	12.06 ± 0.37	0/10	13.68 ± 0.93	0/9		
Water control	60	11.40 ± 0.79	0/10	14.22 ± 0.52	0/9	7.53 ± 0.78	0/7
LSD ($P=0.05$)		1.97		2.39		1.98	

[a]Plants were grown 14 days. Values for LSD are for means with 10 (experiment 1) or seven (experiment 3) replications.
[b]Plants grown 21 days.
[c]Values are means followed by standard errors.
[d]Values are means of surviving plants.
[e]Extract was sterilized by filtration (experiments 1 and 2) or autoclaving at pH 4 (experiment 3).
[f]Extract was autoclaved at pH 8 and readjusted to pH 4.

TABLE 2. Effect of 60-min uptake of an extract of mycelium of *Phytophthora cryptogea* grown on glucose-glutamate medium on growth of seedlings of different tobacco cultivars

	Cultivar shoot weight			
Solutions	Burley 21[a] (g)	L8[b] (g)	Virginia 115[c] (g)	Hicks Broadleaf[c] (g)
Mycelial extract[e]	2.0 ± 0.4[d]	5.0 ± 1.2	8.8 ± 0.7	10.5 ± 1.6
Inactivated mycelial extract control[e,f]	9.5 ± 1.1	11.4 ± 1.2	15.5 ± 0.7	15.3 ± 0.7
Water control	9.4 ± 1.0			
LSD ($P = 0.05$)	2.7	1.6	2.1	4.0

[a]Burley 21 is a burley cultivar. Plants, five replications per treatment, were grown 14 days.
[b]Cultivar L8 is a burley cultivar with black shank disease resistance derived from *Nicotiana longiflora*. Plants grown 18 days, six replications per treatment.
[c]These are flue-cured tobacco cultivars, Virginia 115 with Florida-301-type resistance to black shank. Plants grown 18 days, nine replications per treatment.
[d]Values are means, followed by standard errors.
[e]Extract was sterilized by filtration.
[f]Mycelial extract was adjusted to pH 8 with NaOH and autoclaved.

and were either dead or close to death at the termination of the experiment.

Tobacco plants treated with an extract of the oat culture extract of *P. cryptogea* developed severe veinal necrosis and laminar collapse (Table 4, Fig. 1-3). Only one plant of 20 treated survived; the remainder died within a few days. Tobacco plants treated with the oat culture extract of *P. parasitica* var. *nicotianae* were indistinguishable from the controls.

DISCUSSION

The significance of these findings cannot be determined at present because of lack of information on the distribution and ecology of *Phytophthora cryptogea*. On the gallic acid selective medium, the growth characterisitcs of *P. cryptogea* resemble those of *Pythium* spp. more than those of related fungi such as *Phytophthora parasitica* var. *nicotianae*. Therefore, *P. cryptogea* would not be detected in natural soils unless individual colonies were isolated and grown on media appropriate for propagule formation. Furthermore, the presence of enough mycelium of *P. cryptogea* in soil to inhibit plant growth must be accounted for if we hypothesize that *P. cryptogea* may be a cause of poor survival of tobacco transplants or poor growth of those that survive (5) without parasitizing tobacco plants (1) or increasing in soilborne populations (8). *Pythium* spp. occur in tobacco soils in high populations (3), but whether they are there because of parasitism of tobacco or other plants or by saprophytism is not known. Similarly, nothing is known of the saprophytic capabilities of *Phytophthora cryptogea*. The present studies show that a short exposure (20 min) to a small amount of fungal material can have long-lasting effects on plant growth.

These experiments show that both *P. cryptogea* and *P. parasitica* var. *nicotianae* can kill or inhibit the growth of tobacco plants, but the mechanisms of pathogenesis are different. A mycelial toxin can produce all the symptoms produced by living cultures of *P. cryptogea*, but extracts

TABLE 3. Effect of soil infested with *Phytophthora cryptogea* and *P. parasitica* var. *nicotianae* on growth and survival of tobacco plants

	Cultivar shoot weight and survival			
	Burley 21[a]		White Burley[a,b]	
Soil amendments[c]	Shoot weight (g)	Plants killed/ plants treated (no.)	Shoot weight (g)	Plants killed/ plants treated (no.)
P. cryptogea oat cultures	4.9 ± 0.4[d]	0/10	2.5 ± 0.2	0/10
P. parasitica var. *nicotianae* oat cultures	2.0 ± 0.4[e]	1/10	2.4 ± 1.2[e]	7/10
Noninoculated oat medium	9.8 ± 0.6	0/10	8.5 ± 0.7	0/10
LSD ($P = 0.05$)	1.4		1.6	

[a]Plants were grown 21 days. Values for LSD are for means with 10 replications.
[b]"White Burley, mosaic strain," was maintained for virus research in the Netherlands.
[c]Soil was amended with 2.5 petri dish cultures/kg soil.
[d]Values are means, followed by standard errors.
[e]Values are means of shoot weight of surviving plants.

TABLE 4. Effect of 60-min uptake of extracts of oat cultures of *Phytophthora cryptogea* or *P. parasitica* var. *nicotianae* on growth and survival of tobacco plants

	Cultivar shoot weight and survival			
	Burley 21[a]		White Burley[a]	
Extract[b]	Shoot weight (g)	Plants killed/ plants treated (no.)	Shoot weight (g)	Plants killed/ plants treated (no.)
P. cryptogea	1.5[c]	9/10	0	10/10
P. parasitica var. *nicotianae*	13.7 ± 0.8[d]	0/10	15.5 ± 0.6[d]	0/10
Noninoculated oats	14.1 ± 0.5	0/10	16.0 ± 0.9	0/10
LSD ($P = 0.05$)	2.0		2.4	

[a]Plants were grown 21 days. Values for LSD are for means with 10 replications.
[b]Extracts were sterilized by filtration.
[c]Value is the weight of the surviving plant.
[d]Values are means followed by standard errors.

of *P. parasitica* var. *nicotianae* cultures were not toxic. These experiments do not eliminate toxins in the mode of pathogenesis by *P. parasitica* var. *nicotianae*. However, if this fungus produces a toxin, it does not appear to be the same as that produced by *P. cryptogea*.

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