Oxamyl-Treated Soil Protects Tobacco Against Black Shank

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ABSTRACT

Oxamyl (methyl *N'* ,*N'*-dimethyl-*N*[(methylcarbamoyl) oxy]-1-thiooxamimidate) at rates of 5.58, 11.16, and 22.32 kg active ingredient (a.i.)/hectare (= 1, 2, and 4 lb a.i./acre, respectively) was applied to field plots of sandy loam, pH 5.5, in May. Soil samples were removed to the greenhouse at 30-day intervals over a 4-month period, planted with tobacco Windsor Shade 117 (WS 117), and inoculated 1 week later with approximately 10^4 zoospores of a Connecticut isolate of *Phytophthora parasitica* var. *nicotianae*. Seedlings of WS 117 tobacco transplanted into soils that were treated up to 90 days earlier with 22.32 kg (a.i.) oxamyl/hectare did not develop black shank symptoms, while 11.16 kg (a.i.)/hectare gave intermediate protection and 5.58 kg (a.i.)/hectare afforded no protection over this time period. Results from greenhouse studies indicated that a 2-week period is required after soil is treated with oxamyl before seedlings are protected against the pathogen. Tobacco seedlings grown up to 21 days in oxamyl-treated soil and then transplanted into nontreated soil for 1 week prior to inoculation developed black shank symptoms. In vitro studies showed that oxamyl concentrations as high as 100 μg active ingredient/ml (= 548.34 kg active ingredient/hectare) are not fungicidal or inhibitory to zoosporangia production. Results suggest that protection against black shank is due to breakdown products of oxamyl.

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Additional key words: nematicide, soil-borne pathogen.

Black shank of tobacco, caused by *Phytophthora parasitica* (Dast.) var. *nicotianae* (Breda de Haan) Tucker, is a destructive disease which occurs in all warmer climates where tobacco is grown (11), and has recently been identified in Connecticut (20). The use of moderately resistant cultivars (3, 8, 17), crop rotation (4, 5, 6), and chemical soil treatments (14) have been used to minimize the effects of the pathogen. Difficulty in obtaining both high resistance and suitable commercial value in the same tobacco cultivar (9), the development of new races of the pathogen which overcame resistance to black shank in certain tobacco cultivars (1, 2, 10, 16, 19), and the impracticality of crop rotation in areas where tobacco is intensely grown limit the effectiveness of these methods.

Chemical treatments for black shank control, usually with preplant soil fumigants, have been only marginally successful (18), although Noveroske (13) has recently shown that Dowco 269, [2-chloro-6-methoxy-4-(trichloromethyl) pyridine] as a transplant treatment in combination with topical spray treatment, is apparently capable of providing near season-long protection. Soil treatment with oxamyl (methyl *N'*,*N'*-dimethyl-*N* [(methylcarbamoyl) oxy]-1-thiooxamimidate), a contact nematicide-insecticide (15), appears to protect tobacco against *P. parasitica* var. *nicotianae* (12), and current studies indicate that this material may provide almost season-long control of this pathogen.

MATERIALS AMD METHODS.—A Connecticut isolate of *P. parasitica* var. *nicotianae* (isolate M-15 T) was used. The fungus was grown on fresh lima bean agar for 1 week. The agar containing fungal growth was cut into 5 cm square slabs and transferred to water agar plates and incubated until abundant zoosporangia were present. Zoospores were released using a modification of the method of Dukes and Apple (7). A sample of the zoospore suspension was inactivated by heating at 60 C for 15 seconds, and was counted with a hemacytometer.

Field plots (fine sandy loam soil, pH 5.5) were treated 15 May 1974 with oxamyl (= Vydate 10 G, E. I. DuPont de Nemours and Co., Wilmington, Del.) at rates of 0, 5.58, 11.16, and 22.32 kg active ingredient/hectare (= 0, 1, 2, and 4 lb active ingredient/acre). The soil was rototilled to a depth of approximately 7.5 cm. The treated plots were planted with Consolidated L, a tobacco cultivar which is moderately resistant to the Connecticut isolate of *P. parasitica* var. *nicotianae*, on June, 1974. Rainfall during June and July was near normal (6.05 and 9.08 cm, respectively), but early August was extremely dry, so the plot was irrigated with approximately 2.0 cm of water on 14 August 1974, with an additional 1.25 cm on 16 August 1974. Rainfall during the remainder of August was 11.0 cm.

Soil samples were removed from the field plots every 30 days for 120 days after oxamyl treatments. The samples were placed separately in 355-ml (12-ounce) styrofoam cups, and each cup was planted with a single 6- to 8-week-old seedling of Windsor Shade (WS) 117, a tobacco cultivar which is susceptible to the Connecticut isolate of the pathogen. Five plants were used for each soil sample, and were inoculated 1 week later by pipetting 1 ml of a fresh zoospore suspension (10^4/ml) around the base of each plant. The plants were maintained in the greenhouse (26 ± 3 C) for an additional 4 weeks, during which time they were observed for black shank symptom expression. At the termination of each experiment, plants were removed from the soil and observed for root symptoms. In all experiments plants showing any black shank symptoms were counted as diseased plants.

In November 1974, field soil which previously had been fallow was brought into the greenhouse and portions

TABLE 1. Effect of oxamyl soil treatment on the development of black shank of tobacco caused by *Phytophthora parasitica* var. *nicotianae*

Time between soil treatment in field and planting in a greenhouse[a] (days)	Oxamyl (kg active ingredient per hectare)	Number of plants with black shank symptoms at indicated weeks after inoculation		
		1	2	4
30	0	5	5	5
	5.58	1	5	5
	11.16	1	3	3
	22.32	0	0	0
60	0	5	5	5
	5.58	3	5	5
	11.16	1	2	2
	22.32	0	0	0
90	0	5	5	5
	5.58	4	5	5
	11.16	1	2	2
	22.32	0	0	0
120	0	5	5	5
	5.58	5	5	5
	11.16	2	5	5
	22.32	0	5	5

[a]Field soils were treated with oxamyl 10 G and rototilled to a depth of approximately 7.5 cm. Samples were taken to the greenhouse at 30-day intervals, planted with a single 6- to 8-week-old tobacco seedling of cultivar WS 117, and inoculated 1 week later with 10^4 zoospores from *P. parasitica* var. *nicotianae*.

[b]Five plants per treatment.

TABLE 2. Effect of oxamyl-treated soil in the greenhouse on the development of black shank of tobacco, caused by *Phytophthora parasitica* var. *nicotianae*, 4 weeks after inoculation[a]

Time of inoculation after soil treatment (weeks)	Number of plants with black shank symptoms in soil treated with oxamyl[b]		
	0 mg/kg	2 mg/kg	4 mg/kg
0	10	10	10
1	10	10	10
2	8	8	2
4	10	10	0
8	10	10	10

[a]Soil mixed with oxamyl 10 G in the greenhouse was planted with 6-week-old tobacco seedling of tobacco cultivar WS 117, and inoculated with 10^4 zoospores of *P. parasitica* var. *nicotianae*.

[b]Ten plants per treatment, and the experiment was repeated one time. Data are presented as the average number of plants with black shank symptoms for the two experiments. Oxamyl concentrations were based on active ingredient.

mixed with oxamyl at rates of 0, 2, and 4 mg active ingredient/kg (= 0, 11.16, and 22.32 kg active ingredient/hectare). Samples were placed separately in 473-ml (16-ounce) styrofoam cups and immediately planted with 6-week-old WS 117 seedlings, one plant per cup. At transplanting time and 1, 2, 4, and 8 weeks later, plants were inoculated by the method described in the previous experiment. Plants were maintained in the greenhouse (26 ± 3 C) under supplemental fluorescent lighting (12-hour daylength) for the duration of the experiment. Plants were observed for black shank symptoms, and 4 weeks after inoculation they were removed from the soil and observed for root damage. Ten plants were used per treatment, and the experiment was repeated one time. Variation in the number of plants with black shank symptoms was ± 1 for the two experiments. Therefore data are presented as the average number of plants with black shank symptoms for all observations.

To determine if oxamyl protected WS 117 against black shank after plants were removed from oxamyl-treated soil, 6-week-old WS 117 seedlings were planted singly into 237-ml (8-ounce) styrofoam cups containing a sterile potting mixture of soil:peat:sand (1:1:1,v/v) pH 5.5, which had been treated that same day with oxamyl at rates of 0, 4, or 8 mg a.i./kg (0, 22.32, or 44.64 kg active ingredient/hectare). Plants were grown in the greenhouse (26 ± 3 C, 12-hour photoperiod with supplemental fluorescent lighting) for 2 or 4 weeks, at which time they were removed from the soil, washed free of soil debris, and transplanted into cups that contained a sterile potting mixture without oxamyl. One week after they were transplanted, the plants were inoculated and observed as previously described. Controls were plants remaining in untreated or treated soils. Ten plants were used per treatment and the experiment was repeated one time.

The effect of oxamyl on the growth and zoosporangia production of *P. parasitica* var. *nicotianae* was studied in vitro. Oxamyl was added to water agar (1.2%, 45 C) to give concentrations of 0, 1, 10, 100, 500, and 1,000 μg a.i./ml, and poured separately into petri plates (60 × 15 mm). The plates were inoculated 24 hours later with 5-mm squares cut from 4-day-old cultures of *P. parasitica* var. *nicotianae* grown on lima bean agar. The inoculated plates were cultured in the dark at 25 C. After 4 days, the average mycelial growth was measured from the edge of the inoculum block and the cultures were observed at × 30 magnification for the production of zoosporangia. Five plates were used for each concentration of oxamyl.

TABLE 3. Effect of oxamyl 10 G concentration on the growth and zoosporangial production of *Phytophthora parasitica* var. *nicotianae*[a]

Oxamyl (μg active ingredient/ml)	Radial growth (mm)[b]	Zoosporangia produced
0	5.0	yes
1	4.5	yes
10	2.2	yes
100	0.6	yes
500	0	no
1000	0	no

[a]Oxamyl was incorporated into water agar and plates were poured. Agar were seeded with a 5-mm square piece from a lima bean culture of *P. parasitica* var. *nicotianae* (4 days old), and incubated in the dark at 25 C. After 4 days, mycelial growth from the edge of the inoculum block was determined and plates were observed at × 30 magnification for zoosporangia production.

[b]Radial growth is presented as the average for five observations.

RESULTS.—Tobacco cultivar WS 117 did not

develop black shank symptoms when inoculated after being transplanted into field soils treated up to 90 days earlier with 22.32 kg (a.i.) per hectare oxamyl (Table 1). The rate of 11.16 kg (a.i.) per hectare gave partial control of black shank, while 5.58 kg (a.i.) per hectare caused a delay in symptom expression, but did not control the disease. Oxamyl did not control black shank when plants were inoculated after being placed in field soils treated 120 days earlier with oxamyl.

The greenhouse studies indicated that at least 4 mg of oxamyl/kg soil is required to control black shank on WS 117 (Table 2). The period of effectiveness against inoculation was restricted to 2 to 4 weeks after soil treatment.

Tobacco cultivar WS 117 succumbed to black shank when removed from oxamyl-treated soil and transplanted into oxamyl-free soil 1 week prior to inoculation. Plants that remained in oxamyl-treated soil did not express black shank symptoms when inoculated at the same time as the transplanted tobacco.

Growth of *P. parasitica* var. *nicotianae* was inhibited completely on water agar containing oxamyl in excess of 100 μg/ml (Table 3). Oxamyl at 10 and 100 μg/ml partially inhibited mycelial growth, but 1 μg/ml oxamyl had no effect on growth. Zoosporangia were formed on all cultures which permitted mycelial growth.

We also tested the fungitoxicity of oxamyl in water agar against the following: race 0 (isolate 1587) and race 1 (isolate 1668-1) isolates of *P. parasitica* var. *nicotianae*, from C. C. Litton, University of Kentucky, Lexington; *P. cactorum* (isolate C-2) and *P. cambivora* (isolate Ap 23), from H. S. Aldwinckle, New York State Agricultural Exp. Sta., Geneva; *P. capsica* (isolate P 505 S), *P. cinnamomi* (isolate P 97), *P. megasperma* (isolate P 844), and *P. megasperma* var. *sojae* (isolate P 174), from G. A. Zentmyer and D. C. Erwin, University of California, Riverside; *P. citrophthora* (isolate 743 E) and *P. citricola* (isolate 738), from G. Grimm, U. S. Horticultural Research Lab., Orlando, Florida; *P. drechsleri* (isolate 201), from C. A. Thomas, Plant Science Research Division, U. S. Department of Agriculture, Beltsville, Md.; and *P. palmivora* (isolate 18 F-2P), from W. H. Ko, University of Hawaii, Hilo. The fungitoxicity of oxamyl to these strains and species of *Phytophthora* was comparable to its fungitoxicity to the Connecticut isolate of *P. parasitica* var. *nicotianae*.

DISCUSSION.—Soil treated up to 90 days earlier in the field with oxamyl (22.32 kg active ingredient/hectare) protected transplanted tobacco seedlings against black shank. Plants transplanted into soil treated in the greenhouse with 4 mg active ingredient oxamyl/kg (= 22.32 kg a.i./hectare) were protected against the pathogen when inoculated 28 days after soil treatment, but developed black shank symptoms when inoculated 56 days after soil treatment. Daily watering may have leached the active material from soils treated in the greenhouse faster than those treated in the field. This difference also may have been caused by post-treatment differences in moisture, temperature, or microbiology between soil treated in the field and soil treated in the greenhouse.

Our results do not indicate the mode of action for black shank control. Plants grown in soil previously treated with oxamyl, and then transplanted to soil not treated with oxamyl, were not protected against inoculation with zoospores of *P. parasitica* var. *nicotianae*. This suggests that plants growing in oxamyl-treated soil either are not made resistant to black shank, or are resistant only when they are in direct contact with the oxamyl-treated soil. The lack of black shank control until 2 weeks after plants have been placed into oxamyl-treated soil, the evidence that oxamyl controls black shank up to 90 days after soil treatment, and the need for high concentrations of oxamyl to inhibit the growth of *P. parasitica* var. *nicotianae* and other *Phytophthora* spp. in vitro suggests that oxamyl is not the active compound in disease control. A breakdown product or products of oxamyl could be the active constituent. The reduction or elimination of certain soil-borne microbes by oxamyl could allow the increase of microbes which are antagonists of *P. parasitica* var. *nicotianae*. The use of sterilized soil does not preclude the latter since microbes antagonistic to *P. parasitica* var. *nicotianae*, if present, could be introduced after oxamyl-treatment by its presence as a natural population, either in the greenhouse, or by its association on the roots of the tobacco cultivars transplanted into the treated soil. Possibly the products of the interaction between oxamyl and soil could be fungitoxic to the black shank organism. Soil type, soil pH, tobacco cultivars, and race of the pathogen may also be factors in the control of black shank in oxamyl-treated soils.

Treatment of field soils with oxamyl would appear to offer a means of protecting tobacco against black shank. Field trials will be performed to determine disease control potential.

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