Evaluation of Host Resistance and Soil Fumigation for the Management of Black Root Rot of Tobacco in Ontario

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ABSTRACT

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Black root rot of tobacco, caused by the soilborne fungus *Thielaviopsis basicola*, is a serious problem in many tobacco (*Nicotiana tabacum* L.)-growing regions of the world. In Ontario, the disease is favored by cool, wet soil conditions and heavy textured or poorly drained soils. Yield loss can be severe under these conditions and fumigants containing chloropicrin are used extensively for controlling the disease. Usually, fumigants control the disease reasonably well, but they are costly and could cause a negative environmental impact. A 2-year study was conducted to evaluate the performance of resistant (AC Gayed) and moderately susceptible (Delgold) tobacco cultivars and soil fumigation to black root rot. *T. basicola* reduced yield of the susceptible Delgold cultivar. The interaction between genotype and fumigation was significant for most traits examined, indicating that the two genotypes responded differently. Orthogonal comparisons indicate that yield from nonfumigated AC Gayed was higher than that of nonfumigated Delgold. Yield of nonfumigated AC Gayed was also not significantly different from the yield of AC Gayed treated either with Vorlex Plus (1,3-dichloropropene+methyl isothiocyanate) or with Vorlex Plus CP (1,3-dichloropropene+methyl isothiocyanate+chloropicrin). In contrast, the yield of nonfumigated Delgold was lower than Delgold treated with Vorlex Plus CP.

Black root rot, caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris, is a common soilborne fungus found in many major tobacco (*Nicotiana tabacum* L.)-growing regions of the world. The disease, characterized by black lesions on the roots and hypocotyl, causes stunting, late maturation, and uneven stands, all of which reduce yield and quality of tobacco (15). In Canada, crop loss in any given year is usually low, around 0.08%; however, the cost of control using fumigants is high, amounting to \$2.3 million Canadian per year. At high infestation levels, plant leaves turn pale green to yellow and stunting is severe (11). The level of damage from the disease is generally associated with several soil and environment factors. Conditions such as heavy textured or poorly drained soils and cool, wet soils favor disease development and spread. Soil temperatures affect black root rot development by retarding growth of the host relative to that of the fungus (8,10,12). Temperatures between 17 and 23°C are considered optimal for disease development (2). Disease severity is greatly reduced at 26°C and is negligible at soil temperatures above 30°C (9). Soil pH

affects yield losses due to black root rot. The disease rarely occurs when soil pH is below 5.4 and is most severe above pH 5.6 (4). Use of cultivars with low levels of partial resistance and continuous planting of tobacco or other susceptible crops can increase the severity of the disease (1,11,16,17).

Several soil fumigants are widely used to control black root rot (20). Fumigants containing chloropicrin (Telone C-17R and Vorlex Plus CP) are more effective than others (6). Fumigants are injected into the soil (15 cm deep) 3 weeks before transplanting. Generally, the effectiveness of fumigation depends on the fumigant used and the environmental conditions present at the time of fumigation. Fumigants are least effective in cool, wet soil because they fail to disperse adequately through the soil (5). These same conditions favor development of severe disease early in the growing season by *T. basicola* (11).

Host plant resistance is among the primary means of controlling black root rot on flue-cured tobacco in Ontario. The first flue-cured cultivar resistant to black root rot was released in 1949 (10). Since then, several other resistant cultivars have been registered. About 95% of the flue-cured tobacco cultivars grown in Ontario have low to moderate levels of resistance derived from *N. tabacum* sources. The remaining 5% have a very high level of monogenic resistance derived from *N. debneyi*. Despite the availability of resistant cultivars with monogenic sources of

resistance that are well adapted to the growing conditions of Ontario, information on the performance of these cultivars with and without fumigation treatments is still lacking. This study was undertaken to evaluate host plant resistance and chloropicrin-containing fumigants.

MATERIALS AND METHODS

Field trials were conducted in 1996 and 1997 at Paloot's farm. The soil type is Granby sandy loam consisting of 84% sand, 6.5% clay, and 9.5% silt. The farm is located 5 km east of the Southern Crop Protection and Food Research Centre, Delhi (latitude 42°52', longitude 80°33'), Ontario. This site has been continuously cropped to tobacco for the last 45 years and has a long history of severe and uniform black root rot. The fumigation treatments were 1,3 dichloropropene+methylisothiocyanate (Vorlex Plus) at 56.7 liters ha⁻¹, 1,3 dichloropropene + methylisothiocyanate + chloropicrin (Vorlex Plus CP) at 66.7 liters ha⁻¹, and a nonfumigated control. All fumigation treatments were applied on 4 May 1996 and 5 May 1997 at a depth of 15 cm by chisel placement, using a single-row gravity flow applicator. The fumigant was sealed immediately after the application by ridging the row by means of two disks attached to the tractor. The seedlings were raised in a greenhouse with a uniform muck layer 7.5 cm thick and with a seeding rate of approximately 110 to 120 seed per 930 cm². Nine-week-old seedlings of the susceptible cv. Delgold and resistant cv. AC Gayed were transplanted on 4 June 1996 and 5 June 1997 into plots of three rows, 23 m long. The rows were spaced 107 cm apart, containing 38 plants with 61 cm between plants, resulting in a planting density of 15,441 plants ha⁻¹. Production practices were those normally employed for flue-cured tobacco grown in southwestern Ontario (20).

In both years of the experiment, *T. basicola* and nematode populations were evaluated. Soil was randomly sampled twice (prior to fumigation and midseason) for nematode counts and four times (prior to fumigation in the first week of May and three times during the growing season in the first weeks of June, July, and August) for *T. basicola* populations. Each soil sample was a composite of six 3-cm-diameter soil cores taken to a depth of 20 cm. *T. basicola* populations in each treatment were estimated using the technique de-

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scribed by Mayer et al. (17) and Specht and Griffin (22), in which 10 ml of soil suspension (1:100, wt/wt) in deionized water was pipetted into 300 ml of a malten medium containing 50 ml of canned carrot juice, 18 g of agar, 950 ml of deionized

water, and the antibiotics described by Specht and Griffin (22). The agar-soil suspension was mixed thoroughly on a magnetic stir plate, divided equally into 10 plastic petri dishes, and incubated for 14 days at room temperature (22 to 25°C) in

the dark. Nematode populations were estimated using the Baermann pan method (23).

Data were recorded on yield (leaf weight), grade index, days to flowering, topping height, and leaf area. Leaves were harvested over a 5-week period. Two to three ripe leaves were harvested every 4 to 7 days. The leaves were flue-cured and weighed to determine yield per hectare and were graded according to the Ontario Farm Products Grades and Sales Act (19) to determine the grade index in cents per kilogram on the average market price. Plots were topped when 50% of the plants had three open florets. Topping height of four randomly selected plants was measured from the ground level to the tip of the stem. Length and width of the eighth leaf was measured in four randomly selected plants to calculate the leaf area. To obtain the best estimate, the leaf area measured was multiplied by 0.675.

Two cultivars and three fumigation treatments were evaluated in a factorial randomized complete block design with four replications. *T. basicola* population count were transformed by log ($X + 1$) prior to analysis. The analysis of variance procedure described by Comstock and Moll (3) was used to test the significance of year, genotype, fumigation, and first and second order interactions. The year effect was assumed to be random, whereas genotype effects and fumigation effects were considered fixed. The significance of interaction terms were tested

Table 1. Individual analyses of variance of *Thielaviopsis basicola* population (log [$X + 1$] transformed) for 1996 and 1997 trials conducted at Paloot's farm in southwestern Ontario

Source of variation	df	Mean square ^z			
		1996		1997	
		May 4	June 4	May 5	June 5
Rep	3	0.054	0.164*	0.263**	0.029
Treatment	5	0.038	0.188**	0.058	1.682**
Genotype	1	0.000	0.000	0.003	0.233
Fumigation	2	0.043	0.469**	0.111	3.443**
Genotype × fumigation	2	0.053	0.002	0.034	0.646*
Error	15	0.056	0.031	0.046	0.161

^z May dates are prior to fumigation and June dates are postfumigation; * and ** significant at $P < 0.05$ and $P < 0.01$ respectively.

Table 2. *Thielaviopsis basicola* population (log [$X + 1$] transformed) prior to and after fumigation in 1996 and 1997 at Paloot's farm in southwestern Ontario

Treatments	Mean <i>T. basicola</i> population ^z			
	1996		1997	
	May 4	June 4	May 5	June 5
Delgold-NF	2.00 a	1.82 a	1.79 a	1.26 ab
Gayed-NF	1.92 a	1.79 a	1.97 a	1.68 a
Delgold + VO	1.73 a	1.73 a	1.72 a	0.69 bc
Gayed + VO	1.91 a	1.75 a	1.67 a	0.00 d
Delgold + VO + CP	1.91 a	1.35 b	1.94 a	0.49 cd
Gayed + VO + CP	1.80 a	1.37 b	1.88 a	0.16 cd

^z May dates are prior to fumigation and June dates are postfumigation. Different letters in the same column indicate statistical differences, ($P = 0.05$) according to the least significant difference test.

Table 3. Combined analyses of variance of yield and agronomic traits for 1996 and 1997 trials conducted at Paloot's farm in southwestern Ontario

Source of variation	df	Mean squares ^y				
		Yield (kg/ha)	Grade index (c/kg)	Days to flower	Topping height	Leaf area (8th leaf)
Year	1	1,388,560**	6,097.5**	50.02**	1,507.41**	2,185,843**
Rep (year)	6	58,210*	388.6	4.83*	64.54**	27,915
Treatment	5	545,029**	2,328	90.37**	60.98**	136,456**
Genotype	1	2,325,840**	682.5	275**	138.8**	483,034**
Fumigant	2	17,418	187.5	70**	1.29	4,505*
Year × genotype	1	99,554*	93.5	4.69	3.19	56,183
Year × fumigant	2	27,477	588.6	1.89	14.18	27,682
Genotype × fumigant	2	182,234**	53.1	17.77**	81.76*	95,116**
Year × Genotype × fumigant	2	19,005	516.6	6.44*	4.76	7,589
Error	30	2,365,754	233.9	1.44	12.41	13,892
CV% ^z	...	5.59	3.79	1.75	3.67	9.8

^y * and ** significant at $P < 0.05$ and $P < 0.01$, respectively.

^z CV% = coefficient of variation.

Table 4. Minimum and maximum temperatures and rainfall during the 1996 and 1997 growing seasons at the Southern Crop Protection and Food Research Centre-Delhi (Ontario, Canada)

Month	Temperature (°C)						Rainfall (mm)	
	Maximum		Minimum		Mean		1996	1997
	1996	1997	1996	1997	1996	1997		
April	9.8	11.8	0.6	-0.7	5.2	5.6	149	36
May	17.7	15.2	4.9	4.0	11.4	9.6	153	158
June	24.8	25.8	13.7	12.9	19.3	19.4	147	103
July	26.2	26.6	13.6	14.2	19.9	20.4	85	72
August	27.7	24.0	13.7	12.1	20.7	18.1	33	77
September	21.5	21.3	10.9	9.2	16.2	15.3	221	76
Average	21.3	20.8	9.6	8.6	15.5	14.7	...	...
Total	...	...	...	...	...	...	788	522

using a procedure outlined by McIntosh (13). Data were analysed using the general linear models procedure and Duncan's multiple range test (SAS Institute, Cary, NC). Contrasts calculated from the treatment effects of the analysis of variance were used to compare (i) Delgold and AC Gayed under zero fumigation, (ii) Delgold nonfumigated to fumigated, and (iii) AC Gayed nonfumigated to fumigated.

RESULTS AND DISCUSSION

***T. basicola* populations.** Individual analyses of variance prior to fumigation of *T. basicola* population count in 1996 and

1997 (Table 1) suggest no differences in the main and interaction effects. However, the postfumigation count was significant among treatment means in both years. The postfumigation differences in the treatment effect were mainly attributed to effects contributed by fumigation. The genotype-fumigation interaction was significant in 1997 postfumigation. In 1996 and 1997, postfumigation counts of *T. basicola* were significantly lower on Vorlex Plus CP fumigated treatments (Table 2). However, in contrast to the results of 1996 postfumigation counts, treatments with Vorlex Plus fumigation in 1997 significantly reduced the numbers of *T. basicola* propagules.

Similar results were reported by Gayed (6). He found that Vorlex at a rate of 23 liters ha⁻¹ reduced black root rot severity in 1 year out of 2 years of testing. Similarly, Telone C at a rate of 12 liters ha⁻¹ reduced black root rot severity in 1975, but 15 liters ha⁻¹ in 1968 was not effective. Also, chloropicrin at 5 liters ha⁻¹ neither stimulated tobacco growth nor reduced disease lesions in 1 year out of six seasons of testing. Gayed concluded that the failure of chloropicrin and other fumigants to increase tobacco growth and reduce black root rot severity in 1969 was attributed to rainfall in April and May, consisting of about 2.7 in. during the week before and 1.9 in. during the week after fumigation. Soil temperature is also known to influence the effectiveness of soil fumigants on soil organisms (7,14) and the severity of black root rot of tobacco (9,11).

The error values for the late-season *T. basicola* population counts were too high in both years to account for the actual differences among treatments. Therefore, the discussion is confined to dates prior to and the first count after fumigation.

Yield and agronomic traits. Significant differences in yield, grade index, days to flowering, topping height, and leaf area were present between the 2 years of testing

Table 5. Contrasts between various treatment effects for yield, days to flowering, topping height, and leaf area

Contrast ^z	Mean squares ^y			
	Yield (kg/ha)	Days to flower	Topping height	Leaf area (8th leaf)
D versus G	1,067,605**	156.25**	0.25	53,282.5*
D versus D + Vorlex Plus	3,721	18.06**	55.02*	85,042.22*
D versus D + Vorlex Plus CP	196,249**	150.6**	0.01	2,120.6
G versus G + Vorlex Plus	13,398	10.56*	85.51**	55,142.78*
G versus G + Vorlex Plus CP	36,864	20.25**	4.7	954.96

^y * and ** significant at $P < 0.05$ and $P < 0.01$, respectively.

^z D = Delgold, G = AC Gayed.

Fig. 1. Yield, leaf area, days to flowering, and topping height of a susceptible cultivar (Delgold) and a resistant cultivar (AC Gayed) to black root rot under different fumigation conditions at Paloot's farm in 1996 and 1997. NF = nonfumigated, VOR = fumigated with Vorlex only, VOR + CP = fumigated with Vorlex and Chloropicrin. Vertical bars indicate standard errors.

(Table 3). Warm weather conditions early in the season and above-average precipitation during the 1996 growing season may have contributed to early crop establishment and faster crop growth and development (Table 4).

The difference among treatments and between the genotypic main effect were highly significant for all characters except the grade index (Table 3). The difference among the fumigation main effect was only significant for days to flowering and leaf area. The first order interaction effect of genotype-year was significant for yield, but the genotype-fumigation effect was significant for most of the traits, thus suggesting that the two genotypes reacted differently to the fumigation treatments. Orthogonal contrast (Table 5) showed that yield of nonfumigated AC Gayed was significantly higher than that of nonfumigated Delgold. Yield of nonfumigated Delgold was not different from the yield of Delgold treated with Vorlex Plus, but was significantly lower than the yield of Delgold treated with Vorlex Plus Chloropicrin (Table 5). This suggests that the nematode effect was not severe enough to cause differences in yield and quality. Root-lesion nematode counts ranged from 60 to 140 kg⁻¹ of soil for 1996 and from 60 to 85 kg⁻¹ soil for 1997. These figures are considered too low to cause any appreciable damage to tobacco roots. Nematode economic threshold for tobacco is 1,100 kg⁻¹ soil (18). Yield of nonfumigated AC Gayed was not different from the yield of AC Gayed treated either with Vorlex Plus or with Vorlex Plus CP. This result is in agreement with the results reported by Shew and Shoemaker (21). They found that yield between the fumigated and non-fumigated treatments was similar for cultivars with high level of resistance to *T. basicola* disease. In contrast, however, there was significant yield increase in the fumigated treatment of the susceptible cultivar.

Fumigation with Vorlex Plus and Vorlex Plus CP had no effect on the yield and leaf

area of AC Gayed (Fig. 1A and B). The fumigation treatments decreased the number of days to flowering for both cultivars. In contrast to AC Gayed, topping height of Delgold fumigated with Vorlex Plus was significantly reduced (Fig. 1C and D). At present, almost 95% of the tobacco cultivars grown in Ontario have a moderate level of resistance controlled by a group of recessive genes derived from *N. tabacum* sources. However, AC Gayed falls into the first category of black root resistance described by Clayton (2), also known as immunity. The immunity to black root rot in these cultivars is controlled by a single dominant gene.

Results in this study suggest that *T. basicola*-resistant cultivars can be used to manage black root rot grown in Ontario without the use of chloropicrin as a soil fumigant. However, the application of Vorlex Plus could reduce black root rot population, depending on soil conditions.

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