Verticillium Wilt, a Limiting Factor for Tobacco Production in Chile

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ABSTRACT

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A wilt and orange-to-yellow discoloration observed in recent years in burley types of tobacco in Chile was confirmed to be caused by *Verticillium dahliae*. Disease incidence as high as 25.6% has occurred. Significant yield losses were estimated in nine of 11 selected plots during the 1987–1988 growing season. Quality also was significantly affected in diseased versus symptomless cultivar Burley-49 tobacco plants. Isolates of *V. dahliae* from tobacco were pathogenic to plants of tobacco cultivar Burley-21. However, only mild symptoms occurred in cultivar Coker-86; flue-cured lines V-1 and V-3 appeared to be resistant. All isolates were pathogenic to the susceptible tomato cultivar Earlypak-7, and some isolates caused symptoms on tomato seedlings of the resistant cultivars San Remo, Flora Dade, and Cal-Ace. Lower disease incidence was observed in the latter cultivars. Consequently, isolates from tobacco appeared to be race 1 of *V. dahliae*.

Verticillium wilt of tobacco (*Nicotiana tabacum* L.) was recently described in Chile (6). Symptoms were evident by blossom time and ranged from the development of leaf wilting during hot weather, followed by interveinal chlorosis, to a complete bright-yellowing of the leaf blade. A yellow-to-orange chlorosis always appeared on the lower leaves and progressed rapidly throughout the entire plant. Initially, leaf symptoms appeared on one side of the plant and commonly on one side of the leaf blade. Vascular discoloration was always evident in cross sections of the stalks or petioles of diseased leaves.

Moderate to severe outbreaks have occurred (1984–1988) on burley tobacco. The purpose of this study was to confirm the etiology and the importance of the disease that has occurred in recent years in Chile. A portion of this work has been reported (6).

MATERIALS AND METHODS

Isolation. Isolations were performed on potato-dextrose agar (PDA) acidified with 0.5 ml/L of 1 *N* lactic acid. Small fragments (0.5–1 cm long) of discolored vascular tissue were selected from surfaced-sterilized (95% ethanol for 1 min, 1% sodium hypochlorite for 3–5 min) stalks and/or from leaf petioles. Cultures were incubated for 7–10 days at 22–25 C. Subcultures were grown on PDA from mycelial transfers from the edges of the colonies. Isolates were stored on PDA slant culture tubes at 5 C.

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Growth temperature. Nine isolates of *Verticillium dahliae* Kleb. from tobacco were plated in triplicate on PDA and incubated at 20, 30, and 35 C for 5 days before measuring the radial growth.

Inoculum preparation. Inoculum was prepared from 7- to 10-day-old cultures growing actively on PDA. Cultures were macerated with sterile distilled water in a blender for 5 min, and the final inoculum concentration was adjusted to about 10^6 propagules (conidia, microsclerotia, and mycelium fragments) per milliliter.

Pathogenicity on tobacco. Pathogenicity tests were conducted on tobacco cultivars Burley-21 and Coker-86 and on V-1 and V-3, two different New Zealand lines of a flue-cured type of *N. tabacum*. V-1 and V-3 showed some degree of resistance from previous field observations in Chile. Ten isolates recovered during 1985–1986 and 26 during 1986–1988 were tested for pathogenicity. Each cultivar was seeded on pasteurized soil and two- to three-leaf-stage seedlings (approximately 5–6 cm high) were inoculated by the root-dip method (11,13). Trimmed roots of five seedlings per cultivar per isolate were placed for 30 min in the inoculum suspension before transplanting to clay pots (10 cm diameter) containing a soil mixture (2:1:1, organic soil:sand:loam soil) that was previously sterilized with 122.5 g of methyl bromide plus 2.5 g of chloropicrin. The pots were then covered for 72 hr and aerated for at least 5 days. Pots were randomly arranged and maintained in the greenhouse for approximately 30 days. Noninoculated control plants were always included.

Pathogenicity on tomato. Pathogenicity assays were conducted on tomato cultivars Earlypak-7 (susceptible to *V. dahliae* race 1), San Remo, Cal-Ace, and Flora Dade (resistant to *V. dahliae* race 1). Seventeen isolates recovered between 1985 and 1986 were tested in a first trial on Earlypak-7 and San Remo. In a second trial, 31 isolates obtained during the 1987–1988 growing season were assayed on Earlypak-7, Cal-Ace, and Flora Dade. Inoculations were performed as described by Grogan et al (1), except that trimmed roots of young tomato plants were placed for 30 min in the inoculum suspension. Plants were transplanted to small pots (10 cm diameter) containing a fumigated soil mixture as previously described. Pots were arranged randomly on a bench and grown for at least 30 days under greenhouse conditions. Six or 10 seedlings per cultivar were inoculated with each isolate. Equal numbers of noninoculated plants were included as controls.

Yield losses. The effect of Verticillium wilt on yield under commercial conditions was estimated in 11 field plots of tobacco cultivar Burley-49. Five plots were in Nancagua and six were in Lontué, the most important geographical areas where burley types of tobacco are grown in Chile. Spacings were 1.10 m between rows and 0.50 m within rows. Normal cultural practices for burley tobacco regarding pest control, fertilization, suckering control, weed control, and cultivation were performed at all locations.

Twelve rows were selected randomly in each plot, and groups of eight diseased and eight symptomless plants per row were marked before harvesting. Only those plants showing distinctive symptoms of Verticillium wilt, such as leaf wilting, uneven growth of the laminae, and/or an orange chlorosis, were marked as diseased. Plants were harvested separately and cured following commercial recommendations to determine the dry weight. The results were analyzed statistically using a factorial model with two treatments, both symptomless and diseased plants, 11 plots, and 12 replicates per plot. Means were separated according to Duncan's multiple range test.

Dried tobacco leaves obtained from 48 diseased and 48 symptomless plants per plot were separated into five groups according to the position of the leaves in the stalks. Position one was composed of four or five lower leaves. Position five always was composed of the upper youngest four leaves of each plant. Leaves from each position were classified

for quality according to a 12-degree grade index, with 1 = the poorest and 12 = the best quality. This scale is used commercially and is based on the physical appearance of the cured tobacco leaves. Variance component estimates and their standard errors were obtained according to a complete block design with two treatments, diseased versus symptomless plants, and 11 replicates. Data from each leaf position were analyzed separately because the potential yield or quality varied between each leaf position. The best yield and tobacco quality can only be obtained from leaves of positions three or four. Mean differences were separated according to Duncan's multiple range test.

RESULTS

Isolation. A microsclerotium-forming fungus was consistently isolated in pure culture from diseased samples collected from 1986 through 1988. Colonies were initially white and cottony in appearance, but 7–10 days later they turned black as the microsclerotia developed in the PDA medium. Hyaline and verticillately branched conidiophores with unicellular ovoid to ellipsoid conidia borne on short phialides were produced on PDA and also appeared on the surface of discolored fragments of vascular tissue after 5–6 days of incubation in moist chambers at room temperature.

Nine tested isolates grew at 20 and 30 C, but no growth was observed at 35 C. The mycelial growth varied between 1.35 and 1.8 cm and from 0.9 to 1.35 cm in diameter for cultures incubated at 20 and 30 C, respectively.

Based on morphological characteristics and the absence of growth observed at 35 C, the fungus isolated was identified as *V. dahliae* (2,4). In over 150 samples of diseased tissue collected during the 1987–1988 growing season and seeded on PDA, there was no evidence for the presence of *V. albo-atrum* Reinke & Berth. nor for the presence of the Fusarium wilt fungus (*Fusarium oxysporum* Schlecht. f. sp. *nicotianae* (J. Johnson) Snyd. & Hans.

Pathogenicity. Thirty-six isolates of *V. dahliae* were pathogenic to plants of tobacco cultivar Burley-21. Symptoms appeared on the lower leaves 3–4 wk after inoculation and were characterized by leaf wilting and chlorosis that started as interveinal chlorosis and progressed to encompass the entire leaf (Fig. 1A). A very distinctive vascular discoloration (Fig. 1B,C) was caused by all isolates tested on Burley-21. Five of 10 isolates caused mild symptoms on Coker-86 tobacco plants, characterized by a mild yellowing of the lower leaves that appeared 20–30 days after inoculation. Symptom remission was observed in Coker-86 plants after about 5 wk. Vascular discoloration was not found on this cultivar under greenhouse conditions. Six of 26 isolates caused Verticillium wilt symptoms in one or two of three inoculated V-1 and V-3 tobacco seedlings.

Symptoms in tomatoes first consisted of a premature necrosis of the cotyledonary leaves followed by wilting and yellowing of the leaves. Vascular necrosis was not observed. In trial one, 17 of 17 isolates were pathogenic in tomato seedlings of Earlypak-7, and 15 of 17 isolates also were pathogenic on San Remo. In a second trial, 31 isolates were pathogenic on Earlypak-7, but 16 of 31 isolates from tobacco were pathogenic on tomato cultivars Cal-Ace and Flora Dade, respectively. However, differences in disease incidence were observed. Most isolates caused symptoms in less than 10% of Flora Dade tomato seedlings or in less than 20% of Cal-Ace or San Remo.

Disease incidence and yield loss assessment. Disease incidences of 10.8, 16.5, and 25.6% were estimated in two Burley-49 tobacco plots and one of Burley-64 in over 100 plants randomly selected per plot and evaluated at approximately blossom time.

Dry weight yield reductions varied from 4.4 to 34.6%. This was equivalent to a loss from 150 to 1,440 kg/ha of cured tobacco. Yield reductions were significant ($P = 0.05$) in nine of 11 field plots (Table 1).

Tobacco quality from the five leaf positions also was diminished ($P = 0.05$) on diseased plants relative to symptomless ones. The differences observed on the overall grade index were significant ($P = 0.05$) (Table 2). For example, the quality of cured leaves dropped from 6.8 and 8.0 in symptomless plants to 4.3 and 5.0 in diseased ones, respectively, for leaves harvested from positions three and four (leaves that potentially give the highest quality).

DISCUSSION

The results of this study confirm our previous report showing that *V. dahliae* was the cause of the wilting and yellowing of tobacco observed in Chile (6). The

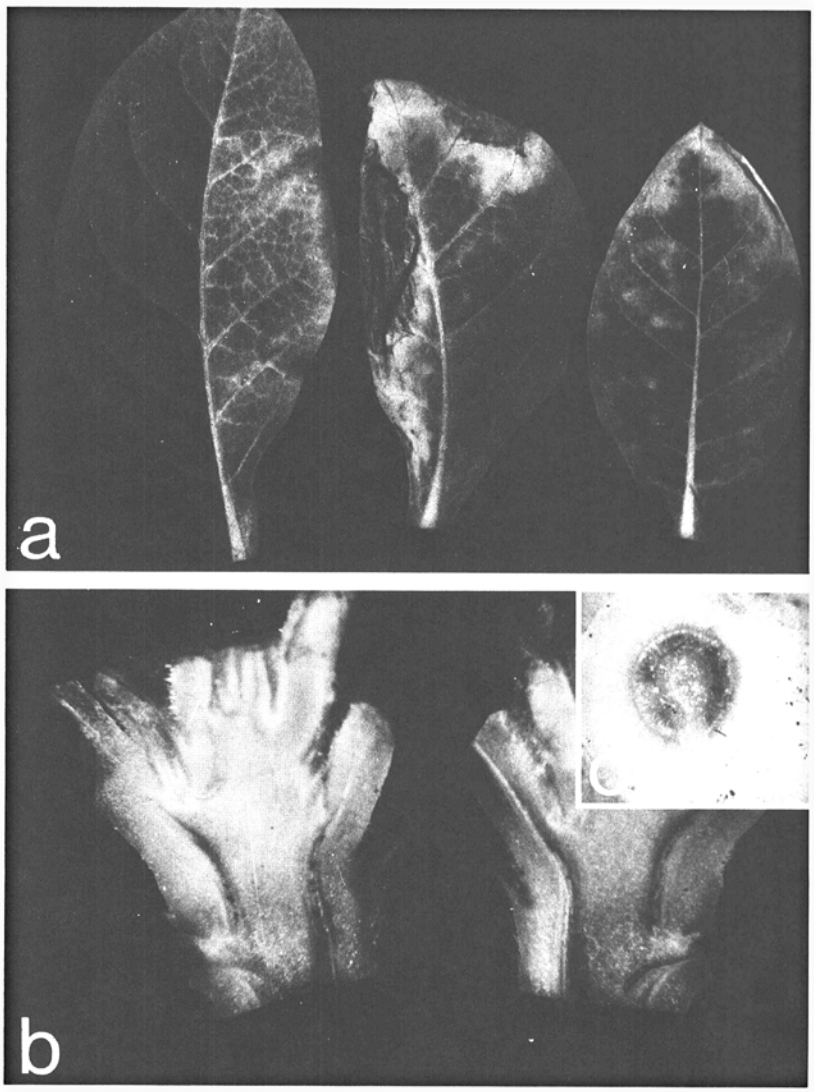

Fig. 1. Symptoms of Verticillium wilt caused by *Verticillium dahliae* on inoculated tobacco cultivar Burley-21. **(A)** Interveinal leaf chlorosis. **(B)** Longitudinal and **(C)** cross-sectional vascular discoloration observed on the main stalk.

Table 1. Yield losses in tobacco cultivar Burley-49 caused by Verticillium wilt during the 1987–1988 growing season

Field plots	Dried weight (g)		Yield loss	
	Symptomless	Diseased	kg/ha[a]	%
		Nancagua[b]		
1	194.4[c]	162.4[c]	581.8	16.4
2	237.0	166.3	1,279.4	29.8
3	211.4	157.7	1,030.9	26.8
4	191.6	147.8	796.4	22.9
5	213.3	200.3	236.3	6.1
		Lontué[b]		
6	186.8	157.0	541.8	16.0
7	245.9	176.4	1,263.7	28.3
8	185.1	176.9	149.1	4.4
9	257.4	219.2	594.5	14.8
10	229.0	149.8	1,440.0	34.6
11	207.0	170.5	663.7	17.6

[a] Estimated for a plantation density of 18,182 plants per hectare.
[b] Most important geographical areas where burley types of tobacco are grown in Chile.
[c] Average of eight plants. All means for dried weight between symptomless and diseased plants for each field plot were significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 2. Effect of Verticillium wilt on quality of tobacco cultivar Burley-49 obtained from 11 field plots relative to the quality obtained from symptomless plants harvested from the same plantations

Leaf position[a]	Condition[b]	Quality grade[c]			F value[d]	Probability of greater F[e]
		Min.	Max.	Mean		
1	S	3.2	5.8	4.2		
	D	1.6	4.2	3.1	25.03	0.0005
2	S	3.0	7.0	5.4		
	D	1.6	5.4	3.7	136.08	0.0001
3	S	4.4	9.4	6.8		
	D	2.6	6.6	4.3	229.53	0.0001
4	S	5.2	11.6	8.0		
	D	3.0	7.2	5.0	192.66	0.0001
5	S	4.8	7.2	5.7		
	D	3.2	5.0	4.0	212.85	0.0001
Average	S	4.2	8.0	6.0		
	D	2.4	5.5	4.0	407.80	0.0001

[a] 1 = Lower three to four leaves, 5 = upper four leaves; 2–4 are intermediate positions where the best quality can be expected. The average number of leaves per plant was 22.
[b] S = symptomless plants and D = diseased plants at harvesting time, approximately 120–130 days after transplanting.
[c] Based on 12 different categories where 1 = poorest quality and 12 = best possible quality (normally obtained on leaves from positions 3 and 4 only).
[d] Calculated F values from analysis of variance.
[e] Probability of obtaining equal or higher F values for 10 degrees of freedom.

results obtained on pathogenicity tests performed on tomato suggest that most isolates of *V. dahliae* from tobacco found in Chile belong to race 1. Verticillium wilt has become established as a very important disease of tobacco and is potentially of increasing importance. High disease incidences with serious losses have already occurred on burley tobacco. The disease may be aggravated by common cultural practices (e.g., a short crop rotation); by the common presence of several host plants in Chile (5), including some weeds (4,9); and by the unavailability of economically feasible control measures. These factors, together with the long survival period (4–8 yr) that has been reported for *V. dahliae* in the soil (3,10), reduce the practicality of crop rotation as a disease-control measure.

Symptoms observed on inoculated plants corresponded with those found in the field and agree with the description of this disease in other countries (6–13). The most reliable symptoms were the yellow-orange chlorosis of the leaves, the uneven growth of laminae, and the vascular browning observed on either the stalks or leaf petioles (Fig. 1).

The New Zealand breeding lines V-1 and V-3 showed resistance to most of the isolates of *V. dahliae* from tobacco in greenhouse evaluations. This was particularly evident after comparing the responses of 26 isolates on either line with that of the susceptible Burley-21 tobacco cultivar. The true nature of this resistance remains to be determined.

Verticillium wilt of tobacco is an important disease for the Chilean tobacco industry, affecting yield and quality of burley types of tobacco. Yield losses as great as 34.6% occurred and resulted in 1,440 kg/ha less yield for symptomless plants in the same field plot. This represented about $1,282 per hectare. These data agree with results reported earlier from New Zealand (8,11,13), where Verticillium wilt caused significant yield reductions and is considered a limiting factor for tobacco production.

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