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Crown and root rots of table grapes caused by *Phytophthora* spp. in Chile

by

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S u m m a r y : *Phytophthora* crown and root rot of grapevine (*Vitis vinifera* L.) occurs frequently in Chile, where grapes are cultivated on their own roots. *P. cinnamomi, P. cryptogea,* and *P. drechsleri* were isolated from diseased root or crown tissue of the table grape cvs Flame Seedless, Italia, Red Globe, and Thompson Seedless with *P. cryptogea* being the most frequently isolated species. These pathogens were identified on the basis of standard morphological and cultural features and were compared by SDS-PAGE profiles of mycelial proteins. All isolates were pathogenic on 1-year-old cane segments and 1-year-old rooted grapevines of cvs Thompson Seedless and Red Globe. The latter cultivar was more susceptible than Thompson Seedless. *P. cinnamomi* and *P. cryptogea* have been associated with root rot of grapevines elsewhere, but this is the first report of *P. drechsleri* as a pathogen of grapevines and the first report of *Phytophthora* spp. affecting table grapes in Chile.

K e y w o r d s : grapevine, Phytophthora, Vitis vinifera.

Introduction

Presently, nearly 50,000 ha of table grape (*Vitis vinifera* L.), primarily seedless cultivars, are cultivated on their own roots in Chile. Vines with crown and root rots are often found singly or in small groups associated with excessive irrigation or imperfect soil drainage. Diseased plants are characterized by reduced growth, small and slightly chlorotic leaves, and poor fruit set. Reddish-brown necrotic cankers, extending from the crown to the roots and partially rotten roots are always associated with the aerial symptoms. Cankers eventually girdle the trunk and kill the plant. This disease has previously been associated with *Phytophthora* spp. in Chile (LATORRE 1988), and has been described in South Africa, India, Oceania and North America (WILCOX and MIRCETICH 1988). The objective of this research was to identify the individual *Phytophthora* spp. affecting table grapes in Chile, to confirm their pathogenicity, and to assess their relative virulence.

Materials and methods

Small pieces (5-10 mm in length) of crown or root tissues were selected at the margins of canker lesions and *Phytophthora* spp. were isolated as described previously (LATORRE and MUÑOZ 1993). Identifications of *Phytophthora* spp. were based on colony morphology, mycelial characteristics, production and morphology of sporangia, oogonia, antheridia, and mycelial growth at 30 and 35 °C (WATERHOUSE 1963, NEWHOOK *et al.* 1978). Sporangia and gametangia were produced as previously described (LATORRE and MUÑOZ 1993). Eight isolates tentatively identified as *P. cryptogea* Pethyb. and Laff., 5 identified as *P. cinnamomi* Rands and one identified as *P. drechsleri* Tucker were compared further by SDS-PAGE profiles of soluble mycelial proteins using previously described techniques (LATORRE *et al.* 1995).

Pathogenicity of individual isolates was determined initially on 15- to 20-cm long segments of 1-year-old canes of *V. vinifera* cvs Thompson Seedless and Red Globe. Cane segments were cut during dormancy and inserted vertically in plastic boxes containing a 2-cm-deep mix of one part infested vermiculite inoculum (LATORRE and MUÑOZ 1993) per two parts of sterile vermiculite. Boxes containing 5 cuttings per isolate were flooded with 1-2 cm of sterile water and incubated for 15 d at 23 °C before determining the length of the lesions that developed. Five cuttings were treated equally in sterile vermiculite as checks.

One-year-old vines of *V. vinifera* cvs Thompson Seedless and Red Globe, rooted in plastic pots (15 x 40 cm) containing a sterile mixture of organic soil and sand (1:1, v/v), were inoculated with each of 4, 2, and 1 grape isolates identified as *P. cryptogea, P. cinnamomi* and *P. drechsleri*, respectively. Two known isolates of *P. cryptogea,* previously isolated from kiwifruit in Chile (K15.2) and stone fruit in New York (NY413) were also included for comparison. Each plant was inoculated by delivering 25 ml of infested vermiculite (produced as before) into two holes bored in the soil at each side of the vine. The soil mix was kept wet constantly by placing the pots into trays containing 1-2 cm of water. Noninoculated control plants were treated equally. Plants were incubated in a screen house from October to December, then they were evaluated by the length of the canker extending from the crown to the main roots and the root fresh weight. Treatments were distributed randomly with 4 replicates and results were analyzed for variance according to a 2 x 10 (grapevine cultivars x *Phytophthora* isolates) randomized factorial design.

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Results

Phytophthora spp. were recovered from 22 of 106 symptomatic grapevines sampled between 1993 and 1995. Successful isolations were accomplished from vineyards of cvs Flame Seedless, Red Globe and Thompson Seedless and from 1-year-old vines of cvs Flame Seedless, Italia, and Red Globe sampled at nurseries.

Isolates from 17 vines were identified as *P. cryptogea.* They produced rosette to radial colonies on ACMA and V-8 agar media (LATORRE and MUÑOZ 1993) and grew at 30 °C but not at 35 °C. Sporangia were non-papillate, ovoid to obpyrifom (33.5 x 23.2 µm) with a mean length:breadth (L:B) ratio of 1.44, and proliferated internally. Oogonia were produced only when each isolate was paired with a known A_1 or A_2 mating type of *P. cinnamomi.* One isolate identified as *P. drechsleri* was similar to *P. cryptogea* except that it grew at 35 °C. Isolates from the remaining 4 vines were identified as *P. cinnamomi.* All grew at 35 °C, produced rosette colonies with aerial growth on ACMA and V-8 agar media, and had mycelia with characteristic coraloid to botryose hyphal swellings. Sporangia were ovoid to elipsoid (44.3 x 30.7 µm) with a mean L:B ratio of 1.44. Oogonia were produced only after pairing with a known A_1 or A_2 mating type of *P. cinnamomi.*

All isolates of *P. cryptogea* formed a homogeneous group based on their SDS-PAGE protein profiles, which were similar to but distinguishable from those of *P. drechsleri* with similarity coefficient (SC) values ranging from 63.6 to 72.7 % in the interspecific comparisons versus 80 to 100 % among intraspecific comparisons of *P. cryptogea* isolates. The SC values for comparisons of *P. cryptogea* versus *P. cinnamomi* isolates varied from 57.0 to 66.7 %.

Phytophthora isolates were pathogenic on 1-year-old cane segments, producing cankers that varied in length from 11.0 to 22.4 mm (Thompson Seedless) and from 7.4 to 25.0 mm (Red Globe) (Tab. 1). On rooted cuttings, isolates identified as *P. cryptogea, P. cinnamomi* and *P. drechsleri* induced slight leaf chlorosis, partially rotted roots, and canker lesions at the crown. No canker developed on control plants and root fresh weights were significantly ($P<0.05$) different between inoculated and noninoculated treatments. Differences between and the cultivar x isolate interaction were also significant at $P<0.05$ for both length of canker lesions and root fresh weight reduction. In general, canker lesions were slightly longer on Thompson Seedless than on Red Globe, whereas root weight reductions were much more pronounced on Red Globe (Tab. 2).

Table 1

Pathogenicity of isolates of *Phytophthora cinnamomi, P. cryptogea* and *P. drechsleri* recovered from diseased table grapes (*Vitis vinifera*) in Chile, determined by a bioassay on one-year-old cane segments

Isolates	Canker length (mm)	
	Thompson Seedless	Red Globe
	P. cinnamomi	
V94.01	22.4 ± 5.1	12.8 ± 4.1
V94.02	12.6 ± 2.1	14.6 ± 3.8
V94.05	12.8 ± 5.0	12.6 ± 1.8
V94.10	18.6 ± 4.7	18.8 ± 10.1
	P. cryptogea	
V94.19	16.8 ± 4.3	14.0 ± 5.4
V94.21	19.6 ± 2.6	25.0 ± 4.4
V94.23	13.2 ± 3.4	12.8 ± 1.3
V94.39	11.0 ± 5.7	11.2 ± 4.0
	P. drechsleri	
V20	12.4 ± 2.8	7.4 ± 3.4
	Uninoculated checks	
	0 ± 0	0 ± 0

Canker length after 15 d incubation at 23 °C in infested vermiculite. Means of 5 replicates ± standard deviation.

Discussion

This is the first study to identify the *Phytophthora* spp. causing root and crown rots of grapevines in Chile and to assess their relative prevalence and virulence. As in other parts of the world (WILCOX and MIRCETICH 1988), *P. cinnamomi* was isolated from diseased vines and shown to be virulent. However, in contrast with previous reports (cited in WILCOX and MIRCETICH 1988), we isolated *P. cryptogea* much more frequently than *P. cinnamomi* and found the two species to be similarly virulent. The single isolation of *P. drechsleri* appears to be the first report of this species as a pathogen of *V. vinifera.* However, the taxonomic distinction between *P. drechsleri* and *P. cryptogea* is often inconsistent (e.g., LATORRE *et al.* 1995).

Only 22 out of 1070 segments (2.1%) of symptomatic root or crown tissues yielded *Phytophthora* spp. on a semiselective agar medium. However, as previously reported (LATORRE and WILCOX 1997), a high proportion of these samples was tested positive for the fungi using a commercial, immunologically based detection kit. This very low frequency of isolation from apparently infected grape tissues may be due to fungal inhibitors within them or to the fungicide and antibiotic components of the agar medium. Regardless of the cause, these results indicate that failure to isolate *Phytophthora* spp. from symptomatic grapevines should not be interpreted as a proof of their absence.

A relatively high incidence of root and crown rots have been observed in Chile on cv. Red Globe, which appeared to be more damaged by *P. cinnamomi, P. cryptogea,* and *P. drechsleri* than Thompson Seedless. Current control strategies integrate sanitation, water management, and fungicide applications. The use of resistant rootstocks, as proposed elsewhere (MARAIS 1979) is currently not practical in Chile, where grapevines are produced exclusively on their own roots.

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Table 2

Pathogenicity of *Phytophthora cinnamomi*, *P. cryptogea* and *P. drechsleri* on potted vines of grape (*Vitis vinifera*) cvs Thompson Seedless and Red Globe

Isolates[1]	Canker, mm[2]		Root fresh weight reduction, %[3]	
	Thompson Seedless	Red Globe	Thompson Seedless	Red Globe
		P. cinnamomi		
V94.01	11.4±1.2	7.8±1.0	5.1 ± 7.3	66.3 ± 9.1
V94.02	11.8±2.3	11.9±2.7	16.6 ± 18.2	48.5 ± 7.4
		P. cryptogea		
V94.19	10.3±2.7	6.8±1.0	12.3 ± 20.0	51.6 ± 8.0
V94.21	13.5±2.1	11.0±2.6	27.7 ± 7.6	50.5 ± 12.6
V94.23	10.0±3.0	9.6±1.4	13.1 ± 12.3	59.6 ± 27.0
V94.39	10.5±4.4	10.9±2.3	27.5 ± 8.7	63.8 ± 15.0
K15.2[4]	11.9±1.6	10.6±2.3	19.3 ± 19.9	47.9 ± 9.8
NY413[4]	11.1±1.6	8.4±2.0	19.3 ± 10.9	62.1 ± 5.1
		P. drechsleri		
V20	11.6±2.9	9.6±1.7	11.4 ± 21.6	58.6 ± 10.7
		Non inoculated checks		
	0	0	0	0

[1] Inoculum of individual isolates was introduced into pots containing 1-year-old vines, which were subsequently maintained in trays filled with 1 to 2 cm of water.

[2] Length of canker lesions on the crown and main roots after 3 months of incubation in a screenhouse. Means of 4 replicates ± standard deviation.

[3] % reduction of root fresh weight relative to the uninoculated checks after 3 months of incubation. Means of 4 replicates ± standard deviation.

[4] K15.3 and NY413 were known isolates of *P. cryptogea* from kiwifruit in Chile and deciduous fruit crops in New York State, respectively.

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