Cause and Control of Decline of Grapevines in British Columbia

R. S. UTKHEDE, Research Scientist, Agriculture Canada, Research Station, Summerland, B.C. V0H 1Z0, and J. VIELVOYE, Grape Specialist, Ministry of Agriculture and Food, Kelowna, B.C., Canada V1Y 4R2

ABSTRACT

Utkhede, R. S., and Vielvoye, J. 1984. Cause and control of decline of grapevines in British Columbia. Plant Disease 68: 820-822.

Pythium ultimum was the fungus most commonly isolated from grapevines that showed symptoms of decline. In pathogenicity tests, *P. ultimum* caused severe root rot and death of vines under greenhouse conditions and large lesions on excised twig vines in vitro. Mycelial growth of *P. ultimum* on cornmeal agar (CMA) was completely inhibited by 50 mg/L of metalaxyl, mancozeb, and a nutrient fertilizer supplement (This), partially inhibited by metalaxyl plus mancozeb, and not inhibited by phosethyl Al. Yield of berries was significantly higher with This treatment in 1982 and with phosethyl Al treatment in 1983. Soil drenches with phosethyl Al around the bases of naturally infected vines showing initial symptoms of decline under vineyard conditions prevented death of grapevines. Metalaxyl or metalaxyl plus mancozeb were ineffective in controlling grape decline and death of vines caused by *P. ultimum*.

A disease referred to as decline of grapevines (*Vitis vinifera* L.) has become a serious problem in irrigated vineyards of the Okanagan Valley of British Columbia. This disease is restricted to cultivar Okanagan Riesling. Typical symptoms on this cultivar included delayed and weak vine growth, sparse yellowish foliage, and little if any berry production. Grapevines are 4–5 yr old when they first show the symptoms of the disease. The disease is more prevalent on heavier soils. Diseased vines show abundant root and trunk rot below the soil line and reduction in feeder roots. Death of the vines usually occurs within 2 yr from the time symptoms appear.

In California, six species of *Phytophthora*, one species of *Pythium*, and two unidentified fungi caused stunting and root decay of young vines (3). *Phytophthora cinnamomi* has been isolated from collars and roots of diseased vines in Australia (10) and India (1). Bumbieris (2) isolated five *Pythium* spp. from roots of diseased vines in Australia. *Cylindrocarpon obtusisporium* was reported to cause a decline of the grapevine hybrid 225 Ruggeri in a Sicilian nursery (6). In South African vineyards, *Phytophthora* and *Pythium* spp. pathogenic to grapevines were isolated, with *P. cinnamomi* being the most frequent (8,9). No previous attempts have been made in the Okanagan Valley to determine the causal agent(s) of the grapevine decline disease. This study, therefore, was undertaken to determine the causal agent of the grape decline and to evaluate fungicides for its control.

Contribution No. 594, Agriculture Canada, Research Station, Summerland, B.C.

Accepted for publication 15 May 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Isolation and identification of causal organism. During summer and early fall of 1981 and spring of 1982, infected bark and root samples were collected from vines showing typical symptoms of grape decline as described earlier. Rotted inner bark and roots were plated on three media as follows:

Medium 1. Ten grams of rotted inner bark from the edge of necrotic area from three samples was added to 100 ml of distilled water, thoroughly mixed, and 1-ml aliquots were dispensed on a selective medium (11) containing 1 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $CaSO_4 \cdot 7H_2O$, 1 g of DL-threonine, 0.02 g of thiamine-HCl, 0.2 g of sodium taurocholate, and 20 g of sucrose, all per 1,000 ml of distilled water. This medium was specially developed for isolation of fungi of the family Pythiaceae. Ten plates were poured per sample. Before pouring the plates, benomyl, dissolved in DMSO, was added to give final concentrations of 20 μg/ml of benomyl and 0.5% DMSO. Plates with bark suspensions were incubated at 18 C. After 48 hr, the suspensions were washed with distilled water from the surface of the medium and 0.2 ml of a solution containing 0.01 g of pimaricin and 0.01 g of gallic acid in 10 ml of sterile distilled water was added to each plate. Three days after application of the bark suspensions, colonies typical of Pythiacius fungi could be recognized at ×40.

Medium 2. Infected roots and root tips were washed in running tap water to remove soil particles and were quick-dipped in 70% ethyl alcohol for surface-sterilization. These roots were placed on blotting paper to remove excess ethyl alcohol. These surface-sterilized roots were plated on a selective medium that contained pimaricin, vancomycin, and PCNB at 10, 200, and 100 ppm, respectively (12). Each plate contained all three compounds. The colonies typical of *Phytophthora* and *Pythium* were identified under binocular microscope.

Medium 3. The infected roots and bark were surface-sterilized as described before and plated on potato-dextrose agar (PDA).

Pathogenicity tests in greenhouse (1981). Twenty-two unidentified fungi and bacteria isolated on PDA (medium 3) were tested for their ability to infect grape cultivar Okanagan Riesling in sterilized soil under greenhouse conditions by using pure cultures grown on PDA. Five 1-yr-old vines of Okanagan Riesling were inoculated with each actively growing fungus/bacterium. A 5-mm hole was made in the vine with a cork borer just below the soil line. Bark was removed and a 5-mm plug containing fungus or bacterium was placed in the hole. The bark was replaced and was covered with transparent Scotch tape. Five vines were left as controls.

Pathogenicity test in greenhouse (1982). The fungus isolated most frequently on all three media, identified as *Pythium ultimum* Trow by D. J. S. Barr of Biosystematic Research Institute, Central Experimental Farm, Ottawa, Ontario, was tested for its ability to infect the 1-yr-old Okanagan Riesling under greenhouse conditions. Ten vines were inoculated with actively growing colonies of *P. ultimum* (5-mm plug) as described earlier. Ten vines were left as controls.

In vitro pathogenicity test. An excised twig assay (7) was also used to test pathogenicity of *P. ultimum* on Okanagan Riesling twigs. Cornmeal agar (CMA) was added to glass jars to give an agar depth of about 10 mm. *P. ultimum* was grown on this agar medium with a 5-mm plug of the fungus. Jars were sealed with Parafilm and stored at 18 C for 48 hr. Segments 75 mm long were cut from the central portion of the healthy shoots obtained from field-grown grapevines. These shoots were surface-sterilized in 0.6% NaOCl for 5 min, rinsed three times in sterile water, and blotted dry. The basal end of each twig was cut tangentially on opposite sides. Ten of these twigs were inserted in the agar in the jar at the periphery of the fungal colony.

Ten twigs were also inserted in the agar without fungal colony to serve as controls. The jars were resealed with Parafilm and incubated at 18 C for 6 days. The length of necrosis on each twig was measured after peeling off the bark.

In vitro effect of fungicides on growth of *P. ultimum*. *P. ultimum* was tested for its ability to grow on CMA containing 50–1,000 mg/L of the fungicides metalaxyl, phosethyl Al, mancozeb, This (flowable copper 4.4% and sulfur 50%), a nutrient fertilizer supplement (Stoller Chemical Inc.), and metalaxyl plus mancozeb (8 + 64%, respectively).

Isolate (S101) of *P. ultimum* used in this in vitro study was obtained from our infected Okanagan Riesling vine. Stock cultures were maintained on CMA at 18 C. The fungicides were suspended in CMA at 48 C and 5 ml of agar was placed in each petri plate (60 × 15 mm). Ten plates of each fungicide treatment were inoculated with a 5-mm plug of active mycelium (48 hr old) of *P. ultimum* in the center of the plate and incubated at 18 C. Growth of *P. ultimum* was measured as the diameter from edge to edge of the fungal growth on the plates three days after inoculation.

Effect of fungicide drenches on grape decline under field conditions. A 7-yr-old planting of Okanagan Riesling in Oliver, B.C., was selected to study the effect of fungicides on disease development. One hundred twenty vines, including some showing symptoms of grape decline, were selected for fungicide treatments. Four fungicides, metalaxyl, phosethyl Al, metalaxyl plus mancozeb, and mancozeb, and a nutrient fertilizer (This) were evaluated as drench applications in a randomized complete-block design with five replicates. Each replicate consisted of four vines per treatment. The fungicides were drenched by hand with 4.5 L of water around the trunk on 18 September 1981, 11 May and 26 August 1982, and again on 11 May and 9 September 1983. Fungicides were applied with a plot sprayer that included a bypass valve, mechanical agitator, and a flow-restrictor valve (9 L/min) on the discharge hose.

Data on yield were collected on 21 September, 26 September, and 29 September of 1981, 1982, and 1983, respectively. Data on pruning weights were collected on 12 May and 8 March of 1982 and 1983, respectively. These vines were balanced-pruned before the first fungicide application. The number of dead vines in each treatment was recorded on 29 September 1983. Original observations on vines showing initial, intermediate, and terminal symptoms of decline were recorded on 18 September 1981. Symptoms of decline were noted as follows: initial = vines beginning to show sparse and yellow leaves, intermediate = vines showing stunted growth, and terminal = vines close to death. Data were subjected to statistical analysis (4). Duncan's new multiple range test at the 5 and 1% levels of significance was used to compare treatments.

Table 1. Effect of fungicides on the mean radial growth (mm) of *Pythium ultimum* after 48 hr of incubation at 18 C

	Concentration of fungicides (mg/L)					
Fungicides	**0**	**50**	**100**	**200**	**400**	**600**
Metalaxyl	50	0.0	0.0	0.0	0.0	0
Metalaxyl + mancozeb	50	9.3	0.4	0.0	0.0	0
Mancozeb	50	0.0	0.0	0.0	0.0	0
Phosethyl Al	50	50.0	50.0	50.0	17.5	0
This	50	0.0	0.0	0.0	0.0	0

Table 2. Effect of fungicides applied as a soil drench on the yield (kg/vine) in a field trial conducted from 1981 through 1983

Treatment	Rate (a.i./vine)	1981	1982	1983	1982 Increase over 1981	1983 Increase over 1981
This	15.0 ml	9.0 a[z]	12.0 a	9.7 ab	3.0 a	0.7 ab
Mancozeb	1.8 g	7.6 a	9.6 abc	8.4 ab	2.0 ab	0.8 ab
Phosethyl Al	5.0 g	8.1 a	9.8 abc	10.5 b	1.7 ab	2.4 b
Metalaxyl	1.0 g	10.6 a	7.3 c	10.2 ab	−3.3 c	−0.4 ab
Metalaxyl + mancozeb	0.1 + 0.9 g	12.1 a	10.1 ab	10.4 ab	−2.0 bc	−1.7 ab
Control	···	10.5 a	8.9 bc	8.0 a	−1.6 bc	−2.5 a
SE		1.4	0.9	0.8	1.5	1.6

[z] Values within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

Table 3. Effect of fungicides applied as a soil drench in 4.5 L of water on the number vines showing initial, intermediate, and terminal symptoms of grapevine decline in 1981 and number of vines dead by fall 1983

		Number of vines[a]					
Treatment	Rate (a.i./vine)	Initial symptoms 1981	Dead by 1983	Intermediate symptoms 1981	Dead by 1983	Terminal symptoms 1981	Dead by 1983
Phosethyl Al	5.0 g	6	0	4	4	2	2
This	15.0 ml	8	8	0	0	1	1
Metalaxyl	1.0 g	5	5	1	1	3	3
Metalaxyl + mancozeb	0.1 + 0.9 g	1	1	1	1	4	4
Mancozeb	1.8 g	6	6	1	1	3	3
Control	···	5	5	1	1	3	3

[a] Number of vines of 20 showing symptoms of grapevine decline.

RESULTS AND DISCUSSION

The fungus most frequently isolated on all three media from infected vines was *P. ultimum*. Although two media (11,12) developed especially for isolation of *Phytophthora* spp. were used in our study, no *Phytophthora* spp. were isolated from infected roots or bark. None of the unidentified fungi and bacteria isolated from infected roots and bark in 1981 were pathogenic to cultivar Okanagan Riesling.

All 10 *P. ultimum*-inoculated Okanagan Riesling vines showed decline after 1 yr under greenhouse conditions. *P. ultimum* was isolated from each infected vine but not from control vines. In the in vitro studies, lesion lengths of inoculated and uninoculated shoots of Okanagan Riesling were 41.6 and 0.0 mm, respectively. All pathogenic tests (in vitro and greenhouse-grown vines) indicated that *P. ultimum* was pathogenic to Okanagan Riesling, causing decline and death of grapevines.

Reduction of growth and severe decay of roots were observed on young Carignane vines when inoculated with *P. ultimum* in California (3). Most isolates from infected grapevines in South Africa were *Pythium* (36.4%), of which *P. ultimum* made up 35.1% (9). It may be argued that the *P. ultimum* is a weak parasite to cause infection to the woody plants. Vineyard operators constantly remove the suckers by hand from the vines at or just below the soil line. This could allow easy access by the fungus into the host.

The mycelial growth of *P. ultimum* was completely inhibited on CMA containing 50 mg/L of metalaxyl, mancozeb, and This (Table 1). Complete inhibition with phosethyl Al required 600 mg/L, and with metalaxyl plus mancozeb, it

required 200 mg/L. No difference in yield of berries was observed among any of the fungicides tested in 1981 (Table 2). In 1982, vines treated with nutrient fertilizer (This) showed significantly higher yield of berries than the untreated control. In 1983, phosethyl Al-treated vines had significantly higher yield than the untreated controls. Data on pruning weights were also collected but no significant differences were found between any of the treatments and the control.

Data on the number of vines showing initial, intermediate, and terminal symptoms in 1981 and number of vines dead by 1983 are presented in Table 3. Phosethyl Al was the only treatment that prevented death of infected vines showing initial symptoms of grapevine decline disease. This indicates that phosethyl Al has the ability to arrest further symptom development of vines infected with *P. ultimum* if the vines are treated as soon as the symptoms of the disease appear. Although the nutrient fertilizer This had provided a significant increase in yield of berries, it failed to protect vines in either initial, intermediate, or terminal stages of disease development. It is interesting to note that the fungicide metalaxyl showed no activity in the field against *P. ultimum*, although complete inhibition of *P. ultimum* was observed with 50 mg/L of metalaxyl on a CMA plate. Recently, Cook et al (5) showed that metalaxyl was ineffective in controlling root rot of wheat caused by *P. ultimum* under field conditions.

ACKNOWLEDGMENTS

We thank B. Hughes and E. Smith for their able technical assistance and J. and M. Fatur for their excellent cooperation in this study. Appreciation is extended to D. J. S. Barr for identification of *Pythium ultimum*.

LITERATURE CITED

1. Agnihotrudu, V. 1968. A root rot of grapes in Andura Pradesh. Curr. Sci. 37:292-294.
2. Bumbieris, M. 1972. Observations on some Pythiaceous fungi associated with grapevine decline in South Australia. Aust. J. Agric. Res. 23:651-657.
3. Chiarappa, L. 1959. The root rot complex of *Vitis vinifera* in California. Phytopathology 49:670-674.
4. Cochran, W. G., and Cox, G. M. 1964. Experimental Design. John Wiley & Sons, New York. 611 pp.
5. Cook, R. J., Zhang, B. X., and Doerr, A. 1983. Failure of metalaxyl to control all of the *Pythium* population pathogenic on roots of Pacific Northwest Wheat. (Abstr.) Phytopathology 73:957.
6. Grasso, J., and Magnano Di San Lio, G. 1975. Infezioni di *Cylindrocarpon obtusisporium* su piante di vite in Sicilia. Vitis 14:36-39.
7. Jeffers, S. N., Aldwinckle, H. S., Burr, T. J., and Arneson, P. A. 1981. Excised twig assay for the study of apple tree crown rot pathogens in vitro. Plant Dis. 65:823-825.
8. Marais, P. G. 1979. Fungi associated with root rot in vineyards in the Western Cape. Phytophylactica 11:65-68.
9. Marais, P. G. 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. Phytophylactica 12:9-13.
10. McGechan, J. K. 1966. *Phytophthora cinnamomi* responsible for a root rot of grapevines. Aust. J. Sci. 28:354.
11. McIntosh, D. L. 1977. *Phytophthora cactorum* propagule density levels in orchard soil. Plant Dis. Rep. 61:528-532.
12. Tsao, P. H., and Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature (Lond.) 223:636-638.