PHYTOPHTHORA CINNAMOMI ROOT ROT OF GRAPEVINES IN SOUTH AFRICA

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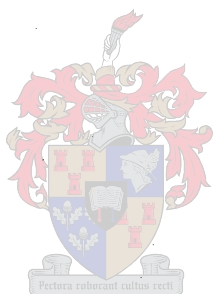

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1 GENERAL INTRODUCTION

Root rot of grapevines (*Vitis* spp.) has become increasingly important in South Africa in recent years. In 1972 the high mortality of vines grafted onto rootstock 99 Richter (*V. berlandieri* P. x *V. rupestris* S.) was attributed to *Phytophthora cinnamomi* Rands (Van der Merwe, Joubert & Matthee, 1972). There have been descriptions of root rot of grapevine caused by *P. cinnamomi* in Australia (McGechan, 1966), India (Agnihothrudu, 1968) and South Africa (Van der Merwe *et al.*, 1972) but, apart from these records, there are no data on *P. cinnamomi* root rot of this crop. Most of the present information on *P. cinnamomi* root rot is based on avocado (Zentmyer, 1980) and is not directly applicable to grapevine. In this present investigation initial surveys showed that *P. cinnamomi* was the most important pathogen associated with root rot in grapevine nurseries and in vineyards in the grape growing areas of the South-Western Cape Province. A detailed study was therefore made of *P. cinnamomi* in relation to its grapevine host.

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2 FUNGI ASSOCIATED WITH ROOT ROT IN VINEYARDS

ABSTRACT

During the period September 1972 to December 1977, 7 287 fungal isolates were obtained from roots and rhizosphere soil of stunted, dying or dead grapevines in South Africa. Most (46,7%) of the isolates belonged to the genera Phytophthora and Pythium. The predominant Phytophthora species was P. cinnamomi (1 952 isolates) followed by P. cactorum (19 isolates), P. parasitica (14 isolates) and P. cryptogea (11 isolates). All four Phytophthora spp. were pathogenic to grapevine rootstocks. P. cinnamomi was the most virulent, killing nearly half the number of test plants. Of the 1 409 isolates of Pythium spp. the dominant were P. ultimum (821 isolates) followed by P. aphanidermatum (230 isolates), P. sylvaticum complex (201 isolates) and P. irregulare complex (157 isolates). All four Pythium spp. were pathogenic to grapevine rootstocks with P. ultimum being the most virulent. Macrophomina phaseolina (Rhizoctonia bataticola) and Rhizoctonia solani were pathogenic to 101-14 Mgt (Vitis riparia x V. rupestris) and V. champini var. Ramsey, respectively.

INTRODUCTION

In the Western Cape Province of South Africa the decline and death of grapevines grafted on various rootstocks have occurred for many years. Death of young vines grafted on the rootstock cultivar 99 Richter (Vitis berlandieri P. x V. rupestris S.) is particularly important, though death of even older vines grafted on the same rootstock also occurs, usually at

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the time of fruiting. In Australia McGechan (1966) consistently isolated Phytophthora cinnamomi Rands from collars and roots of affected vines, and Agnihothrudu (1968) isolated the fungus from affected mature vines in India.

The disease manifests itself in rapid death of vines, and in many cases, also in retarded and weak growth with necrosis of feeder roots. In rootstock Jacquez (V. aestivalis M. x V. cinerea E. x V. vinifera L.), a replant problem is often found when vines grafted on this rootstock are replanted in soil where old vines on Jacquez have been removed. The replants are characterised by weak growth, decline and even death. Chiarappa (1959) found species of Pythium and Phytophthora associated with delayed and weak growth of vines in California. Bumbieris (1972) isolated five Pythium species from roots of vines with this disorder in Australia. Workers in other parts of the world have associated the decline of various other perennial crops with high soil populations of pathogenic species of Phytophthora and Pythium (Klemmer & Nakano, 1964; Hendrix & Campbell, 1966; Campbell & Hendrix, 1967; Hendrix & Powell, 1968; Biesbrock & Hendrix, 1970; Mircetich, 1971).

This study was conducted to determine the identity and numbers of fungal pathogens causing root rot of grapevines in the Western Cape and to assess their pathogenicity under controlled conditions.

MATERIALS AND METHODS

Isolation and identification

A survey of grapevines showing decline and rapid dying in the Western Cape

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was started in September 1972 and completed in December 1977. Root and soil samples were collected at a depth of 300-400 mm from 900 different vineyards in several districts. Roots from dying, dead or stunted vines were surface disinfested in 1% sodium hypochlorite and plated on potato dextrose agar (PDA) containing streptomycin sulphate (100 mg/l). Soil samples were sieved through 0,84 mm mesh screens and pythiaceous fungi were isolated from the soil according to techniques described by Campbell (1949), McIntosh (1964) and Chee & Newhook (1965). Purified cultures were identified to generic level by the keys of Barnett (1955) and Gilman (1957); Pythium and Phytophthora species were identified according to descriptions of various other workers (Middleton, 1943; Waterhouse, 1956, 1963, 1967, 1968; Hendrix & Papa, 1974).

Pathogenicity tests

Rootstock cuttings were rooted in sterilised sand and transplanted to free-draining 18 cm clay pots containing a sterilised potting mixture. Seven to 10-d-old fungus colonies on PDA in 90 mm diameter petri dishes served as inoculum. Four holes were made in the potting mixture around each vine and the culture from one petri dish was macerated in a blender and placed in each hole. The pathogenicity of isolates was tested on rootstocks of the same cultivar. The effect of root rotting fungi was evaluated by comparing the growth of inoculated vines with that of controls, by rating decay on an arbitrary 0-5 scale and by re-isolation on PDA. Four replicates with five vines per replicate were used in all tests.

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RESULTS

Fungi identified

Twenty genera were identified from a total of 7 287 isolates from roots and rhizosphere soil of both stunted and dead or dying grapevines on various rootstock cultivars (Table 1). Phytophthora (27,4%) and Pythium (19,3%) were the most frequently found. The dominant fungus genera from the various rootstocks were Phytophthora (34,7%) and Pythium (18,0%) from 99 Richter, Macrophomina (19,7%), Pythium (19,1%) and Phytophthora (15,9%) from 101-14 Mgt., Pythium (47,5%) and Fusarium (19,8%) from Jacquez, Phytophthora (60,6%) from V. rupestris var. du Lot and Rhizoctonia (71,4%) from V. champini var. Ramsey.

Phytophthora isolates keyed into four species: P. cinnamomi Rands (1 952 isolates), P. cactorum (Lebert & Cohn) Schroeter (19 isolates), P. parasitica Dastur (14 isolates) and P. cryptogea Pethybridge & Lafferty (11 isolates).

A total of 1 409 isolates was separated into two groups based on the presence or absence of protuberances on the oogonial surfaces (Waterhouse, 1967). Further subdivision into species or species groups was done according to the classification suggested by Hendrix & Papa (1974). The sizes of reproductive cells were not taken into account as these can change when isolates are held in culture (Hendrix & Campbell, 1974).

Pythium isolates also keyed into four species: P. ultimum Trow was iso-

TABLE 1 Dominant fungus genera isolated from roots and rhizosphere soil of dying or dead grapevine rootstock cultivars from different areas of the Western Cape Province

Location	Rootstock	Number of isolates	Frequency of fungus genera (%)									
			Phytophthora	Pythium	Fusarium	Cephalosporium	Trichoderma	Penicillium	Cylindrocarpon	Rhizoctonia	Macrophomina	Unidentified or other genera
Stellenbosch	99 Richter	1 600	31,4	15,1	6,6	4,1	3,3	2,9	-	-	-	36,3
	Jacquez	112	-	53,6	21,4	-	-	8,9	10,7	-	-	5,4
	101-14 Mgt	89	34,8	43,8	12,4	6,7	-	-	-	-	-	2,2
	Vitis rupestris var. duLot	42	57,1	21,4	-	-	-	-	-	-	-	21,6
Wellington	99 Richter	1 580	33,8	13,9	2,9	1,8	-	-	-	-	-	47,6
	101-14 Mgt	890	24,7	23,8	-	5,8	-	8,8	9,7	-	-	27,2
	Jacquez	96	-	39,6	22,9	-	16,7	12,5	-	-	-	8,3
	Vitis rupestris var. duLot	26	53,8	34,6	-	-	-	-	-	-	-	8,35
	Vitis champini var. Ramsey	24	-	-	-	-	4,2	12,5	-	83,3	-	-
Paarl	99 Richter	890	47,4	32,1	-	-	-	4,7	6,7	-	-	20,2
	101-14 Mgt	422	26,5	44,5	-	-	4,0	4,7	-	-	17,1	19,1
	Vitis champini var. Ramsey	18	-	-	-	-	-	22,2	-	55,6	-	22,2
Malmesbury	101-14 Mgt	890	-	-	-	-	4,3	2,5	3,0	-	40,6	49,7
	99 Richter	153	40,5	20,3	5,9	11,8	-	10,5	-	-	-	11,1
	101-14 Mgt	80	20,0	11,3	-	-	-	-	-	-	47,5	21,3
Somerset West	99 Richter	64	42,2	17,2	-	-	-	-	-	-	-	40,6
	Jacquez	29	-	48,3	-	-	-	6,9	10,3	-	-	4,5
Worcester	99 Richter	130	50,0	16,2	-	9,2	5,4	-	-	-	-	19,2
	Jacquez	26	-	50,0	23,1	-	-	-	-	-	-	26,9
De Doorns	99 Richter	42	45,2	-	9,5	-	-	-	-	-	-	65,2
Riebeek Kasteel	99 Richter	22	77,3	-	-	-	-	-	-	-	-	22,7
Montagu	Vitis rupestris var. du Lot	26	73,1	-	-	-	-	-	-	-	-	26,9
	99 Richter	18	66,7	-	-	-	-	-	-	-	-	33,3
	101-14 Mgt	18	-	44,4	16,7	-	-	-	-	-	-	38,9

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lated most frequently (821 isolates) followed by P. aphanidermatum (Edson) Fitzpatrick (230 isolates), P. sylvaticum complex (201 isolates) and P. irregulare complex (157 isolates).

The Macrophomina isolates were identified as M. phaseolina (Tassi) Goid (Rhizoctonia bataticola (Taub.) Briton-Jones) whereas the Rhizoctonia isolates were identified as R. solani Kühn.

The three distinct groups of Fusarium isolated showed no pathogenicity to grapevines and were therefore not identified further.

Pathogenicity tests

Pathogenicity tests were made with single representatives of all the Phytophthora and Pythium spp., M. phaseolina, R. solani and three Fusarium isolates (Table 1). All Phytophthora spp. were pathogenic to grapevine roots (Table 2). P. cinnamomi was the most virulent, causing severe root rot, reduction in root mass and death of many plants. P. parasitica caused the death of two out of 20 plants whereas the growth of the remaining plants was poorer than that of non-inoculated controls.

P. cactorum and P. cryptogea caused rotting of fine feeder roots and reduction in root mass. These symptoms were also observed with the four Pythium spp. tested, P. ultimum being the most virulent. A reduction in plant growth and root mass was also observed with M. phaseolina and R. solani. No symptoms were observed with the Fusarium isolates. All isolates recorded as pathogenic were re-isolated from inoculated plants.

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TABLE 2 Influence of different fungal species inoculated on grapevine rootstocks grown for 60 days in a glasshouse at 25°C

Rootstock	Treatment[a]	Average root rot rating[b]	Root mass (g)	Shoot mass (g)	Number of dead plants
99 Richter	Control	0	3,70	2,54	0
	Phytophthora cactorum	2	2,45	1,69	0
	Phytophthora cinnamomi	4	1,85	1,22	8
	L S D	1,29	0,88	0,56	-
101-14 Mgt	Control	0	4,21	3,06	0
	Phytophthora cryptogea	1,5	2,74	2,14	0
	Phytophthora parasitica	3	2,18	1,42	2
	L S D	0,86	1,03	0,36	-
Vitis rupestris var. du Lot	Control	0	3,46	2,41	0
	Phytophthora cinnamomi	4	1,25	1,18	10
	L S D	1,30	0,22	0,96	-
Jacquez	Control	0	2,81	1,96	0
	Pythium aphanidermatum	2	1,52	1,31	0
	Pythium irregulare (complex)	2	1,50	1,20	0
	Pythium ultimum	3	0,92	0,84	0
	L S D	-	1,05	0,93	-
101-14 Mgt	Control	0	4,02	3,01	0
	Pythium sylvaticum (complex)	2	2,51	2,16	0
	Pythium ultimum (complex)	3	2,14	1,96	-
	L S D	-	1,30	0,78	-
99 Richter	Control	0	3,92	2,79	0
	Pythium irregulare (complex)	2	2,36	1,54	0
Vitis rupestris var. du Lot	Control	0	3,52	2,85	0
	Pythium aphanidermatum	2	2,41	1,46	0
	Pythium ultimum (complex)	2,5	2,19	1,30	0
	L S D	-	-	-	-
101-14 Mgt	Control	0	3,52	3,21	0
	Macrophomina phaseolina	2	2,41	1,35	0
	L S D	-	0,64	0,76	-
Vitis champini var. Ramsey	Control	0	4,36	3,40	0
	Rhizoctonia solani	2	2,12	1,51	0
	L S D	-	0,89	0,47	-
99 Richter	Control	0	3,64	2,61	0
	Fusarium sp. (isolate 1)	0	3,58	2,69	0
101-14 Mgt	Control	0	4,12	3,21	0
	Fusarium sp. (isolate 2)	0	3,97	3,26	0
Jacquez	Control	0	2,76	1,72	0
	Fusarium sp. (isolate 3)	0	2,82	1,68	0

[a] Control plants not inoculated.

[b] Rating system: 0 = no root rot; 1 = 1-20% root rot; 2 = 21-40% root rot; 3 = 41-60% root rot; 4 = 61-80% root rot; 5 = 81-100% root rot.

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In most cases where death of grapevines in commercial vineyards was ascribed to P. cinnamomi, the vines were between 2 and 5 years old. The pathogen was less frequently involved with death of vines older than 5 years (18 of all the vineyards surveyed). This was also found with the Pythium spp., although older vines showed stunted growth due to rotting of the fine feeder roots as opposed to dying due to Phytophthora infection.

Isolation of P. cinnamomi from rhizosphere soil of affected vines was successful in a few cases only; Pythium spp. were frequently isolated from rhizosphere soil.

DISCUSSION

P. cinnamomi was shown to be one of the most virulent of the Phycomycetes isolated from diseased grapevine roots. Most of the losses in the field ascribed to this fungus occurred in young vineyards, although vines of up to 10 years old were also killed. The other Phytophthora and Pythium spp. killed young vines in the field whereas older vines showed decline and poor growth due to the rotting of fine roots and root tips. These observations are in line with the findings on other perennial crops by the workers previously mentioned. Results of isolations from rhizosphere soil suggested that in the case of Phytophthora, infection might be due to the planting of infested material rather than to infection from the soil, whereas in the case of Pythium, infection was due to the planting of infested material or to infection from the soil.

Pythium spp. were the dominant fungi (47,5%) isolated from Jacquez. This

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could explain the replant problem experienced with this rootstock. In old Jacquez plantings susceptible roots are constantly regenerated. This could lead to a build-up of Pythium propagules, which remain in the soil after removal of the old vines. Replants of Jacquez can then quickly be killed by extensive infection of the feeder roots and root tips.

This is the first local report that M. phaseolina is a pathogen of grapevines. It was isolated from 101-14 Mgt only, from areas with high summer temperatures.

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3 FUNGI ASSOCIATED WITH DECLINE AND DEATH OF GRAPEVINES IN NURSERIES

ABSTRACT

Fungi most commonly isolated from the roots and rhizosphere soil of dead and dying vines from 223 grapevine nurseries in the Southern and Western Cape Province were Pythium spp. (P. ultimum, P. aphanidermatum, P. sylvaticum complex, P. irregulare complex and P. rostratum complex) comprising 36,4% of the isolates and Phytophthora spp. (P. cinnamomi, P. parasitica, P. cryptogea, P. cactorum and P. megasperma) comprising 23,5% of the isolates. In inoculation studies P. cinnamomi and P. parasitica caused severe root rot and death, whereas P. cryptogea caused rotting of the fine feeder roots and reduction in root mass but not death of the plants. Similar symptoms were also observed with the Pythium spp. tested. P. megasperma was nonpathogenic. Other pathogens isolated were Rosellinia necatrix and Sclerotium rolfsii.

P. cinnamomi colonized 14%, 17% and 8% respectively of vine roots, vine canes, and wheat straw added to inoculated, nonsterilised soil. Colonization was more vigorous (24%, 64% and 19% of the roots, canes and straw respectively) in sterilised soil. The fungus was found in vine debris in soil 3 years after removal of the vines.

INTRODUCTION

The fungi listed by Winkler (1962) as pathogens of grapevine roots are

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Armillaria mellea (Wahl) Quelet, Phymatotrichum omnivorum (Shear) Duggar, Rosellinia necatrix (Hart) Berl. and species of Pythium and Phytophthora. In South Africa, Phytophthora cinnamomi Rands caused rapid death of grapevines grafted on 99 Richter rootstock (Van der Merwe, Joubert & Matthee, 1972). This pathogen has been isolated from collars and roots of diseased vines in Australia (McGechan, 1966) and India (Agnihothrudu, 1968). In California, species of Pythium and Phytophthora have been associated with delayed and weak growth of vines (Chiarappa, 1959). Bumbieris (1972) isolated five Pythium spp. from roots of diseased vines in Australia. Grasso & Magnano Di San Lio (1975) reported that a decline characterized by stunting and a black discoloration of the wood of the grapevine hybrid 225 Ruggeri in a Sicilian nursery was caused by Cylindrocarpon obtusisporium Wollenw.

In a recent survey, different pathogenic Phytophthora and Pythium spp. were isolated from South African vineyards, with P. cinnamomi being the most common pathogen (Part 2). Isolation of P. cinnamomi from rhizosphere soil of diseased vines in commercial vineyards was possible in only a few cases, probably because infection occurred in the nursery rather than in the vineyard soil (see Part 2). The present study was conducted to evaluate this possibility and to determine the identity and numbers of fungal pathogens in grapevine nurseries.

MATERIALS AND METHODS

Isolation and identification of fungi

During 1974 and 1975 plant and soil samples were taken (in collaboration

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with the Division of Plant and Seed Control, Stellenbosch) from 223 grapevine nurseries. Segments of roots from dead, dying and stunted vines of several commercial rootstock cultivars were surface disinfested in 0,5% sodium hypochlorite and plated on potato dextrose agar (PDA) containing streptomycin sulphate (100 mg/l). Representative samples of nursery soils were collected to a depth of 300 mm with a 20 mm diameter Oakfield hand soil auger. Samples were sieved through 0,84 mm screens and pythiaceous fungi were isolated by using techniques of Campbell (1949), McIntosh (1964) and Chee & Newhook (1965).

Pure cultures were identified to generic level by the keys of Barnett (1955) and Gilman (1957). Species of Pythium and Phytophthora were identified according to descriptions of various workers (Middleton, 1943; Waterhouse, 1956, 1963, 1967, 1968; Hendrix & Papa, 1974).

Pathogenicity tests

Fungi belonging to the following groups were tested for pathogenicity to grapevines: Pythium and Phytophthora spp., Rosellinia necatrix and Sclerotium rolfsii. The same rootstock cultivar from which the original isolations had been made was used for inoculation. Rootstock cuttings used routinely in establishing a vineyard were rooted in sterilised sand and transplanted to 18 cm free-draining clay pots containing a sterilised potting mixture. Fungus colonies 7 to 10-d-old growing on PDA in 90 mm petri dishes served as inoculum. Four holes were made in the potting mixture around each vine. The culture from one petri dish was macerated in a Waring blender and added to each hole. Four replicates with five vines per

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replicate were used in all tests. Pathogenicity was evaluated by comparing the growth of inoculated vines with that of uninoculated controls and by rating decay on an arbitrary six-point scale. The identity of the fungus on diseased roots was confirmed by re-isolation on PDA.

Population density of P. cinnamomi and Pythium spp.

A nursery soil with a high mortality of vines due to P. cinnamomi during the previous season was selected. Soils were sampled at weekly intervals from July (immediately after soil preparation for planting) until plants were removed during June of the following year. Unplanted soil and soil within the root zone of the rootstocks 143 B Mgt and 99 Richter, which are tolerant and susceptible respectively to P. cinnamomi (see Part 5), were sampled with the soil auger.

Samples were taken to a depth of 300 mm adjacent to 20 plants of each cultivar. Propagule population densities of P. cinnamomi and Pythium spp. were determined by plating soil on the selective media of Eckert & Tsao (1962) and Hendrix & Kuhlman (1965) respectively. Ten plates per sample were seeded with 1 ml of 1:25 soil suspension and incubated at 25°C in the dark. To study the propagule densities of P. cinnamomi and Pythium spp. at various depths, soil samples were taken at intervals to a depth of 400 mm.

Competitive saprophytic ability and persistence in soil

Saprophytic colonization of P. cinnamomi was determined by the method of

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Zentmyer & Mircetich (1966) using segments of vine roots, vine canes and wheat straw as substrata. Soil was made up to a moisture content of 14% by mass before adding the substrate and then incubated for periods of 10, 20, 40 and 80 d at 25°C. The experiment was replicated five times with 20 substrate segments per replicate.

The persistence of P. cinnamomi in vineyard soil 3 years after the removal of P. cinnamomi infected vines was investigated by plating pieces of grapevine roots and canes from sieved soil (2 mm screen) onto cornmeal agar at 25°C.

RESULTS

Isolation and identification of fungi

Most (36,4%) of the isolates were Pythium spp. (Table 1), including the following: P. ultimum Trow (35,1%), P. aphanidermatum (Edson) Fitzpatrick (21,0%), P. sylvaticum complex (18,9%), P. irregulare complex (14,9%) and P. rostratum complex (10,0%). Phytophthora comprised 23,5% of the isolates. The following species were identified: P. cinnamomi Rands (68,2%), P. parasitica Dastur (19,2%), P. cryptogea Pethybridge & Lafferty (9,4%), P. cactorum (Lebert & Cohn) Schroeter (1,9%) and P. megasperma Dreschler (1,3%). Sclerotium rolfsii Sacc. (2,9%) and Rosellinia necatrix (0,7%) were also recovered.

Phytophthora cinnamomi and P. parasitica caused severe root rot and death of plants, whereas P. cactorum and P. cryptogea caused rotting of the fine

TABLE 1 Fungi isolated from roots and rhizosphere soil of dead, dying and stunted grapevine from different nurseries in the South Western Cape Province

Location	Total number of isolates	Percentage frequency of isolates from each nursery (a) and number of infested nurseries (b)								
		Phytophthora		Pythium		Rosellinia		Sclerotium		Other
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)
Wellington	1 061	30,25	27	32,79	29	1,41	4	2,82	8	32,70
Paarl	432	25,46	6	40,27	10	-	-	6,91	3	28,24
Stellenbosch/Somerset West	361	23,26	4	40,99	7	-	-	3,87	2	31,86
Malmesbury	352	18,46	5	38,06	8	-	-	1,42	1	42,05
Bonnievale	78	-	-	26,92	7	2,56	1	-	-	70,50
Montagu	64	12,50	2	34,37	4	-	-	-	-	53,13
Franschhoek	42	16,67	1	42,85	2	2,38	1	4,76	1	33,30
Vredendal	41	4,87	2	39,02	5	-	-	-	-	56,10
Robertson	40	10,00	2	45,00	4	-	-	-	-	45,00
Porterville	39	17,94	1	41,02	3	-	-	-	-	41,03
Tulbagh	38	21,05	2	50,00	3	-	-	-	-	28,95
Wolseley	12	-	-	41,66	2	-	-	-	-	58,33
Piketberg	10	-	-	30,00	2	-	-	-	-	70,00
Worcester	10	-	-	30,00	2	-	-	10,00	1	60,00
Citrusdal	10	-	-	20,00	2	-	-	-	-	80,00
Villiersdorp	10	-	-	30,00	2	-	-	-	-	70,00
Elgin	5	-	-	20,00	1	-	-	-	-	80,00
Grabouw	4	-	-	25,00	1	-	-	-	-	75,00
Ashton	4	-	-	25,00	1	-	-	-	-	75,00
Ladismith	4	-	-	25,00	1	-	-	-	-	75,00
Oudtshoorn	3	-	-	33,33	1	-	-	-	-	66,67
Swellendam	3	-	-	33,33	1	-	-	-	-	66,67

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feeder roots and reduction in root mass but not death (Table 2). P. megasperma was non-pathogenic. The Pythium spp. tested caused rotting of the fine feeder roots and reduction in root mass but not death, whereas a reduction in plant growth and root mass was also observed with R. necatrix and S. rolfsii. Plants inoculated with S. rolfsii had a typical white fungal growth on the crown area and roots while the bark and underlying tissue were soft and came off easily. All isolates recorded to be pathogenic were re-isolated from inoculated plants.

Population density of P. cinnamomi and Pythium spp.

Populations of P. cinnamomi in the rhizosphere of the susceptible 99 Richter were low during the 6 weeks before planting and increased slowly until October (Fig. 1). From the beginning of October they increased sharply, reaching a peak during November, December and the beginning of January. This was followed by a marked decrease during January. Recovery was lowest during April and June. The first symptoms of P. cinnamomi infection were observed during December and the first dead plants were seen in January. Populations of P. cinnamomi in the rhizosphere of the tolerant 143 B Mgt rootstock also rose slowly in August but thereafter fluctuated at low levels. There was no sharp increase during November and January and no definite symptoms of P. cinnamomi infection were observed.

Populations of P. cinnamomi were highest in the upper 24 cm of soil beneath 99 Richter plants. This was also found with the Pythium spp. (Table 3).

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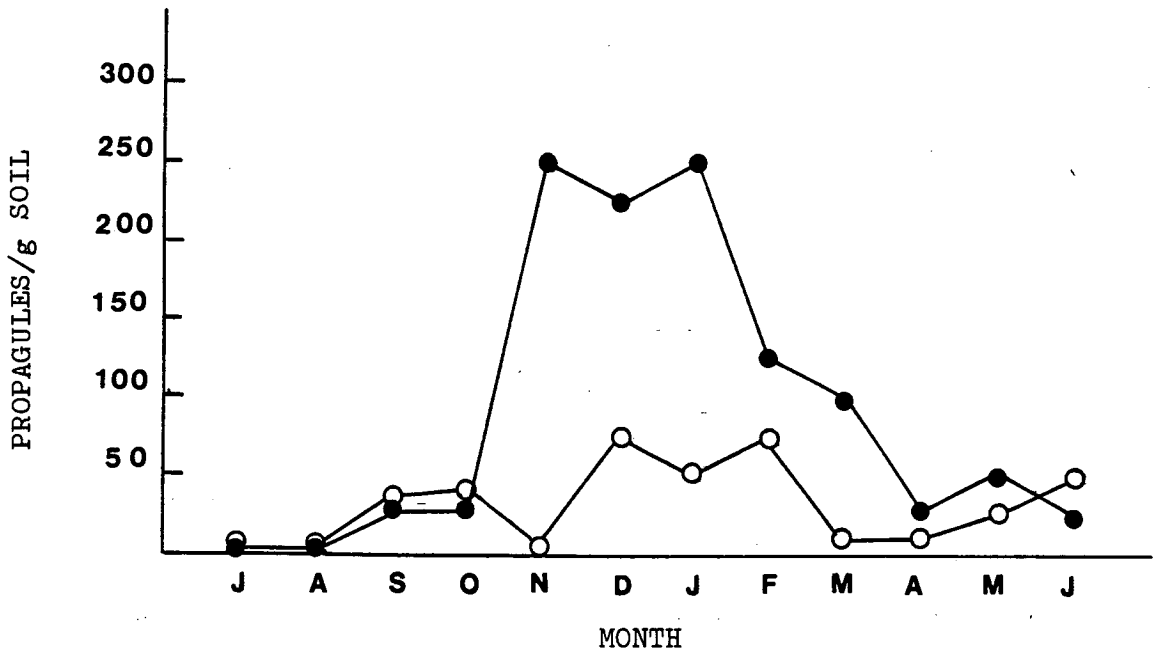

Fig. 1 Populations of Phytophthora cinnamomi in the rhizospheres of susceptible 99 Richter (●—●) and tolerant 143 B Mgt (○—○) grapevine rootstocks in infested nursery soil.

TABLE 2 Influence of different fungi inoculated on grapevine rootstocks grown for 60 d in a glasshouse at 25°C

Rootstock	Treatment[a]	Root rot rating[b]	Root mass (g)	Cane mass (g)	Number of dead plants
99 Richter	Control	0	3,23	2,59	0
	Phytophthora megasperma	0	3,22	2,41	0
	Sclerotium rolfsii	2	2,58	1,42	0
	Phytophthora cactorum	2	2,48	1,41	0
	Pythium irregulare	2	2,45	1,35	0
	Phytophthora cinnamomi	4	1,82	1,14	10
	D-values	-	0,64	0,25	-
101-14 Mgt	Control	0	3,70	3,46	0
	Pythium sylvaticum	2	2,58	2,58	0
	Phytophthora cryptogea	2	2,55	2,33	0
	Rosellinia necatrix	2	2,55	2,32	0
	Pythium ultimum	2	2,42	2,28	0
	Phytophthora parasitica	3	2,01	2,22	1
	Phytophthora cinnamomi	3	2,00	2,10	1
	D-values	-	0,52	0,21	-
Jacquez	Control	0	2,80	2,02	0
	Sclerotium rolfsii	1	1,68	1,79	0
	Rosellinia necatrix	1	1,61	1,78	0
	Pythium aphanidermatum	2	1,55	1,77	0
	Pythium rostratum	2	1,55	1,77	0
	Pythium irregulare	2	1,54	1,76	0
	Pythium ultimum	3	1,00	1,24	0
	D-values	-	0,49	0,19	-
Vitis rupestris	Control				
var. du Lot	*Pythium aphanidermatum*	2	2,43	1,45	0
	Pythium sylvaticum	2	2,40	1,45	0
	Sclerotium rolfsii	2	2,37	1,45	0
	Phytophthora cinnamomi	4	1,49	1,13	9
	D-values	-	0,32	0,30	-

[a] Control plants not inoculated.

[b] Rating system: 0 = no root rot; 1 = 1-20% root rot; 2 = 21-40% root rot; 3 = 41-60% root rot; 4 = 61-80% root rot; 5 = 81-100% root rot.

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TABLE 3 Occurrence[a] of *Phytophthora cinnamomi* and *Pythium* spp. at various soil depths in a grapevine nursery

Depth (cm)	Number of propagules/g dry soil	
	P. cinnamomi	*Pythium*
0 - 6	25	50
6 - 12	50	100
12 - 18	100	200
18 - 24	50	350
24 - 30	25	50
30 - 36	0	25
36 - 40	0	25

[a] Average of five samples.

Competitive saprophytic ability and persistence in soil

P. cinnamomi was recovered from vine canes and roots after 20 d or longer and from wheat straw after 40 d or longer (Table 4). Recovery was greatest from canes (maximum 64%) intermediate from roots (maximum 24%), and least from wheat straw (maximum 19%). Recovery from substrata in infested sterilised soil was higher than from those in nonsterilised soil. After three years' absence of a host, *P. cinnamomi* was isolated from 36% of the roots and 28% of the canes remaining in a vineyard.

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TABLE 4 Recovery of Phytophthora cinnamomi from vine roots, vine canes and wheat straw[a] incubated for different times in infested sterilised and infested nonsterilised soil

Substratum	Recovery (%) from sterilised soil/Recovery (%) from nonsterilised soil			
	Incubation time (d)			
	10	20	40	80
Roots	0/0	12/2	18/7	24/14
Canes	1/0	20/5	40/12	64/17
Straw	0/0	0/0	12/4	19/8

[a] 100 pieces of each substratum used.

DISCUSSION

P. cinnamomi was isolated from 56% of the nurseries in the Wellington district which produces about 50% of the grafted vines in the Western Cape (Archer, 1974). Because it does not always kill young vines in the nursery, it has undoubtedly been widely distributed to commercial plantings on infected nursery stock. Evidence for this is provided by a recent survey (see Part 2) in which it was found that larger numbers of young vines (1-5 years old) were dying from Phytophthora root rot, whereas the fungus was only infrequently isolated from adjacent soil.

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The results also indicate a long persistence of P. cinnamomi in soil and a moderate ability to invade dead vine roots and vine canes. These results correspond with those of Zentmyer & Mircetich (1966) with avocado soil and dead avocado roots. Moderate competitive saprophytic ability allows P. cinnamomi to invade dead plant material and helps the fungus to survive in soil in the absence of a suitable host.

Isolations from the rhizosphere suggest that the population levels of P. cinnamomi are determined by the severity of host infection. In the case of the susceptible 99 Richter rootstock the number of propagules rose rapidly until the plants were killed, and then dropped to a low level. In the case of the resistant 143 B Mgt rootstock the number of propagules fluctuated at a low level, suggesting that little infection and subsequent increase in propagules occurred. The distribution of propagules in the soil also suggests that their number is determined by the location of the roots. More propagules were found in the upper 24 cm of soil in the vicinity of the roots. The same phenomenon was observed with the Pythium spp. There was little regeneration of roots following infection by P. cinnamomi and the more virulent other Phytophthora and Pythium spp. In the case of the less virulent Pythium spp., however, new roots were formed but these became infected in due course, leading to rotting of all the fine feeder roots and finally to retarded growth.

S. rolfsii and R. necatrix were also isolated from roots of nursery vines but appeared to be less important than Phytophthora and Pythium.

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It is concluded that P. cinnamomi is the most pathogenic root rot fungus in grapevine nurseries in the Western Cape, that it has been widely distributed to vineyards in nursery material, and that it can persist on infected roots in such vineyards for long periods after removal of the vines.

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4 SPREAD OF PHYTOPHTHORA CINNAMOMI IN A NATURALLY INFESTED VINEYARD SOIL

ABSTRACT

Phytophthora cinnamomi was isolated from rootstocks of dead or diseased vines in vineyards from 14 districts in the Cape Province of South Africa. It was recovered in vineyard soil to a depth of 320 mm. Downhill spread of the pathogen was more rapid through a soil with a perched water table (Estcourt: Rosmead soil series) than through a freely-draining soil (Clovelly: Blinkklip soil series). Lateral movement of the fungus through soil occurred to a limited extent. The disease potential index of newly infested soil was usually higher than that of areas previously infested. The results indicated the danger of introducing P. cinnamomi to poorly-draining soils by planting infected vines.

INTRODUCTION

The devastation caused by Phytophthora cinnamomi Rands in Australian and New Zealand forests illustrate the ability of the pathogen to spread in both space and time (Newhook & Podger, 1972). In South Africa a single infected tree introduced at planting into a block of over 500 avocado trees resulted in the entire grove being abandoned within 10 years because of root rot (Brodrick & Frean, 1973).

A survey of South African vineyards indicated that P. cinnamomi is one of the most important root pathogens of grapevine (see Part 2). Rootstock 99 Richter is used extensively in this country, especially on heavier-

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textured, slower draining soils and is particularly susceptible to root rot (Marais, 1979). In one locality about 50% of 60 000 vines on 99 Richter rootstock were dead or in various stages of decline within 6 months after planting (see Part 2).

The present study was undertaken to determine the occurrence of P. cinnamomi in vineyards in the Western Cape Province and its spread in a naturally infested vineyard soil.

MATERIALS AND METHODS

Geographic distribution of P. cinnamomi

Data from a previous survey (see Part 2) on the distribution of P. cinnamomi within the Western Cape Province, but not previously reported are now given.

Spread of P. cinnamomi through soil

The spread of P. cinnamomi was determined in naturally infested vineyard soil on the experimental farm Nietvoorbij of the Viticultural and Oenological Research Institute, Stellenbosch. The vineyard, on an 8% slope, had an 8-year-old stand of Cape Riesling vines on rootstock 99 Richter. The dominant soils in the vineyard were classified as Estcourt (Rosmead soil series) and Clovelly (Blinkklip soil series) (MacVicar, 1977).

Vertical distribution. Vertical distribution of P. cinnamomi was determined in soil cores obtained by inserting an auger (diameter 80 mm) to a

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depth of 560 mm, from four sites 15 cm from the stem base of five infected vines. The cores were divided into seven 80 mm subsamples which were pooled according to depth and stored moist until tested. The presence of the pathogen in 10 g soil was determined by the lupin baiting method (Chee & Newhook, 1965).

Horizontal spread. To determine downhill and lateral spread of the fungus the vineyard was divided into 1500 plots of equal size (3 m^2). One soil sample was taken from each plot to a depth of 300 mm with the auger. Sampling was then repeated at 6-monthly intervals for 25 months but only on plots which were initially recorded as being infested, and on bordering plots. Twenty samples were taken from each plot. All samples were stored moist until tested and the presence of the pathogen determined by the lupin baiting method. Development of disease symptoms was recorded and isolations were made from roots of healthy and diseased vines as described in Part 2.

Disease potential index (DPI)

The soil auger was used to collect five soil samples 60 cm from the stem base and to a depth of 300 mm around each of 10 healthy, 10 diseased and 10 dead vines growing within areas of a vineyard known to have been infested for several years. A similar sampling was made of soil around vines growing within areas of the same vineyard where soil infestation had only recently been detected. Samples were taken during winter when soil temperatures were relatively low and soil moisture high. The DPI of each soil sample (reciprocal of the maximum dilution from which the pathogen was isolated) was determined as described by Tsao (1960).

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RESULTS AND DISCUSSION

Geographic distribution of *P. cinnamomi*

P. cinnamomi was isolated from dead or dying grapevines on different rootstocks in vineyards from 14 districts of the Western Cape Province. It was isolated from vines in 69 of 114 vineyards surveyed but was recovered from soil around infected vines in only 11 vineyards (Table 1).

Spread of *P. cinnamomi* through soil

Vertical distribution. The pathogen was isolated to a depth of 320 mm on two sampling sites and to a depth of 240 mm at the other three sites. Vertical distribution of the pathogen thus appears to be restricted to the upper part of the root zone of the grapevine. This agrees with Weste, Cooke & Taylor (1973) who found *P.cinnamomi* to depths of 160-240 mm in soil from under eucalypts. However, it differs considerably from results of Brodrick, Zentmyer & Wood (1976) who found *P. cinnamomi* at depths of 600 to 1050 mm in soil under avocado trees in Southern California.

Horizontal spread. *P. cinnamomi* moved downhill for the first year in the Estcourt soil at a rate of 6 m per 6 months. At that time it reached the Clovelly soil and further downhill movement was progressively slowed in the soil; the fungus advanced only 6 m in 18 months. Gravitational water movement in the perched water table in the Estcourt soil probably contributed to the higher rate of movement of the pathogen. A water table or clay barrier does not exist in the Clovelly soil and water tends to drain away to deeper

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TABLE 1 Presence of *Phytophthora cinnamomi* in soil and grapevine roots from vineyards in the South-Western Cape Province

Location	Rootstock	Number of vineyards:		
		Sampled	Yielding *P. cinnamomi* from	
			Roots	Soil
De Doorns	99 Richter	2	2	1
Franschhoek	99 Richter	2	1	0
Malmesbury	99 Richter	9	6	1
Montagu	99 Richter	6	4	2
	Vitis rupestris var. du Lot			
Oudtshoorn	99 Richter	1	1	0
Paarl	99 Richter	19	11	2
Porterville	99 Richter	3	1	0
Riebeek Kasteel	99 Richter	2	2	0
Robertson	99 Richter	2	1	0
Somerset West	99 Richter	4	2	1
Stellenbosch	99 Richter	25	15	2
	V. rupestris var. du Lot			
Tulbagh	99 Richter	1	1	0
Wellington	99 Richter	32	20	3
	101-14 Mgt			
	V. rupestris var. du Lot			
Worcester	99 Richter	6	2	0
	Totals	114	69	11

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layers.

Little lateral spread of the pathogen occurred in either of the two soils: approximately 1 m during the first year with hardly any further spread during the following 18 months. Lateral spread was not influenced by soil type. Uphill spread was hardly ever detected. This indicated that P. cinnamomi spreads in vineyard soil mainly by water movement. Similar results were previously obtained by Podger (1972) and Weste (1975) in Australian forests and Zentmyer & Ohr (1978) in avocado groves in California.

P. cinnamomi was isolated from all dead or diseased vines. The fungus was always detected in soil before symptoms developed. Not all vines in an infested area developed symptoms at the same time and plants died off in patches. The fungus was isolated from roots of only 2% of seemingly healthy vines within infested areas. The percentage vines showing symptoms in the experimental vineyard increased from 1,5% to 12,5% over a period of 36 months.

Disease potential index

With one exception, the DPI values of recently infested soil were higher or equal compared with those of older infested areas (Table 2). The DPI values of soil from below dead or diseased vines were also higher than those of soil from below healthy vines. Diseased vines therefore act as a source from which inoculum spreads through the soil to healthy vines. Infected nursery material will therefore be an effective source of inoculum when planted into a vineyard, especially in poorly-draining soils where

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TABLE 2 Disease potential index (DPI)[a] of soil below healthy, diseased and dead grapevines

DPI of soil below:					
Healthy vines		Diseased vines		Dead vines	
Old infestation	Recent infestation	Old infestation	Recent infestation	Old infestation	Recent infestation
1	1	2	8	4	8
0	1	4	4	2	16
2	1	8	16	4	8
1	2	4	4	4	4
1	1	2	8	8	8
0	1	2	4	2	8

[a] Determined by the method of Tsao (1960).

horizontal spread of the fungus occurs in soil water.

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5 SUSCEPTIBILITY OF *VITIS* CULTIVARS TO *PHYTOPHTHORA CINNAMOMI*

ABSTRACT

Reactions of different grapevine rootstock cultivars to *Phytophthora cinnamomi* were obtained by inoculating stems, canes and rooted plants in water culture and by growing inoculated rootstocks in both artificially and naturally infested vineyard soil.

Of 24 rootstock cultivars tested, only 2-1 USVIT, 143 B Mgt, 101-14 Mgt, 3-6 USVIT and Jacquez had mortality rates below 20% in soil. The five *Vitis vinifera* cultivars tested were more tolerant than the three hybrids between *V. vinifera* and other *Vitis* spp. Most rootstock cultivars became more tolerant to *P. cinnamomi* when grafted with a *V. vinifera* scion. It was concluded that the laboratory and greenhouse inoculation methods cannot be used to predict disease reactions of rootstocks in the field.

INTRODUCTION

The roots of *Vitis vinifera* L. cultivars are attacked by the vine phylloxera, *Daktulosphaira vitifoliae* (Fitch). It is therefore essential to use *V. vinifera* grafted on rootstocks resistant to the insect. The phylloxera resistant rootstock 99 Richter (*V. berlandieri* P. x *V. rupestris* S.) is used extensively in South Africa, but for nearly four decades it has been subjected to decline and sudden death. Van der Merwe, Joubert & Matthee (1972) attributed this decline to *Phytophthora cinnamomi* Rands, a finding which has subsequently been confirmed (Parts 2 and 3).

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P. cinnamomi can be highly destructive to grapevines. In two vineyards with 99 Richter as rootstock, the author found that 50% of 60 000 vines and 65% of 30 000 vines were dead or in various stages of decline within 6 months of planting. In another case more than 10% of 3 000 vines infected by P. cinnamomi had to be discarded before planting and an additional 20% died within a further 9 months. A previous study (Part 3) also showed that more than half the nurseries in the Wellington district, which produces approximately 50% of all grafted vines in South Africa (Archer, 1974) were infested with P. cinnamomi.

These observations emphasized the need for further information on P. cinnamomi-resistant grapevine rootstocks in South Africa, and motivated the present study.

MATERIALS AND METHODS

Artificial inoculation under controlled conditions

A number of different rootstock cultivars were inoculated (see Appendix).

Stem inoculation. Rootstocks were planted in a sterilised potting mixture in 18 cm clay pots, one plant per pot with five pots per cultivar, and held for a year in a glasshouse at 24°C.

Stems were inoculated with a grapevine isolate of P. cinnamomi. Disks (4 mm diam) of bark were removed with a sterile cork borer to expose the

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cambium approximately 5 cm above soil level. Disks of the same diameter taken from a 14-d-old corn meal agar (CMA) culture of P. cinnamomi were placed on the wounds of four of the five plants of each cultivar. The fifth plant received a sterile CMA disk. The wounds were covered with a waterproof plastic wrapping. The plants were kept in the glasshouse.

Results were recorded 8 weeks after inoculation. The bark around the wound was removed and disease development scored on a five-point scale (Table 1).

Cane inoculation. Canes (8 mm diam) from the different rootstocks were cut into lengths of approximately 60 mm. There were 50 canes per cultivar. They were wounded and inoculated as above, then held on water agar in petri dishes at 25°C.

Disease development was scored after 6 to 8 weeks incubation.

Inoculation in water culture. The method of Zentmyer & Mircetich (1965) was used. Grapevine rootstock cuttings were rooted in steam-sterilised sand and grown in 50 l tanks containing a nutrient solution (10 g of Chemicult (Fedmis (Pty) Ltd., P.O. Box 88, Cape Town 8000) per 5 l water; pH adjusted to 4,5). The vines were supported on a rack on the surface of the nutrient solution so that only the roots were immersed. The tanks were kept in the glasshouse at 24°C.

After 14 d, four cheesecloth bags, each containing two, 14-d-old cultures of P. cinnamomi on potato dextrose agar (PDA), were placed in each tank. Control plants were held in uninfested nutrient solution. Each tank contained

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10 plants and there were four tanks per treatment.

Results were recorded after 6 weeks. Root rot was scored visually on a five-point scale (Table 1).

Inoculation by artificially infesting soil. Vine cuttings rooted in steam-sterilised sand were transplanted into 16 cm free-draining clay pots containing a sterilised potting mixture. Fourteen days later, wheat grain inoculum of P. cinnamomi (Zentmyer & Mircetich, 1966) was added to the potting mixture at 1:50 (v/v). Control pots received sterile uninfested wheat grain. Each rootstock cultivar was replicated four times with 10 plants per replicate. Pots were held in a glasshouse at 24°C and a high soil moisture was maintained by regular watering with an overhead microsprinkler system.

Results were recorded 8 weeks after inoculation. Root rot was scored on the five-point scale and the above-ground health rating on a four-point scale (Table 1). The two values obtained for individual plants of each treatment were combined.

Recovery of P. cinnamomi from grapevine cultivars grown in the field in naturally infested soil

Trials were laid out on a uniform soil type (Glenrosa) where diseased vines had been removed the previous year. The presence of P. cinnamomi in each square meter of soil was confirmed by the lupin baiting technique (Chee & Newhook, 1965). Dormant one-year-old vines were planted 1 m apart in rows spaced 1,5 m.

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As soon as symptoms developed, roots were lifted, washed, and cut into sections. The root sections were surface-disinfested in 0,5% NaOCl for 2 min and plated on CMA containing streptomycin sulphate (100 mg/l). Plates were incubated at 25°C for 24 h and examined for the presence of P. cinnamomi. Roots were also examined for nematodes and phylloxera.

Susceptibility of V. vinifera cultivars and hybrids to natural field infection

V. vinifera cultivars (Grand noir de Calmete, Petite Bouchet, Palomino, Chenin blanc and Alicante Bouchet) and V. vinifera hybrids (Keuka, Ferdinand de Lesseps and Siegfriedrebe) were planted in a randomized block design with three replicates of seven vines each. Mortality was determined.

Effect of V. vinifera graftwood on susceptibility of rootstocks to P. cinnamomi

Twenty-one rootstock cultivars were planted in naturally infested soil. Half of the rootstocks of each cultivar were grafted with Chenin blanc and the other half were left ungrafted. Grafted and ungrafted rootstocks were planted in a randomized block design with three replicates of seven vines each. Results were recorded over a period of 28 months.

RESULTS

Relative susceptibilities of grapevine rootstocks after natural infection and artificial inoculation

The findings are given in Table 1. No control plants developed symptoms and

TABLE 1 Reactions of grapevine rootstock cultivars inoculated with *Phytophthora cinnamomi* by different methods or planted in a naturally infested vineyard soil

Rootstock cultivar	Disease rating				Mortality (%) in naturally infested soil[f]
	Stem inoculations[a,b]	Cane inoculations[b,c]	Water culture[d]	Artificially infested soil[e]	
99 Richter (KWA)	4	4	4	7	80,2
99 Richter (OVRI)	4	4	4	7	78,5
1103 Paulsen	4	4	3,5	7	50,0
2-1 USVIT	4	4	2,75	2	9,5
Prosperi Super 99 Richter	4	4	2,25	5	85,0
3306 C	4	4	2	0	45,2
143 B Mgt	4	4	0	0	11,9
101-14 Mgt	3,5	3,75	3	6	19,1
1 Schabort	3,5	3,75	4	7	-
Rupestris du Lot	3,5	3,5	1,25	7	87,9
Rupestris St. George	3,5	3,5	3,75	7	70,8
Constantia Metallica	3,5	3,5	1	6	30,9
3-6 USVIT	3,5	3,5	1	1	19,1
15 Vivet	3	3,25	2,75	7	-
44-53 Malegue	3	3	2,5	2	60,4
110 Richter	2	2	4	6	50,0
1045 Paulsen	2	2	4	7	95,3
140 Ruggeri	2	1	3,5	5	38,1
Jacquez	0	0	0	0	16,7
1 Orezot	0	0	0	2	30,9
Vitis champini var. Ramsey	0	0	0	0	33,3
3-5 USVIT	0	0	4	1	26,2
420 A Mgt	0	0	1	3	-
504	0	0	0	0	-
0 values (P=0,05)	0,65	0,75	0,51	0,94	15,9
t-values	-	0,1	0,6	2,6	-
r	-	0,26	0,48	0,67	-
Relative information per variable	-	0,02	0,11	0,44	-

[a] Combined average data from four stems.

[b] Disease ratings: 0 = wound covered by callus tissue (i.e. no disease); 1 = lesion less than 25% of stem circumference, with limited vertical extension or callus formation; 2 = lesion occupying 25-50% of stem circumference, with moderate vertical extension and no callus formation; 3 = lesion occupying more than 50% of stem circumference with extensive vertical extension; 4 = stem completely girdled.

[c] Combined average data from five replicate units each of 10 lengths of canes.

[d] Combined average data for 10 plants in each of four tanks. Disease ratings: 0 = 0-20%; 1 = 20-40%; 2 = 40-60%; 3 = 60-80% and 4 = 80-100% root rot.

[e] Combined average data for 10 plants replicated four times. Disease ratings were the sum of root rot ratings (footnote[d]) and ratings of symptoms on above-ground parts where 0 = no disease symptoms; 1 = slightly reduced growth; 2 = stunting and chlorosis; 3 = severe die-back.

[f] Percentage mortality of seven vines replicated six times in a randomized block design.

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no nematodes or phylloxera were found on roots of plants grown in naturally infested soil.

The results of stem and cane inoculations showed that six rootstock cultivars were tolerant (disease rating 0), three intermediate (ratings 1-2,5) and 15 susceptible (ratings 2,75-4). Five of the tolerant rootstocks were also tolerant when tested in water culture. However, the sixth (3-5 USVIT) was highly susceptible. Rootstock 143 B Mgt was tolerant in water culture, but highly susceptible when tested by the other two methods. Further discrepancies are evident in Table 1, e.g. the six rootstocks assigned to the intermediate group in the water culture test were all rated as susceptible following stem and cane inoculations. The reverse held for rootstocks 110 Richter, 1045 Paulsen and 140 Ruggeri.

In artificially infested soil five rootstocks were tolerant (disease rating 0), six intermediate (ratings 1-3) and 13 susceptible (ratings 5-7).

Only three rootstocks (Jacquez, V. champini var. Ramsey and SO4) were rated as tolerant following inoculation by all four methods. Seven rootstocks were rated as susceptible. The response of the other rootstocks varied with the method of inoculation.

Disease mortality in the naturally infested vineyard soil was generally high. Only five (2-1 USVIT, 143 B Mgt, 101-14 Mgt, 3-6 USVIT and Jacquez) of the 20 rootstock cultivars tested showed less than 20% mortality whereas mortalities of 50% or higher were recorded for nine cultivars.

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Prediction of field infection by P. cinnamomi

Different rootstock cultivars showed marked differences in susceptibility in laboratory and glasshouse screening tests, depending on the method of inoculation. A linear, least squares, multiple regression model, as proposed by Daniel & Wood (1971), was therefore used to determine to what extent infection of vines grown in infested soil under field conditions could be predicted from screening tests. Stem inoculations were disregarded because the results were virtually identical to those obtained with cane inoculations.

Disease ratings obtained in artificially infested soil gave a higher t-value, sample correlation coefficient (R^2) and relative information per variable than those obtained with cane inoculation and the water culture test (Table 1).

Results obtained in artificially infested soil and in the field are compared in Fig. 1.

Susceptibility of V. vinifera and hybrids to natural field infection

Mortality rates of the hybrids Keuka (47,6%), Ferdinand de Lesseps (42,2%) and Siegfriedrebe (38,6%) were higher than those of the non-hybrid cultivars Grand noir de Calmete (26,2%), Petite Bouchet (14,3%), Palomino (14,3%), Chenin blanc (11,9%) and Alicante Bouchet (11,9%). The last four values compared favourably with the mortality rates (Table 1) of the more tolerant root-

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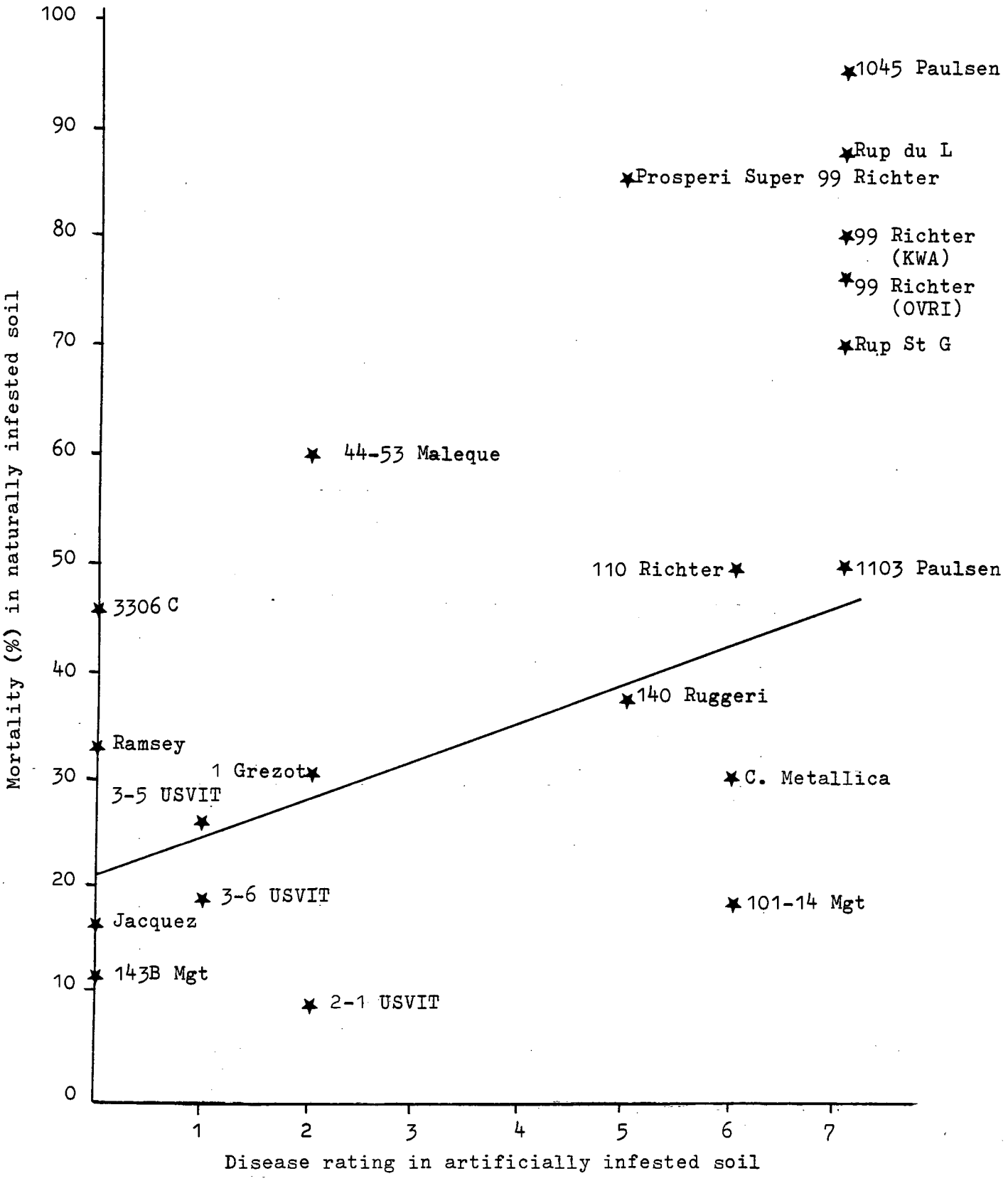

Fig. 1 Relationship between results obtained in artificially infested soil in pots and those from a field trial to determine the susceptibility of different grapevine rootstocks to Phytophthora cinnamomi.

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stock cultivars.

Effect of V. vinifera scions on susceptibility of rootstocks to P. cinnamomi

The mortality rates of grafted and ungrafted rootstocks (Table 2) were significantly different (5% level). Most rootstocks were more tolerant to P. cinnamomi when grafted with V. vinifera.

DISCUSSION

This study has shown that the reactions of different rootstock cultivars to P. cinnamomi under field conditions cannot be predicted accurately from results obtained under laboratory or glasshouse conditions. Cane and water culture inoculations were particularly unreliable. Field infection of roots invariably follows attraction of zoospores to the rhizosphere (Dukes & Apple, 1961; Rai & Strobel, 1966; Khew & Zentmyer, 1973). Infection sites on canes and roots are obviously dissimilar, whereas exudates from roots are greatly diluted in water culture. Although these restrictions did not apply in artificially infested soil, the results obtained in the pot experiment were also disappointing. Data from the artificially infested soil accounted for only 45% of the variation of the results of the field trial.

Results suggest that Jacquez, 143 B Mgt and some new crosses with Jacquez in their parentage may survive in soils containing P. cinnamomi but that 99 Richter, 1045 Paulsen, 1103 Paulsen and Rupestris du Lot should not be planted in infested soil. In general the V. vinifera cultivars compared

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TABLE 2 Pathogenicity in naturally infested soil of *Phytophthora cinnamomi* on 21 vine rootstocks ungrafted and grafted with Chenin blanc

Rootstock	Mortality (%)[a]	
	Grafted	Ungrafted
1045 Paulsen	90,5	100
Rupestris du Lot	85,7	80,9
Prosperi Super 99 Richter	76,2	90,5
99 Richter (KWA)	61,9	95,2
99 Richter (OVRI)	76,2	78,5
Rupestris St. George	52,4	76,2
44-53 Maleque	66,7	46,9
110 Richter	61,9	38,1
1103 Paulsen	33,3	66,7
3306 C	47,6	50,8
140 Ruggeri	28,6	47,6
Vitis champini var. Ramsey	42,8	23,8
1 Grezot	9,5	52,4
Constantia Metallica	33,3	28,6
3-5 USVIT	4,8	47,6
3-6 USVIT	14,3	14,3
101-14 Mgt	0	38,1
De Waal Bosstock	9,5	9,5
Jacquez	19,0	14,3
143 B Mgt	0	9,5
2-1 USVIT	0	19,0
Mean values[b]	38,7	48,9

[a] Based on three replicates of seven vines each of grafted and ungrafted rootstocks in a randomized block.

[b] Mean values differ significantly (P=0,05).

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favourably with the most tolerant rootstocks. This could explain the tolerance of Jacquez, 3-5 USVIT, 3-6 USVIT and 2-1 USVIT which all have V.vinifera in their parentage. The hybrid cultivars were more susceptible than the pure V. vinifera cultivars.

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6 SURVIVAL AND GROWTH OF GRAPEVINE ROOTSTOCKS IN A VINEYARD INFESTED WITH PHYTOPHTHORA CINNAMOMI

ABSTRACT

The survival and growth of 21 grapevine rootstock cultivars in a vineyard naturally infested with Phytophthora cinnamomi was recorded for 7 years. The different rootstocks were rated as tolerant, intermediate and susceptible. Fewer rootstocks grafted with Chenin blanc than ungrafted rootstocks were killed by the pathogen. All tolerant rootstock cultivars had Vitis vinifera in their parentage.

INTRODUCTION

Phytophthora cinnamomi Rands causes decline and rapid death of grapevines. It has been isolated from collars and roots of diseased vines in Australia (McGechan, 1966) and India (Agnihothrudu, 1968). P. cinnamomi was first recovered from grapevines in South Africa in 1972 (Van der Merwe, Joubert & Matthee, 1972) but it was soon recognised as a major cause of grapevine root rot. Nothing was known about susceptibilities of local rootstock cultivars; therefore a study was made of the survival and growth of established grapevine rootstocks in an infested vineyard during a 7-year period. The incidence of root rot of 21 grafted and ungrafted grapevine rootstocks in this vineyard during the 28 months following planting has already been reported (Part 5).

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MATERIALS AND METHODS

A record was kept of the number of plants of each of 21 rootstock cultivars killed yearly by the pathogen between October 1974 and September 1981. The data were expressed as disease indices, i.e. percentages of the numbers of plants alive during the preceding year. Grafted and ungrafted rootstocks of a cultivar were considered together as single entities. On completion of the experiment, surviving plants were uprooted and the dry masses of their roots and shoots recorded. First and second degree polynomials were fitted to the root mass, shoot mass and root rot data and simple and multiple correlation coefficients were calculated. The presence of P. cinnamomi in the roots was also determined (Part 5).

RESULTS

The mortality, root rot and shoot and root mass are given in Table 1. A two-way analysis of variance was conducted on separate data obtained with grafted and ungrafted rootstock cultivars. The statistical significance of differences between means was tested with Bonferroni's method for comparison. The mortality and root rot were lower for grafted than for ungrafted rootstocks. The rootstock cultivars could not be ranked statistically but 143 B Mgt, 2-1 USVIT, De Waal Bosstock, 3-6 USVIT and Jacquez were less severely affected than the others. However, even the roots of these five rootstocks were infected by P. cinnamomi. Shoot and root mass varied considerably and no set pattern was evident.

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Dead rootstocks recorded in each of the 7 years are given in Table 2. Friedman's non-parametric analysis for a total randomized block design was applied to the data. More rootstocks died during the second and third years after planting (1976 and 1977) than at any other stage of the survey. There were no significant differences in the rank totals for 1975, 1978, 1979, 1980 and 1981.

A linear correlation was found for certain selections for some of the rootstocks only (Table 3). However, there was a significant positive correlation between root mass and shoot mass of all five cultivars (Tables 3 and 4).

Multiple correlation coefficients giving acceptable probability levels were obtained with a second degree polynomial regression analysis for some of the selections not showing correlation with a first degree polynomial (Table 4). Both root and shoot mass of Jacquez (Figs. 1 and 2), but only shoot mass of De Waal Bosstock (Fig. 3) were reduced at higher root rot levels.

There was no correlation between root and shoot mass of 143 B Mgt and 3-6 USVIT; with the other cultivars the correlation was negative (Tables 3 and 4; Figs. 1-3).

DISCUSSION

The present study confirmed some of the previous results (Part 5) on the susceptibility of grapevine rootstock cultivars to P. cinnamomi. Although

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TABLE 2 Killing of different rootstock cultivars by Phytophthora cinnamomi over a period of 7 years in a naturally infested soil[a]

Rootstock	Dead vines (%)[b] per year						
	1975	1976	1977	1978	1979	1980	1981
143 B Mgt	0	0	23,8	3,1	0	0	0
2-1 USVIT	0	9,5	0	0	0	0	0
De Waal Bosstock	0	0	14,3	0	0	0	0
3-6 USVIT	0	2,4	12,2	0	0	0	0
Jacquez	0	0	16,7	2,9	0	0	0
3-5 USVIT	2,4	24,4	0	0	0	0	0
101-14 Mgt	9,5	10,5	0	0	0	5,9	6,3
1 Grezot	0	26,6	6,5	0	0	0	0
Vitis champini var. Ramsey	0	0	33,3	0	0	0	0
Constantia Metallica	9,5	15,8	12,5	0	0	0	0
140 Ruggeri	0	28,6	13,3	0	0	0	0
1103 Paulsen	7,1	38,5	12,5	0	0	0	0
110 Richter	7,1	33,3	15,4	9,1	0	0	0
3306 C	2,4	26,8	16,7	8,0	0	0	0
44-53 Maleque	0	35,7	22,2	0	0	0	0
Rupestris St. George	14,3	38,9	31,8	0	0	0	0
99 Richter (OVRI)	14,3	38,9	31,8	0	33,3	0	10,0
99 Richter (KWA)	19,0	41,2	55,0	33,3	0	0	0
Prosperi Super 99 Richter	11,9	57,9	53,3	0	0	0	14,3
Rupestris du Lot	23,8	40,6	63,2	0	14,3	0	37,5
1045 Paulsen	11,9	42,1	90,5	0	0	0	0
Means	6,3	24,4	25,0	2,7	2,3	0,3	3,9
Rank totals as calculated by Friedman's non-parametric analysis[c]	91,5y	139,5z	105,5z	66,5y	83,5y	61,0y	68,5y

[a] Based on three replicates of seven vines each of grafted (with Chenin blanc) and ungrafted rootstocks planted in a randomized block design in October 1974. Ungrafted and grafted rootstocks of a cultivar were considered together as a single entity.

[b] Disease index = number of dead plants/number of plants alive in preceding year.

[c] Values followed by different letters differ significantly (P = 0,05).

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TABLE 3 Linear correlation coefficients for different selections of root rot, root mass and shoot mass of five grapevine rootstocks infected by Phytophthora cinnamomi under field conditions

Rootstock	Criterion	Linear correlation coefficients of:	
		Root rot	Root mass
143 B Mgt	Root mass	-0,513 ns [a]	-
	Shoot mass	-0,608 ns	0,912* [b]
3-6 USVIT	Root mass	-0,145 ns	-
	Shoot mass	-0,163 ns	0,952* [b]
Jacquez	Root mass	-0,701 ns	-
	Shoot mass	-0,796 ns	0,865*
2-1 USVIT	Root mass	-0,905*	-
	Shoot mass	-0,897*	0,938** [c]
De Waal Bosstock	Root mass	-0,941**	-
	Shoot mass	-0,675 ns	0,863*

[a] ns = Not significant.

[b] * = Significant at 95% confidence level.

[c] ** = Significant at 99% confidence level.

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TABLE 4 Multiple correlation coefficients, F-values and probability levels for different selections of root rot, root mass and shoot mass of four grapevine rootstocks infected by Phytophthora cinnamomi under field conditions

Rootstock	Multiple correlation coefficient (R) for selection[a]		F-value for selection		Probability (%) for selection	
	1	2	1	2	1	2
143 B Mgt	0,44	0,45	0,345	0,399	ns[b]	ns
3-6 USVIT	0,26	0,44	0,121	0,376	ns	ns
Jacquez	0,92	0,88	9,142	11,213	93,1	91
De Waal Bosstock	-	0,86	-	9,232	-	92,9

[a] Selection 1 = root mass the dependent variable (Y);
root rot the independent variable (X).
Selection 2 = shoot mass the dependent variable (Y);
root rot the independent variable (X).

[b] ns = not significant.

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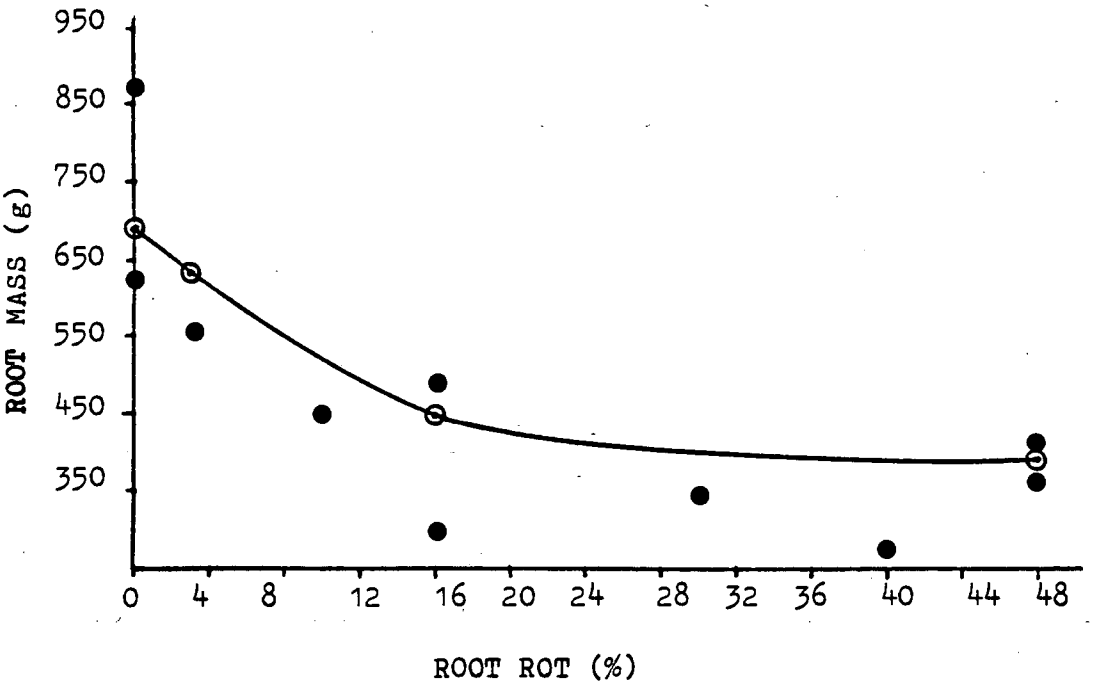

FIG. 1 Fitted curve for grapevine rootstock cultivar Jacquez relating root mass to root rot.

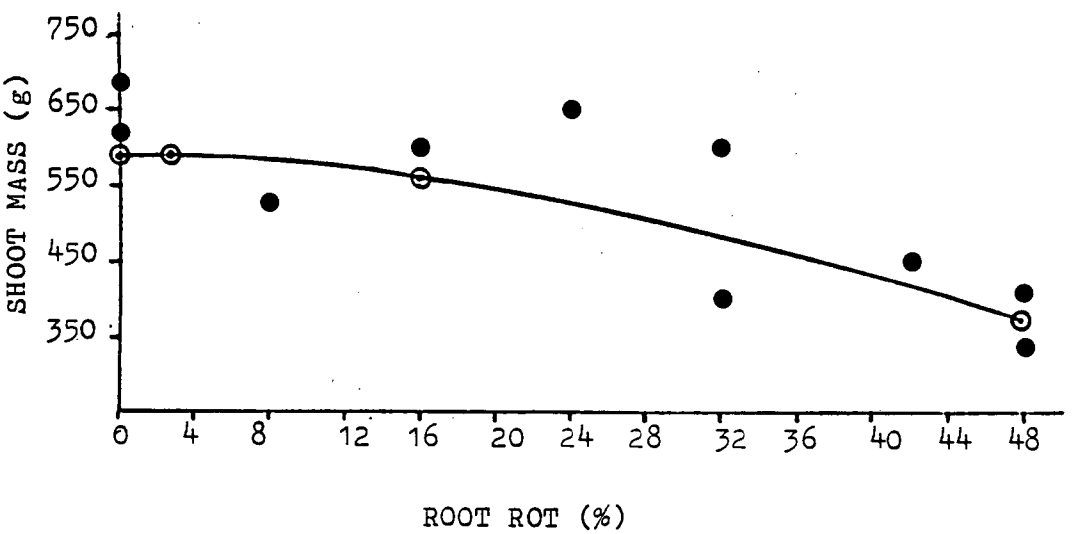

FIG. 2 Fitted curve for grapevine rootstock cultivar Jacquez relating shoot mass to root rot.

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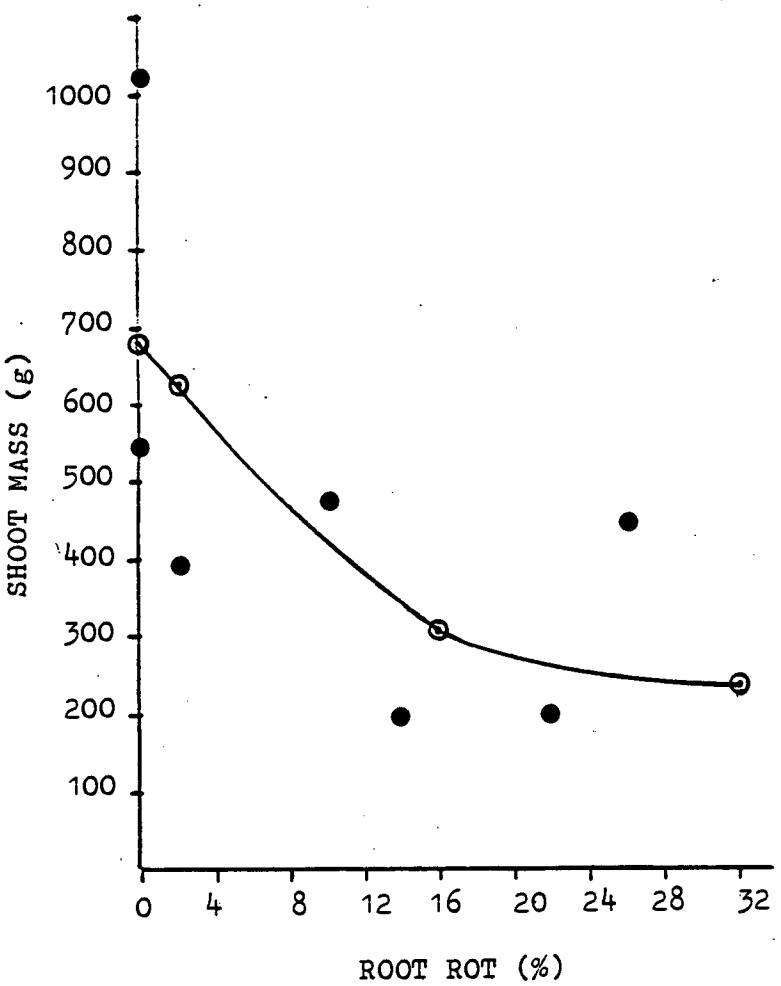

FIG. 3 Fitted curve for grapevine rootstock cultivar De Waal Bosstock relating shoot mass to root rot.

the results were not statistically significant, it was possible to rank cultivars as tolerant (De Waal Bosstock, 143 B Mgt, 3-6 USVIT, 2-1 USVIT and Jacquez), intermediate (V. champini var. Ramsey, Constantia Metallica, 101-14 Mgt, 140 Ruggeri, 3-5 USVIT and 1 Grezot) and susceptible (44-53 Maleque, 110 Richter, 3306 C, 1103 Paulsen, Rupestris du Lot, Rupestris St. George, 99 Richter (OVRI and KWA), Prosperi Super 99 Richter and 1045 Paulsen).

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The most tolerant rootstock cultivars all have V. vinifera in their parentage. This, together with the previously recorded resistance of V. vinifera to P. cinnamomi (Part 5) should be borne in mind in breeding programmes for rootstock resistance. The present study also confirmed earlier findings (Part 5) that most rootstock cultivars become more tolerant to P. cinnamomi when grafted with V. vinifera.

The presence of P. cinnamomi on tolerant rootstock cultivars stresses the importance of hot water treatment (Part 7) to eradicate P. cinnamomi. Unless this is done, the pathogen can unwittingly be spread from nurseries to uninfested vineyard soils on symptomless rootstocks.

P. cinnamomi reduced both root and shoot mass of Jacquez and 2-1 USVIT, only shoot mass of De Waal Bosstock but neither root nor shoot mass of 143 B Mgt and 3-6 USVIT.

It was previously reported that more grapevines were killed by P. cinnamomi in commercial vineyards when the plants are 2 to 5 years old (Part 2). This was confirmed in the present study since climatic conditions did not vary from the norm during the second and third years after planting, when most rootstocks were killed. It is suggested that older vines are more tolerant because they can generate more new roots to compensate for those infected by P. cinnamomi.

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7 EXUDATES FROM ROOTS OF GRAPEVINE ROOTSTOCKS TOLERANT AND SUSCEPTIBLE TO PHYTOPHTHORA CINNAMOMI

ABSTRACT

Zoospores of Phytophthora cinnamomi were more attracted to susceptible 99 Richter roots than to roots of tolerant 143 B Mgt. Root exudates from 99 Richter contained higher concentrations of glutamic acid and arginine than root exudates from 143 B Mgt and the tolerant cultivar Jacquez. The chemotactic index increased with increasing concentrations of glutamic acid, arginine and aspartic acid.

INTRODUCTION

Different grapevine rootstocks show different levels of resistance to Phytophthora cinnamomi Rands (Part 5). Zentmyer (1961) demonstrated a response of zoospores to a concentration gradient of diffusing stimulatory material from roots of avocado seedlings. He also found that zoospores of P. cinnamomi were less attracted to roots of avocado cultivars resistant to P. cinnamomi than to roots of susceptible cultivars. Several investigators have demonstrated chemotaxis of zoospores to compounds in root exudates, particularly amino acids, sugars and organic acids (Dukes & Apple, 1961; Royle & Hickman, 1964; Rai & Strobel, 1966; Chang-Ho & Hickman, 1970). The present study examines the effect of grapevine root exudates on the movement of zoospores of P. cinnamomi and compares the effects of some constituents of the root exudates from tolerant and susceptible grapevine rootstocks.

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MATERIALS AND METHODS

Attraction of zoospores to roots

P. cinnamomi was isolated from grapevine roots and cultured on potato dextrose agar (PDA). After 7 d disks were cut from the growing margin of the culture and transferred to V-8 juice (Chen & Zentmyer, 1970) in petri dishes. After 2 d at 25°C the mycelial mat was washed with sterile distilled water and incubated at 20°C in petri dishes containing nonsterile aqueous soil extract (James, 1958), until sporangia had formed. Mycelial mats bearing sporangia were then chilled at 10°C for 15 min and returned to room temperature for synchronized zoospore release.

Young, vigorously growing shoots (100 mm long) of the rootstocks 99 Richter (*Vitis berlandieri* Planchon x *V. rupestris* Scheele) (susceptible), 143 B Mgt (*V. vinifera* L. x *V. riparia* Michaux) (tolerant) and Jacquez (*V. aestivalis* Michaux x *V. cinerea* Engelman x *V. vinifera* L.) (tolerant) (Part 5) were grown in coarse sand to produce clean roots free from adhering soil particles. Root tips approximately 20 mm long were placed in a suspension of zoospores (approximately 10^2 per cm^3) in special microscope slides with counting grids (Khew & Zentmyer, 1973). After 45 min at room temperature encysted and germinating zoospores 0 - 0,5, 0,5 - 1,0, 1,0 - 1,5 and 1,5 - 2,0 mm from the distal 10 mm of the uncut root tip were counted at 100x magnification.

Root exudates

Green shoot tips 40 mm long were taken from actively growing 99 Richter,

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143 Mgt and Jacquez grapevine rootstocks and surface disinfested by shaking for 2 min in test tubes with 100 ml 2,5% $Ca(OCl)_2$ plus two drops of Tween 80. Apical 10 mm portions of the shoot tips were removed aseptically and placed in 250 ml Erlenmeyer flasks containing 100 ml Murashige & Skoog (1962) medium supplemented with 0,01 mg/l ∝-naphthalene-acetic acid (pH 5,3 after sterilisation). The flasks with the shoot tips were kept for 4 weeks in a growth chamber at 27°C with 16 h daylight at 3000 lux and 8 h darkness. The rooted plants were then transferred aseptically to the apparatus described by Booth (1969) for production and recovery of sterile root exudates. Root exudates were collected at 4 month intervals, concentrated under vacuum at 40°C, frozen and stored until analyzed. For amino acid determinations the exudate was thawed, dried by evaporation under vacuum, redissolved in 10 ml deionized distilled water, dialyzed through a dialysing membrane and brought to pH 2,2 with 18% HCl. Each 5 ml sample was divided into two subsamples. Aliquots (0,5 ml) of each subsample were analyzed for neutral, acid and basic amino acids on a Beckman Unichrom amino acid analyser using a sodium citrate buffer system.

For sugar determinations the exudate was thawed, dried by evaporation under vacuum, extracted with 5 ml pyridine for 10 min at 100°C, cooled, filtered, evaporated under vacuum at 38°C to remove the pyridine and separated by thin layer chromatography on HPT LC-SA silica gel glass sheets (Merck Chemicals, Darmstadt, Germany) using chloroform-glacial acetic acid-water (30:35:5) as solvent system and aniline phthalate for detection (Haer, 1971).

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Attraction of zoospores to different compounds and root exudates

Capillary tubes were drawn and filled with a concentration range of each of the amino acids and sugars detected in the root exudates. The compounds were dissolved in 1% Difco water agar (WA) and the capillary tubes were dropped into the agar solutions kept at 45°C in a water bath to allow the agar to be drawn up. The filled capillaries were stored at 5°C on clean glass slides suspended over sterile water in petri dishes. When required, the capillaries were wiped with filter paper, trimmed to 30 mm and placed on specially prepared microscope slides (Khew & Zentmyer, 1973). The capillary was covered by pipetting 2 cm^3 zoospore suspension into the depressions of the microscope slide. Observations of zoospore behaviour were made at 100x magnification. Each test was replicated four times with capillaries filled with 1% WA serving as controls. The concentrated root exudates of the three rootstocks were incorporated at a rate of 50% (v/v) in the WA.

RESULTS

An average of 38,2 zoospores encysted and germinated at a distance of 0 - 0,5 mm from the 99 Richter roots; at 0,5 - 1,0 mm, 1,0 - 1,5 mm and 1,5 - 2,0 mm the numbers were 18,2, 10,1 and 6,2 respectively. The numbers of encysted and germinated zoospores at the corresponding distances were 9,9, 11,0, 9,4 and 12,1 for 143 B Mgt and 10,4, 8,6, 14,0 and 13,8 for Jacquez. More zoopores thus encysted and germinated near the 99 Richter roots than further away, and germ tubes were directed towards the root pieces. However, zoospores were not attracted to the root pieces of 143 B Mgt and

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Jacquez and they encysted and germinated randomly.

The amino acids and sugars detected in the exudates and their effects on zoospore behaviour are given in Table 1.

Aspartic acid, glutamic acid and arginine exerted the strongest attraction. Although the concentrations of aspartic acid in the exudates of 99 Richter, 143 B Mgt and Jacquez were almost similar, exudates of 99 Richter contained much higher concentrations of glutamic acid and arginine than those of 143 B Mgt and Jacquez. Slight attraction of zoospores was exerted by serine, asparagine, proline, alanine and histidine, whereas the other amino acids and sugars had no effect on zoospore behaviour. The root exudate of 99 Richter attracted zoospores, whereas no such effect was observed with exudates of either 143 B Mgt or Jacquez. No attraction was observed with the control capillaries containing 1% WA.

Capillary root models with different concentrations of aspartic acid, glutamic acid and arginine showed that the chemotactic index (Khew & Zentmyer, 1973) increased with increasing concentrations of all three amino acids, with glutamic acid being the most effective (Fig. 1).

DISCUSSION

From Zentmyer's (1980) review it is evident that there is little data concerning differences in chemotaxis of P. cinnamomi zoospores to roots of resistant and susceptible hosts though Zentmyer (1961) reported that zoospores of P. cinnamomi were less attracted to roots of avocado cultivars

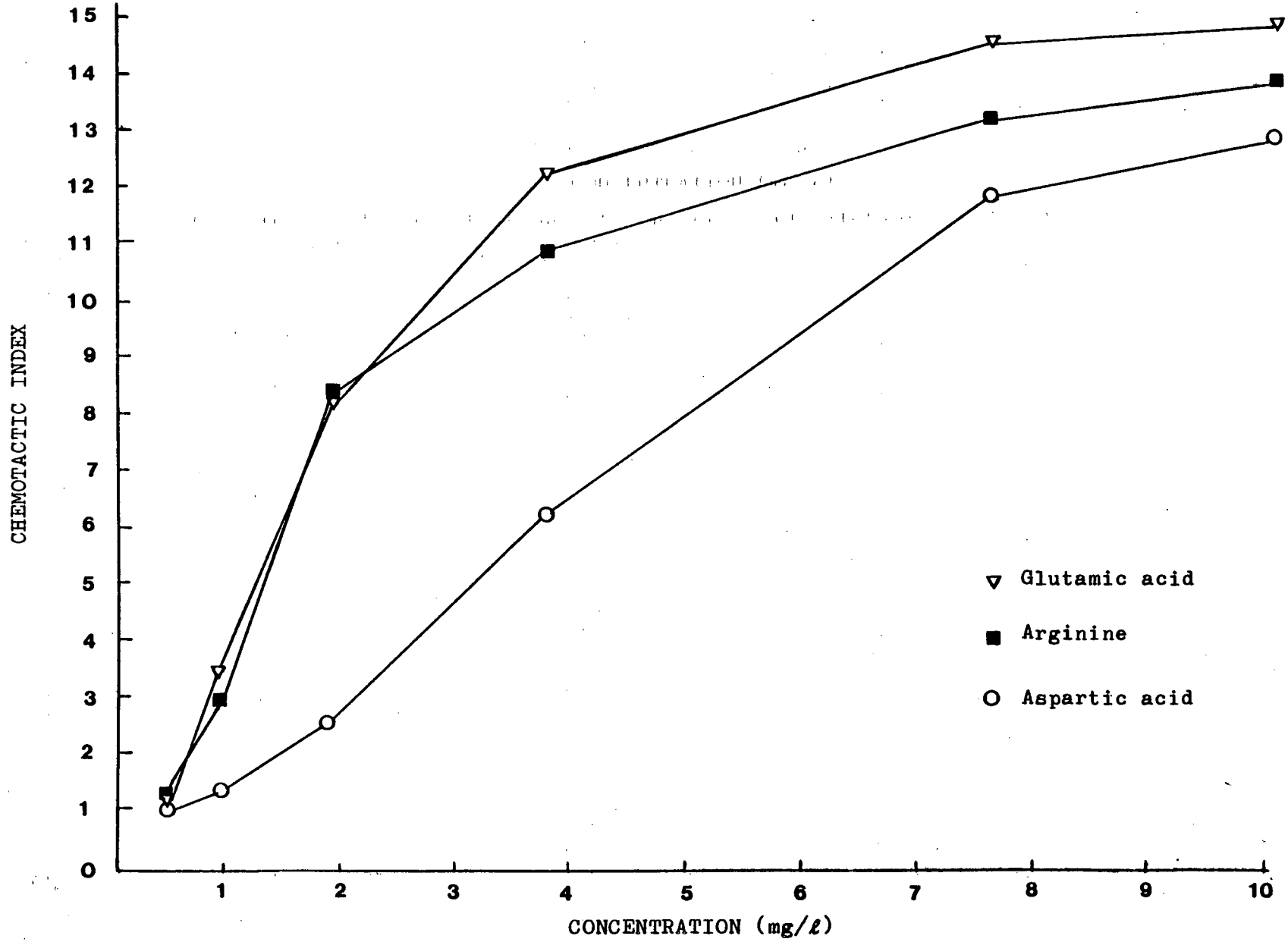

Fig. 1 Effect of different concentrations of aspartic acid, glutamic acid and arginine on attraction of zoospores of Phytophthora cinnamomi.

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TABLE 1 Response of zoospores of Phytophthora cinnamomi to amino acids and sugars in root exudates from three grapevine rootstocks

Compound	Concentration[a] (mg/ℓ) in exudate from rootstock:			Response to compound[b]
	99 Richter	143 B Mgt	Jacquez	
Aspartic acid	3,67	4,15	3,70	+ (1)[c]
Threonine	1,61	0,67	1,65	- (10)
Serine	4,73	2,32	2,89	± (10)
Asparagine	13,18	5,06	35,24	± (1)
Proline	Tr	Tr	N	± (10)
Glutamic acid	10,96	1,57	2,55	+ (1)
Glycine	1,87	1,08	1,02	- (10)
Alanine	5,02	1,58	1,96	± (10)
Valine	2,82	1,35	1,06	- (10)
Tyrosine	2,40	0,36	N	- (10)
Lysine	1,01	0,69	N	- (10)
Histidine	2,00	1,46	0,62	± (5)
Arginine	9,86	3,35	N	+ (1)
Glucose	P	P	P	- (10)
Fructose	P	P	P	- (10)
Abrabinose	P	P	P	- (10)

a Tr = trace; N = not detected; P = present but not determined quantitatively.

b Determined by the method of Royle & Hickman (1964) for attraction of zoospores: + = positive response; ± = slight or sporadic response; - = no response.

c Figures in parentheses indicate the concentration (mg/ℓ) at which the response was recorded.

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resistant to P. cinnamomi than to roots of susceptible cultivars. Turner (1963) found differences in chemotaxis of P. palmivora zoospores to resistant and susceptible cacao seedlings. Khew & Zentmyer (1973) also found that zoospores were more readily attracted to arginine, aspartic acid and glutamic acid than to other amino acids. The present study showed that zoospores of P. cinnamomi were more attracted to the roots of a susceptible grapevine rootstock than to two tolerant rootstocks, and that arginine, glutamic acid and aspartic acid were more attractive than others. The exudation of different amino acids in different concentrations could be an important factor in determining the tolerance or susceptibility of different grapevine rootstocks to P. cinnamomi.

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8 SUSCEPTIBILITY TO PHYTOPHTHORA CINNAMOMI OF TWO GRAPEVINE ROOTSTOCK CLONES AFTER THERMOTHERAPY

ABSTRACT

The grapevine rootstocks 99 Richter clone 1/30/1H74 infected with grapevine fleck and leafroll and Prosperi Super 99 Richter subclone 5 infected with stem pitting and leafroll were more susceptible to Phytophthora cinnamomi without treatment than after heat treatment to free them from the viruses.

Recent field observations by various local workers (unpublished) and claims by local growers suggested that 99 Richter (Vitis berlandieri P. x V. rupestris S.) grapevine rootstock material previously subjected to thermotherapy is more susceptible to Phytophthora cinnamomi Rands than untreated material. This contribution provides data on the effect of thermotherapy on the susceptibility of two 99 Richter rootstock clones to P. cinnamomi.

Two virus-infected clones were used: 99 Richter clone 1/30/1H74 containing leafroll and grapevine fleck viruses and Prosperi Super 99 Richter subclone 5 containing leafroll and stem pitting viruses. After thermotherapy (38°C for ca 150 d) and indexing on a standard range of indicators, both treated clones indexed free of these viruses (D.J. Engelbrecht, Plant Protection Research Institute, Stellenbosch, personal communication).

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Fifty plants each of treated and untreated material of each clone were planted in 30 cm free-draining clay pots containing a sterilised potting mixture. Half the pots of each treatment batch were inoculated with P. cinnamomi by adding a homogenized preparation of a 7 to 10-d-old potato dextrose agar (PDA) culture of P. cinnamomi into each of four holes in the soil around the vines. The pots were randomized in a glasshouse at 25°C ± 5°C and watered weekly. Pathogenicity was evaluated 12 weeks after inoculation by comparing the mass of roots and shoots of inoculated and uninoculated vines, by counting the number of dead plants and by making root isolations from living plants on PDA.

A two-way analysis of variance was applied to the data given in Table 1. The uninoculated treatments (both clones) had no dead vines or vines with infected roots and significantly higher root mass and shoot mass than the inoculated treatments. The inoculated treatments (both clones) had fewer dead heat-treated than untreated vines and also fewer heat-treated vines with infected roots. The shoot mass of inoculated heat-treated vines was also higher than that of inoculated untreated vines. In the case of 99 Richter clone 1/30/1H74 the root mass of inoculated heat-treated vines was significantly higher that that of the inoculated virus-infected material.

The two rootstock clones freed from virus by thermotherapy were less susceptible to P. cinnamomi than the same rootstock clones infected with the respective viruses. This agrees with findings of workers on other crops. Thus Rusell (1966) with sugar beet, and Bovey (1963) and Campbell (1969) with apples, found that infection with virus increased the susceptibility

TABLE 1 Effect of *Phytophthora cinnamomi* on virus-infected grapevine rootstock clones[a] subjected to thermotherapy[b]

Rootstock	Treatment		Dead vines (no)	Living vines with infected roots (no)	Root mass (g)	Shoot mass (g)
99 Richter clone 1/30/1H74	Heat-treated	Inoculated	2	4	2,47	1,41
		Uninoculated	0	0	3,23	2,59
	Control	Inoculated	9	6	1,82	1,14
		Uninoculated	0	0	3,22	2,41
D value (P=0,05)			-	-	0,64	0,25
Prosperi Super 99 Richter	Heat-treated	Inoculated	3	4	2,42	2,46
		Uninoculated	0	0	3,70	3,46
	Control	Inoculated	8	9	2,01	2,22
		Uninoculated	0	0	3,61	3,38
D value (P=0,05)			-	-	0,53	0,20

[a] Fifty rootstocks per treatment.

[b] 38°C for *ca* 150 days.

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of plants to several fungal pathogens.

Under the existing local plant improvement scheme grapevine rootstock and scion material are subjected to thermotherapy and subsequent tip culture to free them from known harmful viruses. The findings in the present study give added motivation for the scheme.

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9 PENETRATION OF 99 RICHTER GRAPEVINE ROOTS BY PHYTOPHTHORA CINNAMOMI

ABSTRACT

Penetration of 99 Richter grapevine roots by Phytophthora cinnamomi was studied by light, scanning and transmission electron microscopy. Zoospores encysted on roots within 30 min of inoculation. More spores encysted and germinated near roots than further away, indicating a response to a stimulus from the roots. Germ tubes were usually not swollen at the point of entry although swollen germ tubes were sometimes observed where intracellular hyphal growth was preceded by direct penetration of epidermal cells. Penetration occurred most frequently at the wall juncture of epidermal cells. Subsequent development was intercellular. There was evidence of hydrolysis of epidermal cell walls, but hydrolysis of cortical cell walls occurred frequently. Shortly after penetration a plug of amorphous material formed in the germ tube to seal the penetration peg in the root. Hyphae occurred in the endodermis within 48 h of inoculation. Invaded epidermal, cortical and endodermal cells were disrupted and underwent plasmolysis. Penetration by vegetative hyphae was essentially the same as penetration initiated by zoospores.

INTRODUCTION

Phytophthora cinnamomi Rands is the causal agent of root rot associated with decline and ultimate death of grapevines grafted on 99 Richter (Van der Merwe, Joubert & Matthee, 1972; Marais, 1978). Different grapevine rootstock cultivars vary in their susceptibility to P. cinnamomi

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(see Part 2), but the mechanism of penetration and infection is unknown. Additional information is required to broaden existing knowledge of resistance to P. cinnamomi. A study was therefore made of the penetration of 99 Richter roots by zoospores and vegetative hyphae of P. cinnamomi.

MATERIALS AND METHODS

Zoospore production

A P. cinnamomi isolate from grapevine roots was cultured for 7 d on potato dextrose agar. Inoculum disks were then cut from the advancing margin of the culture, placed in petri dishes containing V-8 juice broth (Chen & Zentmyer, 1970) and incubated for 2 d at 25°C. The resulting mycelial mat was washed with sterile distilled water and incubated at 20°C in petri dishes containing liquid soil extract (James, 1958). To achieve synchronized release of zoospores, mycelial mats bearing sporangia were chilled at 10°C for 15 min and returned to room temperature. Zoospore concentration was determined with a haemacytometer.

Preparation and inoculation of plant material

Lengths (40 mm) of actively-growing shoots were cut from 99 Richter grapevines, surface-disinfested in 0,5% sodium hypochlorite, washed in sterile distilled water, transferred aseptically to filter paper bridges in 2,5 cm diameter test tubes containing 0,5% Hoagland's nutrient solution, and incubated at 22°C to induce root formation.

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Root tips (20-30 mm) were excised from rooted shoots, washed in distilled water and placed in a suspension containing 10^2 zoospores/cm^3 in an observation cell (Khew & Zentmyer, 1973). The numbers of encysted and germinating zoospore cysts present in 0,5 mm zones extending 2 mm from the root surface were determined microscopically (100 x).

The rooted shoots in each test tube were inoculated with 30 ml of the zoospore suspension (10^2 zoospores/cm^3). Control tubes received 30 ml distilled water. For observation of vegetative hyphal penetration, roots were placed on water agar plates containing 3-d-old P. cinnamomi colonies and incubated in the dark at 25°C.

Light and electron microscopy

Root specimens for light and electron microscopy were removed at intervals, 2-12 h after inoculation.

For scanning electron microscopy, inoculated root sections 1 mm long were fixed in 4% glutaraldehyde (pH 4) for 24 h at 4°C, washed for two 15 min periods in 0,2 M sodium cacodylate buffer (pH 7,2), dehydrated in a graded acetone-water series, subjected to critical point drying with CO_2 and coated with a gold-palladium alloy.

For light and transmission electron microscopy, root sections 1 mm long were fixed for 4 h in 6% glutaraldehyde buffered to pH 7,2 in 0,2 M sodium cacodylate. The sections were postfixed for 12 h in 1% sodium tetroxide and subsequently dehydrated through the acetone-water series.

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Tissue was infiltrated with 30% and 60% Spurr/acetone mixtures for 6 h periods, followed by Spurr's mixture for 24 h. The material was embedded in Spurr's epoxy resin (Spurr, 1969) and polymerized for 8 h at 70°C. Sections for electron microscopy were cut on a Reichert ultramicrotome and stained with 4% uranyl acetate and lead citrate (Reynolds, 1963). Sections (2,0 µm) for light microscopy were stained with 0,2% toluidine blue, pH 9,0.

RESULTS

Within 30 min of exposure to the zoospore suspension zoospores were concentrated in the zones closest to the roots (Table 1) and became clustered around the zone of root elongation. More zoospores also encysted and germinated on and near the root than further away. Germ tubes were directed towards the root.

TABLE 1 Number of encysted and germinating zoospores in four consecutive zones extending from the root tips of 99 Richter grapevine

Zone distance (mm) from root	Number of encysted and germinating zoospores
0 - 0,5	42,6
0,5 - 1,0	42,6
1,0 - 1,5	11,6
1,5 - 2,0	6,2
D-value (P = 0,01)	4,2

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Penetration of grapevine roots by P. cinnamomi is shown in Figs. 1-21. Figs. 1, 2, 5, 7 and 8 show pre-penetration stages and Figs. 3, 4, 6 and 9-21 post-penetration stages.

Zoospores encysted on the root surfaces and cysts began to germinate within a few minutes of zoospore encystment. Fig. 1 shows growth of germ tubes over roots 20 min after germination of the cysts. The single unbranched germ tube first appeared as a conical protuberance (Fig. 2) where the cyst made contact with the root. It grew perpendicularly to the root surface and sometimes extended along the root until it reached a suitable site for penetration. Granular material was detected below the zoospore germ tube at the host-pathogen interface (Fig. 5). Swollen germ tubes (Fig. 7) were usually confined to the sites where penetration occurred directly through the epidermal cell wall. The germ tube wall was often thickened at the point of contact with the epidermal cell wall (Fig. 8). Penetration generally occurred at the wall juncture of epidermal cells (Fig. 3), to establish an intercellular cleavage (Fig. 4). Direct penetration of epidermal cells was occasionally seen (Fig. 6). Walls of cortical cells adjacent to, and in advance of the penetrating hyphae, were usually hydrolyzed (Fig. 9).

During germination, zoospore cysts became progressively vacuolated until they were empty (Figs. 10 and 11). After penetration of the host, a thick plug of amorphous material sealed the hyphae in the root from the empty cyst and germ tube (Fig. 3 enlarged in Fig. 11; Figs. 12 and 13). Vesicles containing electron-dense material were abundant at the growing tip of the penetrating hyphae (Fig. 14).

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Penetration by vegetative hyphae was essentially similar to penetration initiated from zoospores. However, hyphae constricted markedly at the point of penetration. Hyphal cushions were not observed.

The cortex was infected within 4 h after inoculation and both intercellular (Figs. 15 and 16) and intracellular (Figs. 17 and 18) hyphal development was evident. Typical constriction of hyphae occurred where host cells were penetrated directly (Fig. 19).

Hyphae were observed in the endodermis within 48 h of inoculation (Fig. 20). Invaded epidermal, cortical and endodermal cells were disrupted and became plasmolysed (Figs. 20 and 21).

DISCUSSION

More zoospores of P. cinnamomi encysted on and near grapevine roots than further away. Attraction of zoospores by plant roots is well-documented and is attributed to stimulatory chemicals present in the root exudates (Dukes & Apple, 1961; Zentmyer, 1961; Cunningham & Hagedorn, 1962; Rai & Strobel, 1966; Ho & Hickman, 1967; Khew & Zentmyer, 1973).

The function and origin of the granular material detected below the zoospore germ tube on the host surface are uncertain. According to Endo & Colt (1974) other workers have suggested that the granular material acts as an adhesive which attaches the zoospore to the host surface.

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Regular penetration at the wall juncture of epidermal cells rather than directly through the host wall suggests that the fungus followed the path of least resistance. Swollen germ tubes were observed where epidermal cells were penetrated directly. Variation in appressorium morphology is common and all structures adhering to host surfaces to achieve penetration, regardless of morphology, are regarded as appressoria by some authors (Emmett & Parbery, 1975). The present observations agree with reports that appressorium formation by the Pythiaceae is inconsistent (Endo & Colt, 1974).

Many pathogens exert considerable pressure to gain entry into host tissue (Kraft, Endo & Erwin, 1967; Spencer & Cooper, 1967; Marks & Mitchell, 1971; Nemec, 1972; Kim, Kantzes & Weaver, 1974). In the present investigation no evidence was found that epidermal cell walls were indented prior to penetration. Instead, penetration was associated with enzymatic hydrolysis of epidermal cell walls, or with cleavage of the middle lamella of the anticlinal walls of these cells. Hydrolysis of cortical cell walls in infected root tissue also occurred. These observations agree with reports that species of Phytophthora and Pythium produce enzymes capable of degrading cell walls in vivo and in vitro (Ashour, 1954; Winstead & McCombs, 1961; Moore & Couch, 1968; Turner & Bateman, 1968).

The role of the plug which seals the penetration peg and infection hyphae from the empty cyst and germ tube is not clear. It possibly protects the colonizing hyphae in the root against antagonistic micro-organisms present in the rhizosphere.

Hyphal cushions are prevalent on pine roots penetrated by vegetative hyphae

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of P. cinnamomi (Marx & Bryan, 1969). In the present study, roots were penetrated by vegetative hyphae, but no hyphal cushions were seen.

Marx & Bryan (1969) found that vegetative hyphae of P. cinnamomi can be an important source of inoculum. Chlamydospores are important survival units of P. cinnamomi and persist for long periods in dead roots and in soil (Zentmyer & Mircetich, 1966). They are produced over a wide range of moisture regimes (Reeves, 1975), whereas sporangia are formed only at the higher moisture levels. Chlamydospore germination and germ tube growth can also take place at low matric potentials (Sterne, Zentmyer & Kaufmann, 1977). Chlamydospore germ tubes which later become hyphae could therefore play an important role in infection of grapevine roots in the absence of sporangia or conditions favouring their germination. Vegetative hyphae could also be responsible for the secondary spread of P. cinnamomi in the root zones of grapevines.

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Figs. 1-6. Penetration and infection of 99 Richter grapevine roots by Phytophthora cinnamomi. 1. Scanning electron micrograph of zoospores and germinating zoospore cysts (arrows) clustered around a root 40 min after inoculation (x 180). 2. Light micrograph of a zoospore cyst with emerging germ tube (arrow) (x 250). 3. Transmission electron micrograph of penetration from a zoospore infection hyphae (H) between two epidermal cells (E) and cortical cells (C) (x 4 000). 4. Transmission electron micrograph of the intercellular cleavage (arrow) of cortical cell walls (CW) by the invading hyphal tip (HT) (x 3 000). 5. Transmission electron micrograph of granular material (arrows) formed between the zoospore germ tube (Ht) and the cell wall (CW) of an epidermal cell (E) (x 15 000). 6. Transmission electron micrograph of direct penetration of an epidermal cell (E) by an infection hypha (H) (x 8 500).

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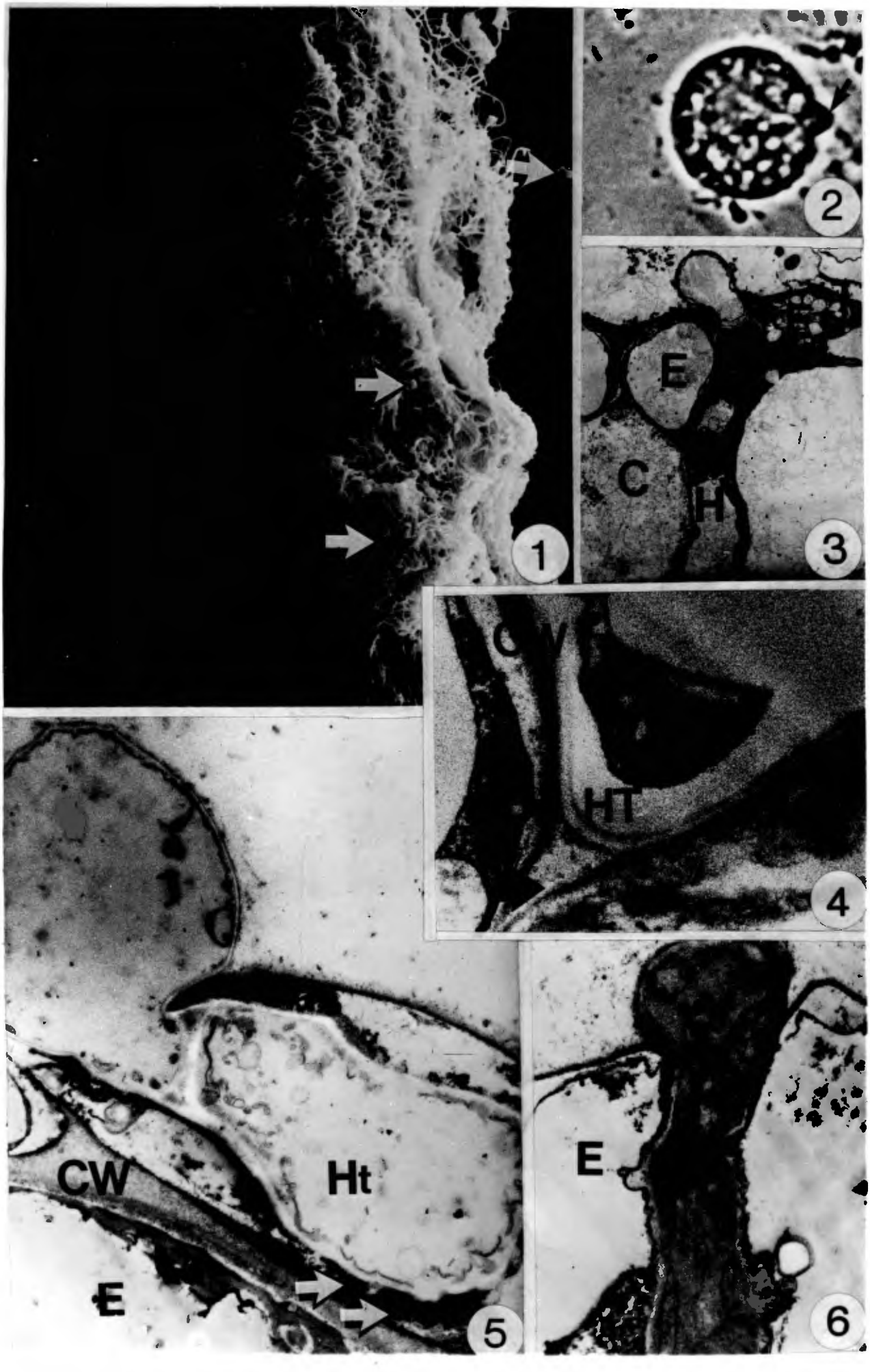

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Figs. 7-14. Penetration and infection of 99 Richter grapevine roots by Phytophthora cinnamomi. 7. Light micrograph of a swelling (arrow) of the germ tube at the point of contact with the epidermal cell (x 250). 8. Transmission electron micrograph of the thickening of the germ tube (Ht) wall (arrow) at the point of contact with the root epidermis (EP) (x 30 000). 9. Transmission electron micrograph of hydrolysis of cortical cell walls (arrow) in advance of an infection hypha (H) (x 36 000). 10. Light micrograph of penetration of epidermal cells (E). Zoospore cysts become vacuolated (small arrows) and infection hyphae (large arrows) are evident between cortical cells (C) (x 150). 11. Transmission electron micrograph of zoospore penetration. A plug (P) is present between the empty cyst (S) and the infection hypha (H) (x 12 000). 12. Transmission electron micrograph of a nearly empty zoospore cyst (S) and a plug (P) sealing the cyst from the internal infection hypha (H) (x 19 000). 13. Transmission electron micrograph of the plug (P) formed between the empty cyst (S) and the infection hypha (H) (x 40 000). 14. Transmission electron micrograph of an infection hypha (H) growing between two cortical cells (C). Vesicles (arrow) are concentrated at the hyphal tip (x 2 400).

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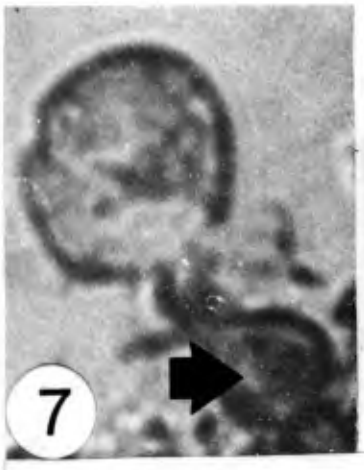

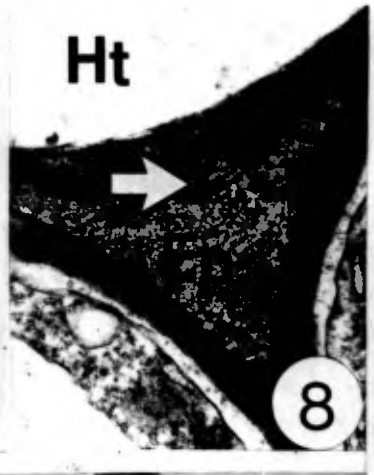

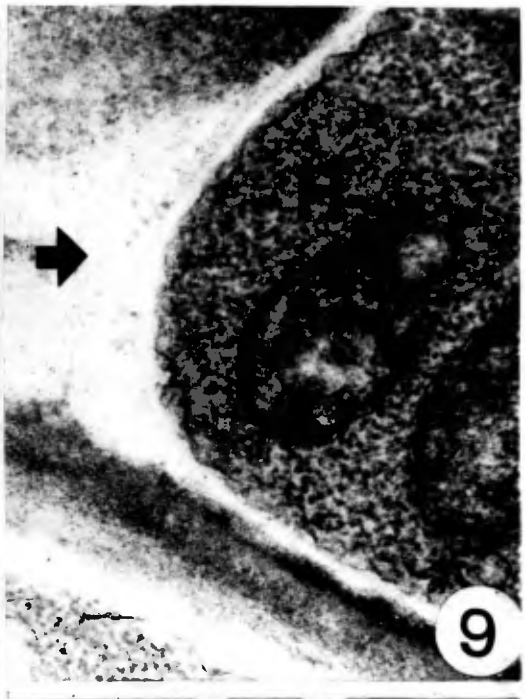

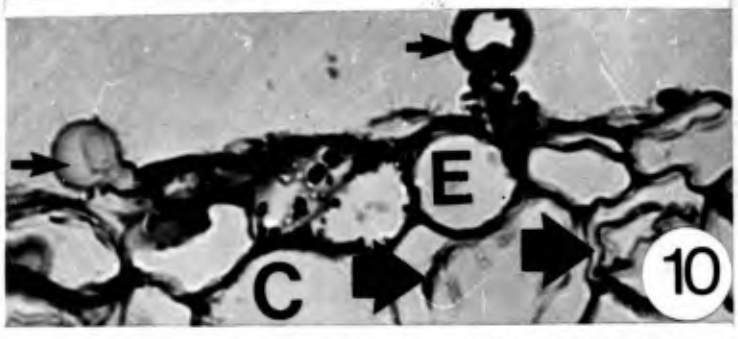

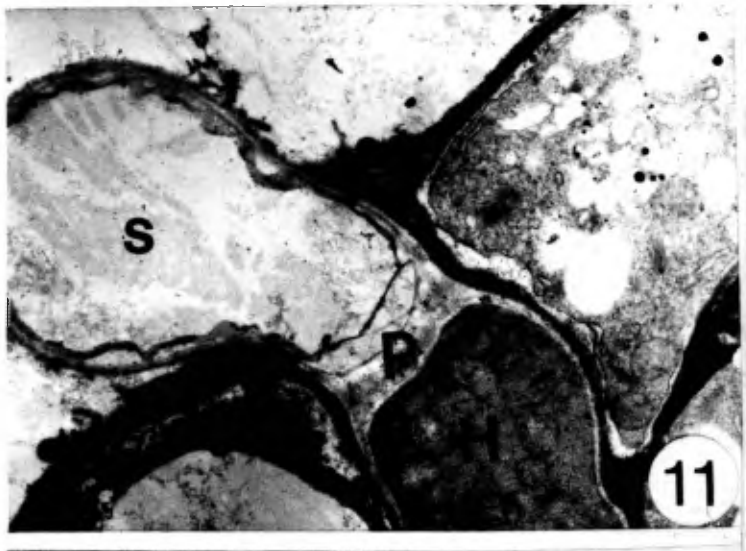

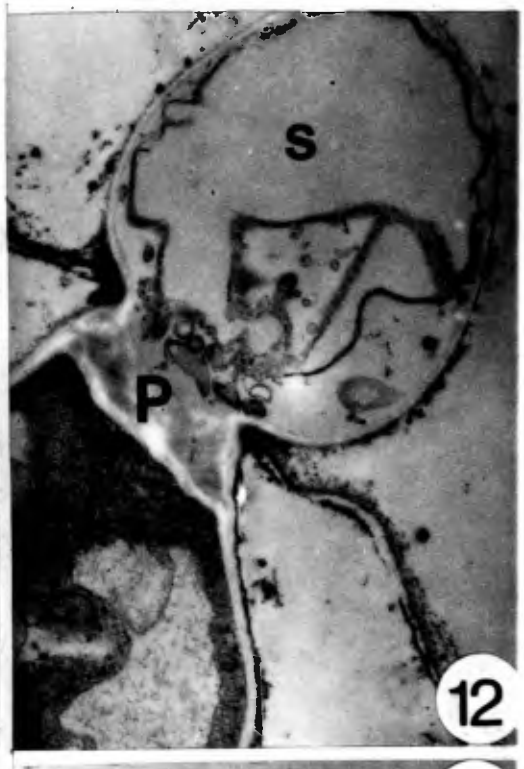

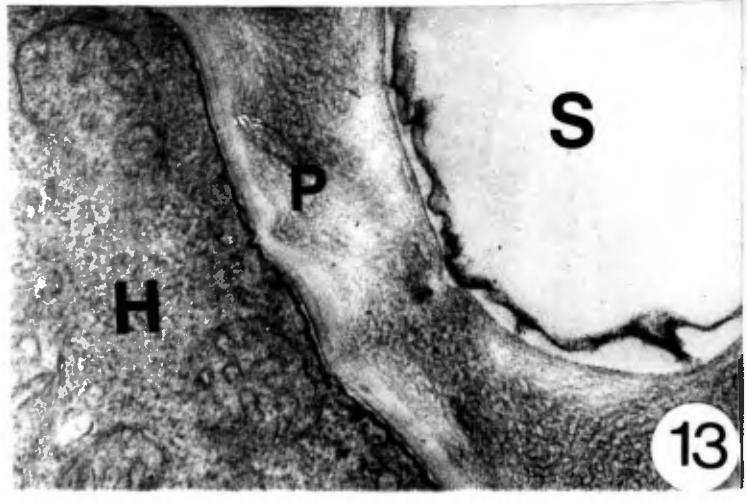

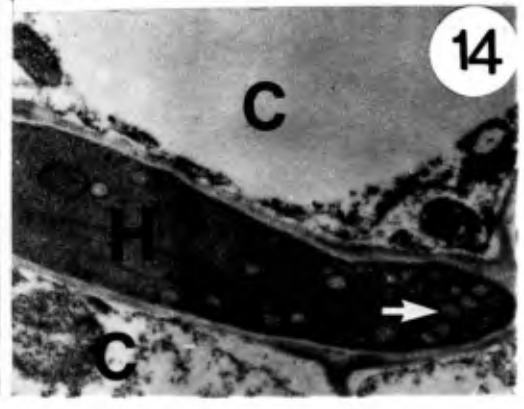

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Figs. 15-21. Penetration and infection of 99 Richter grapevine roots by Phytophthora cinnamomi. 15. Scanning electron micrograph of a hypha (arrow) in the intercellular space of cortical cells (C) (x 2 400). 16. Transmission electron micrograph of hypha (H) in the intercellular space (IN) of cortical cells (C) (x 7 500). 17. Scanning electron micrograph of an intracellular hypha (arrow) in a cortical cell (C) (x 5 400). 18. Transmission electron micrograph of an intercellular hypha (H) in a cortical cell (C) (x 4 000). 19. Transmission electron micrograph of an intracellular hypha (H) growing from one cortical cell (C) to another. Note the constriction of the hypha at the point of cell wall (CW) penetration (arrow) (x 7 500). 20. Light micrograph of a transverse section of a root 48 h after inoculation. The invaded epidermal (E) and cortical (C) cells are disrupted and plasmolysed (small arrows). Invading hyphae are present in the endodermis (large arrows), while spores (S) are visible in the cortical cells (x 100). 21. Light micrograph showing disrupted, plasmolysed epidermal (E) and cortical (C) cells (x 160).

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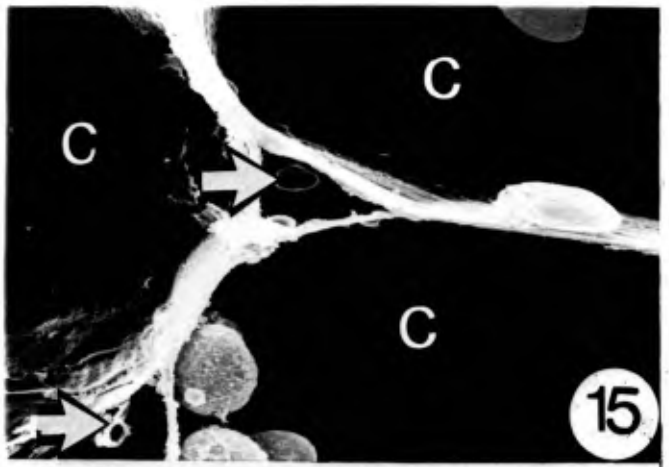

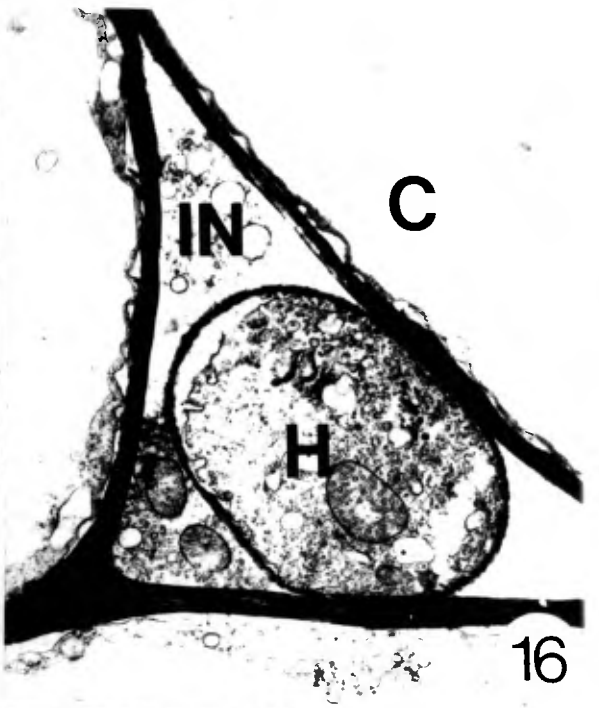

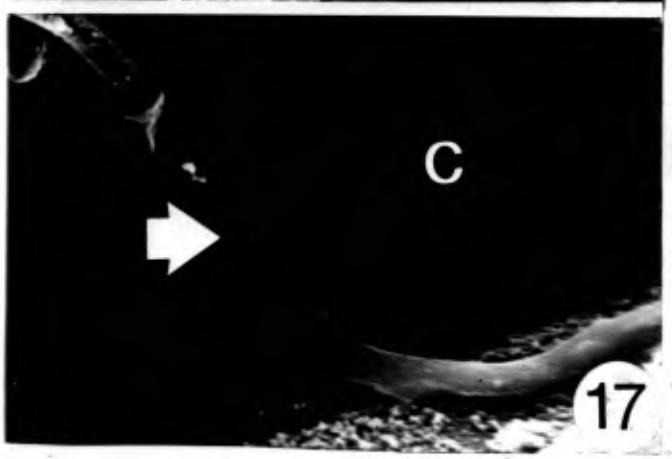

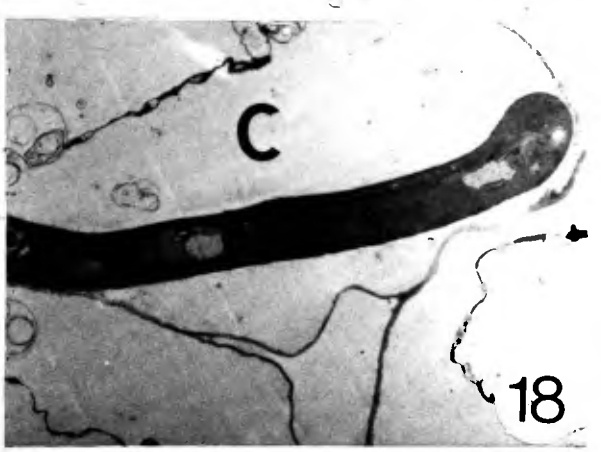

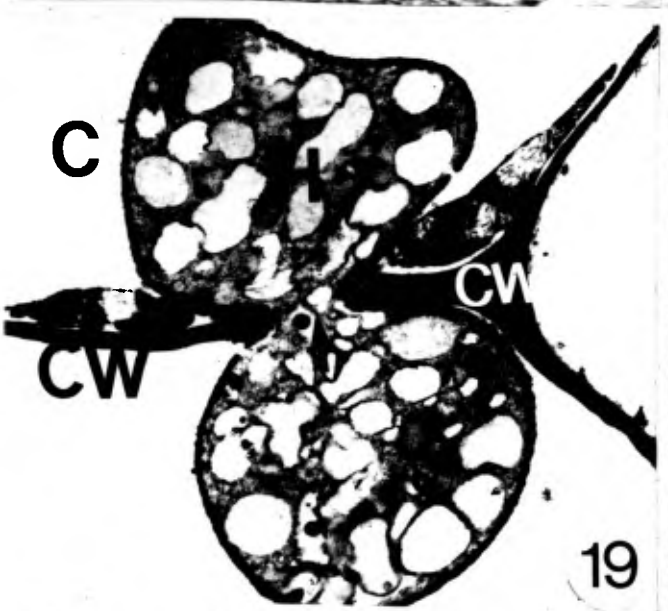

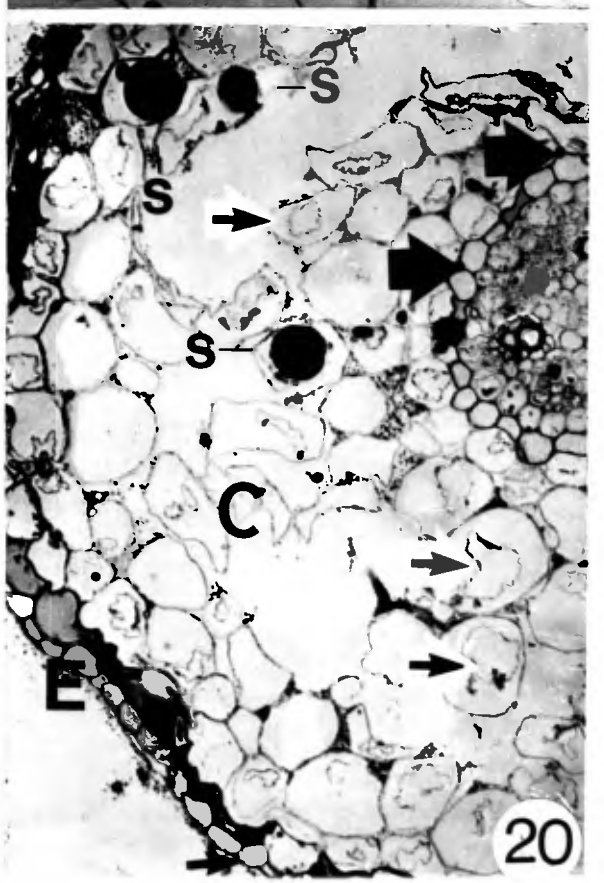

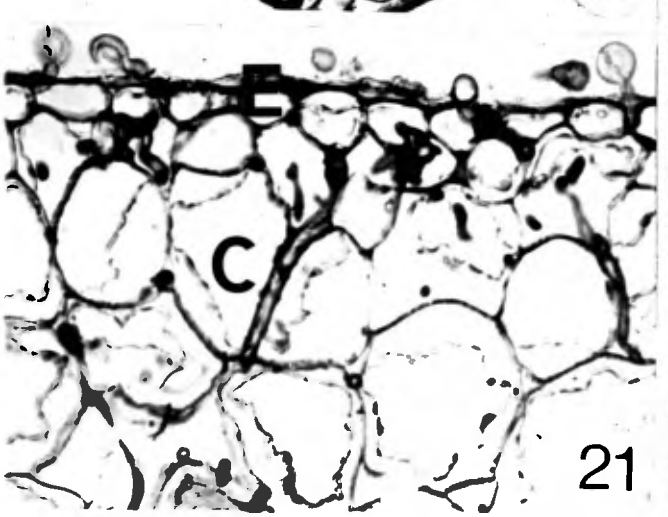

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10 ERADICATION OF *PHYTOPHTHORA CINNAMOMI* FROM GRAPEVINE BY HOT WATER TREATMENT

ABSTRACT

A study was made of the effect of hot water treatment at different temperatures and treatment times on the eradication of *Phytophthora cinnamomi* from dormant vines and canes and from actively growing vines. The harmful effect of hot water treatment on vines and canes was also determined. Hot water treatment at 50°C for 5 min eradicated *P. cinnamomi* from all tested material. When *P. cinnamomi*-infected vines were treated for 5 to 30 min at 50°C and then planted in a nursery, they showed no infection after 8 months. A similar treatment of dormant vines for 15 min resulted in the highest root and shoot mass and the longest shoot growth, whereas a treatment time of 30 min had no harmful effects. Treatment for 15 to 60 min at 50°C resulted in no harmful effects to dormant canes; however, treatment for longer than 15 min at 50°C injured actively growing vines. A hot water treatment at 50°C for 15 min is recommended for all grapevine material for successful eradication of *P. cinnamomi*.

INTRODUCTION

Van der Merwe, Joubert & Matthee (1972) associated *Phytophthora cinnamomi* Rands with the decline and sudden death of grapevines grafted on 99 Richter in South Africa. More recently *P. cinnamomi* has been consistently isolated from the crowns and roots of diseased and dead vines, especially from nurseries and rootstock mother plantations (S. von Broembsen, Plant Protection

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Research Institute, Stellenbosch, personal communication and Part 3). A survey of vine nurseries in the Western Cape showed that P. cinnamomi occurred most frequently in 1 to 5-year-old vine plantations (see Part 2).

Hot water treatment of avocado seeds at 48°C to 50°C for 30 min effectively kills P. cinnamomi without reducing germination of the seeds (Zentmyer, Paulus & Burns, 1967). As early as 1900 the use of hot water at 53°C for 5 min was shown to be a practical treatment of rooted grapevines against phylloxera (Anonymous, 1900). Hot water treatment of vines at 52°C for 5 min was reported to be a safe and effective treatment for nematodes and phylloxera (Raski, Hart & Kasimatis, 1965). Raski & Lider (1960), Moller & Fisher (1961), and Smith (1963) also recommended hot water treatment of vines against nematodes. Furthermore, Goheen, Nyland & Lowe (1973) found that dormant grapevines tolerated an immersion time of up to 2,5 h at 50°C without ill effect.

Because of the occurrence of P. cinnamomi in vine nurseries and rootstock mother plantations, a study was made of hot water treatment of rooted vine material as a method to prevent the spread of this pathogen by rooted nursery material. Because of the possibility of cane infection in mother plantations and heeling-in beds, hot water treatment of such material to eradicate P. cinnamomi was also investigated.

MATERIALS AND METHODS

Isolation

Pieces of vine roots and canes were washed free of soil and loose tissue,

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surface disinfested for 2 min in 1% sodium hypochlorite, rinsed with distilled water and plated on corn meal agar (CMA). Plates were incubated for 24 to 36 h at 24°C and examined microscopically for the presence of P. cinnamomi.

Transmission by canes

More than 300 99 Richter canes were collected at various times from diseased vines in mother plantations in the Stellenbosch, Wellington and Paarl districts. Both above-ground canes and those in direct contact with the soil were examined for P. cinnamomi. Isolations were made from five segments of all canes from 10 dying glasshouse-grown vines which had been artificially inoculated and shown to be infected with P. cinnamomi. In another experiment canes were taken from trellised, infected 99 Richter vines, and were treated as grafted. One hundred suitable pieces were cut, soaked for 1 h in 2,5% chinosol (8-hydroxyquinolene sulphate), placed in a plastic bag with 1% captab (N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboxyamide) and moist perlite, stored at 5°C for 10 weeks, cut into appropriate pieces, waxed on the apical end, placed in callus boxes for 42 d at 25°C and planted in pots in the glasshouse. Isolations were made when the material was collected, after cool storage, and after 6 months in the glasshouse. Plants which failed to root in the glasshouse were also tested.

Infection during heeling-in

Four bundles, each of 100 chinosol-treated 15 cm dormant 99 Richter canes were buried in soil artificially infested with cornmeal-sand inoculum of P. cinna-

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momi as described by Titze et al. (1969). A bundle was removed every 14 d and isolations were made onto CMA plates from all canes in the bundle.

Hot water treatment of root pieces

Twenty large 99 Richter vine roots from which P. cinnamomi had been isolated were placed in a hot water (50°C) bath. After periods of 5, 10, 15, 20, 25 and 30 min, sequential 10 mm pieces of each root were cut with sterile pruning shears and immediately placed in distilled water (20°C). Segments were then cut into smaller pieces and plated on CMA. After 36 h incubation at 24°C the plates were examined for the presence of P. cinnamomi. A similar experiment was carried out with roots treated at 42, 44, 46, 48 and 50°C for 0, 5, 10, 15 and 20 min (five roots for each time interval at each temperature). In an additional experiment temperatures of 32, 34, 36, 38, 40, 42 and 44°C were used.

Hot water treatment of dormant, rooted plants

One-year-old, dormant Cabernet vines grafted onto 99 Richter, showing symptoms of P. cinnamomi infection, were used. All shoots except one 8 cm shoot with three buds were removed from all vines and all roots were cut back to 15 cm. Isolations were made onto CMA from all root and collar regions. Vines were then tagged, numbered and tied into bundles of 10. Two bundles were immersed for 30 min in hot water at each temperature (30, 35, 40, 45 and 50°C) in a Searle thermostatically controlled water bath. After treatment the vines were cooled by immersion in cold water at 20°C. Re-isolations from all roots and collars were then made on CMA.

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Since preliminary results showed the most effective treatment temperature to be 50°C, it was decided to vary the treatment time at this temperature. One-year-old Chenin blanc vines on 99 Richter were treated as previously described, but at 50°C for 5, 10, 15 and 20 min. Treated vines were planted in a nursery. After 8 months, isolations were again made from roots and collars.

Harmful effects

Dormant rooted plants. Healthy, 1-year-old, dormant Chenin blanc vines on 99 Richter were treated for 10, 15, 20 and 30 min in hot water at 50°C. The same procedure was followed as described previously. Fifty vines were treated for each time period and planted out in a randomized block with five replicates and 10 vines per replicate. After 8 months the vines were lifted and assessments made of root mass, shoot mass, and shoot length.

Actively growing plants. Actively growing 1-year old, ungrafted Rupestris St. George vines were treated by submerging the whole plant in water at 50°C for 0, 15, 30 and 45 min (eight plants per treatment) and then transplanted to glasshouse pots. The plants were observed for 6 months for harmful effects.

Canes. Canes were hot-water treated at 50°C for 0, 15, 30, 45 and 50 min (10 canes per treatment), placed in a mist bed for rooting and observed for harmful effects.

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RESULTS

Transmission by canes

P. cinnamomi was isolated from only two of the more than 300 canes treated. Both of these had been in direct contact with the soil. The fungus could not be isolated from any of the canes of the 10 infected plants in the glasshouse. No *P. cinnamomi* was isolated at any stage from the 100 cuttings taken from trellised, infected 99 Richter mother plants.

Infection of canes during heeling-in

Isolations made from canes 2 weeks after heeling-in in *P. cinnamomi*-infested soil, showed no *P. cinnamomi*, but canes removed after 28, 42 and 56 d showed 8, 18 and 41% infection respectively.

Hot water treatment of root pieces

No *P. cinnamomi* was isolated from any root pieces treated at 50°C for 5 min or longer, but it was isolated from 18 of the 20 control pieces. A 5 min treatment at 42, 44, 46 and 48°C also eradicated *P. cinnamomi* (Table 1). At temperatures below 42°C, root pieces remained infected even after the maximum treatment time of 20 min.

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Hot water treatment of dormant, rooted plants

P. cinnamomi was isolated from all the Cabernet vines on 99 Richter prior to treatment and from 20, 20, 20 and 7 vines per 20 vines treated at 30, 35, 40 and 45°C respectively. At 50°C P. cinnamomi was eradicated from all 20 vines.

No P. cinnamomi was isolated from any vine immediately after treatment at 50°C for periods of 5 to 30 min, nor 8 months later after subsequent growth in a nursery. The fungus was isolated from 95 of the 100 vines prior to treatment.

Harmful effects

Dormant rooted plants. Results of hot water treatment of dormant vines are given in Table 2. The root mass of all treatments was significantly higher than that of the control, whereas the shoot masses and shoot lengths of all except the 10 min treatments were significantly greater than the control.

Actively growing plants. When actively growing vines were treated at 50°C, control vines and vines treated for 15 min showed no sign of injury after 6 months, whereas 50% and 62% of the plants treated for 30 and 45 min respectively were dead.

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TABLE 1 Recovery[a] of Phytophthora cinnamomi from infected vine roots after treatment in hot water at 32 to 50°C for various times

Temperature (°C)	Treatment time (min)				
	0	5	10	15	20
32	5	5	5	5	5
34	5	5	5	5	5
36	2	3	4	4	5
38	3	1	3	3	3
40	5	4	2	1	1
42	3	0	0	0	0
44	4	0	0	0	0
46	4	0	0	0	0
48	2	0	0	0	0
50	5	0	0	0	0

[a] Number of roots positive for P. cinnamomi/five root pieces.

TABLE 2 Root mass, shoot mass and shoot length of vines 8 months after treatment of the dormant rooted plants with hot water at 50°C for various times

Treatment period (min)	Root mass (g)	Shoot mass (g)	Shoot length (cm)
0	53,67	22,39	300,62
10	72,25	23,17	306,76
15	126,78	58,31	605,10
20	101,81	45,73	440,20
30	119,16	59,80	508,96
D values (P=0,05)	9,45	9,51	11,82

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Canes. All the unrooted canes treated with hot water budded and came into leaf before the controls. After treatment at 50°C for 0, 15, 30, 45 and 60 min respectively, 9, 8, 9, 9 and 10 canes in each treatment rooted and grew normally.

DISCUSSION

Hot water treatment at 50°C was a safe and effective means of eradicating P. cinnamomi from both rooted and unrooted grapevine material, and therefore of preventing the spread of Phytophthora root and collar rot by either rooted or unrooted grapevine material. A hot water treatment of 15 min at 50°C is recommended for dormant rooted vines and dormant cuttings. This treatment has also been reported to control phylloxera, eelworm and Pierce's disease on grapevine (Anonymous, 1900; Raski & Lider, 1960; Moller & Fisher, 1961; Smith, 1963; Raski et al., 1965; Goheen et al., 1973).

The increase in growth of rooted material treated with hot water could be due to a physiological stimulatory effect of the treatment or to the elimination of other pathogens or to a combination of these factors. Dormancy of both cuttings and rooted material is broken by the treatment. Material must therefore be planted or grafted within a reasonable time after treatment.

There was no transmission of P. cinnamomi by cuttings which had no contact with soil. However, the fungus was isolated from two canes which had been in contact with soil. If such cuttings are used for propagation they should be subjected to hot water treatment.

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P. cinnamomi was isolated from canes which had been buried in infested soil. Care must therefore be taken to ensure that vine material is not buried in infested soil. This has implications in nursery practice, since some nurserymen and most farmers store grafted or rooted vine material in heeling-in beds in the soil.

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11 REDUCTION OF PHYTOPHTHORA CINNAMOMI ROOT ROT OF GRAPEVINES BY CHEMICAL TREATMENT

ABSTRACT

In glasshouse and field trials phosethyl aluminium applied as a spray and metalaxyl applied as a drench significantly reduced Phytophthora cinnamomi root rot of 99 Richter grapevine rootstock. Application of methyl bromide, dazomet or metalaxyl to infested nursery soil before planting significantly reduced root rot, but the pathogen became re-established in the soil. To reduce the disease effectively in nurseries, chemical soil treatment should be followed by regular applications of appropriate fungicides to the growing vines.

INTRODUCTION

Root rot caused by Phytophthora cinnamomi Rands is a serious disease of grapevines in nurseries (see Part 3). The pathogen spreads from transplanted infected nursery material to healthy vines in nurseries and vineyards (see Part 3). These considerations and the wide distribution of the pathogen (see Part 2) complicate the development of effective root rot control measures.

Several fungicides have been tested for the control of P. cinnamomi on crops other than grapevine (Pegg, 1969; Zentmyer, 1973; Bertus & Wood, 1977, 1979). Metalaxyl and phosethyl aluminium are new promising systemic fungicides, specific to oomycetous pathogens (Zentmyer, 1978; Benson,

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1979; Cohen, Reuveni & Eyal, 1979; Staub & Young, 1980; Farih, Tsao & Menge, 1981 a, b).

This study evaluated different fungicidal treatments of 99 Richter grapevine rootstock growing in P. cinnamomi infested soil. The feasibility of treating infested nursery soil with chemical compounds before planting the rootstock was also investigated.

MATERIALS AND METHODS

Fungicides

The following compounds were used: dazomet (98 GRAN), ethazole (24 EC), fenaminosulf (70 WP), metalaxyl (25 WP), phosethyl aluminium (80 WP), prothiocarb (70 EC) and methyl bromide.

Glasshouse test

Cuttings (20 cm) of 99 Richter, rooted in sterilised sand in a glasshouse (ca. 25°C) were transplanted singly to 18-cm pots containing a sterilised potting mixture and inoculated after 3 weeks. Four holes were made 8 cm deep in the potting mixture around each vine. Inoculum was introduced and the holes were refilled. Inoculum applied to each hole was prepared by adding 100 ml water to a 7 to 10-d-old culture of the pathogen growing on potato-dextrose agar in a 90-mm petri dish and macerating the culture in a Waring blender.

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Fourteen days after inoculation, ethazole (5 g ai/m^2), fenaminosulf (12 g ai/m^2), metalaxyl (2 g ai/m^2), phosethyl aluminium (16 g ai/m^2) and prothiocarb (12 g ai/m^2), diluted in 10 l water, were applied to separate pots as soil drenches. Phosethyl aluminium (3,2 g ai/l) was applied as a spray until runoff. Three additional applications of the fungicides were made at 4-week intervals. No fungicide was applied to control vines. A randomized complete block design was used.

After 6 months dead vines were counted and isolations were made from the roots of surviving vines (Part 5).

Field trials

Trials were conducted at two heavily infested nursery sites. The occurrence of the pathogen within the sites was determined by lupin baiting soil samples from 1 m^2 sample areas (Chee & Newhook, 1965).

Trial 1. The experimental area was divided into 20, 4-m^2 plots. One-hundred-and-sixty 20-cm 99 Richter cuttings were planted 8 cm apart in rows spaced 30 cm in each plot.

Eight weeks after planting, soil drenches of ethazole (5 g ai/m^2) and metalaxyl (2 g ai/m^2) and a foliar spray of phosethyl aluminium (3,2 g ai/l) were applied. Fungicides used as drenches were applied evenly over the soil surface. Phosethyl aluminium was sprayed onto the vines until runoff. Untreated control plots were included. Each treatment was replicated five times in a randomized block design. Four additional applications of the fungicides were made at 4-week intervals.

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Only the plots which had received the soil drenches were irrigated (25 mm water) immediately after application of the fungicides. All plots were then watered at weekly intervals for 8 months.

Dead vines were counted 10 months after planting. The presence of the pathogen in roots of surviving vines was determined (Part 5). Root and shoot mass and shoot length of individual vines were also recorded.

Trial 2. Twenty 8-m^2 plots were subjected to four treatments of chemicals and an untreated control, with four replicates each, arranged in a randomized complete block design.

Metalaxyl (2 g ai/m^2) and phosethyl aluminium (16 g ai/m^2) were applied as drenches. Dazomet granules were distributed and worked into the upper soil with a rotavator. Methyl bromide was applied as a gas under a 0,25 mm polythene sheet, which was removed 4 d later. After treatment all the plots, except those treated with methyl bromide, were irrigated (25 mm water).

Four weeks after the chemical treatments, 320 20-cm 99 Richter cuttings were planted 8 cm apart in rows spaced 30 cm. Treatments were evaluated after 10 months. Soil samples from each plot were assayed for *P. cinnamomi* by the lupin baiting method 1, 3 and 9 months after applying the compounds.

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RESULTS

Glasshouse test

Phosethyl aluminium applied as a spray and metalaxyl as a drench effectively reduced root rot (Table 1). Ethazole applied as a drench also reduced mortality significantly, but was less effective than phosethyl aluminium and metalaxyl. No other treatment gave significant control.

Field trials

Trial 1. Phosethyl aluminium and metalaxyl effectively controlled root rot in infested nursery soil (Table 2). Shoot length was also increased significantly. Ethazole reduced mortality to some extent but there was no reduction in infection or increase in shoot length. None of the treatments had a significant effect on root or shoot mass.

Trial 2. Soil treatment with metalaxyl, dazomet or methyl bromide reduced mortality of vines (Table 3). These compounds also reduced root infection and increased root mass. The dazomet and methyl bromide treatments were the only ones giving increased shoot mass and length. The phosethyl aluminium treatment had no effect on the growth of vines.

P. cinnamomi was detected after 1, 3 and 9 months in control plots and plots treated with phosethyl aluminium but only after 9 months in soil treated with metalaxyl, dazomet and methyl bromide.

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TABLE 1 Effect of different fungicidal treatments on the severity of root rot of 99 Richter rootstock inoculated with *Phytophthora cinnamomi* in a glasshouse[a]

Fungicide	Application method	Dosage (a.i.)	Dead vines (%)	Surviving vines with infected roots (%)
Control	-	-	37	41,3
Fenamino-sulf	Soil drench	12 g/m²	28	35,0
Prothiocarb	Soil drench	12 g/m²	27	32,7
Ethazole	Soil drench	5 g/m²	24	29,5
Phosethyl aluminium	Soil drench	16 g/m²	33	38,5
Phosethyl aluminium	Foliar spray	3,2 g/ℓ	9	13,0
Metalaxyl	Soil drench	2 g/m²	13	11,0
D value (P=0,05)			10,2	14,2

[a] Ten replicates per treatment with five vines per replicate arranged in a randomized complete block design.

TABLE 2 Effect of three fungicidal treatments on 99 Richter grapevine rootstock growing in a nursery soil naturally infested with *Phytophthora cinnamomi*[a]

Fungicide	Application method	Dosage (a.i.)	Dead vines (%)	Surviving vines with infected roots (%)	Root mass (g)	Shoot mass (g)	Shoot length (cm)
Control	-	-	18	33,8	111,7	99,8	9,5
Ethazole	Soil drench	5 g/m²	11	26,5	114,3	110,2	10,2
Phosethyl aluminium	Foliar spray	3,2 g/ℓ	4,3	10,6	125,9	114,2	12,8
Metalaxyl	Soil drench	2 g/m²	4,1	11,5	125,3	114,1	12,6
D value (P=0,05)			3,2	12,2	21,0	20,4	2,4

[a] Five replicates per treatment with 160 vines per replicate in a randomized block design.

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TABLE 3 Effect of chemical treatment of a nursery soil naturally infested with *Phytophthora cinnamomi* on the subsequent growth of 99 Richter grapevine rootstock[a]

Compound	Application method	Dosage (a.i.)	Dead vines (%)	Surviving vines with infected roots (%)	Root mass (g)	Shoot mass (g)	Shoot length (cm)
Control	-	-	47,0	20,0	89,1	46,8	6,3
Phosethyl aluminium	Soil drench	16 g/m²	40,0	15,0	104,6	48,2	6,6
Metalaxyl	Soil drench	2 g/m²	26,5	10,0	143,6	53,4	7,2
Dazomet	Granules	50 g/m²	21,6	4,0	159,7	73,6	9,7
Methyl bromide	Fumigant	125 g/m²	17,5	3,0	146,4	62,5	9,7
D value (P=0,05)			19,3	8,2	20,9	11,1	2,7

[a] Four replicates per treatment with 320 vines per replicate in a randomized block design.

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DISCUSSION

Greenhouse and field trials showed that phosethyl aluminium spray and metalaxyl drench significantly reduced root rot of grapevines planted in soil infested with P. cinnamomi. Phosethyl aluminium drench reduced P. cinnamomi root rot of avocado (Zentmyer, 1978) but was not effective on grapevines.

Chemical application to soil before planting is expensive, and is usually limited to nurseries. In the present investigation methyl bromide, dazomet and to a lesser extent metalaxyl, reduced root rot in nurseries. Although less effective, metalaxyl has little effect on micro-organisms antagonistic to P. cinnamomi (Allen et al., 1980), whereas dazomet and methyl bromide are wide-spectrum soil fumigants. The re-establishment of P. cinnamomi in treated nursery soil suggests that soil treatments should be followed by fungicidal treatments of growing vines.

I recommend that grapevine nursery soil be treated with a drench of metalaxyl (2 g ai/m^2) before planting. Planted vines should receive five, monthly drench-applications of metalaxyl or monthly sprays of phosethyl aluminium (3,2 g ai/l). Phosethyl aluminium spray is also effective against Plasmopara viticola (Marais & Van der Walt, 1978; Wicks & Lee, 1982). Metalaxyl is taken up by the roots and translocated upwards in other crops (Zaki, Zentmyer & LeBaron, 1981; Ellis, Grove & Ferree, 1982) but the concentration applied to the soil to control P. cinnamomi is probably too low to have any effect on P. viticola.

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12 GENERAL SUMMARY

Root rot of grapevines (Vitis spp.) has become increasingly important in South Africa in recent years. Initial surveys showed that Phytophthora cinnamomi Rands was the most important pathogen associated with the disease in grapevine nurseries and in vineyards in the grape growing areas of the South-Western Cape Province. A detailed study was therefore made of P. cinnamomi in relation to its grapevine host.

P. cinnamomi was isolated from rootstocks of dead or diseased vines in vineyards from 14 districts in the Cape Province of South Africa. It was recovered in vineyard soil to a depth of 320 mm. Downhill spread of the pathogen was more rapid through a soil with a perched water table than through a freely-draining soil. Lateral movement of the fungus through soil occurred to a limited extent. The disease potential index of newly infested soil was usually higher than that of areas previously infested. The results emphasised the danger of introducing P. cinnamomi to poorly-draining soils on infected planting material.

Reactions of different grapevine rootstock cultivars to P. cinnamomi were obtained by inoculating stems, canes and rooted plants in water culture and by growing inoculated rootstocks in both artificially and naturally infested vineyard soil. Of 24 rootstock cultivars tested, only 2-1 USVIT, 143 B Mgt, 101-14 Mgt, 3-6 USVIT and Jacquez had mortality rates below 20% in soil. The five Vitis vinifera cultivars tested were more tolerant than the three hybrids between V. vinifera and other Vitis spp. Most rootstock cultivars became more tolerant to P. cinnamomi when grafted with a

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V. vinifera scion. It was concluded that the laboratory and greenhouse inoculation methods cannot be used to predict disease reactions of rootstocks in the field.

The survival and growth of 21 grapevine rootstock cultivars in a vineyard naturally infested with P. cinnamomi was recorded for 7 years. The different rootstocks were rated as tolerant, intermediate and susceptible. Fewer rootstocks grafted with Chenin blanc than ungrafted rootstocks were killed by the pathogen. All tolerant rootstock cultivars had V. vinifera in their parentage.

An attempt was made to explain the resistance of some grapevine rootstocks to P. cinnamomi. Zoospores of the pathogen were more attracted to susceptible 99 Richter roots than to roots of tolerant 143 B Mgt. Root exudates from 99 Richter contained higher concentrations of glutamic acid and arginine than root exudates from 143 B Mgt and the tolerant cultivar Jacquez. The chemotactic index increased with increasing concentrations of glutamic acid, arginine and aspartic acid. However, this study should be extended to include analyses of the root extracts of other grapevine cultivars.

Penetration of susceptible 99 Richter grapevine roots was studied by light, scanning and transmission electron microscopy. Zoospores encysted on roots within 30 min of inoculation. More spores encysted and germinated near roots than further away, indicating a response to a stimulus from the roots. Germ tubes were usually not swollen at the point of entry although

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swollen germ tubes were sometimes observed where intracellular hyphal growth was preceded by direct penetration of epidermal cells. Penetration occurred most frequently at the wall juncture of epidermal cells. Subsequent development was intercellular. There was evidence of hydrolysis of epidermal cell walls, but hydrolysis of cortical cell walls occurred frequently. Shortly after penetration, a plug of amorphous material formed in the germ tube to seal the penetration peg in the root. Hyphae were present in the endodermis within 48 h of inoculation. Invaded epidermal, cortical and endodermal cells were disrupted and underwent plasmolysis. Penetration by vegetative hyphae was essentially the same as penetration initiated by zoospores.

Apart from avoiding the introduction of P. cinnamomi to poorly-draining soil and the use of tolerant rootstock cultivars, other measures to control the disease have been investigated. A hot water treatment at 50°C for 15 min is recommended for all grapevine material for successful eradication of P. cinnamomi. In glasshouse and field trials phosethyl aluminium applied as a spray and metalaxyl applied as a drench significantly reduced P. cinnamomi root rot of susceptible 99 Richter grapevine rootstock. Application of methyl bromide, dazomet or metalaxyl to infested nursery soil before planting significantly reduced root rot, but the pathogen became re-established in the soil. To reduce the disease effectively in nurseries, chemical soil treatment should be followed by regular applications of appropriate fungicides to the growing vines.

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14 · APPENDIX

List of cultivars and their affiliation with *Vitis* spp.

99 Richter	(*Vitis berlandieri* P. × *V. rupestris* S.)
1103 Paulsen	(*V. berlandieri* P. × *V. rupestris* S.)
2-1 USVIT	(*V. aestivalis* M., *V. cinerea* E., *V. vinifera* L. × *V. berlandieri* P., *V. rupestris* S.)
Prosperi Super 99 Richter	(*V. berlandieri* P. × *V. rupestris* S.)
3306 C	(*V. riparia* M. × *V. rupestris* S.)
143 B Mgt	(*V. vinifera* L. × *V. riparia* M.)
101-14 Mgt	(*V. riparia* M. × *V. rupestris* S.)
1 Schabort	(*V. rupestris* S.)
Rupestris du Lot[a]	(*V. rupestris* S.)
Rupestris St. George[b]	(*V. rupestris* S.)
Constantia Metallica	(*V. rupestris* S.) × (?)
3-6 USVIT	(*V. aestivalis* M., *V. cinerea* E., *V. vinifera* L. × *V. berlandieri* P., *V. rupestris* S.)
15 Vivet	(*V. rupestris* S. × *V. berlandieri* P.)
44-53 Maleque	(*V. riparia* M. × *V. cordifolia* M. × *V. cordifolia* M., *V. rupestris* S.)
110 Richter	(*V. berlandieri* P. × *V. rupestris* S.)
1045 Paulsen	(*V. berlandieri* P. × *V. rupestris* S.)
140 Ruggeri	(*V. berlandieri* P. × *V. rupestris* S.)
Jacquez	(*V. aestivalis* M. × *V. cinerea* E. × *V. vinifera* L.)
1 Grezot	(*V. aestivalis* M. × *V. cinerea* E. × *V. vinifera* L.)
V. champini var. Ramsey	(*V. champini* P.)
3-5 USVIT	(*V. aestivalis* M., *V. cinerea* E., *V. vinifera* L. × *V. berlandieri* P., *V. rupestris* S.)
420 A Mgt	(*V. berlandieri* P. × *V. riparia* M.)
504	(*V. berlandieri* P. × *V. riparia* M.)
De Waal Bosstock	(*V. vinifera* L. × *V. labrusca* L.?)

[a] French origin

[b] Californian origin.

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