

## SOME ASPECTS OF PHYTOPHTHORA CINNAMOMI RANDS INFECTION OF GRAPEVINES

PG MARAIS and AC DE LA HARPE

OENOLOGICAL AND VITICULTURAL RESEARCH INSTITUTE, STELLENBOSCH,  
RSA

### ABSTRACT

*The mechanisms of penetration of Phytophthora cinnamomi Rands into young grapevine roots were studied by light, scanning and transmission electron microscopy. Culture grown cuttings of root-rot susceptible 99 Richter were inoculated with zoospores of P. cinnamomi. Zoospores encysted on the roots and the germ tubes penetrated without the formation of appressoria.*

*Penetration was mostly intercellular and enlargement of the nucleus as well as granulation of the cytoplasm occurred.*

*The effect of root exudates on zoospore behavior was studied and the strongest attraction of zoospores were obtained with the three amino acids aspartic acid, glutamic acid and arginine.*

Results of chemical control and hot water treatment are also shown.

### INTRODUCTION

There are but a few records in literature of *Phytophthora* spp. associated with root diseases of grapevines. According to Chiarappa (1959) 32 per cent of the fungi isolated from the rhizosphere of grapevines suffering from delayed and weak growth in the San Joaquin Valley of California, consisted of various unnamed species of *Phytophthora*. McGechan (1966) found *P. cinnamomi* Rands associated with root rot of grapevine in New South Wales and in India, Agnihothrudu (1968) isolated *P. cinnamomi* from collars and roots of affected vines.

In South Africa the occurrence of decline and sudden death of grapevines grafted on 99 Richter (*Vitis berlandieri* x *V. rupestris*) has been known for nearly four decades. In 1972 van der Merwe, Joubert and Matthee attributed this sudden dying off to infection with *P. cinnamomi*.

Since these findings were published, a great number of cases where sudden dying off of vines occurred were investigated by the Oenological and Viticultural Research Institute, and in most cases *P. cinnamomi* was involved. The damage caused by this fungus can be really devastating. In one instance 50% of a planting of 60 000 vines on 99 Richter and in another instance 65% of 30 000 vines on 99 Richter was dead or in various stages of decline within six months after planting. In a third typical case more

than 300 of 3 000 vines had to be discarded before planting due to heavy infections with *P. cinnamomi* and a further 20% died within nine months after having been planted.

In a recent survey it was found that more than 50% of the nurseries in the Wellington area which produces approximately 50% of all the grafted vines planted in South Africa, was infected with *P. cinnamomi*. It was therefore not surprising that most cases of *P. cinnamomi* infection of young vineyards could, be traced back to infected nurseries.

## **MATERIALS AND METHODS**

### **Plant and fungal culture methods**

Shoot pieces 400 mm long were cut from actively growing 99 Richter and 143 B Mgt rootstocks. The shoot pieces were surface sterilized in 0,5% sodium hypochlorite, washed in sterile distilled water and aseptically transferred onto filter paper bridges in 2,5 cm diameter test tubes containing 0,5% Hoaglands nutrient solution and incubated at 22°C to induce root formation.

For production of sporangia *P. cinnamomi* isolated from grapevine roots were grown on PDA, inoculum discs cut from the growing margin of the culture after seven d and placed in a petri dish containing a liquid medium made from V-8 juice (CAMPBELL SOUP Co. USA). The liquid medium was prepared by adding 2,5 g CaCO<sub>3</sub> to 170 ml of V-8 vegetable juice and centrifuging the mixture at 5 000 rpm for 10 min. The supernatant was diluted 1:9 v/v with distilled water. The fungus was grown for two d on the liquid medium, the mycelial mat washed with distilled water and placed in a soil extract solution. To achieve synchronised release of zoöspores, mycelial mats bearing sporangia were chilled at 10°C for 15 min and returned to room temperature.

Each test tube containing the rooted shoot pieces were inoculated with 30 ml of the zoöspore suspension containing 10<sup>2</sup> zoöspores per ml. Control tubes were filled with 30 ml distilled water.

### **Light and electron microscopy**

At times ranging from two —196 h after inoculation samples of root were chosen for preparation, embedding and sectioning for light and electron microscopy. For scanning electron microscope studies (ISI 100 A) small pieces of roots (1mm long) were dissected from the inoculated roots and fixed for 24 h in 4% glutaraldehyde, pH4 at 4°C. The roots were afterwards washed twice for 15 min in a 0,2M sodium cacodylate buffer pH 7,2 and dehydrated through an acetone-water series, critical point dried with CO<sub>2</sub> and coated with a gold palladium alloy.

For light and transmission electron microscope (Phillips EM 301) studies small pieces of root (3mm long) were fixed for four h in 6% glutaraldehyde buffered to pH 7,2 in 0,2 sodium cacodylate. The root pieces were then postfixed for 12 h in 1% osmium tetroxide and subsequent dehydration was through an acetone-water series (de la Harpe, 1980).

Infiltration was in 30% and 60% spurr/acetone mixture for six h each followed by a pure

spurr mixture for 24 h. Tissue was then imbedded in Spurr's epoxy resin (Spurr, 1969) and polymerized at 70°C for eight h. Sections for electron microscopy were cut on a Reichert OM U3 ultramicrotome and stained with 4% uranyl acetate and 20% lead citrate. Sections 2,0 µm thick were cut for light microscopy and stained with 0,2% toluidine blue at pH 9,0.

### **Root exudates**

Root exudates of 99 Richter, Jacques and 143B Mgt were collected using the method of Agnihotri & Vaartaja (1966). These exudates were tested for the presence of different amino acids using a Beckman automated amino acid analyzer, while sugars were determined by thin layer chromatography according to the method of Haer (1971). The total root exudate as well as various combinations of amino acids and sugars were tested for their influence on zoospores with a capillary root model technique similar to that of Royle & Hickman (1963).

### **Chemical Control**

Aliette at 3,5 g/l and Ridomil at 2 g/l were applied as foliar sprays on vines in an infested nursery. In the same trial Aliette at 1,75 g/m<sup>2</sup> and Ridomil at 1,00 g/m<sup>2</sup> were applied as soil drenches. All dead plants were removed before commencement while each treatment was replicated four times on plots of two m<sup>2</sup> with 150 vines each. Each chemical was applied four times with 14 d intervals until runoff in the case of the foliar sprays and at a volume of 90 l per plot as soil drenches. Eight days after the final application the number of dead plants were counted and the number of plants infected with *P. cinnamomi* determined by isolation on cornmeal agar. Shoot lengths and root mass of each vine were also determined.

### **Hot water treatment of dormant, rooted plants**

One-year-old, dormant Cabernet vines grafted onto 99R, showing symptoms of *P. cinnamomi* infection, were used. All shoots except one eight 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 the root and collar regions. Vines were then tagged numbered and tied into bundles of ten. Two bundles were immersed for 20 min in hot water at each temperature (30°C, 35°C, 40°C, 45°C 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 onto CMA.

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 99R were treated as previously described, but at 50°C for five, 10, 15 and 20 min. Treated vines were planted out in a nursery. After eight months, isolations were again made from roots and collars.

## **RESULTS AND DISCUSSION**

### **Penetration of grapevine roots**

Indirect germination of sporangia occurred 30 min after cooling (Fig. 1). The zoöspores encysted after a brief phase of sluggish movement on the root surface (Fig. 2). Germination began frequently within a few minutes of zoöspore encystment on the root surface (Fig. 3). The germ tube started to grow rapidly (Fig. 4 & 5) and the cyst was elevated above the root surface (Fig. 6). Intercellular entry of the epidermis was the form of penetration most frequently encountered.

Infection was established rapidly in the cortex, often within eight h after penetration. Penetration was intercellular and penetrating hyphae could be seen wedged in between adjoining cortical cells (Fig. 7, 8 & 9). The protoplast of the cortical cells adjacent to the intercellular hyphae sometimes collapsed and granulation of the cytoplasm occurred (Fig. 10). The nucleus usually enlarged (Fig. 11). Intracellular penetration occurred after eight h and seemed to be either by mechanical pressure or by enzymatic action. This conclusion is based on electron microscope studies which suggest that the cell wall is indented by mechanical pressure exerted by the fungus (Fig. 12). Evidence of enzymatic action of the fungus on the cell wall is illustrated in Fig. 13

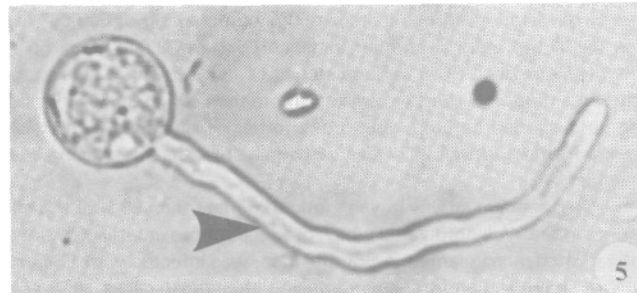
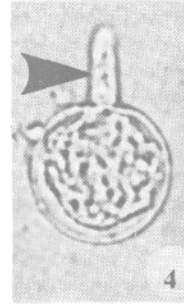
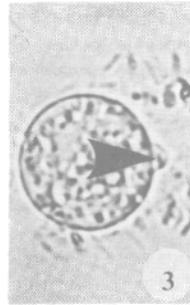
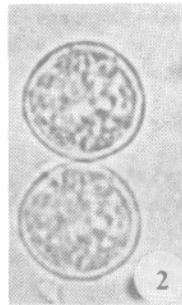
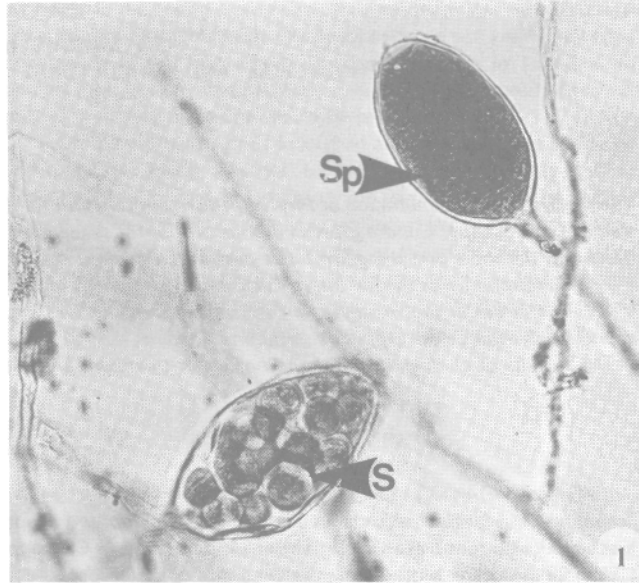
### **Effect of root exudates on zoöspore behavior**

The different amino acids and sugars in the root exudates as well as their effect on zoöspore behavior are listed in Table 1.

The strongest attraction was observed with the three amino acids, aspartic acid, glutamic acid and arginine. Capillary root models with different concentrations of these three amino acids were prepared and zoöspore response tested again. The chemotactic index decreased with increasing concentrations of arginine, aspartic acid with glutamic acid showing the biggest reaction (Fig. 14). As analysis of root exudates showed higher concentrations of glutamic acid and arginine in the roots of susceptible 99R, than did those of the more resistant 143 B vltg and Jacques, it is regarded to be the reason for the greater number of zoöspores attracted and germinating on the 99R roots than on the 143 B Mgt roots.

### **CONTROL**

As far as the control is concerned promising results were obtained with foliar applications of Aliette and soil drenches with Ridomil (Table 2) while hot water treatment (50°C for 15 min) of dormant nursery vines is very effective (Tables 3 and 4).



**FIG. 1:** A light micrograph of zoospores in a sporangium.  
S – zoospores; Sp – sporangia

**FIG. 2:** A light micrograph of encysted zoospores

**FIG. 3:** A light micrograph of a germinating zoospore

**FIG. 4:** A light micrograph of a germtube of a zoospore

**FIG. 5:** A light micrograph of a rapid developing germtube

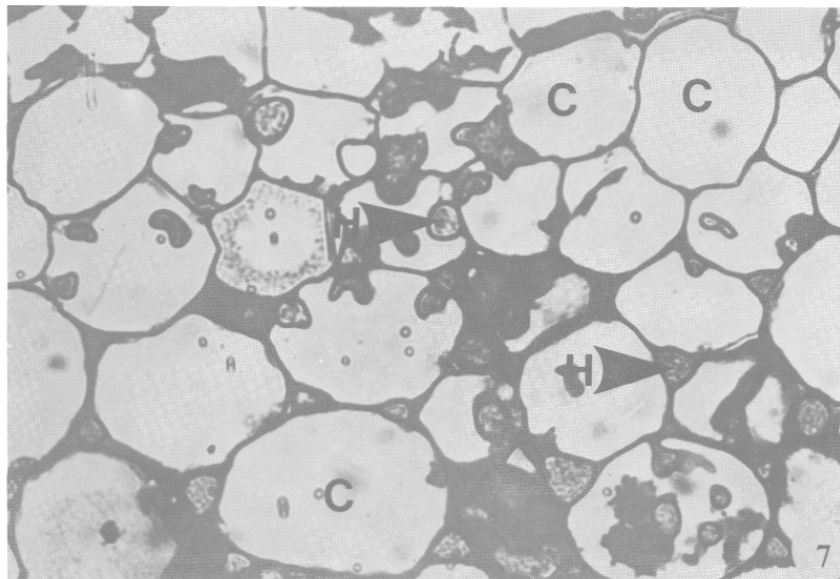
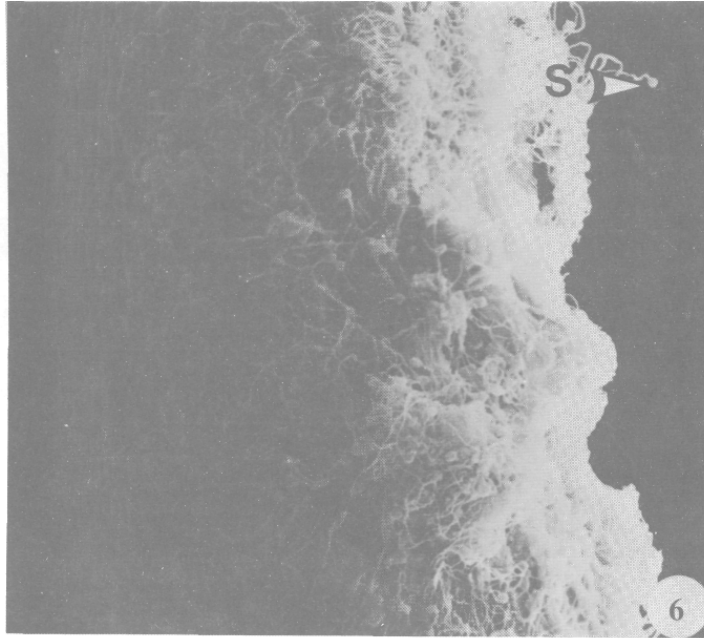


FIG. 6: A scanning electron micrograph of a root of 99R parasitised by *P. cinnamomi*. Note that the spore (S) was elevated above the root surface

FIG. 7: A light micrograph of a cross section of the root of 99 Richter. Note the intercellular penetration of the hyphae. (H = hyphae; C = cortical cells)

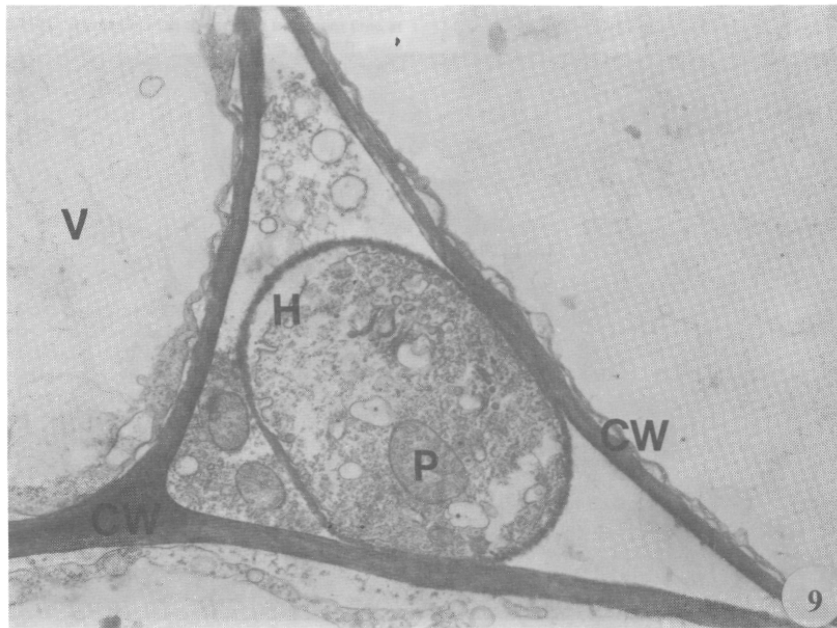
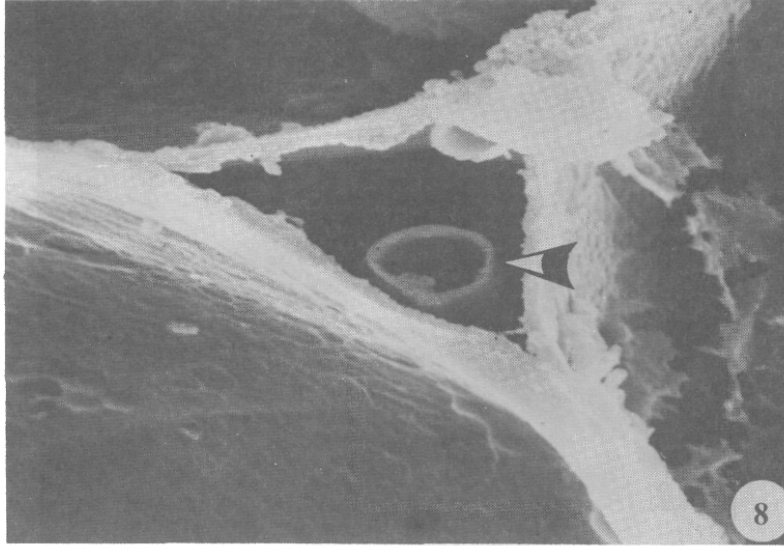


FIG. 8: A scanning electron micrograph of a hyphae in the intercellular space in the cortex (ARROWS)

FIG. 9: A transmitting electron micrograph of a hyphae in the intercellular space in the cortex of the root (CW = cell wall, H = hyphae, P = plastid, V = vacuole)

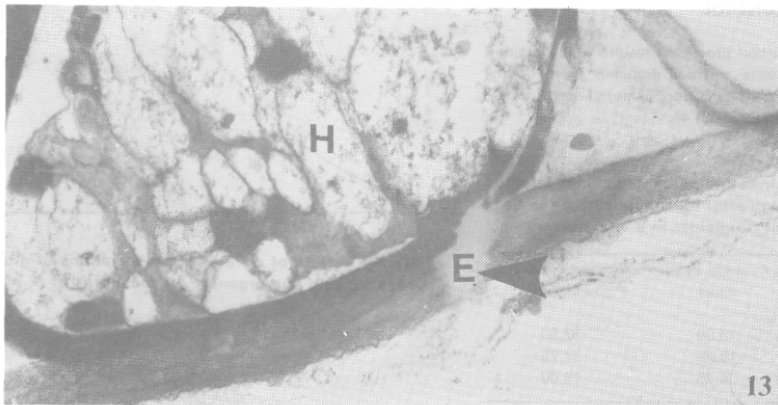
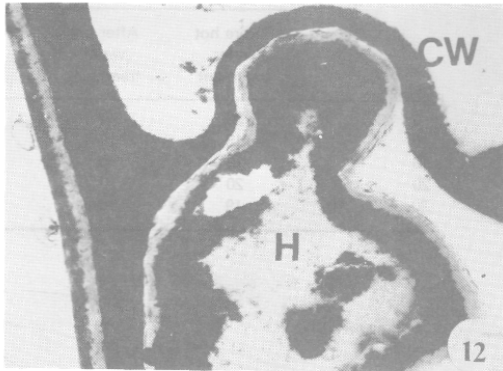
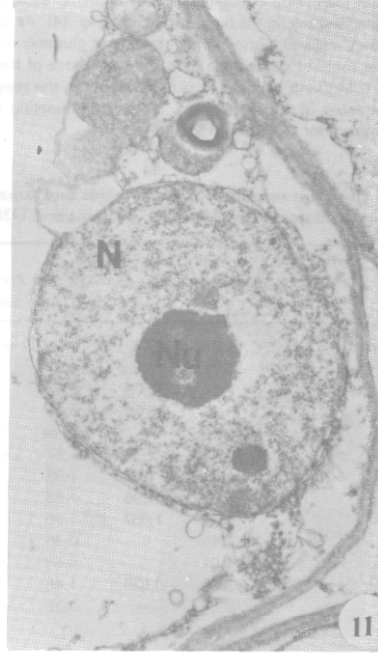
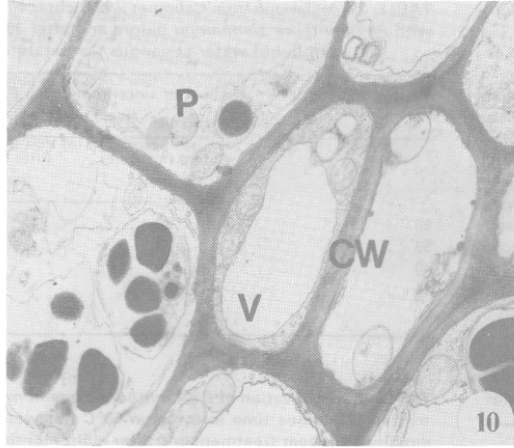


FIG. 10: A transmitting electron micrograph of cells of the root of 99R. Note the granulated appearance of the cytoplasm (P = plastid; V = vacuole, CW = cell wall)

FIG. 12: A transmitting electron micrograph of the penetration of a hyphae through a cell wall (H = hyphae; CW = cell wall)

FIG. 11: A transmitting electron micrograph of an enlarged cell with a granulated appearance of a 99R root (N = nucleus; Nu = Nucleoli)

FIG. 13: A transmitting electron micrograph of a hyphae in the intercellular space of the cortical layer of a root of 99R. Note the enzymatic destruction of the cell wall (H = hyphae, E = enzymatic destruction)



**TABLE 1: Zoospore response to amino acids and sugars detected in root exudates from 99 Richter, Jacquez and 143B Mgt**

Compound	Concentration ppm			Zoospore response
	99 Richter	Jacquez	143B Mgt	
Aspartic acid	3,67	3,70	4,15	+++
Threonine	1,61	1,65	0,67	-
Serine	4,73	2,89	2,32	+
Asparagine	13,18	35,24	5,06	+
Proline	Traces	-	Traces	+
Glumatic acid	10,96	2,55	1,57	+++
Glycine	1,87	1,02	1,08	-
Alanine	5,02	1,96	1,58	+
Valine	2,82	1,067	1,35	-
Tyrosine	2,40	-	0,36	-
Lysine	1,01	-	0,69	-
Histidine	2,00	0,626	1,46	+
Arginine	9,86	-	3,35	+++
Glucose	+	+	+	-
Fructose	+	+	+	-
Arabinose	+	+	+	-

+++ = A strong attraction and accumulation of zoospores

+ = Very slight attraction of zoospores

- = No rection on zoospore behaviour observed

**TABLE 2: Average dead vines and percentage vines infected with *Phytophthora cinnamomi* after foliar applications and soil drenches with different fungicides in a vine nursery**

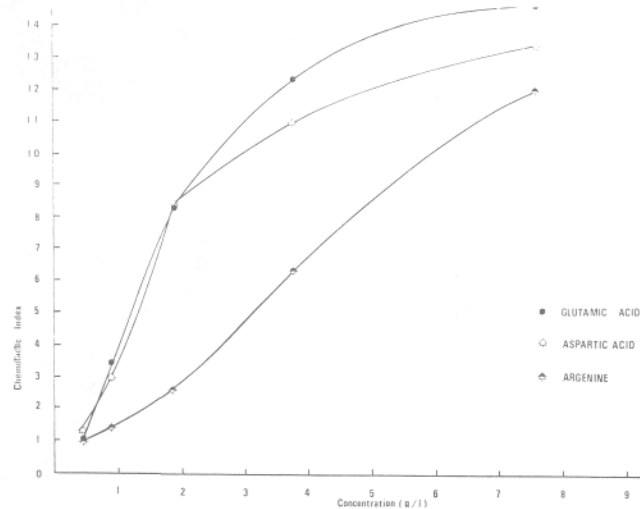
	Treatment	Average dead vines	Vines still yielding <i>P cinnamomi</i> (%)
Foliar applications	Control	18,00	32,50
	Ridomil	18,25	32,75
	Aliette	4,75	13,00
	D value (P = 0,05)	5,86	10,0
Soil drench	Control	33,50	41,25
	Aliette	32,75	38,50
	Ridomil	13,00	11,00
	D value (P = 0,05)	10,00	9,15

**TABLE 3:** Isolations from Cabernet x 99 Richter vines infected with *Phytophthora cinnamomi* before and after 30 min. treatment in hot water at various temperatures

Water temperature °C	Number of vines from which <i>P. cinnamomi</i> were isolated	
	Before hot water treatment	After hot water treatment
30	20	20
35	20	20
40	20	20
45	20	7
50	20	0

**TABLE 4:** Eradication of *Phytophthora cinnamomi* from vines treated at various time intervals in 50°C hot water assessed directly after heat treatment and after eight months growth in a nursery

Treatment	Number of vines from which <i>P. cinnamomi</i> were isolated		
	Before hot water treatment	After hot water treatment	After eight months growing
5	20	0	0
10	18	0	0
15	18	0	0
20	20	0	0
30	19	0	0



**FIG. 14:** Effect of different concentrations of glutamic acid, aspartic acid and arginine on zoospores of *P. cinnamomi*

## REFERENCES

1. AGNIHOTRI, VD en VAARTAJA, P. 1966. Root exudates from red pine seedlings and their effects on *Pythium ultimum*. *Can. J. Bot.* 45: 1031 - 1040.
2. AGNIHOTHRUDU, V. 1968. A root rot of grapes in Andhra Pradesh. *Curr. Sci.* 37, 292 - 294.
3. CHIARAPPA, L. 1959. The root rot complex of *Vitis vinifera* in California. *Phytopathology* 49, 670 - 674.
4. DE LA HARPE, A.C. 1980. Fotosintetiese karakterisering van 'n aantal Suid-Afrikaanse parasitiese blomplante. MSc-thesis, University of Pretoria, Pretoria.
5. HAER, F.C. 1971. An introduction to chromatography on impregnated Glass Fiber. Michigan: Ann Arbor Science Publishers Inc.
6. McGECHAN, J.K. 1966. *Phytophthora cinnamomi* responsible for a root rot of grapevines. *Aust. J. Sci.* 28, 354.
7. ROYLE, D.J. en HICKMAN, C.J. 1964. Analysis of factors governing in Vitro accumulation of zoospores of *Pythium aphanidermatum* on roots. II Substances causing response *Can. J. Microbiol* 10, 210 - 219.
8. SPURR, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Pes.* 26, 31 - 43.