

INFECTION OF SUSCEPTIBLE AVOCADO BY *PHYTOPHTHORA CINNAMOMI*

TAS AVELING & F H J RIJKENBERG

DEPARTMENT OF MICROBIOLOGY AND PLANT PATHOLOGY, UNIVERSITY OF NATAL, PIETERMARITZBURG

SUMMARY

*The mechanism of penetration and infection of **Phytophthora cinnamomi** Rands to susceptible avocado seedlings were investigated with the aid of light and electron microscopy. Zoospore germ tubes penetrated directly after the formation of an appressorium-like structure. A description is given of the subsequent penetration of the epidermis and cortex. Phenolic aggregates were also observed inside cells.*

OPSOMMING

*Die meganisme van penetrasie en infeksie van **Phytophthora cinnamomi** Rands in wortels van vatbare avokadosaailinge is ondersoek deur middel van lig- en elektronmikroskopie. Zoospore kiembuise het direk gepenetreer of na die vorming van appressoriumagtige strukture. 'n Beskrywing word gegee van die daaropvolgende penetrasie van die epidermis en korteks. Fenoliese aggregate binne selle is ook waargeneem.*

INTRODUCTION

Phytophthora cinnamomi Rands causes severe root rot of avocado (Ho and Zentmyer, 1977). Avocado root rot was first described in 1942 (Wager, 1942) and has remained one of the principle problems of the avocado industry in many areas of the world (Zentmyer, 1972), including South Africa. Although much is known about both the disease and the pathogen, *P. cinnamomi* (Zentmyer, 1972), little is known about the infection of avocado roots by the pathogen. To date all published research has been conducted on roots that have been severed from the parent plant. The object of this research was to reduce these artificial conditions by inoculating roots that were still attached to the parent plant and then to follow the infection process.

MATERIALS AND METHODS

Ten-month-old avocado seedlings of a Mexican cultivar which is susceptible to *P. cinnamomi*, were grown in polythene bags filled with bark media. Isolates of *P. cinnamomi* were obtained from Dr. J.M. Darvas, Westfalia Duivelskloof. Cultures were maintained on V8 agar (per liter: 200 ml V8 juice; 2g CaCO₃; 20g agar). Sporangia were induced in the

mineral salt medium described by Chen and Zentmyer (1970) and zoospore release was stimulated by chilling the cultures in sterile deionized water. Approximately 1×10^4 zoospore/ml were produced.

In preparation for inoculation, the bases of polythene bags containing the avocado seedlings were removed. The roots which had tended to accumulate in the bottom of the bags were gently separated from each other and washed free of adhering bark and medium. Two inoculation techniques were then used.

1. The exposed roots were directed outwards into petri dishes containing sterile soil or bark medium or distilled water. Each petri dish was inoculated with 1 ml of a *P. cinnamomi* zoospore suspension. The base of each plant and the petri dishes around it were then covered with tinfoil to prevent moisture loss and to ensure that the roots were in darkness.

2. A 2 l beaker was almost filled with sterile distilled water and immediately inoculated with 5 ml of zoospore suspension. The exposed roots of the seedlings were then immersed in the water by balancing the seedling over the beaker. The beaker was then covered with tinfoil to exclude light.

Five to eight mm root tips from both techniques were harvested at $\frac{1}{2}$ h, 1h, 4h, 8h, 24h and 48h intervals and fixed for 24h in 3% gluteraldehyde buffered in 0.5 M Na cacodylate. The roots were prepared for light microscopy, scanning electron microscopy and transmission electron microscopy,

RESULTS

Zoospores were particularly attracted to the region of elongation above the root tip, or to wound sites. The zoospores encysted on the root surface and the cysts then germinated by germ tubes (Fig. 1). The germ tubes penetrated the roots directly or formed appressoria-like swellings before penetration occurred. The most frequently encountered form of penetration of the epidermis was intercellular entry, the germ tube tip growing down between the anticlinal walls of two epidermal cells. Hyphae were found to penetrate successfully into the cortex in the region of elongation. Initial fungal spread within the cortex was intercellular. Hyphae could be seen wedged in the middle lamella, between adjoining cortical cells. The hyphae were constricted at points of cell wall penetration, but within entered cells resumed their original size. These hyphae branched frequently and eventually occupied much of the cell lumen (Fig. 2). Some of the hyphae within the cells formed vesicle-like structures. Invaded cells and cells adjacent to intercellular hyphae were rapidly disrupted.

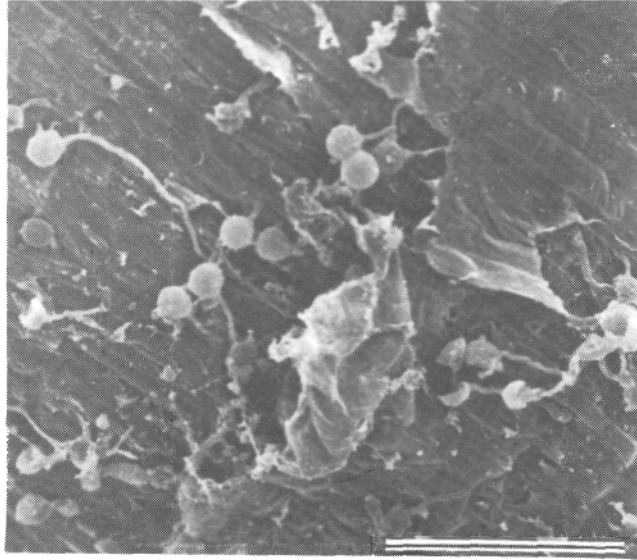


Fig. 1. Scanning electron micrograph of encysted and germinating zoospores of *P. cinnamomi* on avocado root surface (bar represents 60 μm).

The susceptible avocado roots studied showed activated wound responses about 8h after inoculation. In healthy, uninoculated roots only a few phenolic inclusions were observed in isolated cells. In inoculated roots, however, phenolic inclusions accumulated and were found in many of the cells a short distance in advance of the penetrating hyphae.

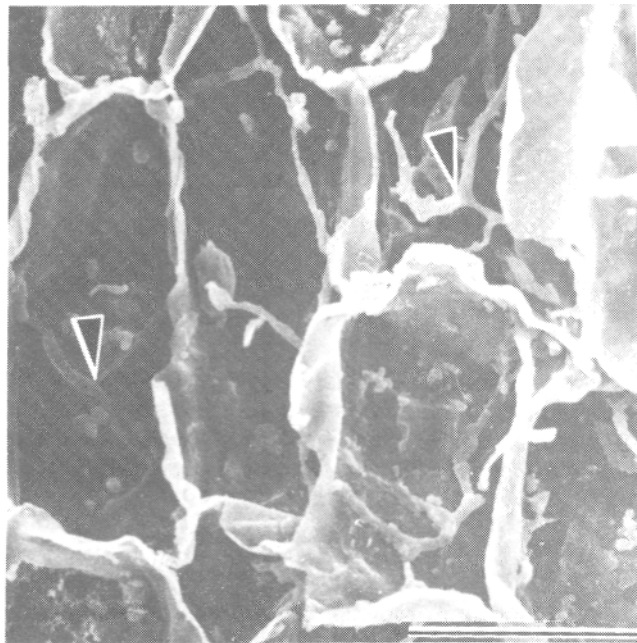


Fig. 2. Scanning electron micrograph of branching hyphae (arrows) of *P. cinnamomi* in host cells (bar represents 25 μm).

DISCUSSION

Young seedlings were used as a convenient system in which to study penetration and infection by *P. cinnamomi*. Pre-penetration behaviour of *P. cinnamomi* towards avocado roots is consistent with that reported by other authors studying *P. cinnamomi* on roots of Australian forest species (Hinch and Weste, 1979; Tippet, Holland, Marks and O'Brien, 1976). Generally germ-tube penetration proceeded rapidly without the germ tubes forming swellings on the root epidermis; infrequently, germ tubes became swollen thus resembling appressoria. Intracellular penetration by hyphae proceeded with constriction at the host wall. These hyphae branched frequently and eventually occupied much of the cell lumen.

It is widely known that phenolic compounds and their oxidation products are accumulated locally in plants in response to infection and injury (Rubin and Artsikhovskaya, 1964). Many of these compounds and their derivatives exhibit antibiotic properties and therefore are considered to play a role in disease resistance (Kosuge, 1969; Kosuge and Gilchrist, 1976). However, the hyphae of *P. cinnamomi* showed no signs of growth impedence or senescence, either in the vicinity of these accumulating phenolics or elsewhere. This indicates that *P. cinnamomi* initially establishes a highly compatible relationship with seedling roots of the susceptible avocado variety and that no obvious barrier is imposed to invasion of the root tip. The phenolics may only express their role in disease resistance later on in the host-parasite interaction.

REFERENCES

- CHEN, D. and ZENTMYER, G.A., 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycologia* 62, 397 - 402.
- HINCH, J. and WESTE, G., 1979. Behaviour of *Phytophthora cinnamomi* zoospores on roots of Australian forest species. *Aust. J. Bot.* 27, 679 - 691.
- HO, H.H. and ZENTMYER, G.A., 1977. Infection of avocado and other species of *Persea* by *Phytophthora cinnamomi*. *Phytopathology* 67, 1085 - 1089.
- KOSUGE, T. and GILCHRIST, D.G., 1976. Metabolic regulation in host-parasitic interactions in *Physiological Plant Pathology* (edited by R. Heitefuss and P. H. Williams). Springer-Verlag, Berlin.
- RUBIN, B.A. and ARTSIKHOVSKAYA, Y.V., 1964. Biochemistry of pathological darkening of plant tissues. *Ann. Rev. Phytopath.* 2, 157 - 178.
- TIPPET, J.T., HOLLAND, A.A., MARKS, G.C. and O'BRIEN, T.P., 1976. Penetration of *Phytophthora cinnamomi* into disease tolerant and susceptible eucalypts. *Archiv. Mikrobiol.* 108, 231 - 242.
- WAGER, V.A., 1942. *Phytophthora cinnamomi* and wet soil in relation to the dying back of avocado trees. *Hilgardia* 14, 519-532.
- ZENTMYER, G.A., 1972. Avocado root rot. *Calif. Avocado Soc. Yrbk.* 55, 29 - 36.