South African Avocado Growers' Association Yearbook 1988. 11:35-37

PRELIMINARY TRIALS TO ASSESS THE RESISTANCE OF THREE CLONAL AVOCADO ROOTSTOCKS TO CROWN CANKER CAUSED BY *PHYTOPHTHORA CINNAMOMI*

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ABSTRACT

Clonal Duke 7, G6 and G755 rootstocks were shown to be highly susceptible to crown and stem infection by P cinnamomi *under glasshouse conditions. The fungus was readily re-isolated from artificially induced lesions. A technique for assessing the resistance of avocado stems to infection by zoosporas of P cinnamomi was developed and tested.*

OORSIG

In glashuisproewe toon klonale Duke 7, G6 and G755 'n hoe mate van vatbaarheid vir stamen krooninfeksie deur P cinnamomi*. Die swam is geredelik uit die kunsmatig-geinduseerde letsels herisoleer. 'n Nuwe tegniek wat die weerstand van avokadostingels teen P cinnamomi -infeksie bepaal, is ontwikkel en getoets.*

Crown and trunk cankers were observed for the first time in 1985 on Duke 7 avocado rootstocks in the Tzaneen area, South Africa. *Phytophthora cinnamomi,* which is the most important pathogen of avocado roots in South Africa (Kotzé, Moll and Darvas, 1987), was associated with these cankers (Lonsdale, Botha, Wehner and Kotzé, 1988).

The use of Phytophthora resistant avocado rootstocks forms an integral part in the control strategy against this pathogen. The purpose of the research done was to screen three clonal rootstocks, namely: G755 renamed Martin Grande (Coffey 1987), G6 and Duke 7 for their resistance to crown canker under laboratory and glasshouse conditions.

A screening technique which is a modification of a method proposed by Zilberstein and Pinkas (1987) was developed to test differences in susceptibility of avocado stems to infection by *P cinnamomi.* The technique measures electrolytes released from excised stem segments suspended in bathing solutions which contain zoospores of *P cinnamomi.* Wheeler and Hanckey (1968) speculated that altered permeability was a characteristic of diseased plant tissue and cells which come into contact with the pathogen, and loose their ability to accumulate salts and other materials. Several workers have observed a relationship between susceptibility and quantities of materials released by exosmosis in plants (Larkin and Scowcroft, 1967; Williams and Keen, 1968 and Zilberstein and Pinkas, 1987).

MATERIALS AND METHODS

Plant material

Three clonal avocado selections were used in all experiments, namely: Duke 7, G6 (both *Persea Americana* Mill) and Martin Grande (a *P schiedeana* Nees selection). Plants were kept at 25-32°C in a glasshouse.

Pathogen isolate

Three isolates of *P cinnamomi* Rands, isolated from crown cankers on young avocado trees in the Hazyview and Tzaneen areas, were used in experiments. Isolates were grown on potato dextrose agar (PDA) at 24°C,

Sporangia production was induced using a technique based on that of Byrrt and Grant (1979). An agar plate containing pea extract and a nylon pad on the surface was inoculated with 10 pieces of agar 2x2 mm taken from a five-day-old PDA culture of the fungus.

The plates were incubated in darkness for five to seven days at 24°C, after which the nylon pads were gently lifted from the agar surface and transferred to 250 mℓ erlenmeyer flasks containing 100 mℓ of clear pea-broth, prepared according to Chen and Zentmyer (1970). The flasks were then placed on a shanking apparatus for 19 hours at 20 - 24°C. Thereafter the mats were washed five times with mineral salt solution (Chen and Zentmyer 1970), suspended in 40 mℓ of this solution and placed on a shaking apparatus for a further 18 hours, while sporangia formed.

Zoospores were released from the sporangia by washing the mats twice with chilled (18°C), distilled water and then incubating in 40 mℓ of water (18°C) for a further 90 minutes. Zoospore concentrations were determined, using a haemocytometer. Concentrations of 10^6 spores/ $m\ell^1$ were used in these tests.

Glasshouse experiment

Five-month-old vegetatively cloned Duke 7, G6 and G755 (Martin Grande) seedlings were inoculated by making a longitudinal slit 10 mm long and 2 mm deep at the base of the trunk of each seedling with a sterile scalpel. Strips containing mycelium, 10x2 mm in size, were cut from six-day-old PDA-cultures of *P cinnamomi* (isolated from crown cankers), placed on the slits in four Duke 7, G6 and G755 trees and secured with waterproof plastic tapes. Control seedlings were treated the same as those inoculated with *P cinnamomi,* but strips of sterile PDA were used instead of the fungus. After inoculation, seedlings were placed in a glasshouse at 25 - 32°C. After 14 days the tapes were removed. Symptom development was observed at regular intervals and isolations were made from the cankers which developed. Segments, 3 mm^3 in size were cut from the periphery of the cankers, surface sterilised with 0,1 per cent $HgCl^2$ for 30 seconds, rinsed three times in sterile distilled water, blot-dried aseptically and plated on PDA. Plates were incubated at 27°C for four to six days, and the fungi that developed from the tissue segments were identified.

Laboratory experiment

Stem segments excised from the crown regions of five-month-old vegetatively cloned Duke 7, G6 and G755 seedlings were weighed and suspended in glass test tubes 2,5 x 15 cm, containing 20 mℓ of sterile distilled water. All glassware was rinsed with water and autoclaved before use. The cut ends of each segment were sealed with lanolin, since increased infection at the cut surfaces may cause an increase in electrolyte leakage and thereby cause a misinterpretation of results. To each tube 4 mℓ of zoospores 1 x 10^5 spore/mℓ^1, were added. The tubes were incubated in the dark and at various time intervals the electrical conductivity (EC) of the bathing solutions was measured, using a conductivity meter. Since the water in which the stem segments were suspended contained ions, and since the concentrated spore suspension may have contained salt remnants, a base reading was taken after one hour of incubation, before significant leakage from stems had taken place. The values recorded were subtracted from those measured later.

Uninoculated stem segments served as controls. A second control was also included to investigate the possibility of electrolyte leakage from the zoospores into the bathing solutions, which would have caused a misinterpretation of the results; 4 mi of zoospores, 1 x 10^5 spore/mℓ^1, were added to glass test tubes containing distilled water. The conductivity of these bathing solutions was taken over the same time intervals as for the inoculated stem segments.

The EC for the stem segments was calculated as a function of weight. In all experiments, treatments were replicated five times. All experiments were repeated twice.

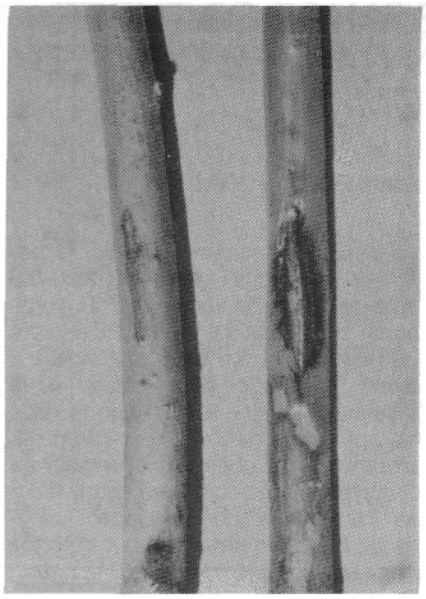

Fig 2 Cankerous lesions on the stem of a vegetatively cloned Duke 7 seedling, artificially inoculated with *P cinnamomi*, three weeks after inoculation (right). An uninoculated seedling is on the left.

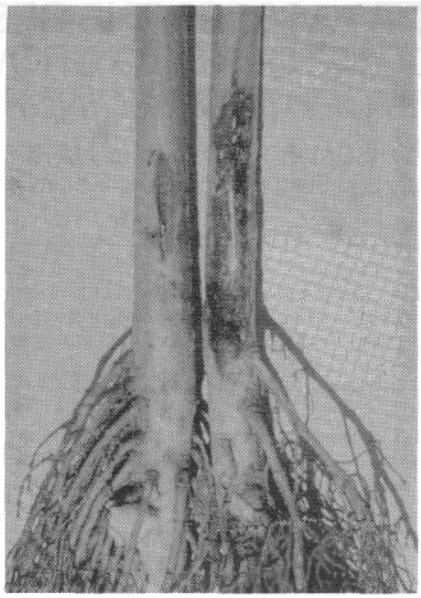

Fig 3 Cankerous lesions on the stem of a vegetatively cloned G6 seedling, artificially inoculated with *P cinnamomi*, three weeks after inoculation (right). An uninoculated control seedling is on the left.

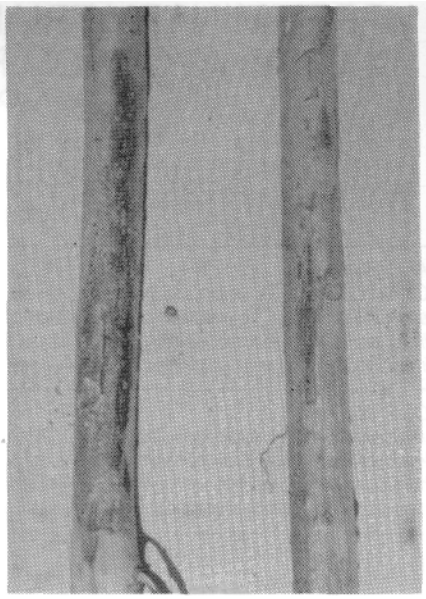

Fig 4 Cankerous lesion on the stem of a vegetatively cloned G755 seedling, inoculated with *P cinnamomi*, three weeks after inoculation (left). An uninoculated seedling is on the right.

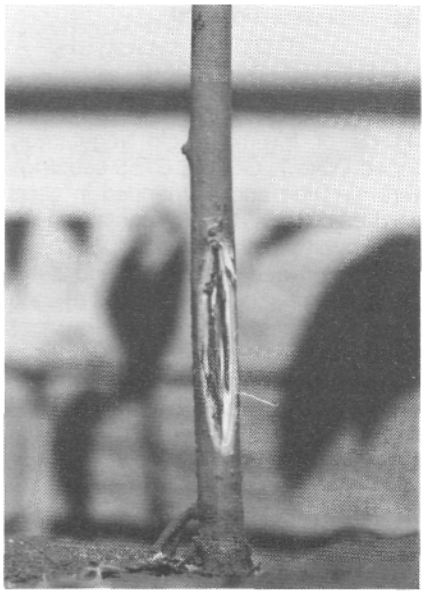

Fig 5 Infected tissue on the stem of a vegetatively cloned Duke 7 seedling, inoculated with *P cinnamomi*, three months after inoculation.

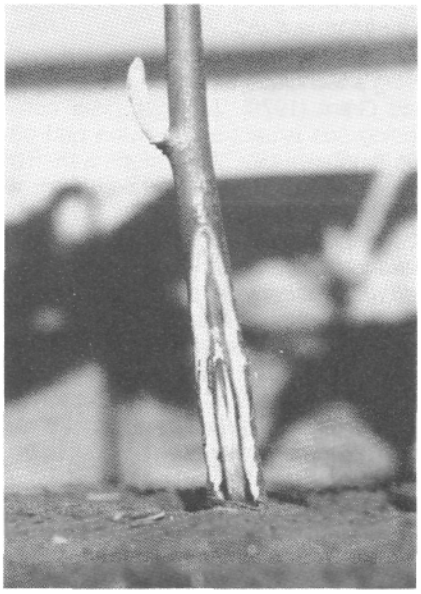

Fig 6 Infected tissues on the stem of a vegetatively cloned G6 seedling, artificially inoculated with *P cinnamomi*, three months after inoculation.

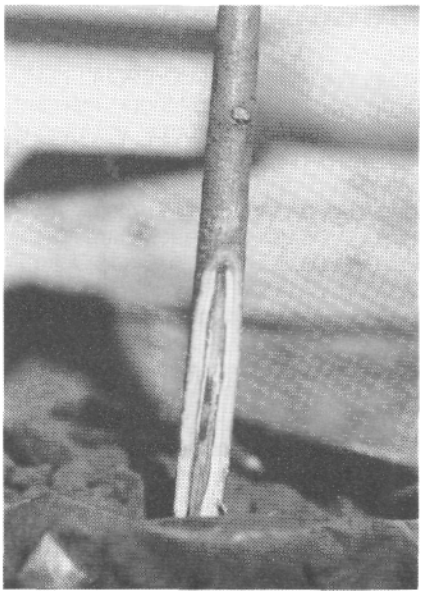

Fig 7 Infected tissue on the stem of a vegetatively cloned G755 seedling, artificially inoculated with *P cinnamomi*, three months after inoculation.

RESULTS

Glasshouse experiment

Cankerous lesions developed in the crown regions of ail inoculated plants. A dark necrotic area surrounding the inoculation site was present in the bark of inoculated trees (Figures 2, 3 and 4). These lesions progressively increased in circumference and with time the outer cortical tissues also became necrotic (Figures 5, 6 and 7). *P cinnamomi* was re-isolated from cankers on inoculated trees. No symptoms developed in any of the controls.

Laboratory experiment

The EC of bathing solutions containing inoculated stem segments was significantly higher than that for non-inoculated controls (Figure 1). No significant electrolyte leakage from the zoospores in the bathing solutions was observed (Table 1).

The EC for inoculated G6 stem segments was significantly higher than those of Duke 7 and G755 segments.

This tendency was however, only observed in one of the repeats (Tables 2 and 3).

DISCUSSION

The roots of Duke 7 and G6 rootstocks have been described by Kellam and Coffey (1987) as being moderately resistant to *P cinnamomi.* The two rootstocks however, were very susceptible to crown and stem infection by *P cinnamomi* in glasshouse experiments. This

suggests a differential susceptibility of stem and root tissue to *P cinnamomi* in these rootstocks.

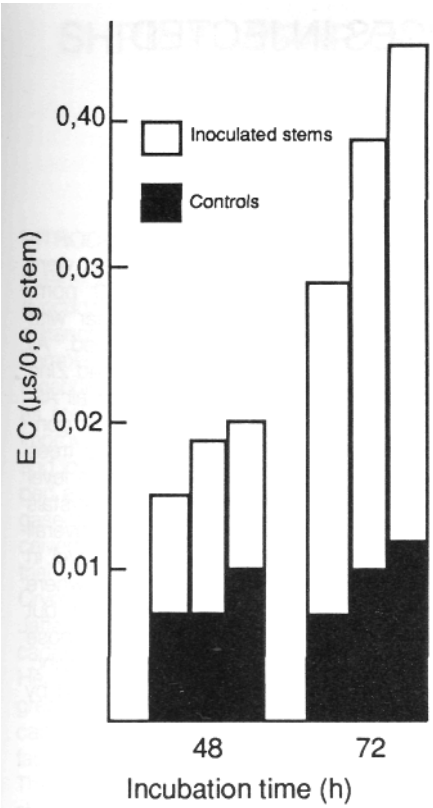

Fig 1 Electrical conductivity of bathing solutions incubated with avocado stem segments inoculated with *Phytophthora cinnamomi*. (Controls were non-inoculated). Values taken after incubation of one hour, were subtracted from those taken later. Data are the means of five replicates.

TABLE 1 Average electrical conductivity of bathing solutions inoculated with *Phytophthora cinnamomi*

Repeat	Electrical conductivity (µs) Incubation time (hour)		
	1	48	72
1	0,15*a	0,05a	0,05a
2	0,08a	0,08a	0,08a

* Each value is a mean of five replications. Values within rows, followed by the same letter are not significantly different (P = 0,05) according to Duncan's multiple range test.

TABLE 2 Average electrical conductivity of bathing solutions incubated with avocado stem segments inoculated with *Phytophthora cinnamomi* (Repeat 1)

Rootstock	Electrical conductivity (µs/0,6 g stem) Incubation time	
	48	72
Duke 7	0,0001*b	0,003b
G6	0,013a	0,01a
G755	0,003b	0,003b

* Each value is a mean of five replications. The values of uninoculated stem segments were subtracted from the inoculated ones. Values within column, followed by the same letter, are not significantly different (P = 0,05) according to Duncan's multiple range test.

TABLE 3 Average electrical conductivity of bathing solutions incubated with avocado stem segments inoculated with *Phytophthora cinnamomi* (Repeat 2)

Rootstock	Electrical conductivity (µs/0,6 g stem) Incubation time	
	48	72
Duke 7	0,007*a	0,021ab
G6	0,010a	0,011b
G755	0,010a	0,034a

* Each value is the mean of five replications. The values of uninoculated stem segments were subtracted from the inoculated ones. Values within column, followed by the same letter, do not differ significantly (P = 0,05) according to Duncan's multiple range test.

Martin Grande (G755) is more tolerant to root rot caused by P *cinnamomi* than either G6 or Duke 7 (Coffey, 1987 and Brokaw, 1987). Dolan and Coffey (1986) showed that etiolated G755 shoots and roots were significantly more resistant to infection by zoospores of *P cinnamomi,* than those of G6 and Duke 7. They observed that lesions on etiolated G755 shoots inoculated with zoospores of *P cinnamomi,* were restricted to the point of inoculation. In the experiments done however, it was found that lesions were not restricted to inoculation sites, but expanded around the inoculation site causing extensive necrosis of the lower stem. It would appear therefore, that G755 stems are highly susceptible to the crown canker isolate of *P cinnamomi.* However, under field conditions, the situation may be different.

The detached stem technique, which is a modification of that proposed by Zilberstein and Pinkas (1987), does not appear to be sufficiently reliable for measuring the susceptibility of avocado stems to infection by *P cinnamomi.* In view of this, the results presented here should be regarded as preliminary.

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