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An improved method to evaluate avocado rootstocks for resistance to *Phytophthora cinnamomi*

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INTRODUCTION

Root rot caused by *Phytophthora cinnamomi* Rands is the most serious disease on avocado (*Persea Americana* Mill) (Zentmyer, 1984). The detached root technique for evaluation of resistance in *Persea* spp was first described by Kellam & Coffey (1985), Dolan & Coffey (1986) and modified by Botha, Wehner & Kotzé (1989). This technique makes use of zoospores of *P. cinnamomi as* inoculum source. However, the production of zoospores is a lengthy and laborious process and sporangium development requires a precise balance of certain nutrients (Chen & Zentmyer, 1970; Gisi, Zentmyer & Klure, 1980), and cannot always be guaranteed.

Mycelium of *P. cinnamomi* has been shown to infect plants (Rodger, 1972; Weste, 1974), and the aim of this study was to compare mycelium fragments and zoo-spores as sources of inoculum for use in the detached root technique.

MATERIALS AND METHODS

Roots from 12 to 14-month-old seedlings of *P. Americana* cultivar Edranol (susceptible) (Snyman, 1989; Snyman & Kotzé, 1984), and vegetatively propagated (Frolich & Platt, 1971) seedlings of *P. Americana* selection. Duke 7 (moderately tolerant) (Coffey, 1987) as well as P. *schiedeana* Nees selection G755 (tolerant) (Coffey, 1987), were used in this study.

Zoospores were obtained as described by Botha *et al* (1989). For the mycelium inoculum, 205 mm^2 potato dextrose agar discs (PDA) previously colonised by *P. cinnamomi* were inoculated into 100 mℓ pea broth prepared as described by Chen & Zentmyer (1970). After incubation at 25°C for four days, the fungal mats were homogenised for 30 s with an ultra turrax to produce a mycelial fragment suspension.

Excised root tips (ca 40 mm in length) from each of the different rootstocks were placed perpendicularly onto two parallel glass rods in petri dishes, containing 15 mℓ water agar in each as described by Botha *et al* (1989). Each root tip was inoculated at the region of elongation with either 10µℓ of zoospore suspension containing 7,9 x 10^3 $mℓ^{-1}$ motile zoospores or 10 mℓ of mycelium homogenate. The root tips were then incubated in the dark at 25°C.

TABLE 1 Lesion length on excised root tips of three avocado rootstocks inoculated with *P. cinnamomi*

Rootstock	Lesion length after 24 h (mm)	
	Inoculum type	
	Mycelium suspension	Zoospore suspension
Edranol	5,8 a	2,6 abc
Duke 7	1,6 bc	1,8 bc
G 755	0,0 c	0,0 c

Each value is the mean of five replicates. Values in horizontal and vertical columns not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

TABLE 2 Linear colonisation of excised root tips of three avocado rootstocks by *P. cinnamomi*

Rootstock	Linear colonisation after 24 h (mm)	
	Inoculum type	
	Mycelium suspension	Zoospore suspension
Edranol	4,0 bcd	4,8 bcd
Duke 7	7,2 abc	12,0 a
G 755	1,6 cd	0,0 cd

Each value is the mean of five replicates. Values in horizontal and vertical columns not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0.05).

TABLE 3 Lesion length on excised root tips of three avocado rootstocks inoculated with *P. cinnamomi*

Rootstock	Lesion length after 48 h (mm)	
	Inoculum type	
	Mycelium suspension	Zoospore suspension
Edranol	15,6 ab	17,0 ab
Duke 7	13,4 b	12,0 b
G 755	0,8 c	0,2 c

Each value is the mean of five replicates. Values in horizontal and vertical columns not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

TABLE 4 Linear colonisation of excised root tips of three avocado rootstocks by *P. cinnamomi*

Rootstock	Linear colonisation after 48 h (mm)	
	Inoculum type	
	Mycelium suspension	Zoospore suspension
Edranol	21,6 a	19,2 ab
Duke 7	21,6 a	30,4 a
G 755	6,4 bc	0,0 c

Each value is the mean of five replicates. Values in horizontal and vertical columns not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

Resistance was determined by measuring lesion length after 24 and 48 hours as well as by aceptically cutting the root tips in 4 mm segments after surface disinfesting for 5 s in 70 per cent ethanol. The roog segments were then plated out sequentially on a PARPH-medium. After incubation at 25°C for three days, the segments from which *P. cinnamomi* developed were counted and multiplied by four to give the total length of root colonisation.

RESULTS

Lesion length after 24 and 48 hours are shown in Tables 1 and 3. Lesion length in the Edranol roots after 24 h was significantly longer than that of Duke 7 and G755 when mycelium was used as inoculum.

No significant differences were detected between the different rootstocks after 24 h when zoospores were used as inoculum. After 48 h the lesion length of Edranol and Duke 7 was significantly longer than that of G755 when mycelium or zoospores were used as inoculum. Tissue colonisation is shown in Tables 2 and 4. The same tendency was found in tissue colonisation as in lesion length. However, tissue colonisation in Duke 7 was greater than in Edranol and G755 after 24 and 48 hours when zoo-spores were used as inoculum source.

DISCUSSION

The same tendency was obtained between lesion length and tissue colonisation when they were used as parameters for determining resistance.

Differences in resistance between the various rootstocks were more obvious after 48 h than after 24 h. Linear colonisation of Duke 7 was generally higher than in Edranol and compared well with the findings of Kellam & Coffey (1985), namely that Duke 7 could possibly support higher populations of *P. cinnamomi* than the susceptible Topa Topa (in this case the susceptible Edranol). Mycelium and zoo-spores compared well as sources of inoculum, as G755 was significantly less susceptible than Duke 7 and Edranol in both instances. Both types of inoculum also reflected known field resistance of the different rootstocks. Mycelium can therefore be used as a source of inoculum instead of

zoospores in the detached root technique.

REFERENCES

BOTHA, T, WEHNER, F C & KOTZÉ, J M, 1989. An evaluation of *in vitro* screening techniques for determining tolerance of avocado root-stocks to *Phytophthora cinnamomi. S A Avocado Growers' Assoc Yrb,* 12: 60 - 63.

CHEN, D W & ZENTMYER, G A, 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycologia,* 62**:** 397 - 402.

COFFEY, M D, 1987. A look at current avocado rootstocks. *California Grower,* 11: 15 - 17.

DOLAN, T E & COFFEY, M D, 1986. Laboratory screening technique for assessing resistance of four avocado rootstocks to *Phytophthora cinnamomi. Plant Disease,* 70*:* 115 - 118.

FROLICH, EF&PLATT, R G, 1971. Use of the etiolation technique in rooting avocado cuttings. *California Avocado Society Yrb,* 56: 97 - 109.

GISI, U, ZENTMYER, GA&KLURE, LJ, 1980. Production of sporangia by *Phytophthora cinnamomi* and *P. palmivora* in soils at different matric potentials. *Phytopathology,* 70: 301 - 306.

KELLAM, M K & COFFEY, M D, 1985. Quantitative comparison of the resistance to Phytophthora root rot in three avocado rootstocks. *Phytopathology,* 75: 230 - 234.

PODGER, F D, 1972. *Phytophthora cinnamomi,* a cause of lethal disease in indigenous plant communities in Western Australia. *Phytopathology,* 62: 972 - 981.

SNYMAN, A J, SNYMAN, C P & KOTZÉ, J M, 1984. Pathogenicity of avocado root rot fungi to Edranol seedlings and Duke 7 rooted cuttings. *S A Avocado Growers' Assoc Yrb,* 7: 80 - 81.

WESTE, G, 1974. *Phytophthora cinnamomi* — The cause of severe disease in certain native communities in Victoria. *Australian Journal of Botany,* 23**:** 67 - 76.

ZENTMYER, G A, 1984. Avocado Diseases. *Tropical Pest Management,* 30(4): 388 - 400.