South African Avocado Growers' Association Yearbook 1990. 13:63-64

Evaluation of field trees for resistance to *Phytophthora cinnamomi* by means of the detached root technique

M de V van der Merwe, Erna M C Maas and J M Kotzé
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002

ABSTRACT

Avocado trees selected by SAAGA for exceptional growth under apparent root rot pressure were evaluated for resistance by means of the detached root technique. As means of comparison root segments from rootstocks such as G755, Duke 7 and Edranol with known responses towards Phytophthora cinnamomi *were used. Nine of the 34 trees evaluated were as resistant as G755 and six were significantly more resistant than Duke 7. Most of the trees, ie 25 were significantly more resistant than Edranol.*

INTRODUCTION

The search for resistance to *Phytophthora cinnamomi* Rands *(P.c.),* the causal organism of avocado root rot, was initiated by Dr G A Zentmyer in 1952 (Zentmyer, 1952). According to him, collections have been made in 18 countries and include 15 species of *Persea* and species of other genera in the Lauraceae. Resistance to *P.c.* in these collections was tested in a nutrient solution test (Zentmyer & Mircetich, 1965), in pots and beds of *P.c.* infested soil and ultimately in the field (Zentmyer, 1952).

In South Africa, no indigenous *Persea* species occur and the search for resistance is thus restricted to orchard trees showing exceptional signs of vigour under apparent root rot pressure. These trees have been termed "escape" trees.

Obtaining clonal material from these trees for use in resistance tests is a long and tedious procedure. The aim of this study was therefore to evaluate the detached root technique described by Botha, Wenher & Kotzé (1990) as a rapid means to assay field trees for resistance to root rot.

MATERIALS AND METHODS

Roots were obtained from trees selected by Mr C Partridge and Mr D Westcott of SAAGA. Locality and tree designation are shown in Table 1.

Root tips excised from the different trees were placed separately in plastic containers, filled with moist, sterile vermiculite to prevent dehydration and contamination. The containers were kept in cool boxes, transported to the laboratory and tested within 24 h.

As control and means of comparison, root tips from two-year-old *P. americana* cultivar Edranol (susceptible) (Snyman, Snyman & Kotzé, 1984) and vegetatively propagated

(Frolich & Platt, 1971) *P. americana* selection Duke 7 (moderately tolerant) (Coffey, 1987) as well as *P. schiedeana* Nees selection G755 (tolerant) (Coffey, 1987) were used.

TABLE 1 Locality and designation of field trees evaluated for resistance to *P. cinnamomi* by means of the detached root technique

Locality	Tree designation
Agatha	A2
Agatha	B1
	B3
	B4
	B12
	B13
	B14
Burgershall	1D1
	1D2
	1D3
	1D4
	1D5
	1D6
	1D7
	1D8
	1D9
	1D12
	1D13
	1D14
Levubu	2B1
	2B2
	2B3
Nelspruit	1Q1
	1Q2
	1Q6
	1Q7
	1Q8
	1Q9
Tzaneen	Z4
Venda	IAV
	2AV
White River	C1
	C2
	1L1

The detached root technique used to test for resistance in avocado rootstocks as described by Dolan & Coffey (1986) and modified by Botha, Wehner & Kotzé (1989) was used. However inoculum of *P. cinnamomi* consisted of 10 $\mu\ell$ of mycelium suspension. For the mycelium inoculum, 20 5 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 shake incubation at 25°C for four days the fungal growth was homogenised for 30 s with an ultra turrax to produce a mycelial suspension.

The excised root tips (ca 40 mm in length) from each of the different trees, as well as those from the control trees, were placed perpendicularly onto two parallel glass rods in petri dishes containing 15 mi water agar in each as described by Botha, Wehner & Kotzé (1989). Each root tip was inoculated at the region of elongation with 10 $\mu\ell$ of the mycelium homogenate after which petri dishes were incubated in the dark at 25°C.

Resistance was determined by aseptically cutting the root tips in 4 mm segments after surface disinfesting for 5 s in 70% ethanol. The root segments were then plated out sequentially on PARPH-medium. After incubation at 25°C for three days the segments from which *P. cinnamomi* developed were counted and multiplied by four to obtain the total length of root colonisation.

To evaluate whether time after field removal of roots affected expression of resistance, the potted control trees were initially taken to the field and the roots were excised at the same time as those of the field trees. Half of the roots from the control trees were immediately taken to a nearby laboratory and tested as described above. The other half of the roots was kept in the same manner as the root tips of the field trees, until the tests were performed 24 h after detachment.

For all further tests on the field trees, the root tips of the potted control trees remained at the University of Pretoria and were excised at approximately the same time as the root tips of the field trees. The root tips were then kept in the same manner as the root tips of the field trees until the tests could be carried out simultaneously!

RESULTS AND DISCUSSION

Time after root detachment (within a 24 h period) did not significantly affect expression of resistance (Table 2). Linear colonisation of the excised roots of field trees from each locality as well as the controls are given in Tables 3 — 6.

Nine of the 34 trees tested showed a higher degree of resistance than G755, although this difference was not significant. Six and 25 trees were found to be significantly more resistant than Duke 7 and Edranol respectively. Three of the 34 trees tested were significantly more susceptible than Edranol.

TABLE 2 Linear colonisation of potted avocado roots by *P. cinnamomi* at different time intervals after detachment

Rootstock	Root colonisation	
	Evaluation 0 h after detachment	Evaluation 24 h after detachment
Edranol	20,45 a	18,00 a
Duke 7	6,00 b	3,40 b
G755	1,67 b	2,00 b

Values not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

TABLE 3 Linear colonisation of excised root tips of field trees by *P. cinnamomi*

Rootstock	Linear colonisation (mm) of roots after 48 h
Edranol	18,0 a
B3	12,45 a b
Z4	12,13 a b
B13	11,00 a b c
B4	9,55 b c d
A2	7,27 b c d
B1	6,42 b c d
B14	4,73 b c d
B12	4,44 b c d
Duke 7	3,40 c d
G755	2,00 d

Values 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 field trees by *P. cinnamomi*

Rootstock	Linear colonisation (mm) after 48 h
Edranol	26,08 a
1Q2	16,71 b c
Duke 7	14,27 b c
1Q7	13,83 b c
1Q6	11,73 b c d
1Q9	7,72 b c d
C2	5,85 c d e
G755	3,81 c d e
C1	2,00 d e
1L1	2,00 d e
1Q8	0,75 e
1Q1	0,50 e

Values not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

TABLE 5 Linear colonisation of excised root tips of field trees by *P. cinnamomi*

Rootstock	Linear colonisation (mm) after 48 h
Duke 7	15,54 a
2B3	14,84 a
1AV	14,00 a
2B2	13,46 a b
Edranol	13,33 a b
2AV	12,00 a b
G755	10,00 a b
2B1	1,54 b

Values not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

TABLE 6 Linear colonisation of excised root tips of field trees by *P. cinnamomi*

Rootstock	Linear colonisation (mm) after 48 h
Edranol	27,5 a
Duke 7	21,0 ab
1D8	20,8 ab
1D12	19,3 ab
1D4	18,0 ab
1D6	15,2 b
1D9	14,7 b
1D13	12,5 bc
1D1	12,4 bc
1D3	11,7 bc
G755	11,5 bc
1D5	11,4 bc
1D2	11,3 bc
1D14	10,6 bc
1D7	1,5 c

Values not followed by the same letter differ significantly according to Duncan's multiple range test (P = 0,05).

DISCUSSION

According to Zentmyer & Mircetich (1965) preliminary tests for resistance of rooted cuttings are conducted in a nutrient solution inoculated with *P.c.* However, due to the time required to obtain rooted cuttings, an alternative method for evaluating resistance of field trees was investigated. It was found that the detached root technique described by Botha *et al,* (1989) could readily be used when controls such as G755, Duke 7 and Edranol were included in each evaluation. Thus results of this study showed some field trees to be as resistant as the highly acclaimed G755. No previous reports on the "resistant status" of existing avocado trees in South Africa could be found.

ACKNOWLEDGEMENTS

We wish to thank Mr C Partridge, Mr D Westcott and Mr N Claassens of SAAGA, for the demarcating of escape trees and assistance.

REFERENCES

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

CHEN, DW & ZENTMYER, G A, 1970. Production of sporangia by *Phytophthora cinnamomi* in axenic culture. *Mycologia* 62: 379 - 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, E F&PLATT, RG, 1971. Use of the etiolation technique in rooting avocado cuttings. *California Avocado Soc Yrb* 56: 97 - 109.
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.
ZENTMYER, G A & MIRCETICH, S M, 1965. Testing for resistance of avocado to *Phytophthora* in nutrient solution. *Phytopathology* 55: 487 - 489.