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An Improved Method to Evaluate Avocado Rootstocks for Resistance to *Phytophthora cinnamomi*

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Abstract. **A detached root technique for evaluation of resistance in avocado root-stocks makes use of zoospores of *Phytophthora cinnamomi* Rands (P.c.) as inoculum source. The production of zoospores is a lengthy process and requires a precise balance of nutrients. Mycelium fragments of *P. cinnamomi* were therefore evaluated as an alternative inoculum source. Resistance was determined by measuring lesion length and tissue colonization after 24 and 48 hours. Zoospore and mycelium inoculum compared well as sources of inoculum, both reflecting known field resistance of the various rootstocks. Mycelium of *P. cinnamomi* can therefore replace zoospores as an inoculum source in the detached root technique.**

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 and Coffey (1985), Dolan and Coffey (1986) and modified by Botha, Wehner and Kotze (1989). This technique makes use of zoospores of *P. cinnamomi* as inoculum source. However production of zoospores is a lengthy and laborious process and sporangium production requires a precise balance of certain nutrients (Chen and Zentmyer, 1970; Gisi, Zentmyer and Klure, 1980), and cannot always be guaranteed.

Mycelium of *P. cinnamomi* have been shown to infect plants (Rodger, 1972; Weste, 1974), and the aim of this study was to compare mycelium fragments and zoospores 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, *et al.,* 1984), and vegetatively propagated (Frolich and 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, 20 5 mm^2 potato dextrose agar discs (PDA) previously colonized by *P. cinnamomi* were inoculated into 100 mL pea broth prepared as described by Chen and Zentmyer (1970). After incubation at 25C for four days, the fungal mats were homogenized 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 1 5 mL water agar in each as described by Botha *et al.* (1989). Each root tip was inoculated at the region of elongation with either 10 *µL* of zoospore suspension containing 7.9 x 10^3 per ml motile zoospores or 10 *µL* of mycelium homogenate. The root tips were then incubated in the dark at 25C.

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

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 root-stocks 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 colonization is shown in Tables 2 and 4. The same tendency was found in tissue colonization as in lesion length. However, tissue colonization in Duke 7 was greater than in Edranol and G755 after 24 and 48 hours when zoospores were used as inoculum source.

Discussion

The same tendency was obtained between lesion length and tissue colonization 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 colonization of Duke 7 was generally higher than in Edranol and compared well with the findings of Kellam and 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 zoospores 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.

Literature cited

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Table 1. Lesion length on excised root tips of three avocado rootstocks inoculated with *P. cinnamomi* after 24 hours.

	Lesion length after 24 h (mm)	
	Inoculum type	
Rootstock	Mycelium suspension	Zoospore suspension
Edranol	5.8 a[z]	2.6 abc
Duke 7	1.6 bc	1.8 bc
G755	0.0 c	0.0 c

[z] 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, ($P \leq 0.05$).

Table 2. Linear colonization of excised root tips of three avocado rootstocks inoculated with *P. cinnamomi* after 24 hours.

	Linear colonization after 24 h (mm)	
	Inoculum type	
Rootstock	Mycelium suspension	Zoospore suspension
Edranol	4.0 bcd[z]	4.8 bcd
Duke 7	7.2 abc	12.0 a
G755	1.6 cd	0.0 cd

[z] 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, ($P \leq 0.05$).

Table 3. Lesion length on excised root tips of three avocado rootstocks inoculated with *P. cinnamomi* after 48 hours.

	Lesion length after 48 h (mm)	
	Inoculum type	
Rootstock	Mycelium suspension	Zoospore suspension
Edranol	15.6 ab[z]	17.0 ab
Duke 7	13.4 b	12.0 b
G755	0.8 c	0.2 c

[z] 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, ($P \leq 0.05$).

Table 4. Linear colonization of excised root tips of three avocado rootstocks inoculated with *P. cinnamomi* after 48 hours.

	Linear colonization after 48 h (mm)	
	Inoculum type	
Rootstock	Mycelium suspension	Zoospore suspension
Edranol	21.6 [z]	19.2 ab
Duke 7	21.6 a	30.4 a
G755	6.4 bc	0.0 c

[z] 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, ($P \leq 0.05$).