Detached Root Inoculation—A New Method to Evaluate Resistance to Phytophthora Root Rot in Avocado Trees

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ABSTRACT

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A screening method was developed to differentiate between rootstocks of avocado (*Persea americana*) resistant and susceptible to root rot disease; the method is applicable to fruit-bearing trees. Large numbers of horticulturally outstanding trees, or those that survive in infected groves, can be screened quickly and simply without sacrificing or damaging the tested trees. Detached young (white) avocado root tips are suspended in double-distilled water and incubated with zoospore suspension of *Phytophthora cinnamomi* at 24 C in the dark. Electrolyte leakage from inoculated root segments is followed by measuring electrical conductivity of the root-bathing solution at 24, 48, and 72 hr after inoculation. Variability between replicates was reduced by zoospore concentrations above 10^4/10 root segments. The difference in electrolyte leakage between thick and thin roots was offset by expressing electrical conductivity as a function of root weight rather than of length. Avocado Duke 7 and G6 (resistant) root segments leaked significantly less than those of six rootstocks (susceptible) at 48 and 72 hr after inoculation.

Avocado root rot, caused by *Phytophthora cinnamomi* Rands, is the most damaging disorder of avocado trees (*Persea americana* Mill.) (14). Great efforts have been expended in the search for resistant rootstocks. In California, a screening program in progress since 1952 (12) has tested tens of thousands of avocado seedlings. The screening procedure is based upon inoculation of young seedlings kept in nutrient solutions (15). Trees that were exposed to and have withstood high inoculum pressure under natural infection conditions, and that are therefore a potentially important source of resistance, are difficult to identify with this method. In addition, rootstocks from such trees must be vegetatively cloned to obtain young plants, a laborious, long (1.5 yr), and costly procedure (5) that involves sacrificing the parent trees. The new laboratory-screening method developed by Dolan and Coffey (4) does not avoid these problems; vegetative propagation of rootstocks is still required.

The current study was undertaken to develop a rapid and simple procedure by which assumed resistant rootstocks that survived in infested groves or among a population of horticulturally outstanding fruit-bearing trees could be screened in situ without damaging the parent trees. It has been suggested that the changes in tissue permeability that occur during pathogenesis could be used to differentiate between susceptible and resistant genotypes (7–9). We considered it feasible to develop a screening method based upon comparison of electrolyte leakage from avocado root segments excised from mature plants and subsequently inoculated with *P. cinnamomi*.

MATERIALS AND METHODS

Roots from 8-wk-old Topa Topa, Ein Harod, Ashdot 7, Tsrifin 99, Duke, and Binyamina avocado seedlings (susceptible) and from 10-mo-old, vegetatively cloned Duke 7 and G6 (resistant) rootstocks were used. Ten white, freshly cut root tips (15 mm long) of feeder roots were suspended in double-distilled water in test tubes (2.5 × 15 cm); all glassware was rinsed before use. The water was replaced three times at 10-min intervals to wash out ions leaking from the cut surfaces. The roots were then suspended in 10 ml of double-distilled water into which zoospores of *P. cinnamomi* were introduced. Zoospores of a virulent strain (613) isolated from diseased avocado roots from Ein Eron, Israel, were produced by using the method of Byrt and Grant (3). Two milliliters of motile zoospores at the desired concentration—1×10^4/ml (calibrated in a hemocytometer—was introduced into each tube containing the roots. The tubes were incubated at 24 C in the dark. At various time intervals, the bathing solutions were measured for electrical conductivity (EC; conductivity meter CDM3, Radiometer, Denmark).

The differences in the quantity of water required to dilute the spore suspensions to the desired concentration resulted in differences in EC of the initial bathing solution, since the concentrated spore suspension contains salt remnants. To standardize the EC, the solutions in the test tubes were measured 24 hr after inoculation, after zoospores had encysted and before significant leakage from the infected roots had begun. The values recorded were subtracted from those measured later.

Cuts and wounds on the root segments may attract zoospores in a manner similar to the chemotactic attraction found in the elongation zone of the root apex (13). The increased infection at the cut surfaces may cause increased electrolyte leakage, leading to incorrect interpretations. To analyze the effect of the cuts, 15-mm-long nonapical root segments (two cut ends) were prepared. One cut end of half of the segments was covered with lanolin. In addition, apical segments (one cut end), with and without a 2-mm portion removed from the apical end, were also tested. In all experiments, treatments were replicated five times. All experiments were repeated at least twice.

RESULTS

Inoculation with low zoospore concentrations resulted in great variability in solution conductivity between replicates (Table 1). With 1×10^4 zoospores per test tube and above, uniformity was much better and the EC reached a relatively stable value 72 hr after inoculation. The bathing solution became turbid with bacteria between 72 and 96 hr; consequently, subsequent experiments were terminated after 72 hr.

Diameters of young avocado roots, even from the same seedling, are not identical. Feeder roots equal in length but differing in diameter were inoculated with *P. cinnamomi*. The average diameter and weight of 10 thin root segments, each 2 cm long, were 0.48 mm and 50 mg, respectively, as opposed to 0.75 mm and 200 mg with thick roots. Forty-eight hours after inoculation, the EC of the bathing solution of thicker roots was five times higher than that of the thinner roots (Fig. 1A). However, when EC was calculated as a function of root weight, the values for infected thick and thin roots were almost equal (Fig. 1B). In subsequent experiments, the results were expressed on a weight basis.

To compare changes in EC between inoculated root segments, the contribution of the cut area to the overall results was assessed by comparing root segments differing in number of exposed cuts (Fig. 2). After 48 hr of incubation, all inoculated segments leaked significantly more electrolytes than the uninoculated ones. The inoculated nonapical segments with two exposed wounds leaked significantly more electrolytes than those with one end sealed with lanolin because of a larger zoospore-attraction zone at the cut surfaces. Inoculated apical segments with one or two exposed ends, on the other hand, leaked similar quantities of electrolytes, perhaps because the apex, whether intact or severed 2 mm from the end, still contains a nearby, very large zoospore-attracting site at the elongation zone.

When roots of avocado trees are uncovered, brown and white roots are discernible. The youngest roots are white but rapidly undergo suberization and turn brown. Electrolyte leakage from inoculated and uninoculated white and brown feeder root tips was followed (Fig. 3). Forty-eight hours after inoculation, there was much more leakage from uninoculated brown roots than from white roots. However, inoculation of brown roots increased their leakage by only 180%, whereas the EC of inoculated white roots was 310% greater than that of the uninoculated controls.

In the previous experiments only seedling roots were used, despite the fact that resistant rootstocks are vegetatively propagated. We considered it important to verify that differences

TABLE 1. Effect of zoospore concentration of *Phytophthora cinnamomi* on electrical conductivity of solutions bathing Topa Topa avocado root segments

Incubation time (hr)	Electrical conductivity (μS/0.1 g of root)					
	Number of zoospores per 10 root segments					
	0	500	1,000	5,000	10,000	40,000
24	1.1[a] ± 17.2	0.7 ± 34.9	0.6 ± 48.5	1.0 ± 27.3	1.0 ± 33.9	2.0 ± 59.1
48	2.6 ± 32.5	11.4 ± 105.7	24.2 ± 17.7	44.5 ± 16.0	56.7 ± 5.8	60.0 ± 7.5
72	3.8 ± 35.5	49.4 ± 28.4	77.7 ± 27.1	71.0 ± 13.0	76.0 ± 6.5	67.3 ± 10.8
96	4.2 ± 38.4	95.8 ± 14.6	106.0 ± 27.0	85.7 ± 12.9	80.5 ± 5.8	70.5 ± 11.8

[a] Each figure is mean of five replicates followed by standard error expressed as percentage of mean.

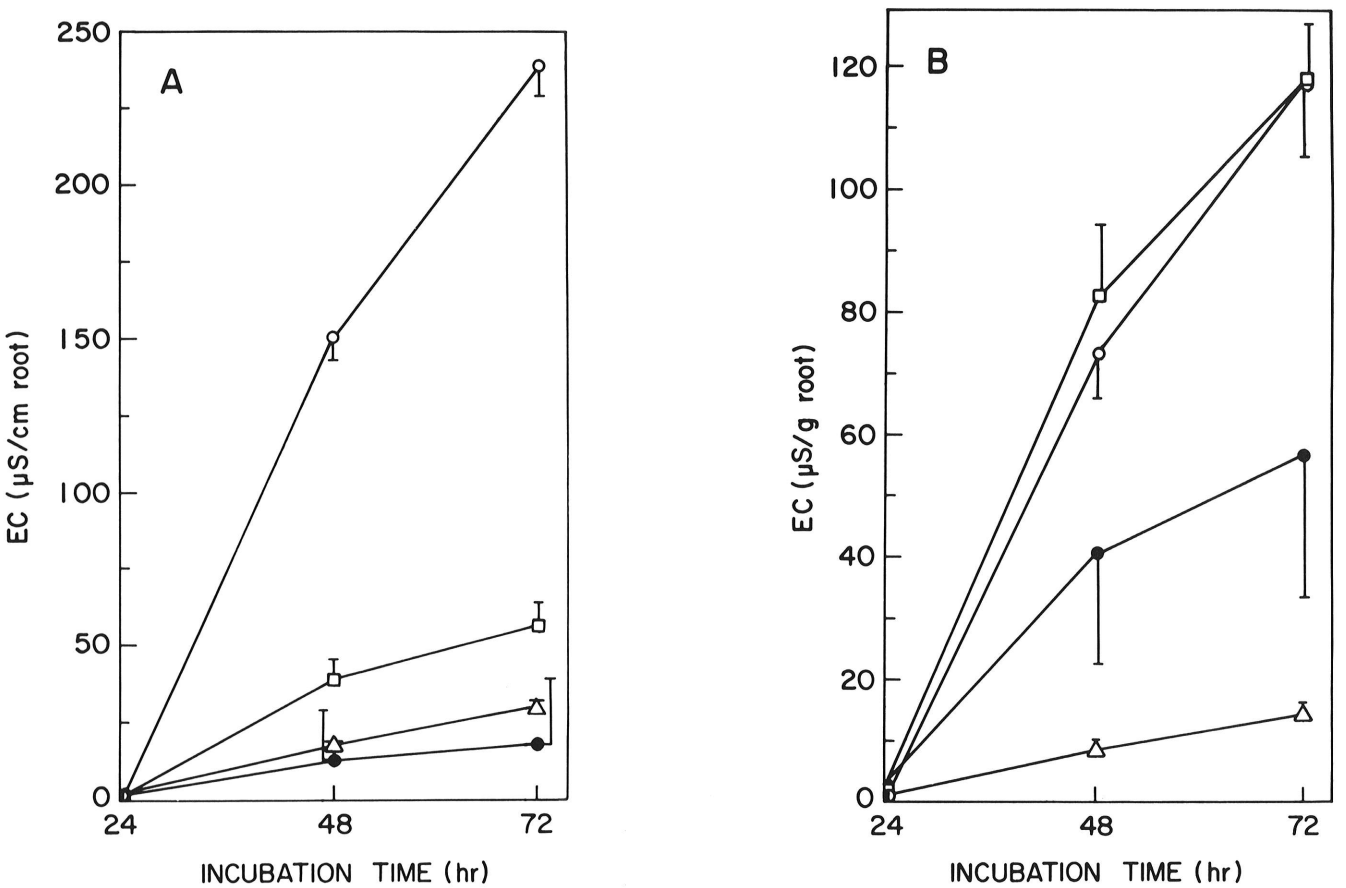

Fig. 1. Electrical conductivity (EC) of bathing solutions with Topa Topa avocado root segments of different diameters inoculated with *Phytophthora cinnamomi*. Data are means of five replicates; vertical lines indicate standard error above or below mean. **A,** Electrical conductivity as function of root length. **B,** Electrical conductivity as function of root weight; □ = thin root, inoculated; o = thick root, inoculated; • = thin root, control; and Δ = thick root, control.

in EC, if they exist, are not a result of root system type. Therefore, Topa Topa seedlings and Topa Topa vegetative clones were included in the next experiment. The EC values of inoculated root segments from Topa Topa seedlings and vegetative clones were similar; however, both were significantly greater ($P = 0.01$) than the moderately resistant Duke 7 and G6 (Table 2).

Duke 7 and G6 were retested along with six susceptible rootstocks—Topa Topa, Binyamina, Duke (Mexican), Ein Harod, Ashdot 7, and Tsrifin 99 (West Indian). The EC of the bathing solution of the inoculated susceptible root segments was nearly double the value of that from the resistant ones (Table 3).

DISCUSSION

An avocado improvement program has been conducted in Israel for more than 20 yr (1,2), and thousands of high-yielding trees grown all over the country—and therefore under various edaphic conditions—were identified. It would be desirable to screen these horticulturally superior trees and locate among them those that also are resistant to root rot. This would be preferable to testing seedlings that, even if found resistant, may not produce well when grafted with commercial cultivars.

Genetic resistance cannot be assumed to be present in all trees that survive in infected groves; many are genetically susceptible and escape the disease because of specific local conditions such as good drainage. With the new screening procedure, a large number of trees can be tested rapidly and still used for crop production because they are not damaged. Only resistant trees, if identified, must be cut back to obtain new growth from the resistant rootstocks to be vegetatively cloned for further greenhouse and field tests.

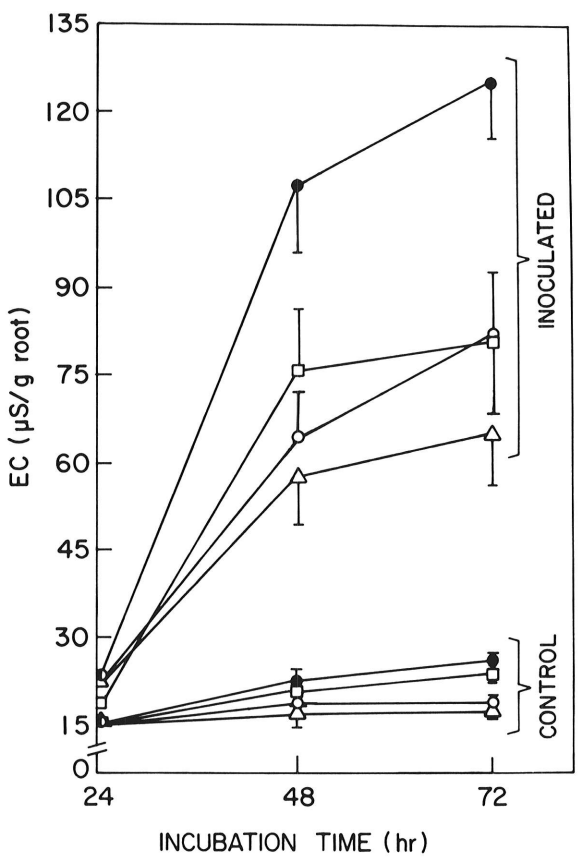

Fig. 2. Effect of wounds on electrical conductivity (EC) of bathing solutions with Topa Topa avocado root segments inoculated with *Phytophthora cinnamomi*. Data are means of five replicates: Vertical lines indicate standard error above and below mean; ● = nonapical root segments (two cuts); □ = nonapical root segments (one cut); o = apical root segments (one cut); and Δ = apical root segments (two cuts).

The reported resistance of Duke 7 and G6 rootstocks under field conditions was confirmed quantitatively in greenhouse tests (6). Both rootstocks were defined as moderately resistant (6,16), and they were the only resistant rootstocks available to us for testing. Based on Zentmyer's water bath system (15), the two could not be differentiated from the susceptible Topa Topa (A. Kariv and Y. Pinkas, *unpublished data*). In the current work, we demonstrated that the new method enables one to distinguish between these two and the other susceptible rootstocks tested. Among the four resistant rootstocks tested by Dolan and Coffey (4), Duke 7 and G6 were rated lowest; however, comparison with a susceptible rootstock was not included in their work (4). The relative EC value

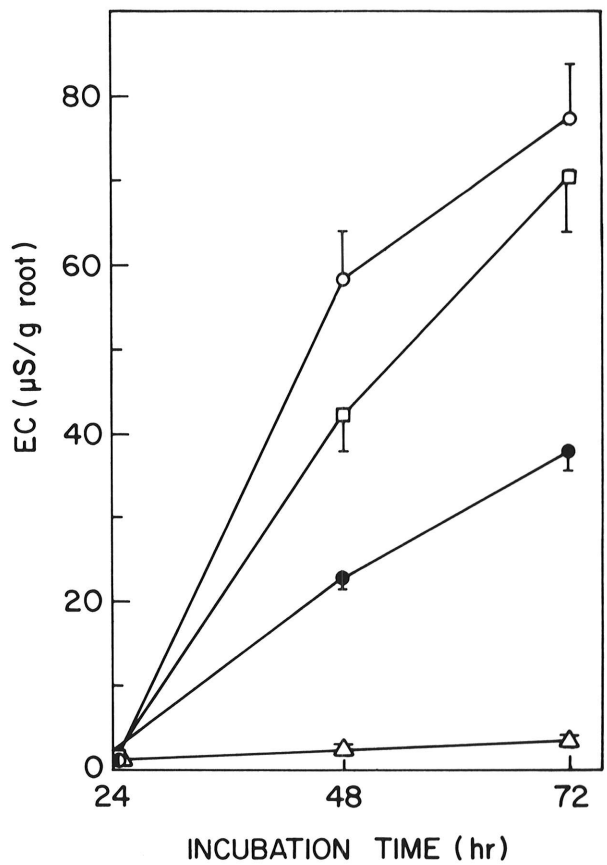

Fig. 3. Electrical conductivity (EC) of bathing solutions with white or brown Topa Topa avocado root segments inoculated with *Phytophthora cinnamomi*. Data are means of five replicates: vertical lines indicate standard error above or below mean; o = white root, inoculated; Δ = white root, control; □ = brown root, inoculated; and ● = brown root, control.

TABLE 2. Relative electrical conductivity of bathing solutions incubated with seedlings (S) as opposed to vegetative clones (VC) of avocado root segments inoculated with *Phytophthora cinnamomi*

	Electrical conductivity (µS/0.1 g of root)	
	Incubation time (hr)	
Rootstock	48	72
Topa Topa (S)	100.0 a[z]	100.0 a
Topa Topa (VC)	117.1 a	122.2 a
G6 (VC)	52.2 a	55.1 b
Duke 7 (VC)	32.6 b	31.6 b

[z] Each value is mean of five replications. Conductivity with Topa Topa root segments raised from seeds was calculated as 100%. Values of uninoculated root segments were subtracted from inoculated ones before calculating percentages. Values within column not followed by same letter are significantly different ($P = 0.01$) according to Duncan's multiple range test.

TABLE 3. Relative electrical conductivity of bathing solutions incubated with susceptible and resistant avocado root segments inoculated with *Phytophthora cinnamomi*

	Electrical conductivity (μS/0.1 g of root)		
	Incubation time (hr)		
Rootstock	24	48	72
Topa Topa	100.0 b[y]	100.0 b	100.0 ab
Ein Harod	73.4 b	123.6 b	126.9 ab
Binyamina	69.6 b	97.0 b	94.3 b
Ashdot 7	91.6 b	152.2 a	132.1 b
Tsrifin 99	86.9 b	112.5 b	111.2 ab
Duke	138.4 a	110.0 b	106.1 ab
Duke 7[z]	71.3 b	54.3 c	42.5 c
G6[z]	81.4 b	67.2 c	59.9 c

[y] Each value is mean of five replications. Conductivity with Topa Topa root segments (susceptible) was calculated as 100%. Values of uninoculated root segments were subtracted from inoculated ones before calculating percentages. Values within column not followed by same letter are significantly different ($P = 0.01$) according to Duncan's multiple range test.

[z] Vegetatively cloned plants; other rootstocks were seedlings.

that separates susceptible from resistant rootstocks depends upon the resistance level desired. In our test, EC of the moderately resistant rootstocks was characterized by a statistically significant lower value as compared with susceptible rootstocks. It is expected that more resistant rootstocks will have much lower EC values. In spite of our attempts to standardize the experimental system, variability between experiments still existed. In a few repeated tests, electrolyte leakage of all tested rootstocks (including the susceptible one) started earlier than anticipated (a very early leakage may denote a hypersensitive response [9]). It is therefore necessary to include in each test a susceptible rootstock as a standard.

The new procedure is based upon previous observations that, with certain diseases, tissue permeability changes at the earliest stages of pathogenesis (11) and later, if and when there are gross disruptions of plasma membrane structure (10). Changes in tissue permeability are normally correlated with changes in EC of the solution bathing the infected tissue. The changes in EC that we measured could probably be correlated with the number of diseased cells in the infected roots.

Electrolyte leakage values from inoculated thick and thin roots were similar when EC was plotted as a function of root weight rather than length. Therefore, the differences in diameter that occur in populations of roots may be compensated for by comparing electrolyte leakage on a weight basis. The extent of leakage from an uninoculated thin root increases when plotted as a function of weight. It is likely that the extent of electrolyte leakage in healthy roots depends upon the number of cells on the surface of the roots. With infected roots, however, the rate of leakage depends upon the number of cells damaged on the surface as well as inside the roots.

Roots of young avocado plants raised from seed differ morphologically from roots of rootstocks that were propagated vegetatively. It can be assumed that other differences, not related to resistance, may exist and may be responsible for the differences in EC. This possibility was ruled out by comparing Topa Topa vegetative clones and seedlings. In both treatments, similar EC values of inoculated root segments were obtained.

As with most screening procedures, some resistant forms may appear to be susceptible. Some forms of field resistance will also be difficult to identify, as was demonstrated in a similar experimental system where seedlings of forest trees differing in susceptibility were inoculated with *P. cinnamomi* and the EC of the bathing solutions was compared (9).

The described screening method offers a means of exploring the resistance potential of avocado rootstocks in mature trees. To stimulate the production of the new feeder roots needed for the test, the soil surface should be covered with sawdust. After a few months, the cover layer contains enough young roots for the assay. With this technique, highly productive avocado trees with known horticultural characters, or those known to have survived in infested groves, can be tested for their resistance in a simple, rapid, and inexpensive manner.

LITERATURE CITED

1. Ben-Ya'acov, A. 1972. Avocado rootstock-scion relationships: A long term, large-scale field research project. I. Preparation of the experimental set-up in the planting of commercial orchards in Israel. Yearb. Calif. Avocado Soc. 55:158-161.
2. Ben-Ya'acov, A. 1985. Selections of avocado rootstocks. Yearb. S. Afr. Avocado Growers Assoc. 8:21-23.
3. Byrt, P., and Grant, B. R. 1979. Some conditions governing zoospore production in axenic cultures of *Phytophthora cinnamomi* Rands. Aust. J. Bot. 27:103-115.
4. Dolan, T. E., and Coffey, M. D. 1986. Laboratory screening technique for assessing resistance of four avocado rootstocks to *Phytophthora cinnamomi*. Plant Dis. 70:115-118.
5. Frolich, E. F., and Platt, R. G. 1972. Use of the etiolation technique in rooting avocado cuttings. Yearb. Calif. Avocado Soc. 56:97-109.
6. Kellam, M. K., and Coffey, M. D. 1985. Quantitative comparison of the resistance to Phytophthora root rot in three avocado rootstocks. Phytopathology 75:230-234.
7. Larkin, P. J., and Scowcroft, W. R. 1981. Eyespot disease of sugarcane. Induction of host-specific toxin and its interaction with leaf cells. Plant Physiol. 67:408-414.
8. Thatcher, F. S. 1943. Cellular changes in relation to rust resistance. Can. J. Res. C21:151-172.
9. Weste, G., and Cahill, D. 1982. Changes in root tissue associated with infection by *Phytophthora cinnamomi*. Phytopathol. Z. 103:97-108.
10. Wheeler, H. E. 1976. Permeability alteration in diseased plants. Pages 413-429 in: Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Encyclopedia of Plant Physiology, vol. 4. Springer-Verlag, Berlin.
11. Wheeler, H. E., and Hanchey, P. 1968. Permeability phenomena in plant disease. Annu. Rev. Phytopathol. 6:331-350.
12. Zentmyer, G. A. 1952. Collecting avocados in Central America for disease resistance tests. Yearb. Calif. Avocado Soc. 37:107-111.
13. Zentmyer, G. A. 1961. Chemotaxis of zoospores for root exudates. Science 133:1595-1596.
14. Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the Diseases It Causes. Phytopathol. Monogr. 10. American Phytopathological Society, St. Paul, MN. 96 pp.
15. Zentmyer, G. A., and Mircetich, S. M. 1965. Testing for resistance of avocado to *Phytophthora* in nutrient solution. Phytopathology 55:487-489.
16. Zentmyer, G. A., and Ohr, H. D. 1978. Avocado root rot. Calif. Agric. Sci. Leafl. 2440. 15 pp.