TIEGHEM, P. VAN. 1869. Anatomie comparée de la fleur femelle et du fruit des Cycadées, des Conifères et des Gnétacées. Ann. Sci. Nat.—Bot. V. 10: 269–304.
WODEHOUSE, R. P. 1935. Pollen grains. McGraw-Hill Book Co. New York.
YU, C. H. 1948. The wood structure of *Metasequoia disticha*. Bot. Bull. Acad. Sinica 2: 227–230.
ZIEGENSPECK, H. 1929. Die Nadeln der erwachsenen und keimenden Koniferen und die Phylogenie dieser Klasse, eine phylogenetisch-anatomische Studie. Bot. Archiv. 26: 257–348.

EFFECT OF OXYGEN SUPPLY ON PHYTOPHTHORA ROOT ROT OF AVOCADO IN NUTRIENT SOLUTION [1]

Denzel S. Curtis and George A. Zentmyer

ROOT ROT of avocado, a disease of considerable economic importance in California and other avocado-growing regions, has been considered to be favored in its development by wet, poorly drained soils (Crandall, 1948; Tucker, 1929; Wager, 1942; Zentmyer and Klotz, 1947a, b). The causal fungus, *Phytophthora cinnamomi* Rands, also attacks roots of a number of other plants, including chestnut, pineapple, rhododendron, heather, cinnamon, cinchona, and coniferous and broadleaved nursery stock (Crandall *et al.*, 1945; Mehrlich, 1936; Oyler and Bewley, 1937; Rands, 1922; White, 1937).

The fact that wet, poorly drained soils have a limited oxygen-supplying power (Hutchins, 1926) suggested the advisability of investigating the effect of oxygen supply on the development of avocado root rot. Observations in this connection are reported herewith.

EXPERIMENTAL PROCEDURE.—Seeds of two avocado varieties, the Dickinson (*Persea americana*) and Topa Topa (*P. americana* var. *drymifolia*), were allowed to germinate in sand in a greenhouse. Seedlings were selected for uniformity in size and vigor, and transferred with great care, to avoid root injury, to 25-l. pyrex battery jars, where they grew in well-aerated complete nutrient solution until new root development had started. Each seedling was then transferred to a 20-l. pyrex bottle filled with complete nutrient solution (table 1). Each bottle was covered with paper to exclude light, and was fitted with a two-hole rubber stopper to hold both the seedling and the aeration tube in place and to reduce gas exchanges with the atmosphere.

The nutrient solution was provided with concentrations of dissolved oxygen ranging from 7.2–0.05 p.p.m. The highest oxygen level (7.2–6.8 p.p.m.) was obtained by aeration with compressed air. The oxygen concentration was decreased by mixing increasing proportions of nitrogen gas with the air, as described in an earlier paper (Curtis, 1949a). At the lowest oxygen level (0.1–0.05 p.p.m.), where nitrogen gas alone was used, the small amounts of oxygen remaining in solution are attributed to contamination from the atmosphere.

The water used in preparing the nutrient solution and in replacing losses due to evaporation and transpiration was taken from an exchange-resin de-ionizing installation. Iron was added as ferrous sulfate at the rate of 0.1 p.p.m. three times weekly. The solution reaction was adjusted daily to *p*H 5.5 with either HNO_3 or KOH, as required.

Samples of cultures were taken daily, and analyzed in order to adjust the different oxygen levels and observe the accumulation of nitrites. Oxygen measurements were made by the Winkler method, and nitrites were measured colorimetrically by the use of alpha-naphthalamine (A.O.A.C., 1945).

Inoculations were made from an isolate of *P. cinnamomi* from avocado roots, which had been transferred to tubes of sterilized alfalfa stems. As soon as the seedlings were in place in the 20-l. bottles, a tube of inoculum was added to half of the bottles, and a tube of sterile medium was added to the other half, at each oxygen level. At the termination of the experiment, the presence or absence of the fungus was verified for each seedling by placing excised root tips on cornmeal-agar medium, incubating the cultures, and examining the mycelial growth under magnification.

Treatment at various oxygen levels was given for periods of 2, 4, and 16 days, after which aeration with compressed air alone was resumed for further observations. The Dickinson seedlings were treated at six different oxygen levels for a period of 2 days; then all cultures were aerated with compressed air. The Topa Topa seedlings were treated at three oxygen levels, with half the cultures at each level receiving treatment for 4 days and the other half receiving treatment for 16 days before full aeration was resumed.

EXPERIMENTAL RESULTS.—*Effect of the fungus.*—Regardless of the oxygen level or duration of the treatment, all seedlings growing in solutions inoculated with *P. cinnamomi* died after varying lengths

[1] Received for publication February 12, 1949.
Paper No. 594, University of California Citrus Experiment Station, Riverside, California.

TABLE 1. *Composition of nutrient solution.*

Milliequivalents per liter								Parts per million					
Ca	Mg	K	Na	NO_3	PO_4	SO_4	Cl	B	Mn	Al	Zn	Fe	Cu
6.0	2.0	2.0	0.1	7.0	1.0	2.0	0.1	0.5	0.5	0.2	0.5	0.1	0.01

TABLE 2. *Effect of inoculation with Phytophthora cinnamomi on avocado seedlings of Dickinson and Topa Topa varieties grown in nutrient solution at various oxygen levels.*

Culture number	Oxygen supply: Maximum (p.p.m.)	Oxygen supply: Minimum (p.p.m.)	Duration of treatment, days	Inoculation with *P. cinnamomi*[a]	Effect on roots: Tips constricted 2nd day	Effect on roots: Tips discolored 4th day	Effect on roots: New growth 16th day	Leaf wilt: 4th day	Leaf wilt: 8th day	Leaf wilt: 16th day	Nitrites in solution (p.p.m.)
					Dickinson						
1	7.2	6.8	2	—	none	none	good	none	none	none	0.1
2	7.2	6.8	2	+	none	brown	none	slight	slight	severe	0.2
3	2.3	1.9	2	—	none	none	good	none	none	none	0.2
4	2.3	1.9	2	+	none	brown	none	slight	slight	severe	0.2
5	1.2	0.9	2	—	none	none	fair	none	none	none	0.2
6	1.2	0.9	2	+	none	brown	none	slight	slight	severe	0.3
7	0.5	0.4	2	—	all	purple	fair	none	slight	none	0.1
8	0.5	0.4	2	+	all	purple	none	none	slight	slight	0.2
9	0.3	0.3	2	—	all	purple	poor	none	slight	none	0.2
10	0.3	0.3	2	+	all	purple	none	none	slight	slight	0.5
11	0.1	0.05	2	—	all	purple	poor	none	slight	none	0.2
12	0.1	0.05	2	+	all	purple	none	none	slight	slight	0.5
					Topa Topa						
1	7.2	6.8	4	—	none	none	good	none	none	none	0.2
2	7.2	6.8	4	+	none	brown	none	slight	slight	severe	0.2
3	7.2	6.8	16	—	none	none	good	none	none	none	0.2
4	7.2	6.8	16	+	none	brown	none	slight	slight	severe	0.2
5	1.5	1.4	4	—	none	none	fair	none	none	none	0.4
6	1.5	1.4	4	+	none	brown	none	none	slight	severe	0.4
7	1.5	1.4	16	—	none	none	fair	none	none	none	0.4
8	1.5	1.4	16	+	none	brown	none	none	slight	severe	0.4
9	0.1	0.05	4	—	all	purple	poor	none	slight	slight	0.6
10	0.1	0.05	4	+	all	purple	none	none	slight	slight	0.4
11	0.1	0.05	16	—	all	purple	none	none	slight	slight	0.8
12	0.1	0.05	16	+	all	purple	none	none	slight	slight	0.8

[a] — = noninoculated; + = inoculated.

of time. Root attack by the fungus was most rapid and severe at the highest oxygen levels. Exposure to very low oxygen levels (0.5–0.05 p.p.m.) for 4 days or more was eventually lethal to seedlings even in the absence of the fungus (see below).

The presence of *P. cinnamomi* was manifested by brown discolorations which first appeared along the fleshy white feeder roots of the avocado seedlings and then spread in uneven patterns throughout the entire root system. The physical texture of the affected roots was soft and brittle. At the highest oxygen level (7.2–6.8 p.p.m.), the root tips were brown after 2 days; at intermediate oxygen levels (2.3–0.9 p.p.m.), the brown discolorations did not appear until after 4 days (table 2); and at low oxygen levels (0.5–0.05 p.p.m.), the characteristic brown color could not be detected until many days later.

Low oxygen supply appeared to delay or hinder root injury by *P. cinnamomi,* and the delay became more evident as the period of exposure to low oxygen supply was increased. All the inoculated seedlings finally died, however.

At oxygen levels ranging from 7.2–0.9 p.p.m., the inoculated seedlings showed their first signs of wilting 4 days after inoculation, and further wilting progressed rapidly until all the leaves hung down in a vertical position (fig. 1). Noninoculated seedlings did not wilt at these oxygen levels (fig. 2).

Effect of oxygen level.—The characteristic effect of oxygen deficiency, as described in previous studies (Curtis, 1949), was observed on all seedlings exposed to the lowest oxygen levels (0.5–0.05 p.p.m.), regardless of the presence or absence of *P. cinnamomi.* The roots became shrunken or constricted in the region immediately behind the root tip, turned light shades of yellow, pink, and purple, and eventually turned rather dark. On the fourth day, the predominating color was purple (table 2). Injured and uninjured root tips are shown in fig. 3.

Seedlings that were not inoculated with the fungus and were subjected to 0.5–0.05 p.p.m. of oxygen for only 2 days produced new roots following several weeks of aeration by compressed air (fig. 2). Root growth was retarded in these seedlings, in comparison with those subjected to higher levels of oxygen (table 2), but good root growth began again 16–30 days after the treatment at the low oxygen levels. After the 4-day treatment at the lowest oxygen levels, new root growth was so poor that the seedlings did not survive, and after the 16-day treatment, new root growth was entirely lacking.

The immature leaves of inoculated and noninocu-

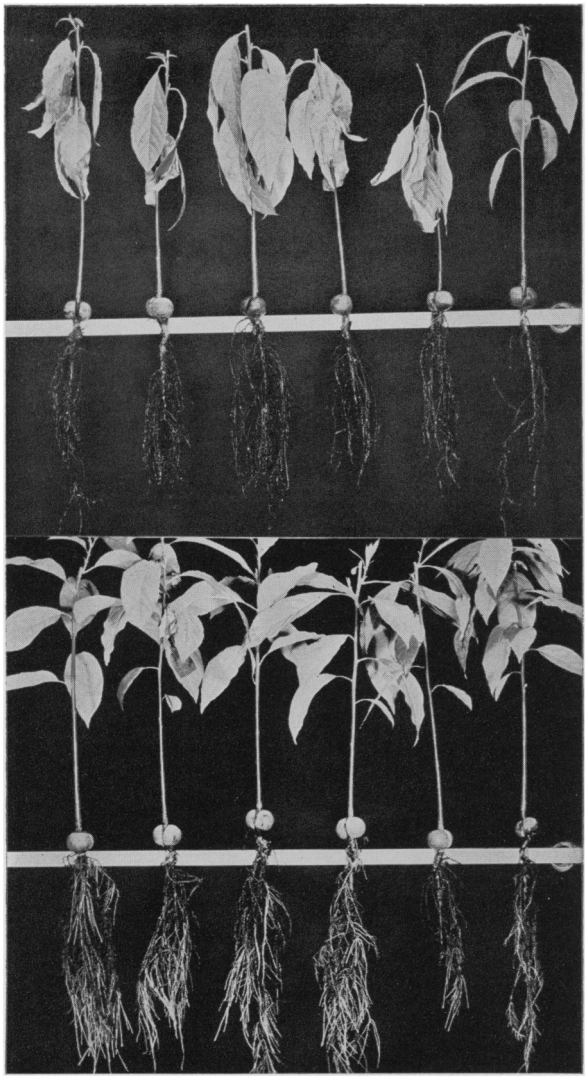

Fig. 1–2. Avocado seedlings (Dickinson variety) grown for a 2-day period in nutrient solutions having minimum oxygen concentrations, left to right (both figures), of 6.8, 1.9, 0.9, 0.4, 0.3, and 0.05 p.p.m.—Fig. 1 (above). Inoculated with *Phytophthora cinnamomi*. Leaves wilted; roots blackened and dead.—Fig. 2 (below). Noninoculated. Note turgid leaves and new root growth. (Photographed 30 days after exposure to the various oxygen levels.)

lated seedlings exposed to low oxygen levels (0.5–0.05 p.p.m.) passed through a period of temporary wilting followed by recovery during the first day of treatment. No further wilting of these seedlings was observed until 8 days later, when both mature and immature leaves began to lose turgor. Thereafter, wilting slowly increased until all plants died except those noninoculated seedlings whose exposure to low oxygen supply had been limited to a 2-day period. These seedlings produced new root growth and regained their turgor (fig. 2). There was no permanent injury to noninoculated seedlings exposed to oxygen levels ranging from 7.2 p.p.m. (full aeration) down to 0.9 p.p.m.

Nitrite accumulations.—Small amounts of nitrite nitrogen were found in the nutrient solution of all cultures, but the maximum concentration was less than 1 p.p.m., which, according to previous studies (Curtis, unpublished), is not toxic to avocados cultured at *p*H 5.5.

It has been shown (Curtis, unpublished) that, under similar conditions, higher nitrite concentrations accumulated at *p*H 4.5, but that a rapid decrease followed, probably owing to nitrite transformations by microorganisms. These microorganisms evidently had remained relatively inactive until they were supplied with nitrites.

SUMMARY

Avocado seedlings were treated in culture solution at various oxygen levels ranging from 7.2–0.05 p.p.m. Half of the seedlings at each oxygen level were inoculated with *Phytophthora cinnamomi* Rands, and the other half were cultured without addition of this fungus. Regardless of the oxygen level of the solution or of the duration of the treatment, all seedlings inoculated with *P. cinnamomi* died. Root injury by the fungus was manifested by brown discolorations which first appeared along the fleshy white feeder roots and then spread in uneven patterns over the entire root system. The fungus attack was most rapid at the highest oxygen level (7.2–6.8 p.p.m.), where nearly all the roots became discolored within a period of 2 days. At low oxygen levels (0.5–0.05 p.p.m.), the root tips showed the characteristic symptoms of oxygen deficiency, but indications of fungus injury were slow to appear, although all inoculated seedlings finally died. Regardless of the presence or absence of *P. cinnamomi*, all seedlings subjected to low oxygen levels (0.5–0.05 p.p.m.) for 4 days and for 16 days were severely damaged and did not recover when returned to full aeration with compressed air. There was no permanent injury to noninoculated seedlings exposed to these oxygen levels for 2 days and then returned to full aeration. Noninoculated seedlings were not visibly injured at oxygen levels ranging from 7.2–0.9 p.p.m. Two types of injury to avocado trees in the field are therefore suggested: (1) injury from root attack by *P. cinnamomi* under conditions of ample moisture and at any level of oxygen from full aeration down to nearly total lack of oxygen, and (2) injury from nearly total exclusion of oxygen from the soil under severely waterlogged conditions. On the basis of the present investigation, no estimate of the relative importance of the two types of root injury to trees in the field is warranted.

Division of Soils and Plant Nutrition and
Division of Plant Pathology,
University of California
Citrus Experiment Station,
Riverside, California

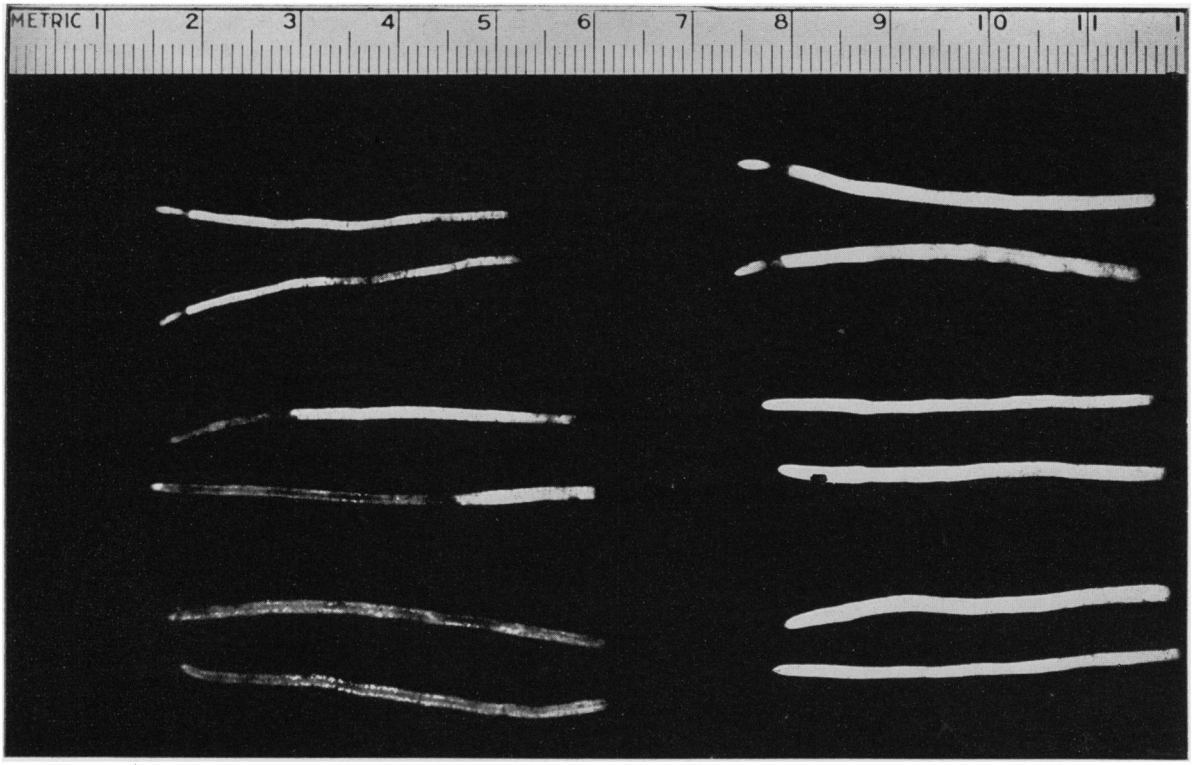

Fig. 3. Root tips of avocado seedlings (Topa Topa variety) grown in nutrient solution at various concentrations of dissolved oxygen. Left, inoculated with *Phytophthora cinnamomi;* right, noninoculated. Top pair (left and right) treated at low oxygen levels (0.05–0.1 p.p.m.) by bubbling the nutrient solution with nitrogen gas alone. Constricted root tips and discolorations are caused by lack of oxygen. Center pair (left and right) treated at intermediate oxygen levels (1.5–1.4 p.p.m.) by bubbling with mixture of air and nitrogen gases. Bottom pair (left and right) treated at high oxygen level (6.8–7.2 p.p.m.) by bubbling with compressed air. Brown discolorations (left, center and bottom) caused by the fungus.

LITERATURE CITED

Association of Official Agricultural Chemists. 1945. Official and Tentative Methods of Analyses. 6th Ed. Washington, D. C.

Crandall, B. S. 1948. *Phytophthora cinnamomi* root rot of avocados under tropical conditions. Phytopath. 38: 123–130.

Crandall, B. S., G. F. Gravatt, and M. M. Ryan. 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi.* Phytopath. 35: 162–180.

Curtis, D. S. 1949. Effect of oxygen supply in nutrient solution on avocado and citrus seedlings. Soil Sci. 67: 253–260.

Hutchins, L. M. 1926. Studies on the oxygen-supplying power of the soil together with quantitative observations on the oxygen-supplying power requisite for seed germination. Plant Physiol. 1: 95–150.

Mehrlich, F. P. 1936. Pathogenicity and variation in *Phytophthora* species causing heart rot of pineapple plants. Phytopath. 26: 23–43.

Oyler, Enid, and W. F. Bewley. 1937. A disease of cultivated heaths caused by *Phytophthora cinnamomi* Rands. Ann. App. Biol. 24: 1–16.

Rands, R. D. 1922. Streepkanker van kaneel, veroorzaakt door *Phytophthora cinnamomi* n. sp. Dept. Landbouw., Nijv. Handel, Buitenzorg Inst. v. Plantenziekten Meded. 54. 53 p.

Tucker, C. M. 1929. Report of the plant pathologist. Avocado root disease. Puerto Rico Agric. Exp. Sta. Rept. 1928: 29–35.

Wager, V. A. 1942. *Phytophthora cinnamomi* and wet soil in relation to the dying-back of avocado trees. Hilgardia 14: 519–532.

White, R. P. 1937. Rhododendron wilt and root rot. New Jersey Agric. Exp. Sta. Bull. 615: 1–32.

Zentmyer, G. A., and L. J. Klotz. 1947a. *Phytophthora cinnamomi* in relation to avocado decline. (Abst.) Phytopath. 37: 25.

———, and ———. 1947b. The cinnamon fungus and avocado decline. California Avocado Soc. Yearbook 1947: 40–42.