Importance of Phytophthora spp. and Aeration in Root Rot and Growth Inhibition of Orange Seedlings

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ABSTRACT

Infection of sweet orange seedling roots by *Phytophthora* spp. greatly increased the rate of decay of roots and depressed the rate of production of healthy roots, stems, and leaves, the rate of increase in height of stems, and rate of water use. Reduced aeration of root environment significantly decreased rate of roots produced, height of stem, and water use, but did not affect rate of root decay or rate of increase in weight of stem and leaves. The effects of *Phytophthora* parasitism on the vegetative growth of the seedlings and on root decay were greater than those of aeration.

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Additional key words: soil aeration, *Citrus sinensis*.

This experiment, which is relevant to investigations previously reported (2, 4, 7, 9, 10, 11, 12), was designed to evaluate the importance of infection by two *Phytophthora* spp. under three levels of aeration on destruction of feeder roots, growth of plant parts, and on water usage of sweet orange seedlings. Since oxygen available to both roots and fungi is reduced, it was of value to investigate the relative effects of restricted aeration on health and growth of the citrus host and the activity of its root parasites under controlled conditions.

MATERIALS AND METHODS.—Seeds freshly extracted from fruit of the sweet orange, *Citrus sinensis* Osbeck 'Bessie', were immersed in water at 52 C for 10 min, and in 5% Formalin at 25 C for 4 min to assure their freedom from citrus fungus pathogens (1, 5). They were then planted in 38- x 37- x 10-cm flats filled with chloropicrin-treated soil (greenhouse mix: 5 parts top soil, 3 parts silt, and 2 parts peat). The surface inch of this soil had 50 g/flat of aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] to inhibit damping-off by the fungus *Rhizoctonia solani* Kuehn (13). Healthy 6-week-old seedlings were transplanted into milk cartons (473 ml) filled with greenhouse mix soil. The cartons had the outer surfaces covered with aluminum paint, and drainage holes cut out at two diagonal corners of the bottoms.

The plant roots were inoculated with *Phytophthora parasitica* Dastur (isolate 6810B) by inserting into the soil one 3-inch piece of alfalfa stem culture of the fungus having abundant, discharging sporangia. The same plants were similarly inoculated with *P. citrophthora* (Sm. & Sm.) Leonian (isolate 1309A). Twenty-five seedlings received two pieces of sterile alfalfa stem, and served as checks.

Eleven days later, 15 of the inoculated and 15 of the noninoculated cultures were transplanted from the cartons to 11-liter wax-coated porcelain pails containing greenhouse soil mix. After 6 days, each pail culture was inoculated with four isolates of *Phytophthora* spp. (*P. parasitica* isolates 6972, 6973, and 6810B; and *P. citrophthora* 1309A); and each of the checks received the same number of sterile alfalfa stem pieces. The pails were tightly fitted with wooden lids having openings for entrance and exit of gases and for placement of tensiometers (Fig. 1) (9).

Differential aeration treatments consisting of air, 2% oxygen plus 98% nitrogen, and 1% oxygen plus 99% nitrogen were begun on 28 July 1970 and continued to the end of the experiment on 28 September 1970. From a distribution and mixing

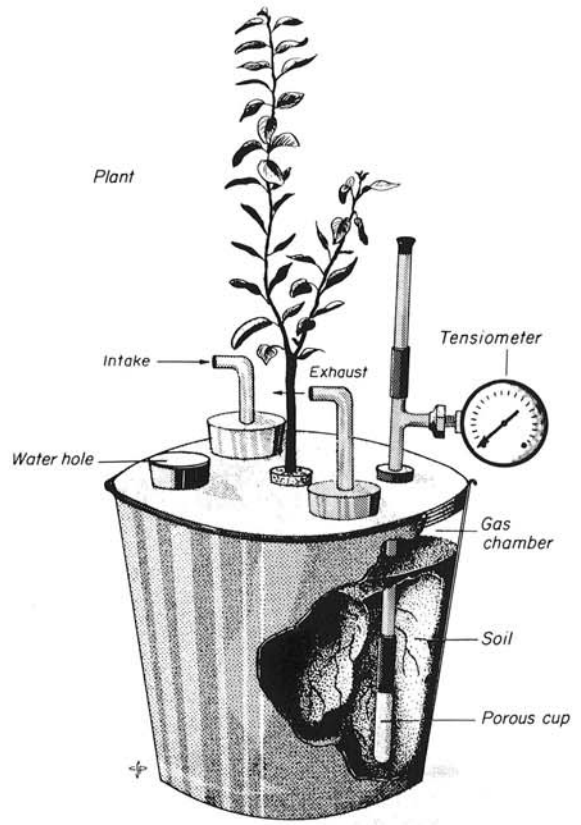

Fig. 1. Covered container with tensiometer permitting control of water supply, and portholes for control of flow of oxygen-nitrogen mixtures to soil-root system of sweet orange seedling culture.

TABLE 1. Effects of root infection by *Phytophthora* spp. on fresh weight of roots, stems, and leaves, on increase in stem height, and on water usage of sweet orange seedlings[a]

	Root rating[b]	Fresh wt (g) of plant parts			Increase in height of stem (cm)	Liters of water used during 2-month period
		Roots	Stem	Leaves		
–Phytophthora	92.0	152	97	104	42	13.9
+Phytophthora	79.7	63	53	58	7	5.0
Level of significance	**	**	**	**	**	**

[a] Each value is a mean of 15 individual determinations. ** = F value significant at 1% level.
[b] Root rating = % of perfect condition as influenced by ± Phytophthora infection and degree of aeration.

manifold, the gas mixtures were made to flow over the soil surfaces, as described by Letey et al. (6). Soil and root temperatures were maintained at 25± 2 C in constant-temperature control tanks. Aboveground parts of seedlings were exposed to night-day air-temperature ranges of 19 to 39 C during the 2-month period in the greenhouse temperature tanks. The mean day-length for this period was 13 hr 11 min.

Water usage, as guided by tensiometer readings, was measured for the entire period (16 December 1969 to 28 September 1970). This included the pre-aeration period of 16 December 1969 to 28 July 1970, during which all the plants were under the same conditions of atmospheric temperature and aeration, and the 2-month period of differential aeration (28 July 1970 to 28 September 1970). Only the water used by the plants during the latter period is reported in the tables. At no time did the water added cause the suction to drop below 2 centibars in any of the cultures. Good nutrition of seedlings was maintained throughout the experiment by regular additions of Hoagland's solution minus copper. Since that element in the soil could inhibit parasitism by the *Phytophthora* spp., a foliar spray of copper sulfate having 1.0 mg Cu^{++}/liter was applied once a week to prevent copper deficiency symptoms (exanthema). There were six different treatments each replicated 5 times.

At the end of the experiment, the plants were cut off at the top of the taproot, the soil was removed from the root system by careful washing, and determinations were made of the height and fresh weight of stems, fresh weight of leaves, and fresh weight and condition of roots (estimation of root rot). Data obtained were analyzed statistically, and treatment differences were evaluated by Duncan's multiple range test (3, 8).

RESULTS AND DISCUSSION.–The effect of the root pathogens in increasing decay of roots and reducing fresh weight of roots, stems, height of plants, and amount of water used is shown in Table 1. As shown by root rating, the infected root systems, recoverable by careful washing-out at the end of the experiment, had significantly more rot than those of noninoculated cultures (Fig. 2). Reducing the rate of aeration of roots from the 21% of oxygen in air to either 2 or 1% significantly reduced production of roots, growth in height, and water consumption, but did not affect significantly the percentage of root decay or the fresh weight of stems and leaves (Table 2).

Root growth in the absence of infection was markedly reduced by the two lower levels of aeration, but, in the presence of the root pathogens, decreasing

TABLE 2. Effects of soil aeration on root decay, fresh weight of roots, stems, and leaves, increase in stem height, and water usage of sweet orange seedlings[a]

	Root rating[b]	Fresh wt (g) of plant parts			Increase in height of stem (cm)	Liters of water used during 2-month period
		Roots	Stem	Leaves		
Air	82.5	135^{y}[c]	79	87	35^{y}	13.1^{y}
2% O_2,98% N_2	90.0	110^{y}	77	79	22^{xy}	9.7^{xy}
1% O_2,99% N_2	85.0	77^{x}	69	78	17^{x}	7.3^{x}
Level of significance	NS	**	NS	NS	**	**

[a] Each value is a mean of 10 individual determinations. ** = F value significant at 1% level. Mean values are statistically significant only if they do not have a superscript letter in common. NS = mean values not significantly different from each other.
[b] Root rating = per cent of perfect condition as influenced by ± Phytophthora infection and degree of aeration.
[c] x,y,z indicate statistical populations. Mean values in column for each element are statistically different only if they do not have letter in common.

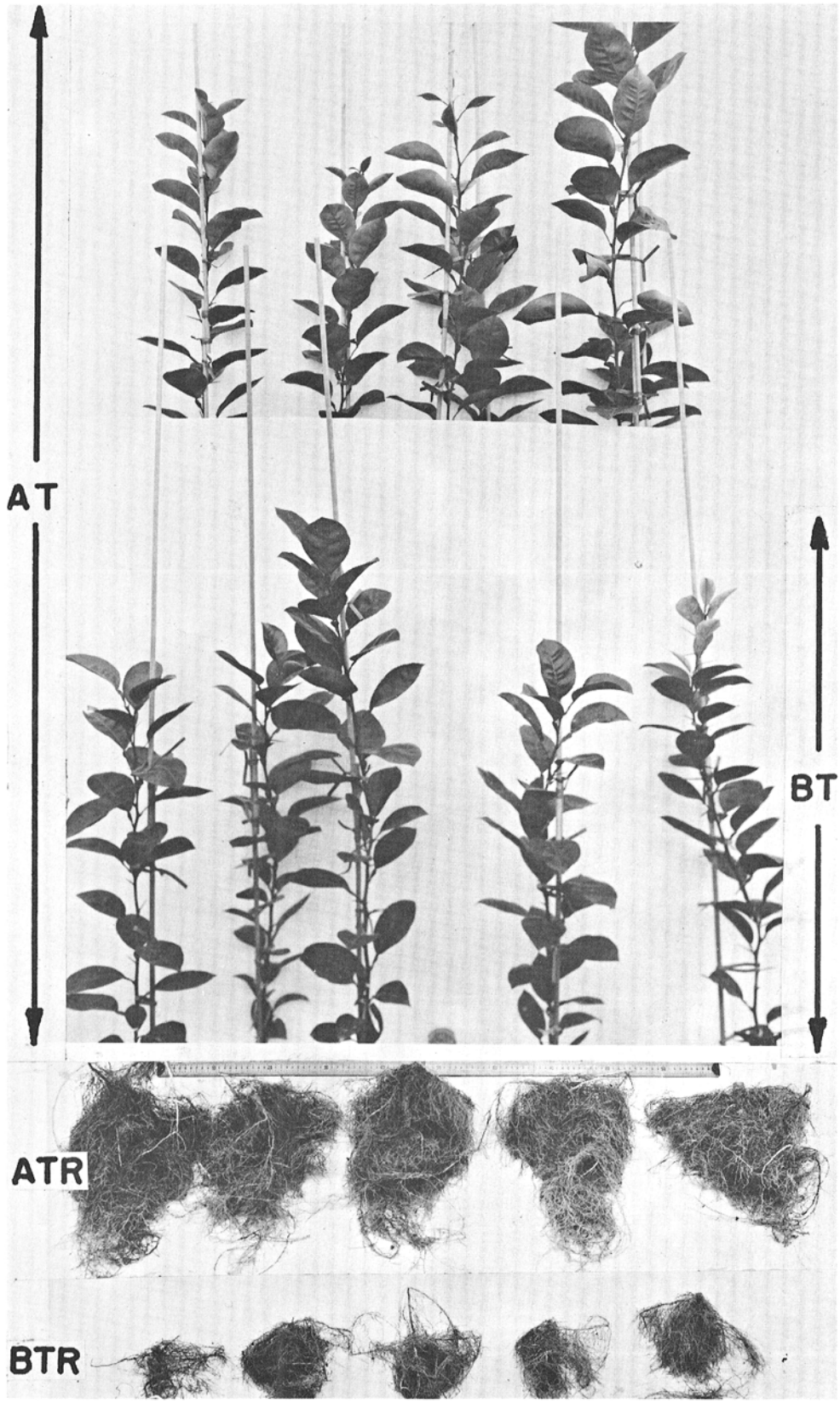
AT
BT
ATR
BTR

TABLE 3. Interaction effects between *Phytophthora* spp. and amount of aeration on fresh weight of roots, height increase, root condition, and water usage by sweet orange seedlings[a]

	Fresh wt (g) of roots		
	–Phytophthora	+Phytophthora	Level of significance
Air	202[z]	67	**
2% O_2, 98% N_2	162[y]	59	**
1% O_2, 99% N_2	93[x]	61	NS
Level of significance	**	NS	
	Root condition, % healthy roots		
Air	95.0	70.0	**
2% O_2, 98% N_2	95.0	85.0	NS
1% O_2, 99% N_2	86.0	84.0	NS
Level of significance	NS	NS	
	Height increase (cm)		
Air	62[y]	8	**
2% O_2, 98% N_2	38[x]	6	**
1% O_2, 99% N_2	25[x]	9	NS
Level of significance	**	NS	
	Water usage, liters used during 2-month period		
Air	16.8[y]	5.9	**
2% O_2, 98% N_2	15.1[y]	4.3	**
1% O_2, 99% N_2	9.9[x]	4.7	**
Level of significance	**	**	

[a] Each value is a mean of five individual determinations; x, y, z = statistical populations; mean values in column for each element are statistically different only if they do not have letter in common. ** = F value significant at 1% level. Mean values are statistically significant only if they do not have a superscript letter in common. NS indicates that mean values are not significantly different from each other.

the concentration of oxygen reaching roots did not retard root growth significantly (Table 3). Thus, effects caused by the fungi greatly overshadowed those from aeration. However, parasitism of roots by the *Phytophthora* spp. was greatest in the two higher levels of aeration, and not significant in the lowest (1%) level. The data in Table 3, moreover, supported orchard observations that parasitism by *Phytophthora* spp. is severest in well-aerated, but thoroughly watered, sandy soils. Table 3 reports similar effects on height increase.

There were no significant effects of the three oxygen concentrations on the amounts of root rot either in the presence or absence of infection. The data showed, however, that the *Phytophthora* spp. in the air treatment markedly affected the roots. Where

Fig. 2. Effect of root infection on production of root and tops of sweet orange seedlings. Seedlings grown from 10 June 1969 to 28 July 1970 in air, and from 28 July 1970 to end of experiment (28 Sept. 1970) with root zone aerated by 2% oxygen-98% nitrogen. AT is the length of tops of seedlings grown from 10 June 1969 to 28 Sept. 1970 with no *Phytophthora* spp. in root zone; lower part of the AT seedlings down to containers obscured by white background placed between AT and BT plants. BT plants grown from 10 June to 26 Nov. 1969 without root parasites and from 26 Nov. 1969 to 28 Sept. 1970 with *Phytophthora citrophthora* and *P. parasitica* in root zone. Entire height of BT plants, from container to top, shown. ATR, roots of AT plants, were produced in absence and BTR, roots of BT plants, in presence of *Phytophthora* spp. in the root zone.

soil aeration was restricted, parasitism of the roots was reduced, illustrating that good aeration favors parasitism by these fungi.

Root aeration with 1% oxygen in the absence of root infection significantly reduced water use by the seedlings as compared to water use when roots received 21% oxygen (Table 3). With the root systems parasitized, none of the three levels of aeration had a significant effect on water use. However, there were highly significant differences in water use between inoculated and noninoculated plants under each aeration treatment.

Interaction effects between aeration and infection on fresh weight of leaves, stems, and roots were not statistically significant, and are not included in tabular form.

The four *Phytophthora* isolates used in this experiment caused more destruction of roots than the two isolates used in previous investigations (9, 10). This may be related to any of several factors, such as more virulence in isolates of the fungi or a physiological state of the orange seedlings that made them more susceptible. One may assume that with the possibility of change in fungus virulence through mutation or hybridization, strains evolve which can cause extensive destruction of roots where conditions of moisture and aeration are favorable.

The results in this report show that good aeration of roots favors high incidence of infection by the *Phytophthora* spp. and agree with field observations. The drip method of irrigation, which permits good aeration and which is currently being promoted, should be investigated under field conditions. The work of McIntosh & O'Reilly (7) with pot cultures of pears suggests that this method of watering may be conducive to rootlet infection by *P. cactorum*. The effect of the drip method on incidence of root rot and gummosis in California and in Israel citrus orchards, where the method has been under field test for 6 years, has not yet been reported.

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