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Growth, Mineral Uptake, and Evapotranspiration of Air Layers from Blight-affected and Healthy Grapefruit Trees with Two Nutrient Regimes[1, 2]

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Abstract. **Air layers from 6 blight-affected and 6 healthy grapefruit, *Citrus paradisi* Macf., trees were grown in a greenhouse with low N, low S, and lime and high N, high S, and no lime regimes, one air layer from each tree in each treatment. Shoot fresh weight of air layers from healthy trees was 25%, and root fresh weight was 38% greater than that of air layers from blighted trees, after 8 months of treatment (shoot/root ratios of 1.59 and 1.90). Shoot weight was the same with both nutrient treatments; root weight was 40% smaller with high N, high S, and no lime than with low N, low S, and lime (shoot/root ratios of 2.12 and 1.37). Air layers from blighted trees had higher concentrations of N, P, and water-soluble phenolics, and lower Ca and Na in the wood; more S, Fe, Zn, Cu, and Mo in the bark; more N and K, and less Mg, Na, and Cl in the roots, and more P and less B and Cl in the leaves than air layers from healthy trees. Low N, low S, and lime induced higher K and Mo in the wood, higher K in the bark, and lower Na and Cl in the roots of air layers from blighted trees; high N, high S, and no lime increased Mg and Zn in the roots, Fe in the wood, and Zn in the leaves of air layers from blighted trees above the levels of healthy air layers. There were curvilinear relationships between evapotranspiration and root weight and the shoot/root ratio; air layers from blighted trees transpired more water than those from healthy trees on a per unit shoot and root weight basis.**

Blight, Florida's most serious citrus production problem, remains unexplained and with unknown cause (4, 12, 13). Visual symptoms are not specific, and water injection into the trunk and analysis of the wood for Zn and water-soluble phenolics are used as diagnostic tests (4, 14). Lower S and Cl levels have been found in the soil under blight-affected than under healthy trees, and lime has been implicated in the appearance of blight (2, 9). The present report is on a greenhouse experiment where paired grapefruit air layers propagated from blighted and healthy trees were grown under 2 regimes: one designed to imitate the lower S levels found under blighted trees in the field and to test the effect of high levels of lime and the other with higher N and S levels and no lime. The method of air-layer propagation was chosen so that any preconditioning or a disease organism would affect the plants in both treatments equally.

Materials and Methods

Two 1- to 2-cm-thick branches on each of 6 blight-affected and healthy 'Marsh' grapefruit trees on rough lemon, *C. limon* (L.) Burm. f., rootstock were air-layered between Sept. 1979 and May 1980 in a 53-year-old commercial grove near Clermont, Fla. A ring of bark was removed, the wound was treated with 1% indolebutyric acid powder, and then wrapped with wet sphagnum moss and plastic, with an outer covering of aluminum foil. The status of the trees was determined by analysis of the outer 2.5 cm of the trunk wood for Zn (blight 8–21 ppm; healthy 2–4 ppm) (4, 14, 15).

The air layers were grown for 3 months in small pots (18 cm wide, 18 cm deep) in a soil mix (50% sand, 25% sphagnum peatmoss, 25% perlite v/v, pH 5.6), and then transplanted into larger (26 cm wide, 26 cm deep) pots containing the same soil mix. They were fertilized every 2 weeks with a solution, pH 5.1, containing 200 ppm N as ammonium nitrate (77% NO_3, 23% NH_4), 10 ppm P, 100 ppm K, 100 ppm Ca, 60 ppm Mg, 2 ppm Fe, 2 ppm Mn, 0.4 ppm Zn, 0.2 ppm Cu, 0.2 ppm B, 0.1 ppm Mo, 20 ppm Cl, and 250 ppm S. All plants were grown in a greenhouse; shade cloth reduced incoming sunlight by 45%. The trees were watered as needed with tap water (pH 7.3, 37 ppm Ca, 8 ppm Mg, 14 ppm Cl, 5 ppm S, and traces of Zn and Cu).

Treatments were started 6 months after transplanting, in Jan. 1981. The trees were arranged on 2 benches and trunk diameters were measured at a paint-marked point 3 cm above the soil line. The cross-sectional area was calculated. One of the 2 air layers from each tree was included in each of the 2 nutritional treatments. One air layer propagated from a healthy tree was lost; therefore, there were 6 pairs of trees propagated from blighted trees and 5 pairs and 1 extra tree propagated from healthy trees, 12 trees in treatment 1 (low N, low S, and lime) and 11 trees in treatment 2 (high N, high S, and no lime).

In treatment 1, the soil pH was raised from the original 5.6 to about 8.0 by 3 additions, at 30-day intervals, of 15 g CaO to the 13 kg of soil in each pot in the first 3 months of treatment. The pH remained constant during the rest of the experiment. The nutrient solution applied in treatment 1, pH 6.8, contained 100 ppm N (50% NH_4-N, 50% NO_3-N, a combination of ammonium carbonate and ammonium and magnesium nitrate), 10 ppm P, 100 ppm K (as potassium tartrate), 40 ppm Ca, 30 ppm Mg, 2 ppm Fe, 0.1 ppm Mn, 0.4 ppm Zn, 0.2 ppm Cu, 0.2 ppm B, 0.1 ppm Mo, 15 ppm Cl, and 5 ppm S. The same amount of minor elements was added to both treatment solutions, but less Mn remained in solution in treatment 1. In treatment 2,

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the solution applied was the same as the one used before the start of differential nutrient treatments and no lime was added. Both treatments were applied every 7 days.

A record was kept of irrigations applied in the 2-week period between June 23 and July 7, 1981. The plants were watered to saturation between nutrient solution applications whenever the soil surface was dry. During this period, the pots were weighed every 24 hours to determine evapotranspiration. When watering was needed, the pots were weighed before and 20 min after watering to establish a base for the next 24 hr. A pot containing soil only was weighed to determine the soil evaporation component of evapotranspiration.

Before harvest on Sept. 8, 1981, the trunk diameters of the trees were measured again at the paint marks. The shoots were cut off 3 cm above the soil line and weighed. The root systems were removed from the pots, washed thoroughly, and weighed after air-drying for 4 hr. Thirty 4- to 7-month-old leaves and about 70 g of feeder roots were collected from each tree for analysis for 14 elements. The basal 10 cm of the trunk were cut off and the bark separated from the wood. The center of the trunk was removed with a drill, because blight affects primarily the outer layers of trunk wood (11); the outer 0.5 cm of the wood were analyzed.

The 10- to 15-cm section above the basal part of the trunk was cut off and stored in a water-vapor-saturated container for water conductivity measurement the same day. The system used was described by Garnsey and Young (7). Water was drawn through the trunk sections over 3-min periods and stem conductivity was calculated as ml $\times$ cc^{-1} trunk $\times$ hr^{-1}.

The dried and ground leaf, bark, wood, and root samples were analyzed for N by micro Kjeldahl, for P and B colorimetrically (1, 5), for S turbidimetrically (10), for Cl by electrometric titration, for K and Na by flame emission, and for Ca, Mg, Fe, Mn, Zn, Cu, and Mo by atomic absorption spectroscopy. Water-soluble phenolics in the wood were determined spectrophotometrically (14). Samples of the soil medium in each pot were extracted with water (1:1 w/v) and analyzed as published recently (16). The data were analyzed statistically using analysis of variance and correlation.

Results and Discussion

It was surprisingly easy to root air layers from the 53-year-old grapefruit trees with the technique used. The success rate was 100%; however, one of the air layers did not survive in the greenhouse. The plants propagated from blight-affected trees were at first visually distinguishable from those propagated from healthy trees because they had fewer and smaller leaves, but after 6 months in the greenhouse, all trees looked uniformly healthy. At the start of nutrient treatments, the air layers from blighted trees tended to be somewhat smaller than their healthy counterparts, but the difference in trunk cross-sectional area was not significant (Table 1).

The growth rate during the 8-month experiment, measured as increase in trunk cross-sectional area, was the same for trees propagated from blighted and healthy trees, but the plants in treatment 1 grew faster than those in treatment 2 (Table 1). Trunk cross-sectional area apparently was not the most sensitive measure of growth of the small trees in the experiment. The shoots of trees propagated from blighted trees weighed 25% less, and the roots 38% less than those for air layers from healthy trees (Table 1). Shoot weights were almost identical with the 2 treatments, but the root systems of trees in treatment 2 were 40% smaller than those of the trees in treatment 1. The difference in size of the root systems can be explained by the high N level of treatment 2, which tends to reduce the root system (6) and the high Ca level in treatment 1, which enhanced root growth (8).

Trees in treatment 1 showed iron deficiency chlorosis and manganese deficiency symptoms after the lime applications, and they had sparser foliage. They apparently produced more wood and fewer leaves than the trees in treatment 2, which were uniformly dark green and looked very healthy. Five trees in treatment 2, 4 propagated from healthy trees and 1 from a blighted tree, flowered and set fruit in Aug. 1981; none of the trees in treatment 1 set flowers.

There was little difference in pH and water-extractable cations and anions between the soil media of the trees propagated from blighted and healthy trees at the end of the experiment (Table 2), but a comparison of the soils of treatments 1 and 2 showed that the soil medium in treatment 1, with a pH of 8.2, was essentially saturated with Ca. Water-extractable cations and anions were both lower in treatment 1 than in treatment 2, and total cations (K, Ca, Mg, and Na) exceeded total anions (SO_4 and Cl). These conditions are found in the field under blight-affected trees (16), and the solution used in treatment 1 was designed to create these conditions.

With the exception of a slight rise in K in the wood (Table 3), treatment 1 did not produce the changes in nutrient concen-

Table 1. Growth and water conductivity of the trunk and evapotranspiration of air layers propagated from blight-affected and healthy trees.

Treatment	Shoot fresh wt (g)	Root fresh wt (g)	Shoot/root ratio	Original trunk cross-sectional area (cm^2)	Increase in trunk cross-sectional area (cm^2)	Water conductivity of trunk (ml/cc-hr)	Evapotranspiration[z] ml/culture/24 hr	Evapotranspiration[z] ml/kg shoot fresh wt -24 hr	Evapotranspiration[z] ml/kg root fresh wt -24 hr
Blight[y]	924b[x]	561b	1.90a	1.9a	2.2a	7.2a	889b	978a	1833a
Healthy[w]	1262a	900a	1.59b	2.4a	2.5a	10.4a	1064a	866b	1345b
Treatment 1[y] (low N, low S, lime)	1106a	914a	1.37b	2.3a	2.6a	12.1a	1125a	1062a	1680a
Treatment 2[w] (high N, high S, no lime)	1101a	546b	2.12a	2.0a	2.0b	5.5a	827b	781b	1498b

[z]Means of 14 days.
[y]Means of 12 trees.
[x]Different letters indicate significant differences at P = 0.05 between trees propagated from blighted and healthy trees and between treatments 1 and 2.
[w]Means of 11 trees.

Table 2. Water-soluble cations and anions and pH of soil medium at the end of the experiment.

Treatment	pH	Water-soluble cations (meq/100 g)	Water-soluble anions (meq/100 g)	Cation/anion ratio
Blight[z]	6.7a[y]	0.994a	1.770a	0.56
Healthy[x]	6.9a	0.996a	1.951a	0.51
Treatment 1[z] (low N, low S, lime)	8.2a	0.551b	0.301b	1.83a
Treatment 2[x] (no lime)	5.5b	1.480a	3.255a	0.45b

[z]Means of 12 trees.
[y]Different letters indicate significant differences at P = 0.05 between trees propagated from blighted and healthy trees and between treatments 1 and 2.
[x]Means of 11 trees.

tration in the various tissues of the trees observed with blight in the field (15). There were interactions, however, differences in response to the nutrient treatments between the air layers propagated from blighted trees and from healthy trees. Like blight-affected trees in the field (15), they accumulated more K in the bark and the wood with treatment 1 than with treatment 2; there was no difference in air layers from healthy trees. Treatment 2 raised Mg in the roots, Fe in the wood, and Zn in the leaves and roots in air layers from blighted trees to levels higher than those with treatment 1. Because of the high pH, Mo levels were generally much higher with treatment 1 than with treatment 2, but this effect was stronger in trees propagated from blighted trees than in those propagated from healthy trees. Leaf Cu was slightly higher with treatment 2 in plants derived from blighted trees than with treatment 1. The opposite was true for air layers from healthy trees. Air layers from healthy trees also accumulated more Ca in the roots with treatment 2 than with treatment 1, and more Na and Cl with treatment 1 than those from blight-affected trees. Treatment 1 lowered Mn in all tissues, in both types of air layers. In spite of the uniformly healthy appearance of the air layers at the beginning of nutrient treatments, there was apparently still a physiological difference between the 2 types of trees, which is reflected in the varying response to nutrient treatments.

Water conductivity of the trunk (Table 1) tended to be higher with treatment 1 and in healthy trees, but the results were too variable for the differences to be statistically significant.

Soon after differential nutrient treatment was started, the trees in treatment 1 needed more frequent irrigation than the trees in treatment 2. Records taken during the 2-week evapotranspiration test in June and July 1981 showed that in addition to the weekly application of nutrient solutions, air layers from blighted trees

Table 3. Concentration of 14 elements in the leaves, bark, wood, and roots and water-soluble phenolics levels in the wood.

Treatment	Element concn N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Na (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Cl (ppm)	Mo (ppm)	Water-soluble phenolics (mg/g)
Leaves															
Blight[z]	3.18	0.195*	2.40	2.85	0.55	0.793	471	72	33	22	6	157*	333*	9	
Healthy[y]	3.25	0.181	2.34	2.83	0.56	0.842	576	72	34	23	6	175	392	6	
Treatment 1[z]	2.59*	0.124*	2.26	3.12*	0.53*	0.438*	586	65*	12*	21*	6	171	400*	13*	
Treatment 2[y]	3.85	0.252	2.47	2.56	0.58	1.200	461	79	55	24	6	161	325	2	
Wood															
Blight	0.81*	0.199*	0.46	0.44*	0.04	0.067	230*	38	4	7	5	13	209	5	3.5*
Healthy	0.76	0.187	0.41	0.45	0.04	0.068	296	35	3	6	5	13	262	5	3.0
Treatment 1	0.58*	0.100*	0.47*	0.44	0.04	0.038*	312*	32*	2*	5*	4*	13	278	7*	3.0*
Treatment 2	0.98	0.287	0.40	0.45	0.04	0.097	214	41	5	7	6	12	193	3	3.5
Bark															
Blight	1.51*	0.084	0.99	3.31	0.21	0.211*	419	70*	16	54*	73*	74	452	6*	
Healthy	1.38	0.083	1.06	3.31	0.21	0.185	403	57	14	46	30	67	594	4	
Treatment 1	1.19*	0.064*	1.07*	3.50*	0.20	0.140*	414	47*	6*	43*	40	67*	541	6*	
Treatment 2	1.69	0.102	0.98	3.13	0.21	0.256	408	80	24	62	63	74	506	4	
Roots															
Blight	3.16*	0.392	2.28*	1.15	0.46*	0.854	1533*	679	276	173	31	45	9044*	34	
Healthy	3.02	0.478	1.87	1.29	0.60	0.923	2346	687	363	174	36	42	9786	24	
Treatment 1	2.21*	0.148*	2.27*	1.01*	0.51	0.454*	2568*	639	37*	163	39*	42	13960*	52*	
Treatment 2	3.97	0.722	1.87	1.43	0.55	1.324	1311	727	602	184	28	45	4870	6	

*Differences significant at 5% level.
[z]Means of 12 plants.
[y]Means of 11 plants.

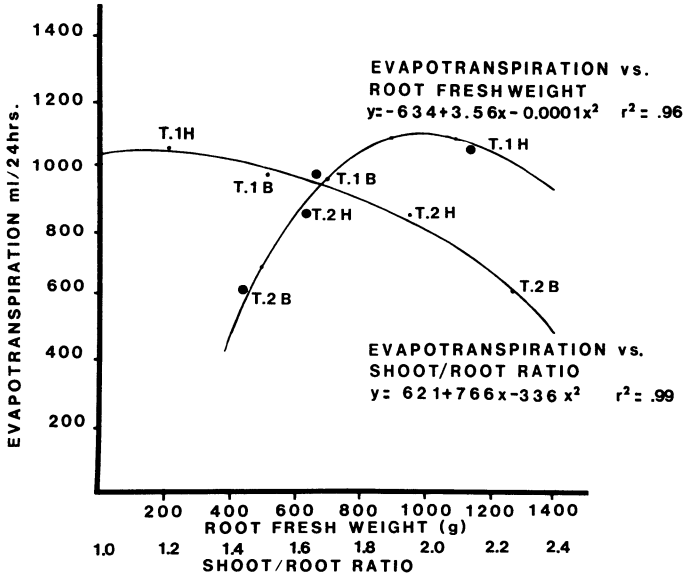

Fig. 1 Relationship of evapotranspiration with root fresh weight and shoot/root ratio. T.1B = treatment 1, trees propagated from blighted trees; T.1H = treatment 1, trees propagated from healthy trees; T.2B = treatment 2, trees propagated from blighted trees; and T.2H = treatment 2, trees propagated from healthy trees.

in treatment 1 needed 3.7, and those from healthy trees needed 5.0 irrigations. In treatment 2, both types of air layers needed only 2.8 irrigations. The evaporation, as measured on a soil-filled pot without a plant, made up 10–17% of the evapotranspiration measured. Air layers propagated from healthy trees transpired more water than those propagated from blighted trees (Table 1), and treatment 1 increased evapotranspiration 36% over that of trees with treatment 2. The relationships of evapotranspiration, root fresh weight, and the shoot/root ratio are shown in Fig. 1. The quadratic curves show that the weight of the root system was related to the amount of water transpired and that increases in the shoot/root ratio were linked to decreases in evapotranspiration. On a per-unit root or shoot weight, however, air layers propagated from blight-affected trees transpired more water than air layers from healthy trees, which could provide an alternative to the explanation of the wilt observed with blight through blockage of the xylem vessels (3).

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