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Interaction of Simulated Acid Rain with Ozone on Freeze Resistance, Growth, and Mineral Nutrition in Citrus and Avocado[1]

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Abstract. **The combined effects of O_3 and acid rain on freeze resistance, growth, and mineral nutrition were studied using broadleaf-evergreen citrus and avocado trees. Using a factorial design, 'Ruby red' grapefruit *(Citrus paradisi* L.) trees on either Volkamer lemon *(Citrus volkameriana* Ten. & Pasq.) or sour orange *(Citrus aurantium* L.) rootstocks and 'Pancho' avocado trees *(Persea americana* Mill.) on 'Waldin' rootstock were exposed to O_3 and acid rain for 8 months in open-top chambers under field conditions. The O_3 treatments were one-third ambient (0.3X), ambient (1X), twice ambient (2X), or thrice ambient (3X). Ambient O_3 concentrations averaged 39.1 nl-liter-^{1} over a 12-hour day. The acid rain treatments had a pH of 3.3, 4.3, or 5.3 and were applied to simulate long-term rainfall averages. In general, the effects of acid rain on growth and freeze resistance were small. Rain of high acidity (pH = 3.3) offset the negative effects of O_3 on growth (total leaf mass) in avocado and grapefruit/Volkamer lemon trees. In contrast, rain of high acidity magnified the detrimental effects of O_3 on electrolyte leakage of leaf disks at subzero temperatures, especially for citrus. Freeze resistance, determined by stem and whole-plant survival following freezing temperatures, was lower in the most rapidly**

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growing trees. Consequently, for trees exposed to a combination of O_3 and acidic rain, leaf electrolyte leakage did not correlate significantly with stem survival of freezing temperatures. We conclude that the danger of acid rain to citrus and avocado in Florida is rather slight and would only present a potential problem in the presence of extremely high O_3.

Plant injury resulting from environmental stresses is commonly attributable to more than a single factor. This problem has been highlighted in the recent forest decline syndrome, where many environmental factors, including anthropogenic air pollution, apparently have interacted to kill many trees (e.g., Schulze, 1989). One interaction that is receiving increased attention is the association between winter injury and exposure to air pollutants. Loss of freeze resistance can be induced by elevated levels of O_3 (Barnes and Davison, 1988; Barnes et al., 1988; Brown et al., 1987; Eissenstat et al., 1991; Fincher et al., 1989), sulfur dioxide (Davison and Bailey, 1982), and acidic mist (Fowler et al., 1989). However, the situation is complicated by the fact that plants often are exposed to more than one pollutant at a time. The effects of a combination of air pollutants may be considerably worse than the sum of the component effects of each pollutant if applied singly (e.g., Ormrod, 1978; Thompson et al., 1972). The effects of a single air pollutant, O_3, on citrus and avocado growth and freeze resistance have been studied (Eissenstat et al., 1991). In the study reported here, we focused on the combined effects of chronic exposure to O_3 and acid rain on freeze resistance, growth, and nutrition in citrus and avocado. Both of these subtropical evergreens are grown in areas that are periodically subjected to freezing temperatures that can cause disastrous economic losses (Yelenosky, 1985).

The effects of acid rain on citrus and avocado have not been extensively investigated (Kender et al., 1983), and there has been only limited study on the effects of O_3. Thompson et al. (1972), using fumigated greenhouses in the Los Angeles Basin, Calif., examined the effects of O_3 alone and its interaction with the polluted ambient air, which included oxides of N and peroxyacyl nitrates, on fruit drop and fruit yield in navel orange [*Citrus sinensis* (L.) Osb.] trees. They found that the total photochemical-smog complex, including O_3, substantially reduced yields compared with O_3 alone. Although there has been some investigation of avocado response to acute O_3 exposure (Olsyzk et al., 1987), we found no information on the response of avocado to chronic O_3 and acid rain exposure.

Methods

EXPERIMENTAL PROCEDURE. Grafted trees of commercial citrus and avocado cultivars growing in 12-liter pots were exposed to O_3 and simulated acid rain in 24 open-top chambers from Apr. to Dec. 1989. Avocado trees were ≈6 months old and citrus trees were 1 year old at the beginning of the experiment. The avocado scion was 'Pancho', a relatively cold-hardy cultivar of the Mexican race, on 'Waldin' rootstock. The citrus scion was 'Ruby red' grapefruit (RG) on the rootstocks, Volkamer lemon (VL), and sour orange (SO). Trees on SO typically are more freeze resistant than those on VL (Yelenosky, 1985).

The treatment design was a factorial with four levels of O_3, one-third ambient (0.3X), ambient (1X), 2X, and 3X, along with three levels of acid precipitation (pH = 3.3, 4.3, and 5.3) replicated twice in a completely randomized design. Each of the 24 chambers contained three to four trees of each cultivar. The effects of pollutants on freeze resistance, growth, and mineral nutrition were analyzed separately for grapefruit and avocado; for grapefruit, species of rootstock were analyzed as a split plot within the overall factorial design. In cases such as growth responses, where there was a significant species x O_3 x acid interaction, we simplified the analysis by examining the O_3 x acid effect separately for each rootstock.

CHAMBER DESIGN AND TREATMENTS. The chamber site was located in north central Florida (Austin Gary Forest), ≈15 km northeast of Gainesville, Fla. Chambers were 4.6 m in diameter and 4.3 m tall with an umbrella-like cover. Design is described in detail by Heagle et al. (1989). Ozone doses were set by monitoring the concentration of ambient O_3 and applying a constant multiple to the chambers using a computer-controlled electrical discharge-type generator and pure O_2 as the seed gas (Heagle et al., 1979; Hogsett et al., 1985; Shafer and Heagle, 1989). Ozone supplementation began at 0800 and ended at 2000 HR. Ozone treatments were charcoal-filtered (≈0.3 X ambient O_3 = 0.3X), ambient nonfiltered air (1X, particulate filter only), and ambient air with O_3 added to provide target doses of two (2X) and three (3X) times ambient O_3 concentrations 12 h-day^{-1}. Daily maxima (hourly average) from 1989 are shown in Fig. 1.

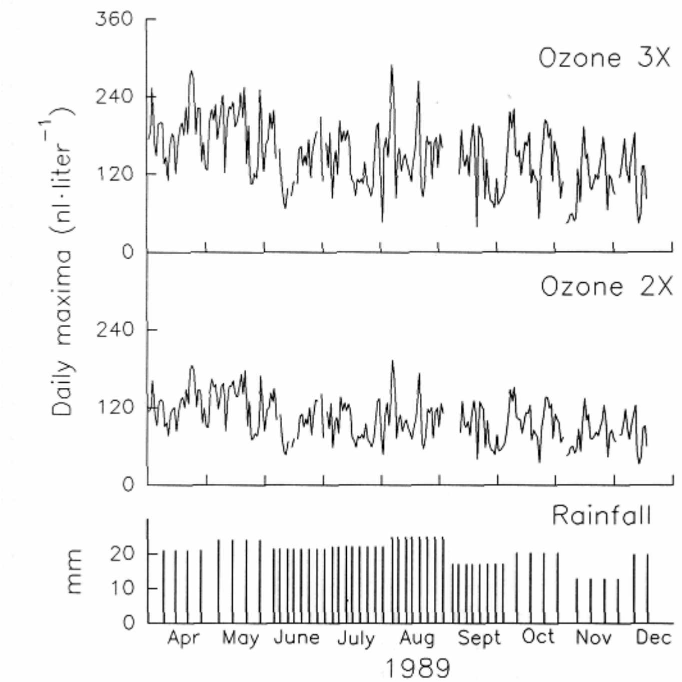

Fig. 1. Ozone daily maxima (hourly averages) for multiples of ambient O_3 and daily rainfall deposition in the open-top chambers during 1989.

There were no days during the exposure period (data for 244 days) when ambient O_3 exceeded 120 nl-liter^{-1} (National Ambient Air Quality Standard for human exposure to O_3). Ambient O_3, based upon 12 h-day^{-1}, averaged 39.1 nl-liter^{-1} during the exposure period. For 2X and 3X O_3 treatments, there were totals of 331 h during 65 days and 1148 h during 143 days when O_3 levels exceeded 120 nl-liter^{-1}, respectively. Average 12-h season-long O_3 concentrations in the 2X and 3X treatments were within 7% of the targeted doses.

Natural precipitation was excluded from the chambers. Simulated rainfall was maintained at a pH of 3.3, 4.3, or 5.3. Acid solutions were prepared by adjusting the pH of deionized water containing selected background ions (Shafer et al., 1985) with a 1-N mixture of sulfuric and nitric acids in a ratio of 70S:30N. Simulated rain was applied at 0.16 mm-min^{-1} through stainless steel solid core nozzles at the top of the chamber 2 days-week^{-1} June-September and 1 day-week^{-1} in the low rainfall months (Fig. 1). Total amount of rain was based on monthly average rainfall in the study area for the past 56 years. In general, rates of precipitation were more uniform than the rates typical of the intense summer thunderstorms in Florida.

PLANT CULTURE. Trees were replanted in a peat-perlite commercial potting mixture (Vergro, Verlite Co., Tampa, Fla.) in 38-cm-tall 12-liter pots before they were placed in the open-top O_3-exposure chambers. A slow-release 14N-6.2P-11.6K fertilizer (Osmocote), a fungicide drench (metalaxyl), and a broad spectrum systemic insecticide (aldicarb) were added when the trees were replanted. Trees of each cultivar were placed in the chambers from 1 Apr. until either 2 Dec. 1989 for the avocado or 13 Dec. for the citrus. A separate experiment with slash pine was conducted in the central portion of the chambers.

In addition to the simulated acid rain, trees were irrigated twice a week with well water having a pH of 8.2 with 46 mg Ca^{++} and 19 mg Mg^{++}/liter. Water was added directly to the pots without wetting the leaves. Every 3 months, trees were treated with fungicide, insecticide, and slow-release fertilizer. In addition, each tree was fertilized monthly with soil application of 5 g of a soluble 20N-8.9P-16.6K (+ microelements) commercial fertilizer (Tracite, Helena Chemical, Memphis, Tenn.). Trees were watered as needed, which by late fall meant watering every other day. At harvest, leaves representing a cross section of leaf ages were sampled from each tree, dried, ground, and analyzed for total N (semi-micro-Kjeldahl) and other major nutrients using plasma spectrophotometry after digestion in perchloric-nitric acid.

GROWTH AND FREEZE RESISTANCE. Growth was assessed by measuring total stem length, number of leaves, and average area and leaf dry mass at the end of the experiment. When trees were placed in the chambers in April, they had between 20 and 30 leaves. In December, 10 leaves per tree representing a cross section of leaf ages were sampled and their area (LI-COR Li 3000; LI-COR, Lincoln, Neb.) and dry mass (70 °C for 48 h) measured. The total number of leaves on each tree, which by the end of the growing season for any cultivar averaged > 200 leaves, was multiplied by the average dry mass to estimate total leaf dry mass per tree. The ratio of leaf area:leaf mass or specific leaf area (SLA) was also determined.

Freeze resistance was assessed in two ways: 1) the critical freezing temperatures at which electrolytes leaked from leaf disks at various subzero temperatures in a water-methanol bath and 2) percent stem survival following whole-plant exposure to either simulated freezes in a temperature-controlled freeze chamber or a natural freeze in the field.

To assess critical leaf freezing-point temperature, electrolyte leakage was determined from disks of 10 leaves of each tree for each of six successively lower temperatures. For each tree, 10 leaves were randomly selected and one 8-mm-diameter disk was punched from each leaf and placed in the same test tube after being rinsed with deionized water. Six replicate test tubes, one for each temperature and with 10 disks each, were inoculated with ice to prevent supercooling. The test tubes were then placed in a controlled-temperature water-methanol bath at -2 °C. Temperature in the water bath was then decreased ≈2 °C/h in six 1 h steps. At each step, one replicate tube was removed from the bath and 2 ml of deionized water added. Leaf tissues thawed slowly since ice surrounding the tissue required 2 to 5 min to melt into the solution. After all the tubes had been removed from the bath, the tubes were gently shaken overnight at room temperature and the electrical conductivity of the leaf tissue solution determined the following morning. Electrical conductivity was then expressed as a percentage of total electrical conductivity of the solution determined following freezing overnight at -14 °C in a freezer and shaken overnight as before. The precise temperature at which test tubes were removed from the water-methanol bath varied by ± 1 °C between runs. The temperatures at which 25%, 50%, and 75% of the total electrolytes were lost (LT_{25}, LT_{50}, and LT_{75}) were then calculated by interpolation. The use of three critical temperatures was more informative than just relying on one (e.g., LT_{50}) for characterizing the relationship between temperature and electrolyte leak age.

Whole-plant freeze tests were conducted in freeze chambers (Yelenosky, 1978) programmed to simulate a natural freeze by starting at 4 °C for 2 h, followed by °IC/h decrease to a temperature expected to provide discrimination of pollution effects based on stem and whole-plant survival. Two of the four replicate citrus trees and two to three replicate avocado trees from each open-top chamber were tested in the whole-plant freeze. Potential differences in supercooling of tissues were avoided by spraying trees with water when air temperature reached 0 °C. Plants were kept at 20 °C for 3 h following each freeze test. The stem survival, expressed as a percent of total stem length, was assessed 2 weeks following the test. Dead stem tissue was based on visible browning and shriveling of the bark.

Weather conditions also permitted testing freeze resistance of citrus (but not avocado) during a natural freeze in central Florida. Before the freeze, the remaining two replicate citrus trees of each rootstock from each chamber were taken to the field to a location within 30 m of a weather station. Pots were buried in the soil so that the soil surface in the pots was about level with the ground surface. On the night of the freeze (23-24 Dec. 1989), temperatures reached a minimum of -7.2 °C for l h and were less than -4 °C for ≈8 h.

Results

GROWTH. Total leaf dry mass of 'Ruby red' grapefruit on Volkamer lemon (RG/VL) was ≈59% greater than 'Ruby red' grapefruit on sour orange (RG/SO) ($P < 0.001$, Table 1). Total stem length was ≈45% greater for RG/VL than for RG/SO ($P < 0.001$). The type of rootstock also influenced the specific leaf area of the grapefruit leaves ($P < 0.01$).

Since there was a significant O_3 x acid x rootstock interaction, the O_3 and acid rain effects were analyzed for each rootstock separately (Table 1). Leaf mass of RG/VL was significantly less at 2X O_3 than at either lower or higher O_3 concentrations.

In avocado, plants exposed to 3X O_3 had substantially less leaf dry mass than those at ambient (O_3 effect: $P < 0.01$). Most of the older leaves in avocado trees in the 3X treatment had dropped by December. Therefore, average leaf age was less on 3X than ambient avocado trees, which was reflected by higher SLA in the 3X treatment (Table 1, $P < 0.01$).

In both RG/VL and avocado, O_3 interacted with acid rain ($P < 0.05$), as the total leaf dry mass of plants exposed to rain with pH 3.3 was less affected by 3X O_3 than those exposed to precipitation with pH 5.3 (Table 1). There was also evidence in RG/VL (but not avocado) that, at 0.3X O_3, rain of pH 3.3 diminished growth more than rain at pH 4.3 or 5.3. In RG/SO, however, the interaction of O_3 and acid rain on leaf dry mass was not significant (*P* = 0.32). Indeed, in RG/SO, simulated acid rain tended to increase rather than decrease the harmful effects of high O_3.

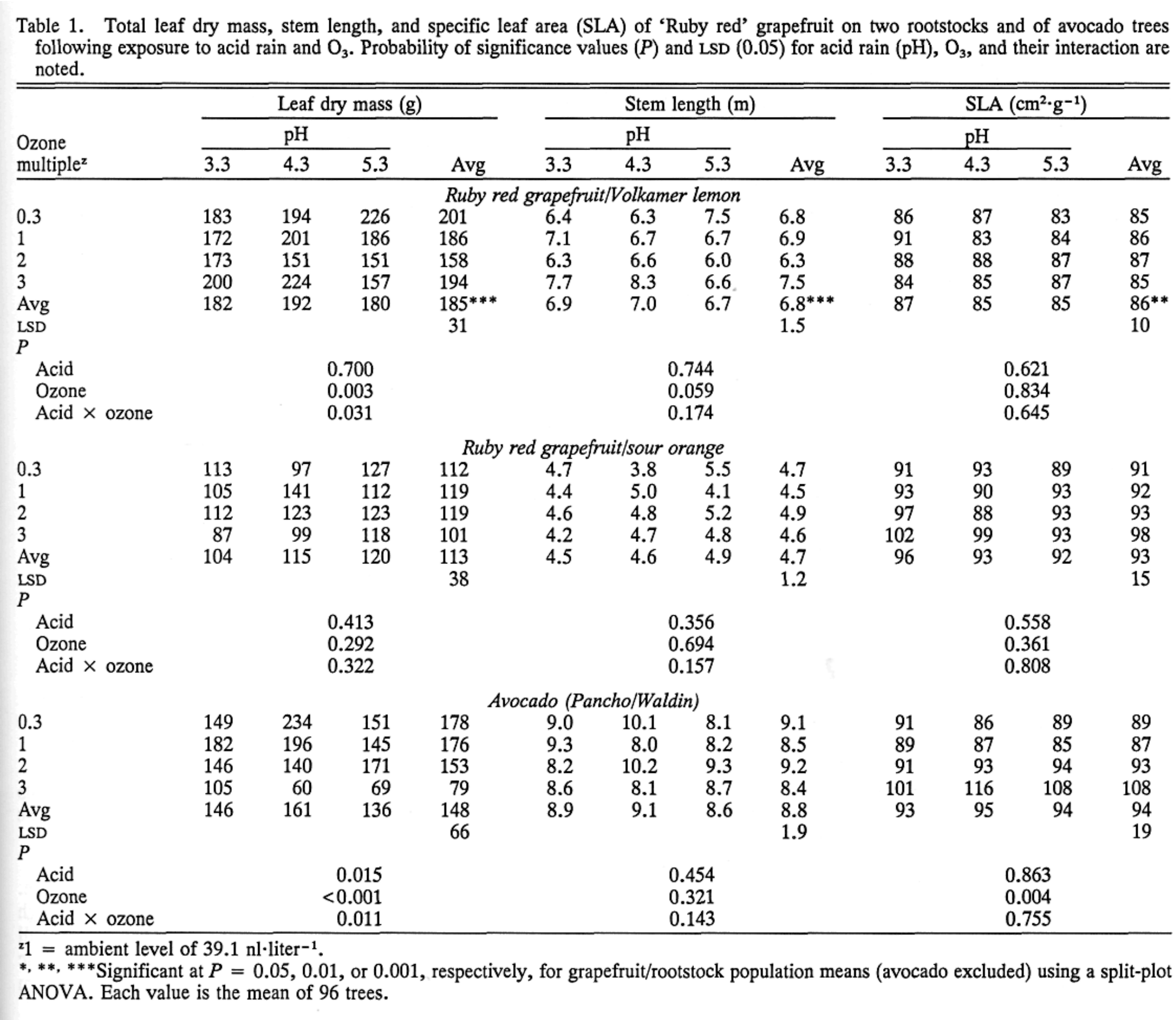

Table 1. Total leaf dry mass, stem length, and specific leaf area (SLA) of 'Ruby red' grapefruit on two rootstocks and of avocado trees following exposure to acid rain and O_3. Probability of significance values (*P*) and LSD (0.05) for acid rain (pH), O_3, and their interaction are noted.

Ozone multiple[z]	Leaf dry mass (g)				Stem length (m)				SLA ($cm^2 \cdot g^{-1}$)			
	pH				pH				pH			
	3.3	4.3	5.3	Avg	3.3	4.3	5.3	Avg	3.3	4.3	5.3	Avg
Ruby red grapefruit/Volkamer lemon												
0.3	183	194	226	201	6.4	6.3	7.5	6.8	86	87	83	85
1	172	201	186	186	7.1	6.7	6.7	6.9	91	83	84	86
2	173	151	151	158	6.3	6.6	6.0	6.3	88	88	87	87
3	200	224	157	194	7.7	8.3	6.6	7.5	84	85	87	85
Avg	182	192	180	185***	6.9	7.0	6.7	6.8***	87	85	85	86**
LSD				31				1.5				10
P												
Acid			0.700				0.744			0.621		
Ozone			0.003				0.059			0.834		
Acid × ozone			0.031				0.174			0.645		
Ruby red grapefruit/sour orange												
0.3	113	97	127	112	4.7	3.8	5.5	4.7	91	93	89	91
1	105	141	112	119	4.4	5.0	4.1	4.5	93	90	93	92
2	112	123	123	119	4.6	4.8	5.2	4.9	97	88	93	93
3	87	99	118	101	4.2	4.7	4.8	4.6	102	99	93	98
Avg	104	115	120	113	4.5	4.6	4.9	4.7	96	93	92	93
LSD				38				1.2				15
P												
Acid			0.413				0.356			0.558		
Ozone			0.292				0.694			0.361		
Acid × ozone			0.322				0.157			0.808		
Avocado (Pancho/Waldin)												
0.3	149	234	151	178	9.0	10.1	8.1	9.1	91	86	89	89
1	182	196	145	176	9.3	8.0	8.2	8.5	89	87	85	87
2	146	140	171	153	8.2	10.2	9.3	9.2	91	93	94	93
3	105	60	69	79	8.6	8.1	8.7	8.4	101	116	108	108
Avg	146	161	136	148	8.9	9.1	8.6	8.8	93	95	94	94
LSD				66				1.9				19
P												
Acid			0.015				0.454			0.863		
Ozone			<0.001				0.321			0.004		
Acid × ozone			0.011				0.143			0.755		

[z] 1 = ambient level of 39.1 $nl \cdot liter^{-1}$.

*, **, ***Significant at $P = 0.05$, 0.01, or 0.001, respectively, for grapefruit/rootstock population means (avocado excluded) using a split-plot ANOVA. Each value is the mean of 96 trees.

ELECTROLYTE LEAKAGE. In grapefruit, electrolyte leakage increased curvilinearly with successively lower temperatures; whereas in avocado, the relationship was about linear (Fig. 2). Excessive O_3 significantly ($P \leq 0.01$) diminished freeze resistance as indicated by the LT_{25}, LT_{50}, and LT_{75} in citrus and the LT_{50} and LT_{75} in avocado (Table 2). Compared with 0.3X O_3 exposure, 3X exposure (averaged over pH) caused LT_{50} to increase an average of 0.6 and 2.2 °C in citrus and avocado, respectively. There was also evidence, especially in grapefruit, that pH had a negligible effect on LT_{25}, LT_{50}, and LT_{75} at 0.3X and 1X O_3 exposure; at 2X O_3 exposure, a pH of 4.3 tended to induce the highest electrolyte leakage, and at 3X O_3 exposure, electrolyte leakage was highest with the highest acidity (Table 2; Fig. 2, acid x O_3 interaction). For example, at 3X O_3 exposure, citrus LT_{75} at pH 3.3, 4.3, and 5.3 was -5.36, -6.76, and -6.83 °C, respectively. Species of citrus rootstock in general had no effect on electrolyte leakage (Table 2). However, for LT_{75}, SO was more sensitive to O_3 exposure than VL.

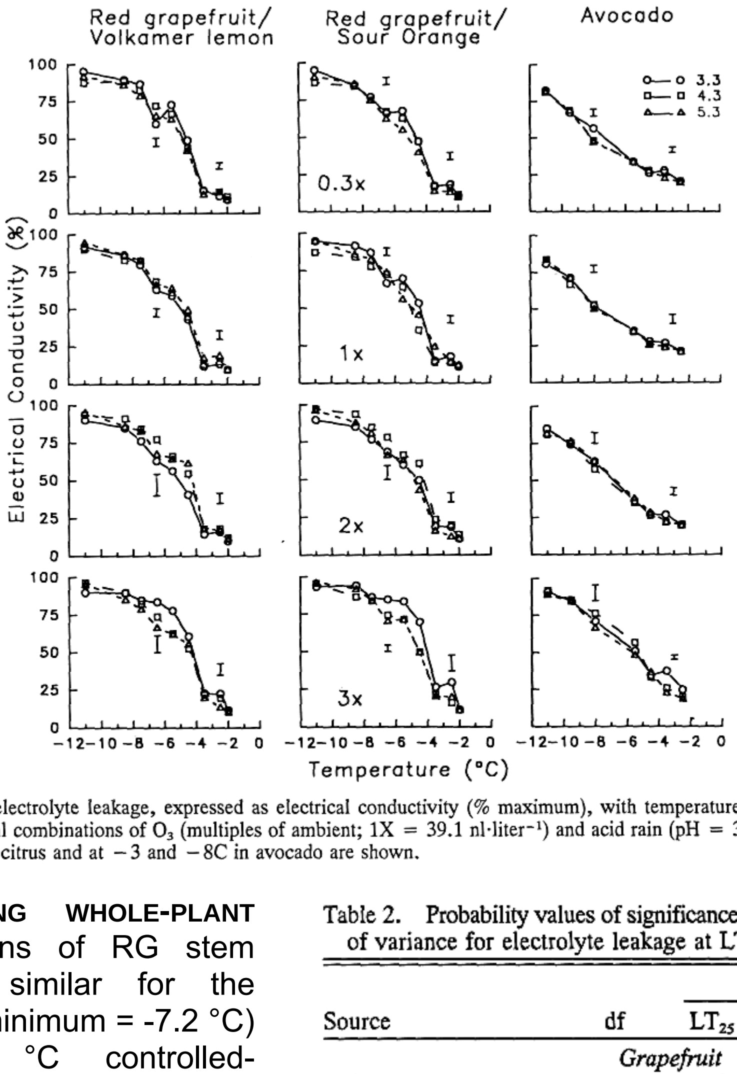

Fig. 2. Relationship of electrolyte leakage, expressed as electrical conductivity (% maximum), with temperature in grapefruit and avocado trees exposed to factorial combinations of O_3 (multiples of ambient; 1X = 39.1 nl·liter^{-1}) and acid rain (pH = 3.3, 4.3, or 5.3). Pooled SE at −2.5 and −6.5C in citrus and at −3 and −8C in avocado are shown.

SURVIVAL DURING WHOLE-PLANT FREEZES. Patterns of RG stem survival were similar for the natural freeze (minimum = -7.2 °C) and the -8 °C controlled-temperature freeze; consequently, these data were combined (Fig. 3). In general, stem survival at sub-zero temperatures was variable. As expected, because of root stock effects on freeze resistance (Yelenosky, 1985), RG/SO had higher stem survival than RG/VL (SO = 38%, VL = 25%, $P < 0.01$). In RG, species of rootstock, O_3, and acidic precipitation interacted in a complex fashion ($P < 0.01$). In VL, greater O_3 exposure generally reduced stem survival, with the acidity of the rain having little effect (O_3 effect of just VL: $P < 0.01$). However, in SO, rain with a pH of 3.3 enhanced

Table 2. Probability values of significance (*P*) for treatments in analysis of variance for electrolyte leakage at LT_{25}, LT_{50}, and LT_{75}.

		P		
Source	df	LT_{25}	LT_{50}	LT_{75}
		Grapefruit		
Acid	2	0.821	0.837	0.277
Ozone	3	0.001	0.014	0.011
Acid × ozone	6	0.025	0.181	0.035
Rootstock (R)	1	0.639	0.906	0.729
R × acid	2	0.540	0.484	0.548
R × ozone	3	0.925	0.797	0.041
R × acid × ozone	6	0.573	0.934	0.925
		Avocado		
Acid	2	0.606	0.831	0.904
Ozone	3	0.194	<0.001	<0.001
Acid × ozone	6	0.950	0.894	0.729

stem survival at low O_3 exposure (0.3X and 1X), diminished stem survival at 2X exposure, and again enhanced stem survival at 3X exposure when compared with a pH of 4.3 and 5.3 (acid x O_3 interaction of SO alone: $P = 0.02$). Avocado was similar to VL in that O_3 generally diminished stem survival with little effect of acid rain (O_3 effect: $P = 0.06$), although avocado trees exposed to ambient O_3 (1X) tended to exhibit greater stem survival than those exposed to charcoal-filtered air (0.3X) (Fig. 3).

Table 3. Leaf nutrient concentration ($mg \cdot g^{-1}$) of 'Ruby red' grapefruit on Volkamer lemon (VL) and sour orange (SO) rootstocks following exposure to acid rain and O_3. Data represent the mean nutrient concentration combined over four levels of O_3[z] (n = 8). The probability values of significance (*P*) of acid rain (pH), O_3, and their interaction are noted. There were no significant interactions of rootstock with O_3, acid, or O_3 × acid ($P > 0.25$).

	N			P			K		
	Rootstock			Rootstock			Rootstock		
	VL	SO	Avg	VL	SO	Avg[y]	VL	SO	Avg
pH 3.3	23.7	22.9	23.3	1.41	1.23	1.32 a[y]	29.0	24.8	26.9
4.3	24.8	25.4	25.1	1.66	1.58	1.62 b	27.7	24.2	26.0
5.3	25.2	25.4	25.3	1.66	1.62	1.64 b	28.5	25.6	27.0
Avg	24.6	24.6		1.58	1.48		28.4 a	24.8 b	
P									
Acid			0.146			0.027			0.584
Ozone			0.876			0.586			0.705
Acid × ozone			0.660			0.387			0.949

	Ca			Mg		
	Rootstock			Rootstock		
	VL	SO	Avg[y]	VL	SO	Avg
pH 3.3	28.6	29.3	29.0 a	5.37	4.81	5.09
4.3	25.9	24.6	25.2 b	5.51	4.45	4.98
5.3	25.6	24.3	24.9 b	5.12	4.41	4.41
Avg	26.7	26.1		5.35 a	4.55 b	
P						
Acid			0.028			0.270
Ozone			0.576			0.910
Acid × ozone			0.135			0.409

[z] 0.3X, 1X, 2X, 3X (X = ambient, 39.1 $nl \cdot liter^{-1}$).

[y] Mean separation from pH 3.3 by Dunnett's test, $P < 0.05$.

LEAF NUTRIENT CONCENTRATIONS. In grapefruit, leaves exposed to precipitation with a pH 3.3 contained less P and more Ca than leaves exposed to more neutral precipitation ($P \leq 0.05$) (Table 3). There was a tendency for N concentrations to also be less at pH = 3.3 than at pH = 4.3 or 5.3. Grapefruit leaf nutrient concentration was not affected by O_3 alone or O_3 in combination with acid rain (Table 3). In general, avocado exhibited similar nutrient concentration patterns in relation to acidity as RG for P, although differences in concentration between plants from pH = 3.3 and those from more neutral pH values were only significant at $P < 0.10$ (Table 4).

Ozone alone affected Ca and possibly Mg leaf concentrations, which may simply reflect the large effect of O_3 on avocado growth (Table 1) and leaf age. The significant interaction ($P < 0.05$) of O_3 and acid for N concentration in avocado leaves may have occurred by chance. There was no influence of pH at 0.3X or 3X O_3 exposure. At ambient (1X) O_3 exposure and at pH 3.3, 4.3, and 5.3, respectively, N concentrations were 18.7, 20.1, and 18.1 $mg\text{-}g^{-1}$. Nitrogen concentrations within the 2X O_3 exposure were 19.0, 18.6, and 22.1 $mg\text{-}g^{-1}$ for pH 3.3, 4.3, and 5.3, respectively. Consequently, we suspect that the acid x O_3 interaction for avocado N concentration was a statistical anomaly.

Table 4. Leaf nutrient concentration of 'Pancho' avocado following exposure to acid rain and O_3. Data represent the mean nutrient concentration combined over four levels of O_3 or three levels of acid. The probability values of significance (*P*) of acid rain (pH), O_3, and their interaction are noted.

Variable	N	P	K	Ca	Mg
		(mg·g⁻¹)			
pH					
3.3	19.4	1.59	10.4	18.7	6.15
4.3	20.3	1.67	10.6	16.9	5.63
5.3	20.0	1.78	10.8	17.0	5.87
Ozone multiple[z]					
0.3	20.2	1.70	10.7	18.0 a[y]	6.16
1	18.9	1.68	10.1	18.8 a	6.09
2	19.9	1.61	10.8	18.1 a	5.98
3	20.6	1.74	10.8	15.3 b	5.30
P					
Acid	0.211	0.098	0.546	0.160	0.188
Ozone	0.063	0.582	0.279	0.036	0.051
Acid × ozone	0.022	0.515	0.490	0.186	0.489

[z] 1 = ambient level of 39.1 nl·liter⁻¹.
[y] Mean separation in column by protected LSD ($P < 0.05$).

Since acidity only strongly influenced electrolyte leakage at 3X O_3 exposure in citrus, we examined the correlation of electrolyte leakage with P and Ca concentrations at this level of O_3. Although electrolyte leakage was unrelated to Ca (data not shown), it was negatively correlated *(P < 0.05)* with RG leaf P concentration for the 3X O_3 treatment (Fig. 4).

Discussion

Compared with O_3 (Eissenstat et al., 1991), the effects of simulated acid rain in citrus and avocado were generally subtle (Tables 1 and 2). In other studies, acid rain has shown greater effects (e.g., Schulze, 1989). The supplemental irrigation with water of high pH and a regular fertilization program in this study should have essentially eliminated potential indirect effects of acid rain on soil chemistry or mineral nutrient availability. Such irrigation and fertility practices are typical in Florida, where the source for irrigation water is typically groundwater in the limestone strata. We found a general decrease in leaf P concentration, an increase in Ca, and to a lesser extent, an increase in Mg at pH 3.3 compared with pH 4.3 or 5.3.

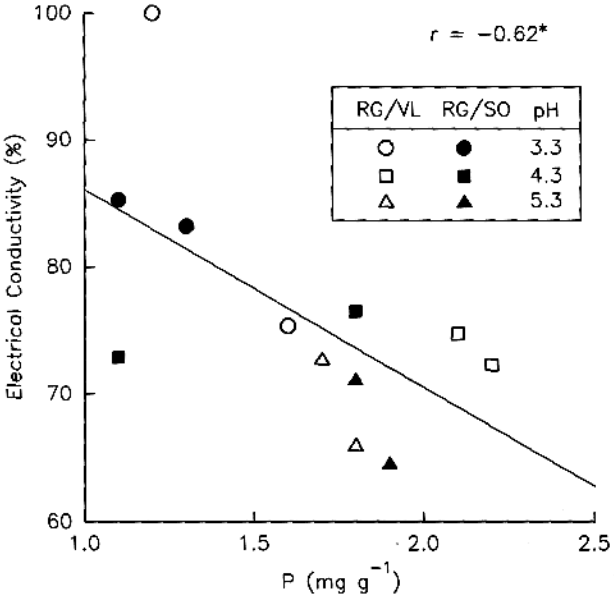

Fig. 4. Relationship of phosphorus (P) concentration in 'Ruby red' grapefruit leaves and their electrical conductivity at −6.5C for trees exposed to acid rain with a pH of 3.3, 4.3, or 5.3 at 3X O_3 (1X = 39.1 nl·liter⁻¹). Each point represents the mean response of three red grapefruit/Volkamer lemon trees or three red grapefruit/sour orange trees in a chamber. Correlation significant at $P < 0.05$ (denoted by *).

Studies in conifers frequently indicate increases in P as well as N concentrations with acid rain (Reich et al., 1988; Schulze, 1989; Westman and Temple, 1989). Presumably, the different response of leaf P concentration to acid rain exposure in our subtropical broadleaf species is attributable to our well-fertilized conditions as opposed to the nutrient-limiting condition in the studies with conifers. In addition, leaves of subtropical broad leaf evergreens commonly have thick, waxy cuticles that render the leaves relatively resistant to leaching by rain (Kriedemann and Barrs, 1981). Studies in conifers often indicate a reduction in leaf Ca and Mg concentrations with low pH (Prinz and Krause, 1988; Schulze, 1989; Westman and Temple, 1989).

However, Reich et al. (1988) found an increase in needle Ca and Mg concentrations in white pine *(Pinus strobus* L.) at pH 3.0 compared with 4.0 or 5.0. Besides possible species differences, the variable effects are likely due to differences in soil conditions, the amount of precipitation, and the acidity of the precipitation. In our study, the supplemental irrigation water, which was high in Ca, Mg, and pH, was required every 2 to 3 days in the fall when the trees were large and the amount of simulated acidic precipitation was relatively low (in accordance with long-term rainfall averages).

Besides the effect of acid rain on leaf nutrient concentration, acid rain generally had appreciable effects on growth and freeze resistance of RG and avocado only when trees were also exposed to high O_3 concentrations. Under conditions of 3X O_3, avocado and RG/VL trees exposed to rain with a pH of 3.3 had more leaf mass than trees exposed to rain with a pH of 5.3 (Table 1). In citrus, higher acidity enhanced electrolyte leakage at high O_3 (Fig. 2). Acid rain had essentially no effect on growth and electrolyte leakage at ambient O_3 concentrations. These data underscore the apparent complex plant responses associated with exposure to multiple pollutants.

The ability of precipitation of pH 3.3 to ameliorate the harmful effects of 3X O_3 on plant growth and yield may be related to acid rain causing reduced stomatal conductance, thereby limiting O_3 entry into the leaf (Ormrod, 1978). That this interaction of O_3 and acid rain on leaf mass was only found in the relatively fast-growing avocado and RG/VL but not in RG/SO (Table 1) may also relate to the greater susceptibility of fast-growing species to O_3 (Ormrod, 1978).

In contrast to growth, high acidity in the precipitation tended to exacerbate the negative effects of O_3 on electrolyte leakage in citrus, especially at LT_{25} and LT_{75} (Fig. 2; Table 2). This effect may be partly related to the reduction in leaf P concentration by rain with pH 3.3 (Table 3). Like O_3, poor P nutrition can diminish freeze resistance (Koo, 1985; McAinsh et al., 1990a, 1990b). Low P concentrations and O_3 may affect freeze resistance by reducing the integrity of the cytoplasmic membranes by diminishing their phospholipid content (Mackay et al., 1987; Sakaki et al., 1985). Consequently, the diminished P nutrition caused by acid rainfall (pH = 3.3) by itself may have been insufficient to affect electrolyte leakage. When combined with 3X O_3, however, low P may have increased susceptibility of leaves to electrolyte leakage at subzero temperatures (Fig. 4). Other possibilities for enhanced electrolyte leakage by a combination of high O_3 and acidic precipitation may be related to the O_3-induced formation of toxic organic hydroperoxides (ROOH), whose stability is enhanced by acid rain (Hewitt et al., 1990) or, in a general sense, to the detrimental effect of a combination of stresses on freeze resistance.

Although high acidity combined with high O_3 diminished freeze resistance in RG, based on electrolyte leakage of leaf disks (Fig. 2; Table 2), this effect was only weakly indicated by stem survival following whole-plant freezes in RG/VL (Fig. 3). In RG/SO, stem survival was in a direction opposite what would be expected from the electrolyte leakage results. There is conflicting evidence on the use of electrolyte leakage from leaves as an indicator of plant resistance to freezing temperatures (Scorza and Wiltbank, 1976; Wolf et al., 1986; Yadava et al., 1978). As discussed by Eissenstat et al. (1991), electrolyte leakage and stem survival indicated general patterns of an O_3-induced reduction in freeze resistance in citrus and avocado. However, measurement of electrolyte leakage of leaves (Fig. 2) may not reflect stem freeze resistance in trees of variable growth rate (Table 1).

Freeze resistance may be enhanced by moderate stress, since rapidly growing trees are commonly less freeze resistant than slower growing, more dormant trees (Syvertsen and Yelenosky, 1988; Wilcox et al., 1983; Yelenosky, 1979). Furthermore, our data support the generalization that rootstocks that produce vigorous growth, like VL, commonly cause trees to be less freeze resistant than less vigorous rootstocks like SO (Yelenosky, 1985). Such relationships between tree vigor/growth rate and freeze resistance also may have contributed to the differential stem survival of RG/VL and RG/SO to a combination of high O_3 and high acidity.

In conclusion, freeze resistance is affected by many physiological processes that may be influenced by environmental pollutants. The overall effect of O_3 and acid rain appears to depend on the concentration of the pollutant, its duration, and the growth rate of the particular cultivar. In this study, acid rain had little direct effect on subtropical evergreens exposed to low concentrations of O_3. At high O_3 concentrations, acid rain can subtly interact with O_3 by ameliorating the negative effects of O_3 on growth, especially for fast-growing species. Rain with pH 3.3 may also affect citrus freeze resistance, although whether the effect is positive or negative apparently depends on other factors that are poorly understood.

Additionally, at least in Florida, acid rain is not likely to appreciably affect growth and freeze resistance in well-managed citrus and avocado in the near future for two reasons. First, sustained rains with a pH of 3.3 are unlikely in south and central Florida (Brezonik, 1983). Second, rain of pH 3.3 was generally only important when coupled with 3X *chronic* O_3 exposure, a level that was intended to be extreme and about the worst that could be expected in the near future (Fig. 1). Daily peak exposures of 120 nl-liter^{-1} infrequently occur and only then for several hours per day near major cities (State of Florida, Dept. of Environmental Regulation, unpublished data). Thus, the danger of acid rain to citrus and avocado in Florida appears to be rather slight.

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