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Influence of Paclobutrazol in the Soil on Growth, Nutrient Elements in the Leaves, and Flood/Freeze Tolerance of Citrus Rootstock Seedlings

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Abstract. Paclobutrazol [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3ol] was applied to soil at 0, 100, or 250 mg/3.78-liter pot containing seedlings of Swingle citrumelo, Carrizo citrange, Cleopatra mandarin, sour orange, rough lemon, and Sun Chu Sha. All cultivars were sensitive to paclobutrazol, which caused a proliferation of shorter/thicker roots, and top growth showed shorter internodes and lower dry weight. Induced changes resulted in greater root/shoot ratios, and paclobutrazol treatments showed higher concentrations of nitrogen, calcium, boron, iron, and manganese in the leaves of different cultivars. Paclobutrazol-treated seedlings did not show a greater ability to tolerate flooded soil for 60 continuous days under greenhouse conditions nor survive −6.7°C controlled freeze tests. Paclobutrazol is a potentially useful plant growth regulator to dwarf citrus, but it apparently is not a strong candidate for increasing flooding and freezing tolerance in citrus rootstock seedlings.

Paclobutrazol (PP333) is a strong growth inhibitor of citrus. Foliar sprays of 10 ppm concentrations reduce internode length of rough lemon seedlings by 33%, and 10^3 ppm sprays cause a reduction of 61% (Bausher and Yelenosky 1986). Concentrations of 10–10^5 ppm in the soil progressively reduce the germination of rough lemon seed, inhibit later root formation, enlarge root cells, and tend to reduce shoot extension growth before inhibiting primary root extension (Yelenosky et al. 1993). Oxygen uptake is decreased in both root and leaf tissues, photosynthetic enzyme activity is reduced, carbon exchange levels are decreased, and chlorophyll concentrations are lower in the leaves. All of these changes contribute to marked reductions in the total biomass of citrus plants treated with paclobutrazol. Among the observations in previous work, it was noted that paclobutrazol-treated trees may express increased tolerance to flooded soil conditions because of paclobutrazol-induced aerenchyma-like roots (Vu and Yelenosky 1992). Inhibition of internode elongation and compact appearance reflect reduced growth that is associated with growth cessation and greater freeze survival in citrus (Yelenosky et al. 1987).

This report extends our observations on the effects of paclobutrazol on citrus seedlings to determine changes in root shoot ratios, mineral nutrients in leaves, and flooding and freeze tolerance of six different selections of citrus rootstock seedlings.

Abbreviations: PPFD, photosynthetic photon flux density; ANOVA, analysis of variance.

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Materials and Methods

All evaluations were done on plants germinated from seed in 3.78-liter pots containing Astatula fine sand (hyperthermic, uncoated topic quartzipsamments). Seedlings were screened for uniformity and thinned to three/pot within test populations of rough lemon (*Citrus limon* [L.] Burm. f.), sour orange no. 2 (*Citrus aurantium* L.), Sun Chu Sha (*Citrus reticulata* Blanco), Cleopatra mandarin (*Citrus reticulata*), Carrizo citrange (*Citrus sinensis* [L.] Osb × *P. trifoliata*), and Swingle citrumelo (*Citrus paradisi* Macf. × *P. trifoliata*). Plants were maintained in a 50% shaded greenhouse (approximately 1,000 μmol · s^{-1} · m^{-2} maximum photosynthetic photon flux density (PPFD) of natural daylight) with daily watering and monthly applications of fertilizer, 12N-2.6P-5K with micronutrients. Paclobutrazol, at 0, 100, or 250 mg/pot, was applied to the soil when the seedlings were 3 months old and approximately 15 ± 7 cm tall. Nine months after treatment, growth observations and leaf mineral nutrient concentrations were analyzed, and soil flooding and cold hardening tests were started.

Growth Observations

These were limited to three pots (three seedlings/pot)/concentration of paclobutrazol/citrus selection. Measurements included seedling height above soil level, diameter at midstem, and ovendry weight of stem and root for shoot/root ratios. Height and diameter were measured and calipered respectively to the nearest centimeter. Separated stems and roots at soil level were dried for 120 h at 65°C in a forced air convection oven. Means were tested for significance in ANOVA.

Nutrient Elements

Thirty leaves, 10 each from top, mid, and lower stem areas, per treatment were randomly taken from seedlings used for growth observations. Leaves were prepared and analyzed according to established procedures (Wutscher 1989). Concentrations of 13 elements were determined from duplicate leaf samples and compared with recommended levels for citrus (Wutscher and Smith 1993). Values were analyzed using ANOVA in a randomized complete block design.

Soil Flooding

Three pots/treatment were submerged in tap water in 11.3-liter pots keeping the water level approximately 5–8 cm above the soil level with daily additions. Water temperature, monitored with soil thermometers, lagged 2–3 h behind ambient greenhouse temperatures, which reached 32.2°C during the day and 20°C during the night. Relative humidity ranged from a low of 35% during the day to 98% during the night. Root systems were submerged in standing water for 60 days (12 April to 11 June 1993), and entire seedlings were observed for damage and recovery for 3 months after flooding.

Cold Hardening

These were limited to two pots/each of three paclobutrazol concentrations and four freeze intensities. Cold hardening was done under 10 continuous h of light (450 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPFD) every 24 h from incandescent/fluorescent lights in a controlled environment room for 2 consecutive weeks of 21°C days and 10°C nights, immediately followed by 2 weeks of 15°C days and 4°C nights (Yelenosky 1978). Seedlings were watered daily and relative humidity ranged from 65% to 75%. Nonhardened seedlings in an equal number of pots and treatments remained in the greenhouse until freeze tests.

Freeze Tests

Test seedlings were temperature equilibrated at 0°C for 1 h prior to lowering the temperature 5°C/h to −6.7°C for 4 h. Past freeze trials indicated that the 5°C/h decrease does not significantly contribute to any more freeze damage than that resulting with slower rates of temperature decreases. Thawing rates also were approximately 5°C/h, and seedlings were kept at about 25°C room temperature for 4 h before returning to greenhouse conditions. Seedlings were observed for damage for 5 weeks.

Results and Discussion

Growth

All seedlings showed marked growth reduction with paclobutrazol in the soil (Table 1). Mean height was approximately 37–69% less, and stem diameters were approximately 12–50% thinner. Differences among cultivars on avoidance/tolerance to paclobutrazol were not discernible in this study. That all cultivars were susceptible to the growth regulator in the soil was supported by similar responses to concentration by all cultivars. There were fewer flushes, shortened internodes, smaller leaves, smaller stem diameters, and smaller root systems with larger diameter and less fibrous roots. Increasing the level of paclobutrazol in the soil resulted in higher root/shoot ratios, which in some instances doubled over the period of the study. This was due to greater dry weight decreases in shoots than roots. None of the cultivars tested showed a strong ability to overcome the inhibitory influence in pots, although in some instances rough lemon was observed to produce new top growth while other seedlings remained quiescent. This is in line with the long lasting effect of paclobutrazol in the soil (Yelenosky et al. 1993), which probably decreases as new roots occupy soil spaces that have low concentrations of the chemical. Paclobutrazol is relatively immobile in soils (Anderson and Aldrich 1987, Yelenosky et al. 1993). This would help to maintain the inhibitory influence of paclobutrazol in field applications. We know from personal experience that scion bud grafting on potted rootstock seedlings, which were previously exposed to paclobutrazol in the soil, will result in dwarfed trees. The inhibitory effect of paclobutrazol apparently does not prevent callus formation and successful scion healing. The results of this study support paclobutrazol as one of the more potent growth regulators of citrus germplasm (Yelenosky et al. 1987); fast growing seedlings used as rootstocks, like rough lemon, are more likely to overcome the inhibitory influence. If different levels of gibberellins exist in these rootstocks, then one might suspect different abilities to overcome the antigibberellin growth-retarding effect of paclobutrazol (Saidha et al. 1983).

Flooding Tolerance

There was no strong evidence to support any benefit of altering citrus root systems with paclobutrazol to increase the chances of survival during pro-

Table 1. Effect of paclobutrazol (PP333) in soil on the growth of seedlings of different citrus cultivars in pots under greenhouse conditions.[a]

Cultivar and amount of PP333 (mg pot^{-1})	Height (cm)	Midstem diameter (cm)	Dry weight Roots (g)	Dry weight Shoots (g)	Dry weight Root/shoot ratio
Rough lemon					
0	81	0.07	67	48	1.4
100	36	0.5	37	20	1.8
250	30	0.6	35	16	2.1
ANOVA	**	**	*	**	*
r	−0.90	−0.89	−0.66	−0.96	0.63
Sour orange					
0	74	0.8	52	37	1.4
100	26	0.4	22	14	1.6
250	23	0.04	25	11	2.3
ANOVA	**	**	**	**	*
r	−0.93	−0.88	−0.83	−0.94	0.60
Swingle citrumelo					
0	58	0.7	31	24	1.3
100	33	0.5	22	9	2.4
250	33	0.5	20	7	2.8
ANOVA	**	**	**	**	**
r	−0.95	−0.91	−0.91	−0.96	0.92
Cleopatra mandarin					
0	71	0.6	23	25	0.9
100	39	0.5	20	17	1.5
250	34	0.4	19	9	2.2
ANOVA	*	*	NS	*	**
r	−0.74	−0.60	−0.22	−0.55	−0.82
Carrizo citrange					
0	86	0.9	53	41	1.3
100	54	0.6	46	16	2.9
250	45	0.5	29	12	2.5
ANOVA	**	**	*	**	*
r	−0.95	−0.94	−0.62	−0.97	0.67
Sun Chu Sha					
0	73	0.7	34	28	1.2
100	29	0.4	18	11	1.6
250	29	0.4	16	7	2.3
ANOVA	**	**	**	**	**
r	−0.95	−0.91	−0.91	−0.96	0.92

[a] Data are the means of 12 plants for each dosage. Responses significant at $P = 0.05$ (*) or 0.01 (**). Correlation of coefficient (r) reflects quadratic regression fit. NS, not significant.

longed and continuous soil flooding (Table 2). The suggestion that paclobutrazol-induced aerenchyma-like roots may increase the tolerance of seedlings to flooded soil conditions (Vu and Yelenosky 1992) is seriously limited by the rate of degradation of the original root system. Paclobutrazol-induced roots in excellent condition were found after 60 days of soil flooding provided the degradation of the main roots had not progressed beyond their point of attachment. Otherwise, the paclobutrazol-induced roots were killed. There were no instances in which flooding killed paclobutrazol-induced roots when the original roots were still alive. These observations suggest that the flood tolerance of paclobutrazol-induced roots is largely academic in citrus survival under prolonged soil flooding since any practical benefit would depend on the flooding tolerance of the original roots.

Flooded plants of each rootstock not treated with paclobutrazol were rated according to their overall appearance and prognosis to survive after flooding. Swingle citrumelo and Carrizo citrange were rated as excellent, large flower trifoliate orange as good, sour orange as fair, rough lemon and Cleopatra mandarin as poor to fair, and Sun Chu Sha as poor with the most damage and essentially no chance to survive once the water was drained from the soil. Ratings are generally in line with earlier ratings by

Table 2. Flood and freeze damage to citrus rootstock seedlings growing in soil with different amounts of paclobutrazol (PP333).

	Flood damage (%)[a,b]				Freeze damage (%)[a,c]			
	Flooded		Nonflooded		Cold hardened		Nonhardened	
Rootstock and amount of PP333 (mg pot^{-1})	Leaves lost	Stem dieback	Leaves lost	Stem dieback	Leaves lost	Stem dieback	Leaves lost	Stem dieback
Rough lemon								
0	70 ± 30	36 ± 36	0	0	100	75 ± 5	100	100
100	41 ± 41	27 ± 27	0	0	100	51 ± 37	100	100
250	51 ± 18	30 ± 25	0	0	100	44 ± 38	100	100
Sour orange								
0	36 ± 13	0	0	0	80 ± 20	23 ± 12	100	100
100	26 ± 26	7 ± 7	0	0	90 ± 10	14 ± 14	100	100
250	76 ± 24	5 ± 5	0	0	74 ± 16	20 ± 20	100	100
Swingle citrumelo								
0	47 ± 26	0	0	0	80 ± 5	0	100	100
100	22 ± 10	0	0	0	25 ± 10	0	100	100
250	37 ± 15	0	0	0	56 ± 44	0	100	100
Cleopatra mandarin								
0	86 ± 12	22 ± 22	0	0	100	30 ± 2	100	100
100	90 ± 10	75 ± 25	0	0	50 ± 36	0	100	100
250	100	84 ± 16	0	0	100	74 ± 7	100	100
Carrizo citrange								
0	31 ± 24	0	0	0	60 ± 10	0	100	100
100	77 ± 23	17 ± 17	0	0	25 ± 5	0	100	100
250	73 ± 27	0	0	0	43 ± 21	0	100	100
Sun Chu Sha								
0	98 ± 2	71 ± 29	0	0	100	1 ± 1	100	100
100	100	99 ± 1	0	0	100	56 ± 31	100	100
250	95 ± 5	79 ± 21	0	0	100	0	100	100

[a] ±S.D.
[b] Roots submerged 2 inches above soil level for 60 continuous days.
[c] −7°C for 4 h.

other researchers (Ford 1964). Flooding variables such as low soil pH, the presence of root rot organisms, the production of toxic sulfides, low oxygen content of the flooded soil condition, and high temperatures often interact with root development and make flood injury evaluations difficult in citrus groves (Tucker et al. 1992). Sun Chu Sha, a relatively new rootstock in the industry, may merit some caution in "wetland" plantings regardless of its apparent tolerance to *Phytophthora* spp. (Davenport and Rouse 1992). Roots of Sun Chu Sha were more active in oxygen uptake than those of rough lemon, Cleopatra mandarin, and other citrus rootstocks in a recent study (Wutscher et al. 1993).

Freeze Tolerance

There was also no strong evidence to suggest that paclobutrazol contributes significantly to increased cold acclimation of the seedlings in this study (Table 2). This does not necessarily detract from instances in which triazole growth retardants are associated with increased cold hardiness in *Actinida* (Tafazoli and Bey 1993) and ancymidol and AMO-1618 with citrus (Yelenosky 1987).

Foliar Mineral Elements

Leaf analyses for 13 different mineral elements showed changes in concentrations associated with paclobutrazol soil treatments (Table 3). Causes of these changes are not known, but numerous physiologic factors such as differences in absorption, translocation, and redistribution could be involved (Syvertsen et al. 1983). Leaves from paclobutrazol-treated citrus seedlings had significantly higher concentrations of nitrogen, calcium, and boron for all of the rootstocks. Concentrations of iron and manganese increased in five of the six rootstocks. There was no strong indication that paclobutrazol would increase plant toxicity through greater uptake of sodium, chlorine, and copper. Overall, changes noted

Table 3. Concentration of mineral elements in leaves of citrus rootstock seedlings growing in soil with paclobutrazol (PP333).

Rootstock and amount of PP333 (mg pot^{-1})	% Dry weight								ppm				
	N	P	K	Ca	Mg	S	Cl	Na	Fe	Mn	Zn	Cu	B
Sour orange													
0	1.33[a]	0.11	0.98	1.12	0.30	0.21	0.03	0.01	81	13	24	4	48
100	2.70**	0.12	1.15*	1.62*	0.28	0.25	0.05	0.05	123*	35**	22	6	82**
250	3.10*	0.10	0.92	1.35*	0.17*	0.20	0.04	0.04	158*	33**	21	6	97**
Rough lemon													
0	1.33	0.12	1.00	2.44	0.40	0.21	0.05	0.04	133	11	42	9	69
100	2.56**	0.17	1.10*	2.52*	0.39	0.35*	0.05	0.02	183**	29*	36	9	76
250	2.72*	0.15	1.11*	2.80*	0.37	0.35*	0.05	0.02	155*	37**	36	9	121**
Swingle citrumelo													
0	2.26	0.13	1.34	2.05	0.34	0.46	0.03	0.02	149	18	24	9	79
100	3.12*	0.16	0.94**	3.11**	0.29	0.47	0.04	0.02	175*	36*	21	7	148**
250	3.13*	0.14	0.78**	3.20**	0.28	0.46	0.05	0.04	195**	31*	19	8	158**
Cleopatra mandarin													
0	1.46	0.11	1.10	1.24	0.26	0.16	0.01	0.02	60	13	19	4	45
100	2.41**	0.17*	1.02*	2.36**	0.35*	0.24*	0.03	0.03	55	15	19	4	68*
250	2.43**	0.16*	1.05	2.37**	0.28	0.23*	0.03	0.05*	66	20*	16	5	92**
Carrizo citrange													
0	2.42	0.14	1.16	2.55	0.32	0.56	0.09	0.02	266	18	35	8	99
100	3.40*	0.16	0.78**	3.20**	0.31	0.63*	0.17*	0.01	236*	21	24	6	140**
250	3.34*	0.16	0.81**	2.86*	0.29	0.50*	0.05	0.01	217**	16	30	8	153**
Sun Chu Sha													
0	1.92	0.11	1.00	1.42	0.37	0.25	0.02	0.07	93	19	33	8	54
100	2.64**	0.19*	0.92*	1.90**	0.45*	0.24	0.03	0.07	77*	23	20*	6	68*
250	2.76**	0.19*	0.72**	1.52*	0.24**	0.28	0.04	0.22**	186**	42**	23*	7	116**

[a] ANOVA, * significant at the 5% level, ** significant at the % level.

in this study show no indications toward strong deficiency or excess (Wutscher and Smith 1993). Apparently, paclobutrazol may alter the concentrations of mineral elements in citrus leaves through changes in root morphology, but such changes do not appear harmful.

In contrast to the above, flooded sour orange and rough lemon seedlings without paclobutrazol showed definite nutrient element losses when compared with mineral element levels found in nonflooded seedlings. Calcium and manganese showed the greatest percentage loss (Fig. 1). However, these data only pertain to postflooding since no preflooding measurements were made. In other studies the initial effect of flooding is usually reduced ion absorption that results in decreases of mineral elements in the leaves of plants (Kozlowski and Pallardy 1984). Such losses seemingly are secondary to the deterioration of cell membranes and the root system, which will largely determine plant recovery following flooding.

In summary, paclobutrazol continues to be one of

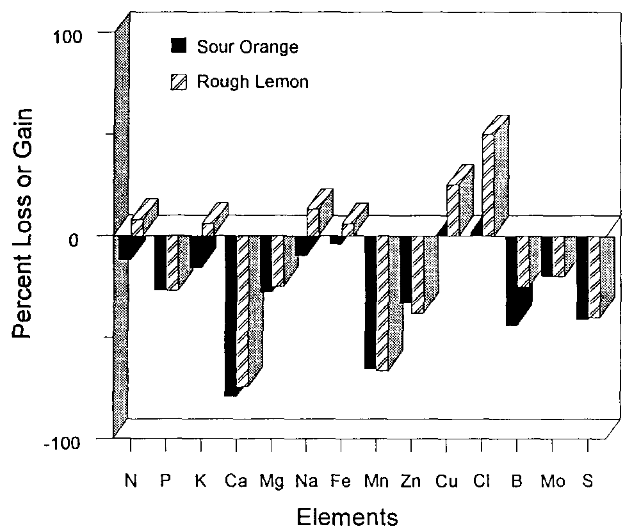

Fig. 1. Relative percent change in concentrations of mineral elements in leaves of two citrus rootstock seedlings flooded for 30 continuous days.

the most potent regulators of citrus growth and development. Paclobutrazol-induced, dwarf-like plants have been suggested in creative uses of citrus for space-limiting landscape situations (Yelenosky et al. 1993). The observations made in flooding and freeze trials of citrus seedlings, commonly used as rootstocks, in this report do not support any practical benefit of using paclobutrazol to combat flooding and freeze stress. On the other hand, the use of paclobutrazol apparently does not diminish the inherent tolerance to prolonged flooded soil conditions and severe freezes, nor does it result in drastic excesses and deficiencies in leaf nutrients.

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