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Growth and Photosynthesis of Sweet Orange Plants Treated with Paclobutrazol

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Abstract. Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], formulated as GFU 265, applied at 100, 250, and 500 mg plant^{-1} to the soil of container-grown sweet orange [*Citrus sinensis* (L.) Osbeck cv. Valencia], suppressed plant weight, stem height, leaf size, and total leaf area. At the 500-mg dosage, total plant dry weight was reduced by 61%, stem height by 74%, and both leaf biomass and area by 80%, as compared to control plants. All paclobutrazol dosages induced fibrous root thickening and increased their soluble sugar and starch content. Fresh root biomass was 14 to 40% higher and root:shoot ratios were increased three- to sixfold for treated plants. Paclobutrazol applications of 250 and 500 mg plant^{-1} reduced leaf photosynthetic rate, ribulose bisphosphate carboxylase activity, total nonstructural carbohydrates, and dark respiration 70 to 80% of the control plants. Reductions of leaf photosynthetic rate, carboxylase activity, and photosynthate by paclobutrazol contributed to biomass reduction in treated sweet orange.

Plant growth regulators have potential for manipulating growth of many agricultural crops. In citrus, compounds eliciting responses in tree growth and acclimatization to environmental stresses are of potential importance to commercial growers (Yelenosky 1985). Several plant growth regulators have been examined for their potential for controlling excessive shoot growth and for enhancing cold acclimation in citrus trees (Aron et al. 1985, Krezdorn and Cohen 1962, Lima and Davies 1984, Yelenosky 1985). Such compounds can influence a variety of physiological and biochemical processes; however, there is limited information available on these processes in citrus.

The present study was conducted to evaluate the responses of sweet orange plants to paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], an inhibitor of gibberellin synthesis. We focus on growth and some critical physiological processes associated with the tolerance of sweet orange to soil-applied paclobutrazol. Reduction of vegetative growth by this compound has been reported in a number of fruit tree species (Davis et al. 1988).

Materials and Methods

Plant Materials and Growth Conditions

Seeds of sweet orange [*Citrus sinensis* (L.) Osbeck cv. Valencia] were germinated in 2.5-L plastic pots containing washed sand. Potted plants were grown in a greenhouse, watered daily, and fertilized biweekly with 500 ml of a 1.5% solution of 15-7-7 (N-P-K) liquid fertilizer per pot. During the study period, maximum day and minimum night temperatures inside the greenhouse were 32 and 20°C, respectively, and relative humidity fluctuated from 50 (day) to 90% (night). The photosynthetic active radiation (PAR, 400–700 nm) inside the greenhouse was about 900–1000 μmol m^{-2} s^{-1} at plant shoot levels during midday.

Paclobutrazol Treatment

Plants having similar appearance and height were selected for the experiment 90 days after seed planting. Plants were arranged into four groups of 24 plants each. Paclobutrazol, formulated as GFU 265, was obtained from ICI Americas Inc. (Goldsboro, NC, USA) as a liquid formulation (50% methanol and 50% Renex 30, a nonionic surfactant) containing 50 g L^{-1} of active ingredient. Appropriate dilutions were made using the blank liquid (50% methanol and 50% Renex 30) as provided by the same company.

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Twenty-five milliliters of the diluted solutions containing 0 (control), 100, 250, and 500 mg paclobutrazol were applied around the base of each plant for each dosage group. All measurements and analyses were performed 4 months after treatment.

Measurements of Leaf Photosynthesis

Photosynthetic CO_2 exchange rates (CER) of the uppermost, fully expanded leaves were measured inside the greenhouse where plants were grown and maintained, from 0900–1200 EST, using the closed gas-exchange LI-6200 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA), as previously reported (Vu and Yelenosky 1988). During a 3-h morning measurement, an average of 32 plants, or eight plants for each dosage, were measured. Data on CER of field-grown sweet orange from previous studies indicate maximum CER light saturation occurred at about 600–800 μmol m^{-2} s^{-1} solar PAR; further increases in solar PAR did not enhance leaf CER of sweet orange (Vu and Yelenosky 1988). For paclobutrazol-treated sweet orange plants, leaf CER measurements were made on two clear mornings, during which PAR levels inside the greenhouse increased from about 500 (0900 EST) to 1000 (at 1200 EST) μmol m^{-2} s^{-1}. Only CER values at PAR of 600 μmol m^{-2} s^{-1} or higher were used. The values of CER were expressed on the basis of leaf area, determined after each measurement with the LI-COR LI-3000 Portable Area Meter.

Determinations of Leaf Rubisco and Chlorophyll

Leaf sampling procedures and storage conditions for ribulose bisphosphate carboxylase-oxygenase (Rubisco) and for chlorophyll determinations have previously been described (Vu and Yelenosky 1988). Briefly, uppermost, fully expanded leaves were detached near midday from six plants for each treatment, plunged immediately into liquid N_2, ground to a powder with a mortar and pestle, and continuously stored in liquid N_2 until analysis. The extractions and radiochemical assays of HCO_3^-/Mg^{2+}-activated Rubisco were performed as reported previously (Vu and Yelenosky 1988). Total chlorophyll was extracted with 80% (vol/vol) acetone and its concentration determined by the method of Arnon (1949).

Nonstructural Carbohydrates and Dark Respiration

Uppermost, fully expanded leaves were harvested near midday from six plants for each treatment, oven-dried at 55°C for 72 h, ground to a powder, and subsampled for carbohydrate determinations. Starch and soluble sugars were extracted and analyzed according to previously reported procedures (Vu and Yelenosky 1989). Dark respiration was determined on leaf discs (0.6-cm diameter) and fibrous root tips (0.5-cm long) with a Gilson differential respirometer, using standard Warburg procedures (Umbreit et al. 1966). Discs were removed with a hole puncher from uppermost, fully expanded leaves of five plants for each dosage, and four discs per leaf replicated three times per dosage were arbitrarily assigned to flasks. Root tips were sampled from three plants for each dosage after roots were washed and rinsed thoroughly with water. The flasks, which contained 0.2 ml of 10% (wt/vol) KOH in the center well, 1 ml of 67 mM potassium phosphate buffer (pH 6.7), and 40 leaf discs, were equilibrated for 1 h at 30°C before readings were taken. Dosage effects were based on the volume of O_2 uptake at 15-min intervals for 1 h.

Biomass Partitioning

Eight plants of each dosage were harvested individually and weights of stems, leaves, and roots were determined before and after drying at 55°C for 72 h. Total leaf area of each plant was measured with the LI-3000 Area Meter.

Results

Paclobutrazol inhibited growth of sweet orange plants at all dosages (Table 1). The 500-mg level reduced total plant fresh weight by 44% and total plant dry weight by 61%. Leaf fresh and dry weights and area were reduced by about 80%. Shoot development of plants treated with paclobutrazol was severely depressed (Fig. 1), with stem height and weight reduction of up to 82% of the control plants (Table 1). Root biomass on a fresh weight basis, however, was 14–40% higher for plants treated with paclobutrazol, as compared to control plants (Table 1). Increases in root fresh weight of treated plants were mostly associated with abnormal thickening of the fibrous root system (Fig. 2). On the dry weight basis, however, there was a 17% reduction in root biomass for plants treated with 250 and 500 mg paclobutrazol (Table 1).

Paclobutrazol treatment reduced total number of leaves as well as leaf size (Table 2). Leaf weight and area reduction averaged 39% for the 100-mg level, and 68% for both the 250- and 500-mg levels (Table 2). Specific leaf weight changes (FW) were mostly confined to the 500-mg level where it was reduced about 18%.

There was no apparent difference in leaf CER between the 100-mg paclobutrazol-treated and control plants (Table 3), but leaf photosynthetic assimilation rates of plants treated with 250 and 500 mg paclobutrazol were depressed by 12 and 27% of the control, respectively. We noted no chlorotic symptom development on leaves of treated plants. Pigment extraction and analysis indicated no differences in leaf chlorophyll concentration among the dosages (Table 3). Activities of Rubisco were, however, decreased in extracts from leaves of paclobutrazol-treated plants. Rubisco activity inhibition averaged 18% for the 100-mg level, and 30% for both the 250- and 500-mg levels.

Soluble sugars, starch, and dark respiration were lower in leaves of plants treated with 250- and 500-mg paclobutrazol (Table 4). At these dosages, inhibitions averaged 15, 24, and 29% for leaf soluble

Table 1. Fresh and dry weights of plant parts, stem height, and leaf area of sweet orange plants treated with paclobutrazol.

Paclobutrazol (mg plant^{-1})	Fresh weight				Dry weight				Stem height (cm)	Leaf area (cm^2 plant^{-1})
	Leaf (g plant^{-1})	Stem	Root	Total	Leaf (g plant^{-1})	Stem	Root	Total		
0	11.3	5.2	10.8	27.3	3.4	1.8	2.4	7.6	39.1	498.3
100	4.9	1.3	15.1	21.3	1.6	0.5	2.5	4.6	16.2	205.2
250	3.0	0.9	12.3	16.2	0.9	0.3	2.0	3.3	11.9	129.5
500	2.2	0.8	12.2	15.2	0.7	0.3	2.0	3.0	10.3	92.8

Data are the means of eight plants for each dosage. Quadratic regression equations for the responses as functions of paclobutrazol dosages (x), with correlation coefficient (r) and nonsignificant (NS) or significant at p = 0.05 (*) or 0.01 (**), are presented below. Leaf fresh wt = 1.07E1 − 5.14E-2(x) + 6.93E-5(x^2), (r = 8.52E-1; **); stem fresh wt = 4.66E0 − 2.76E-2(x) + 4.02E-5(x^2), (r = 8.01E-1; **); root fresh wt = 1.18E1 + 1.44E-2(x) − 2.82E-5(x^2), (r = 1.66E-1; NS); total fresh wt = 2.72E1 − 6.52E-2(x) + 8.21E-5(x^2), (r = 6.03E-1; **); leaf dry wt = 3.26E0 − 1.55E-2(x) + 2.11E-5(x^2), (r = 8.60E-1; **); stem dry wt = 1.64E0 − 9.48E-3(x) + 1.38E-5(x^2), (r = 8.22E-1; **); root dry wt = 2.48E0 − 1.41E-3(x) + 7.20E-7(x^2), (r = 2.61E-1; NS); total dry wt = 7.38E0 − 2.64E-2(x) + 3.56E-5(x^2), (r = 7.58E-1; **); stem height = 3.65E1 − 1.73E-1(x) + 2.44E-4(x^2), (r = 8.72E-1; **); leaf area = 4.68E2 − 2.30E0(x) + 3.13E-3(x^2), (r = 8.53E-1; **).

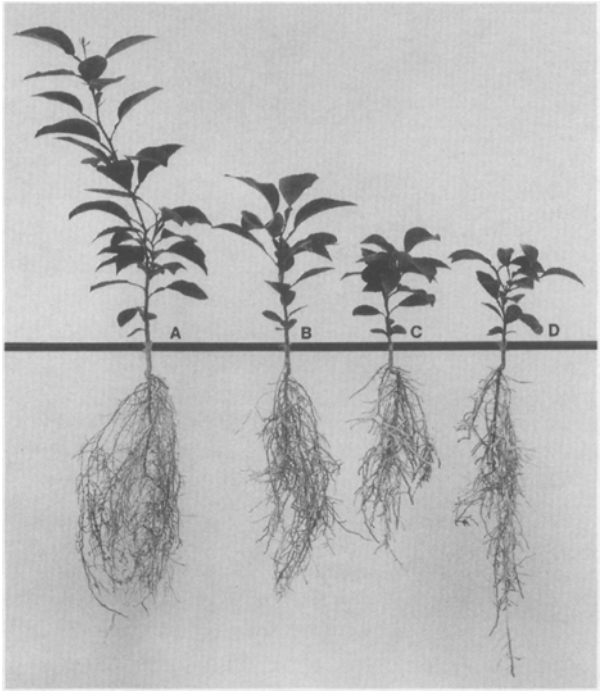

Fig. 1. Paclobutrazol effects on sweet orange plants 4 months after soil treatment with 0 (**A**), 100 (**B**), 250 (**C**), and 500 (**D**) mg plant^{-1} of the inhibitor. Paclobutrazol in a liquid form (50% methanol and 50% Renex 30) was applied directly to the root zone of 3-month-old plants.

Fig. 2. Morphological changes in the fibrous root systems of sweet orange plants 4 months after soil treatment with 0 (**A**) and 500 (**D**) mg plant^{-1} of paclobutrazol. The inhibitor in a liquid form (50% methanol and 50% Renex 30) was applied directly to the root zone of 3-month-old plants.

sugars, starch, and dark respiration, respectively, and total nonstructural carbohydrates were reduced about 22%. Paclobutrazol, however, induced accumulation of soluble sugars and starch in fibrous roots (Table 4), where sugar concentration was as much as 50% higher in treated vs. control roots. Although sugar concentrations were higher in paclobutrazol-treated fibrous roots, dark respiration rates were depressed to almost 50% of controls.

Discussion

Data from this and related studies (Aron et al. 1985, Swietlik and Fucik 1988, Yelenosky et al. 1987) show that paclobutrazol effectively retarded growth of citrus. In the case of sweet orange, application of paclobutrazol to leafy branches at an early reproductive stage restricted leaf and fruit growth (Yelenosky et al. 1987). Foliage sprays or soil applications of paclobutrazol to citrus reduced shoot

Table 2. Fresh and dry weight, area, and specific leaf weight (SLW) for sweet orange plants treated with paclobutrazol.

Paclobutrazol (mg plant^{-1})	Fresh wt (mg)	Dry wt (mg)	Area (cm^2)	SLW FW (mg cm^{-2})	SLW DW (mg cm^{-2})
0	540.2	192.8	24.1	22.4	8.1
100	318.3	122.3	14.6	21.7	8.4
250	169.3	64.2	8.3	20.3	7.8
500	160.1	56.8	8.6	18.5	6.7

Data are the means of 10 uppermost expanded leaves for each dosage. Quadratic regression equations for the responses as functions of paclobutrazol dosages (x), with correlation coefficient (r) and nonsignificant (NS) or significant at p = 0.05 (*) or 0.01 (**), are presented below.
Fresh wt = 5.31E2 − 2.25E0(x) + 3.03E-3(x^2), (r = 8.91E-1; **); dry wt = 1.91E2 − 7.59E-1(x) + 9.80E-4(x^2), (r = 8.66E-1; **); area = 2.37E1 − 9.68E-2(x) + 1.34E-4(x^2), (r = 9.04E-1; **); SLW (FW area^{-1}) = 2.23E1 − 8.11E-3(x) + 9.18E-7(x^2), (r = 7.85E-1; **); SLW (DW area^{-1}) = 8.33E0 + 1.64E-3(x) − 9.08E-6(x^2), (r = 2.33E-1; NS).

Table 3. CO_2 exchange rates (CER), Rubisco activities, and total chlorophyll concentration in uppermost, fully expanded leaves of sweet orange plants treated with paclobutrazol.

Paclobutrazol (mg plant^{-1})	CER (μmol m^{-2} s^{-1})	Rubisco (μmol g^{-1} FW h^{-1})	Chlorophyll (mg g^{-1} FW)
0	9.7	719.1	3.2
100	9.3	587.9	3.2
250	8.5	501.7	3.1
500	7.1	502.9	3.1

Data are the means of 10–14 measurements dosage^{-1} for CER and three measurements dosage^{-1} for Rubisco and chlorophyll. Quadratic regression equations for the responses as functions of paclobutrazol dosages (x), with correlation coefficient (r) and nonsignificant (NS) or significant at p = 0.05 (*) or 0.01 (**), are presented below.
CER = 9.65E0 − 3.97E-3(x) − 2.12E-6(x^2), (r = 7.06E-1; **); Rubisco = 7.12E2 − 1.25E0(x) + 1.50E-3(x^2), (r = 9.68E-1; **); chlorophyll = 3.23E0 − 2.51E-4(x) + 1.53E-7(x^2), (r = 4.02E-1; NS).

Table 4. Concentrations of soluble sugars, starch, and total nonstructural carbohydrates (TNC) and dark respiration rates in leaves and fibrous roots of sweet orange plants treated with paclobutrazol.

	Leaf				Roots			
Paclobutrazol (mg plant^{-1})	Soluble sugars	Starch (mg g^{-1} DW)	TNC	Dark respiration (μl O_2 mg^{-1} DW h^{-1})	Soluble sugars	Starch (mg g^{-1} DW)	TNC	Dark respiration (μl O_2 mg^{-1} DW h^{-1})
0	82.3	142.8	225.2	1.4	88.1	27.5	115.5	5.9
100	74.6	148.2	222.8	1.4	120.6	34.7	155.3	6.8
250	69.2	111.9	181.1	1.0	133.1	41.4	174.2	3.3
500	70.1	103.7	172.8	1.0	131.3	33.5	164.8	3.1

Data are the means of three determinations dosage^{-1}. Quadratic regression equations for the responses as functions of paclobutrazol dosages (x), with correlation coefficient (r) and nonsignificant (NS) or significant at p = 0.05 (*) or 0.01 (**), are presented below.
Leaf soluble sugars = 8.21E1 − 8.19E-2(x) + 1.16E-4(x^2), (r = 9.44E-1; **); leaf starch = 1.49E2 − 1.39E-1(x) + 9.15E-5(x^2), (r = 9.07E-1; **); leaf TNC = 2.31E2 − 2.19E-1(x) + 2.0E-4(x^2), (r = 9.43E-1; **); leaf dark respiration = 1.45E0 − 2.05E-3(x) + 2.36E-6(x^2), (r = 8.95E-1; **); root soluble sugars = 9.06E1 + 2.87E-1(x) − 4.14E-4(x^2), (r = 9.72E-1; **); root starch = 2.72E1 + 9.58E-2(x) − 1.66E-4(x^2), (r = 9.06E-1; **); root TNC = 1.18E2 + 3.83E-1(x) − 5.80E-4(x^2), (r = 9.70E-1; **); root dark respiration = 6.53E0 − 1.16E-2(x) + 8.62E-6(x^2), (r = 7.92E-1; **).

growth and plant biomass (Aron et al. 1985, Swietlik and Fucik 1988, Yelenosky et al. 1987).

Paclobutrazol reduced Rubisco activity at all concentrations evaluated (Table 3). However, there was little detectable effect on leaf CER at the 100-mg dosage (Table 3). Because CER measurements were made on single leaves and at a single growth stage, our data do not imply that net photosynthesis of the whole plant throughout the experimental period was unaffected by the 100-mg paclobutrazol application. Total biomass accumulation on a dry weight basis was depressed by 40%, and stem height and leaf area were reduced by 60% for the 100-mg dosage (Table 1). The accumulation of plant biomass, which reflects a summation of effects throughout the experimental growth period, is effective for evaluating whole plant response to a treatment. Even though inhibition of leaf CER was not as obvious for the 100-mg as for the 250- and 500-mg levels, reductions in both individual and total leaf area per plant apparently contributed to a significant reduction in whole plant photosynthetic carbon assimilation capability (Tables 1 and 2). Using CER data (Table 3) and total leaf area per plant (Table 1), the rates of photosynthetic carbon assimilation of the whole plant were estimated, by assuming that CO_2 exchange rates were uniform over the entire plant. On this basis, nmol CO_2 fixed/s were 481 (control), 190 (100-mg level), 110 (250-mg level), and 66 (500-mg level), which represent reductions 60–86% in whole plant photosynthetic capability.

Reduced leaf CER and Rubisco activity appar-

ently resulted in lower soluble sugar and starch concentrations in leaves of paclobutrazol-treated plants, which also showed lower leaf dark respiration rates (Table 4). In contrast, fibrous root tissues of paclobutrazol-treated plants contained more starch and soluble sugars, and had lower dark respiration rates than those of control roots (Table 4). As in apple seedlings, reduced dark respiration resulted in less carbohydrate utilization which may partially account for the carbohydrate accumulation in paclobutrazol-treated roots (Steffens et al. 1983, Swietlik and Miller 1983). In apple seedlings, treatment with paclobutrazol via the nutrient solution resulted in manyfold increases in total nonstructural carbohydrates in stem and root tissues (Wang et al. 1985), indicating a profound effect of this compound on partitioning and utilization of carbon assimilates. Application of paclobutrazol to the root zone via soil treatment of sweet orange also caused thickening of fibrous roots (Fig. 2), resulting in three- to sixfold increases in the root:shoot ratios for the treated plants (Table 1). Increased root tip diameters, weights, and root:shoot ratios have been reported in peach and apple plants following treatment with paclobutrazol (Steffens et al. 1983, Williamson et al. 1986). In sweet orange plants, correlation between root thickening and accumulation of carbohydrates as a result of paclobutrazol treatment needs more study. Such an effect, however, may enable this cultivar to better tolerate adverse environmental stresses. In a preliminary soil-flooding test, three paclobutrazol-treated (500-mg per plant soil treatment) sweet orange plants survived total root system submergence in standing water under greenhouse conditions for 60 days without apparent injury to tops or roots (unpublished data). In contrast, the three control plants died and no live tissues could be found in the tops or roots. Cell volume enlargement has been found in cross-sectioned roots of paclobutrazol-treated sweet orange (Yelenosky et al. 1987). It may be that paclobutrazol induced the formation of "aerenchyma-like" roots in sweet orange which increased the ability of the plants to withstand flooded soil conditions.

In apple, paclobutrazol has shown considerable promise for controlling excessive shoot growth and improving fruit set and quality (Williams and Edgerton 1983, Williams 1984, Miller and Swietlik 1986, Greene 1991). Similarly, this growth retardant decreases vegetative growth in citrus and may be useful for developing high-density, dwarf-tree citrus orchards, as well as reducing pruning costs. However, more studies are needed to assess the practical value of paclobutrazol in commercial operations.

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