J Plant Growth Regul (1994) 13:15–19

Journal of
Plant
Growth
Regulation

Application of Paclobutrazol and GA_3 to Adult Peach Trees: Effects on Nutritional Status and Photosynthetic Pigments*

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August 16, 1993; January 4, 1994

Abstract. Soil applications of 1 or 2 g paclobutrazol (Pbz) and foliar sprays of 1000 mg L^{-1} GA_3 to adult peach trees [*Prunus persica* (L.) Batsch] had opposite effects: vegetative growth was inhibited by paclobutrazol and promoted by GA_3, whereas mean fruit yield was greater in Pbz-treated trees and less in GA_3-sprayed trees. Compared to controls, differences in the concentration of different mineral nutrients occurred in leaves collected shortly after fruit harvest: in Pbz-treated trees, the concentration of N and K significantly decreased, whereas that of Mg and Mn increased. Leaves of trees sprayed with GA_3 had a significantly lower concentration of N and Ca and Mn, and a slightly greater concentration of K. In spite of these changes, element concentrations were within accepted ranges and therefore the nutritional status of peach trees did not change with the application of both growth regulators. Pbz did not alter the concentrations of chlorophylls, whereas GA_3 significantly reduced the concentrations of chlorophylls a and b. Neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, and β-carotene concentrations were unaffected by all treatments.

Paclobutrazol (Pbz), inhibitor of gibberellin biosynthesis (Hedden and Graebe 1985), is a plant growth inhibitor which is being used to control the size of fruit trees and other agronomic crops (Davies and Curry 1991). In peach [*Prunus persica* (L.) Batsch], Pbz inhibited vegetative growth, promoted fruit growth, retarded leaf abscission, increased flower bud formation, and advanced bloom in the following spring (Blanco 1988, Coston 1986, Martin et al. 1987), for which its commercial use may become widespread.

Changes in nutrient concentrations in certain plant organs may be related to effects induced by modifications in the rates of tissue growth, nutrient uptake, and nutrient translocation (Swietlik and Miller 1984). Rieger and Scalabrelli (1990) reported that Pbz induced an inhibition of root growth, from which differences in mineral element uptake may be expected. Alterations of one or several essential nutrient elements is one of the most common causes affecting chlorophyll (Chl) and other photosynthetic pigments, and hence carbon assimilation (Monge et al. 1991).

Sun plants, grown under normal light conditions, have about 30% of their total Chl contained in photosystem I complexes (PSI), the distal antenna of photosystem II (PSII) contains 40%–60%, and the other subunits of PSII contain 10%–15%. The PSI contains mainly Chl a and β-carotene with traces of lutein and a very low concentration of Chl b (Goodwin 1976, Barber 1983). The reaction center of PSII contains Chl a, β-carotene, and pheophytin, the proximal antenna consists of Chl a and β-carotene, but no Chl b, and the distal antenna contains Chl a, Chl b, and xanthophylls (Hansson and Wydzynsky 1990).

Dalziel and Lawrence (1984) found a large increase in cell volume in sugar beet treated with Pbz, but the number of chloroplasts per cell did not increase. This, together with the increase in Chl per unit leaf area, indicates that Pbz increased the Chl content per chloroplast. Wang et al. (1985), Bonomo and Neri (1986), Coston (1986), Steffens and Wang (1986), and Mauk et al. (1987) have reported increased intensity of green color in leaves from fruit trees treated with Pbz. Similar effects

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have been reported following application of other growth retardants as daminozide or ancymidol (Halfacre et al. 1969, Starman et al. 1989). GA_3 effects on mineral nutrient and phosynthetic pigments in fruit tree leaves have been studied less than that on plant growth inhibitors. However, a reduction in Chl concentration in apple leaves has been described by Steffens and Wang (1986).

Most results published on Pbz and GA_3 effects, and, in general, those published on plant growth regulators, mineral nutrition, and Chl concentrations have been obtained in plants grown in pots or solution culture either in greenhouse or growth chambers, under controlled conditions, but few data have been obtained from plants grown under field conditions. Therefore, the purpose of this work was to obtain information on the effects of Pbz in the context of orchard management systems, determine the changes in the concentrations of mineral elements and photosynthetic pigments on adult peach tree leaves, and confirm the results by comparing them with those obtained from plants treated with GA_3, as they should be opposite.

Materials and Methods

Plant Material

Two experiments were performed on adult "Redhaven" peach trees grafted on GF677 rootstock, in an orchard of the Servicio de Investigacion Agraria, Diputación General de Aragon, in Zaragoza (Spain). The orchard was planted on sandy to sandy-loam soil with 0.9% organic matter content and a pH of 8.2, and managed according to the usual cultural practices of the area, including fruit thinning and flood irrigation.

In the first experiment, trees with an average trunk girth of 42.2 ± 2.0 cm were treated with 0 (control), 1, or 2 g active ingredient of Pbz (Cultar, ICI-Zeltia). Treatments were applied 11 days after full-bloom. The appropriate amount of Cultar was dissolved in 1 L of water, and the solution was poured on the soil around the trunk of the trees. In the other experiment, trees of 35.5 ± 2.9 cm mean trunk girth were sprayed to runoff with 1000 mg L^{-1} of GA_3 (Berelex, ICI-Zeltia). Each tree received 1.5 L of solution at 14 days after full-bloom; untreated trees served as controls. The experiments were both randomized block designs, with five replicate trees for Pbz treatments, and six trees for GA_3 treatments.

At harvest, the weight and number of fruits per tree were recorded, and the mean fruit weight calculated. Trunk girths were recorded at the same height in winter, at the beginning and at the end of the experiment, and the lengths of 10 terminal shoots per tree were measured in the winter following treatment application.

On July 31, about 10 days after harvest, samples of 25 leaves per tree were harvested from three blocks per treatment per experiment, and were analyzed for photosynthetic pigments and mineral elements made.

Mineral Analysis

To determine the mineral elements, we washed the leaves with detergent and then rinsed them with tap and deionized water to eliminate surface contamination. Leaves were dry ashed following the method of Pinta and DeWele (1975), and then Ca, Mg, Fe, Mn, Cu, and Zn concentrations were determined by atomic absorption spectroscopy, K concentration by flame emission, and P concentration by the vanodomolibdophosphoric method. Nitrogen was determined by the Kjeldahl procedure. The resulting data were expressed as a percentage of dry tissue (%) for Ca, K, Mg, N, and P, whereas Fe, Mn, Cu, and Zn were recorded as milligrams per kilogram of dry leaves.

HPLC Pigment Determinations

Photosynthetic pigments were extracted from leaf disks freshly cut with a cork borer (0.358 cm^2) and ground with 5 mL of 100% acetone and 10 mg of sodium ascorbate to prevent the formation of phaeophytins. Pigments were quantified with reverse-phase HPLC using the method of Val and Monge (1990). This method resolves neoxanthin, violaxanthin, lutein, zeaxanthin, Chl a, Chl b, and β-carotene. Antheraxanthin and α-carotene were also resolved, but in most samples, their concentrations were negligible. The mobile phase (2 ml min^{-1}) consisted of two isocratic steps: the first (≅6 min) dichloromethane/methanol/water/acetonitrile (1.75:1.75:2:94.5 v/v) and the second acetonitrile/ethyl acetate (50:50 v/v). The column was a 10 cm C_{18} reverse-phase 4 μm Nova-Pack cartridge (Waters, Ass.).

Statistical Analysis

Data were analyzed independently for each experiment. Analysis of variance was performed for both experiments, and regression analysis was used for Pbz treatments.

Results

More and larger fruit were collected from the Pbz treated than from the control trees, but due to the variability in the responses, the differences were not significant. In contrast, GA_3-treated trees had fewer fruits, though not significant, but the mean weight of fruits was significantly less than that in untreated trees (Table 1).

Terminal shoot length was significantly reduced by Pbz ($r = -0.805$; $p \leq 0.001$), as was the relative increase in trunk girth ($r = -0.752$; $p \leq 0.001$), whereas GA_3 increased shoot length and the relative increase in trunk girth (Table 1).

Compared to control trees, leaves from Pbz-treated trees, had smaller concentrations of N and K and greater concentrations of Mg (Table 2). Regression analysis showed a linear trend of both elements: K ($r = -0.545$; $p \leq 0.01$) and Mg ($r = 0.369$; $p \leq 0.05$) with increasing doses of Pbz. Ca showed a slight, although nonsignificant increase, whereas P did not vary.

Except for Mn, microelement concentrations did not differ significantly from the control trees (Table

Table 1. Fruit crop and vegetative growth of 'Redhaven' peach trees treated with paclobutrazol or GA_3.

	Crop weight (kg/tree)	No. fruits per tree	Mean fruit weight (g)	Terminal shoot length (cm)	Relative trunk girth increment (%)
Paclobutrazol					
Control	67.9	569	119	57.4	16.9
1 g	75.2	567	135	23.6	4.5
2 g	80.2	639	126	22.1	4.7
Significance[a]	NS	NS	NS	***	***
GA_3					
Control	52.7	443	117	59.1	20.2
1000 ppm	39.0	411	95	73.5	34.7
Significance[b]	NS	NS	*	**	***

NS, nonsignificant.

[a] Regression analysis.

[b] ANOVA.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 2. Macronutrient concentration (%) in leaves of 'Redhaven' peach trees treated with paclobutrazol or GA_3.

	N^a	P	K^b	Ca	Mg^b
Paclobutrazol					
Control	4.1^c	0.23	2.38	1.79	0.39
1 g	3.8^d	0.21	1.98	2.09	0.44
2 g	3.9^d	0.22	1.83	1.99	0.45
Significance	*	NS	**	NS	*
Ga_3					
Control	3.8	0.22	2.16	1.92	0.43
1000 ppm	3.7	0.22	2.19	1.32	0.43
Significance	*	NS	NS	***	NS

NS, nonsignificant.

[a] In this column, values with the same superscript are not significantly different at $p \leq 0.05$ following Duncan's multiple range test.

[b] Regression analysis of paclobutrazol experiment has resulted in significant differences.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 3. Effects of paclobutrazol and GA_3 on the levels of micronutrients (mg kg^{-1}) in leaves of 'Redhaven' peach trees.

	Fe	Mn^a	Cu	Zn	Fe/Mn^a
Paclobutrazol					
Control	101.6	33.8^b	11.3	29.3	3.44^b
1 g	118.1	50.1^c	12.1	29.8	2.42^c
2 g	120.5	44.7^c	12.7	29.3	$2.87^{b,c}$
Significance	NS	***	NS	NS	*
GA_3					
Control	144.0	39.7	10.9	30.0	3.68
1000 ppm	122.1	16.3	12.0	32.4	7.54
Significance	NS	***	NS	NS	**

NS, nonsignificant.

[a] In this column, values with the same superscript are not significantly different at $p \leq 0.05$ following Duncan's multiple range test.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

3). The concentration of Mn was significantly less than that in the control trees. The ratio of Fe/Mn was reduced by 1 g Pbz and increased by 2 g Pbz.

In leaves of trees sprayed with 1000 mg L^{-1} of GA_3, the concentrations of P, Mg, and K were unaffected, but N and Ca concentrations were reduced (Table 2). For micronutrients, only Mn concentration was affected, being significantly lower in leaves from trees sprayed with GA_3 as compared with that from the control leaves (Table 3). The ratio Fe/Mn was significantly higher in the GA_3-treated trees than for that in the controls.

The effects of the growth regulators on the growth rate of shoots were clearly visible by mid-June in both experiments. Changes in the intensity of the green color in leaves were clearly observed: those on trees treated with Pbz were dark-green whereas leaves on trees sprayed with GA_3 were yellow-green. Chlorophyll was reduced by GA_3 and was nonsignificantly increased by Pbz (Table 4).

Treatments did not affect the concentration of xanthophylls (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin) and β-carotene (Table 4). Lutein was the carotenoid that showed the highest concentration.

Table 4. Effects of paclobutrazol and GA$_3$ on the content of chlorophylls in leaves of 'Redhaven' peach trees ($\mu g \cdot cm^{-2}$).

	Chl a	Chl b	Total xantophyll	β-carotene
Paclobutrazol				
Control	21.82	7.04	8.23	1.35
1 g	25.75	8.16	9.54	1.65
2 g	24.61	7.73	8.70	1.25
Significance	NS	NS	NS	NS
GA$_3$				
Control	23.17	8.81	9.09	1.75
1000 ppm	19.36	5.75	8.13	1.45
Significance	*	*	NS	NS

NS = nonsignificant.
* $p < 0.05$.

Although trends were apparent, the Chl a/b, total chlorophyll/total xantophylls, total xantophylls/β-carotene, total chlorophyll/total carotenoids, Chl a/β-carotene, and xantophylls/Chl b ratios were not significantly affected by the Pbz-treatments. The ratio xantophylls/Chl b (photosynthetic pigments of LCH) of leaves treated with GA$_3$, was significantly greater ($p < 0.01$) than that for control trees (data not shown).

Discussion

The results presented are, in general terms, opposite since Pbz inhibits gibberellin biosynthesis by blocking the oxidation of kaurene to kaurenoic acid (Hedden and Graebe 1985), and its effects can be reversed by the application of GA$_3$ (Steffens and Wang 1986).

The effect of Pbz on peach tree nutrition has been studied with conflicting results. This discrepancy of results may be due to different dosages, application timing, plant material, and environments. Coston (1986) found that leaf N increased with foliar applications of Pbz, Martin et al. (1987) found increases in Ca, K, and P levels, and Rieger and Scalabrelli (1990) obtained similar trends to our results from leaves of "Nemaguard" peach rootstock: a decrease in the levels of N, P, K, and Fe, which showed a quadratic response, and increases in Ca, Mg, and Mn.

Manganese interferes with the absorption and translocation of iron from a growth medium, and several researchers have reported an antagonism between Fe and Mn, which could lead to Fe chlorosis (Rogers et al. 1974, Casero and Carpena 1987, Bindra 1980), probably due to a substitution of Fe by Mn in the biosynthesis of Chl (Clairmont et al. 1986). In our case, the ratio Fe/Mn (Table 3) in leaves subjected to the treatments in both experiments differed from the controls: the ratio was smaller in Pbz-treated trees, and greater in GA$_3$-sprayed trees. These effects were due to a greater concentration of manganese rather than to an antagonistic effect between the two elements.

Using established critical or standard values of peach leaf mineral nutrient concentrations (Jones et al. 1991), the nutritional status of our trees was good except for N, which was slightly above normal levels. Therefore, under our conditions, Pbz and GA$_3$ do not affect the nutritional status of peach leaves.

The effects of GA$_3$ on photosynthetic pigment concentrations in peach has received little attention, but reductions in Chl content have been found in leaves of honeysuckle (Kwack and Kwack 1990), pepper (Aloni and Pashkar 1987), and in apple trees previously treated with Pbz (Steffens and Wang 1986). These papers and our data agree with the report of Daniell and Rebeiz (1986) that showed the involvement of GA$_3$ in the control and regulation of the biosynthesis of Chl. Although in certain cases an increase in Chl levels in leaves and fruits was also shown, this has been related to delays in the senescence of these organs (Goldthwaite 1988).

Except for Chl on GA$_3$-treated trees, the other photosynthetic pigments analyzed in both experiments did not vary in relation to the controls. The reduction in Chl resulting from the application of GA$_3$ was greater for the Chl b concentration (34.6% of the level in the control leaves) than that for Chl a (16.4%). Because Chl b is a pigment of LHC, gibberellins could affect the antenna complexes responsible for the harvest of solar radiation, rather than the reaction centers. In order to study if Pbz or GA$_3$ alters the photosynthetic structures, the ratios of pigment concentrations were calculated, and, except for an increase in the ratio of xantophylls/Chl b of leaves treated with GA$_3$, there was no change. This suggests that under field conditions, Pbz does not alter photosynthetic pigments, the relationships among them, or the specific chloroplast structure. The greater ratio of xantophylls/Chl b suggest that GA$_3$ treatments could affect photosynthetic pigments of antenna complexes, because Chl b and xanthophylls are the main pigments of these structures.

Work in progress by Pequerul (personal communication) to study whether Pbz or GA$_3$ alters the structures of chloroplasts in sugar beet leaves following the method of Anderson et al. (1978), shows no differences between the separation pattern of pigment-protein complexes from Pbz and GA$_3$ thylakoid membranes and the untreated controls.

Davies et al. (1988) have suggested that some alterations produced on leaves treated with growth regulators could be due to an effect on leaf size. Reductions of about 50% leaf area induced by soil application of 2 g of Pbz on peach tree leaves have been recorded (Monge et al. 1993). Consequently, the greater concentrations of photosynthetic pigments and certain mineral elements in Pbz-treated leaves could be due to a greater number of cells per unit area. Further work is consequently needed to elucidate all these questions.

Acknowledgments. This work was carried out under research project INIA 5520. The financial support provided to R. Aguirre by the C.S.I.C.-C.O.N.A.C.Y.T. scheme is gratefully acknowledged. The authors thank the staff of the Dpto. de Fruticultura of the S.I.A. (D.G.A.) for the management of the orchard plot, and ICI-Zeltia for gifts of Pbz. The contribution of Mrs. M. A. Gracia is also acknowledged.

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