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EFFECT OF PACLOBUTRAZOL ON CELL WALL POLYSACCHARIDE COMPOSITION OF THE APPLE TREE

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Key Word Index—*Malus domestica*; Rosaceae; apple; plant bioregulator; cell wall carbohydrates; paclobutrazol.

Abstract—The effect of paclobutrazol, a gibberellin biosynthesis inhibitor, on cell wall carbohydrate composition of apple shoots was determined. Inhibition of apple shoot extension corresponded to a change in wall composition. Paclobutrazol did not inhibit shoot growth during the first year (1983) after the treatment. There was also no significant difference in cell wall carbohydrate composition between control and paclobutrazol treated shoots. In the second year (1984), however, paclobutrazol altered the composition of cell wall polysaccharides and inhibited shoot extension. Paclobutrazol treatment increased rhamnose, arabinose and galacturonic acid but decreased cellulose. The ratio of xylem to phloem was also reduced by paclobutrazol treatment.

INTRODUCTION

Cell division in plants generally takes place in apical regions and plant growth occurs by expansion and elongation of these cells. Therefore, the changes in cell wall constituents are an important part of plant growth [1]. There have been many reports on the composition and the structure of plant cell walls [2]. The presence of hormones is required for cell elongation [2]. Promotion of extension-growth with auxin is mediated by wall loosening and changes in cell wall chemistry [3–9]. Stimulation of lettuce hypocotyl elongation with GA in the dark by enhancing the production of cell wall polysaccharides has been reported [10]. GA has also been suggested to enhance production of cell wall polysaccharides in *Avena* stems [11].

The plant growth regulator, paclobutrazol [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol)], has been found to interfere with gibberellin biosynthesis in plants [12, 13] and has been shown to be an effective growth retardant on apple [14–24]. We previously reported [18, 22] that paclobutrazol treatment of 'Spartan' apple trees reduced shoot growth and leaf area and caused alterations in leaf and shoot non-structural carbohydrate metabolism during the growing season and the winter dormant period.

The objectives of the work reported here were to determine whether the paclobutrazol treatment changed cell wall carbohydrate composition and if the changes could be correlated to the changes in growth.

RESULTS AND DISCUSSION

Paclobutrazol treatment of 'Spartan' apple trees did not inhibit shoot growth in the first year (1983) after treatment but significantly retarded shoot growth during the second year (1984) [23]. However, the increase in non-structural carbohydrate induced by the treatment was similar in both years, regardless of whether or not growth was inhibited. This indicates that paclobutrazol has an effect on non-structural carbohydrate metabolism independent of the growth effect [23].

The non-cellulosic neutral sugars rhamnose, arabinose, xylose, mannose, glucose and galactose were found in cell walls of apple shoots (Tables 1 and 2). Xylose was the predominant sugar followed by arabinose and glucose. Rhamnose, mannose and galactose were present only in trace amounts. The % composition of cell wall neutral sugars were comparable in 1983 and 1984 wood.

In 1983, paclobutrazol did not inhibit shoot growth and there was no significant difference in non-cellulosic neutral sugars between control and paclobutrazol-treated shoots, nor were cellulose and galacturonic acid levels altered by paclobutrazol treatment (Table 1). In 1984, paclobutrazol treatments significantly retarded shoot growth, and increased arabinose, rhamnose and galacturonic acid but decreased cellulose during the Winter dormant period and the growth in the Spring (Table 2). This decrease in cellulose content associated with paclobutrazol treatment was found in both xylem and phloem tissues (Table 3). Differences in cellulose between the control and treated shoots increased as the season progressed (Table 2). This was probably due to a more profound reduction of cellulose content by paclobutrazol in the Spring and the decrease in the ratio of xylem to phloem as Spring approached (Table 3). Since the cellulose content was higher in the xylem than in the phloem, a reduction of xylem tissue of paclobutrazol treated trees resulted in lower cellulose content in the Spring in one year old shoots (Tables 2 and 3). Differences in the cell wall composition of xylem and phloem have been reported in other woody trees [25]. Cellulose and hemicellulosic polysaccharides are typically deposited during primary growth of higher plant cells [1, 2]. Xylose and glucose are present in a polymer, presumbly xyloglucan [26, 27], which binds to cellulose. Xyloglucan and cellulose have been reported to play an important structural role in plant cell walls. They have long been thought to be involved in the control of cell wall elongation in dicots [28, 29]. Xyloglucan and cellulose seem to be required to maintain

Table 1. Effect of paclobutrazol (paclo) treatment on cell wall polysaccharide composition of two year old wood (1983 wood)

Sampling date	Treatment	Length (cm/shoot)	Sugar composition (%)*							
			Rha	Ara	Xyl	Man	Glu†	Gal	Cellulose	Galua‡
12-7-84	Control	30.1 ± 7.1	0.6	2.5	10.8	0.9	2.5	1.7	60.0	21.0
	Paclo	37.7 ± 8.7	0.8	3.0	11.7	1.1	2.4	1.5	57.7	21.8
1-7-85	Control	30.8 ± 7.3	0.8	2.1	11.7	0.8	2.5	1.4	59.1	21.6
	Paclo	36.6 ± 6.1	0.7	2.4	11.6	0.8	2.3	1.3	59.1	21.8
2-4-85	Control	31.2 ± 10.8	0.5	2.5	10.3	0.7	2.0	1.0	61.8	21.2
	Paclo	30.1 ± 4.1	0.6	2.3	9.7	0.6	2.1	1.1	61.6	22.0
3-4-85	Control	32.4 ± 7.4	0.6	2.2	10.4	0.8	1.9	0.8	59.2	24.1
	Paclo	38.9 ± 6.5	0.8	2.7	10.9	0.6	1.9	0.9	58.4	23.8
3-27-85	Control	36.4 ± 8.3	0.5	1.9	9.0	0.7	1.3	0.8	59.6	26.2
	Paclo	41.8 ± 7.8	0.5	2.2	9.3	0.7	1.3	0.5	60.7	24.8
4-17-85§	Control	38.7 ± 9.9	0.5	2.2	8.5	0.7	0.9	0.6	57.6	29.0
	Paclo	41.5 ± 8.2	0.4	2.4	9.2	0.5	0.8	0.6	57.2	28.9

*Data represent the mean of nine analyses.
†Non-cellulosic.
‡Galacturonic acid.
§Full bloom.

Table 2. Effect of paclobutrazol (paclo) treatment on cell wall polysaccharide composition of one-year old wood (1984 wood)

Sampling date	Treatment	Length (cm/shoot)	Sugar composition (%)*							
			Rha	Ara	Xyl	Man	Glu†	Gal	Cellulose	Galua‡
12-7-84	Control	34.4 ± 6.3	0.6	2.4	11.6	0.7	2.6	1.8	59.6	20.7
	Paclo	6.2 ± 1.2	0.8	3.4	9.9	0.7	2.1	2.0	59.3	21.8
1-7-85	Control	29.4 ± 5.7	0.6	2.3	10.5	0.6	2.6	1.4	61.9	20.1
	Paclo	3.9 ± 2.2	0.7	2.5	9.8	0.5	2.1	1.9	60.6	21.9
2-4-85	Control	32.3 ± 6.2	0.7	2.1	9.9	0.6	2.1	1.2	61.7	21.7
	Paclo	3.6 ± 1.0	0.9	3.1	9.7	0.5	1.7	1.7	56.6	25.8
3-4-85	Control	32.0 ± 5.8	0.5	1.4	8.6	0.5	1.7	1.2	56.6	29.5
	Paclo	3.0 ± 0.9	0.8	3.1	8.9	0.6	1.6	1.2	51.9	31.9
3-27-85	Control	33.5 ± 6.4	0.5	2.2	9.8	0.7	1.2	0.8	56.4	28.4
	Paclo	4.1 ± 1.9	0.8	2.7	11.3	0.7	1.0	1.3	38.0	44.2
4-17-85§	Control	30.5 ± 6.4	0.6	2.3	9.0	0.5	1.1	0.7	56.8	29.0
	Paclo	3.9 ± 2.0	0.9	3.2	10.5	0.7	0.9	0.8	36.6	46.4

*Data represent the mean of nine analyses.
†Non-cellulosic.
‡Galacturonic acid.
§Full bloom.

the integrity of the wall frame work, and severe inhibition of its synthesis appears to contribute to inhibiting plant growth. Our data showed that in paclobutrazol-treated trees a reduction in the proportions of glucose and cellulose in shoot tissue were associated with the inhibition of apple shoot extension. Xylose in both control and paclobutrazol treated shoots of one year old wood decreased to a minimum at the end of the dormant period and then increased toward Spring. The content of arabinose, rhamnose and mannose in cell walls did not change significantly from the Winter dormant period through to resumption of growth in the Spring (Tables 1 and 2).

Paclobutrazol-treated shoot showed an increase in the proportion of galacturonic acid in both xylem and phloem tissues (Table 3). An increase in galacturonic acid also was noted while galactose and glucose declined on the resumption of Spring growth (Tables 1 and 2). Galacturonic acid occurs in the backbone of some polysaccharides. Polygalacturonase (EC 3.2.1.15), a hydrolytic enzyme that cleaves 1,4-galacturonosyl linkages between nonesterified galacturonosyl, has been implicated in the softening process [30, 31]. The increase in the Spring of galacturonic acid in both xylem and phloem tissues may indicate softening of the cell wall and active resumption of growth. Therefore, cells of the shoot formed during Spring appear

Table 3. Effect of paclobutrazol (paclo) treatment on cellulose and galacturonic acid content of the cell wall during winter and spring in phloem and xylem tissues of one year old shoot (grown in 1984)

Sampling date	Treatment	Ratio xylem/phloem (dry wt)	Sugar composition (%)*			
			Cellulose		Galacturonic acid	
			Phloem	Xylem	Phloem	Xylem
12-7-84	Control	1.80	20.5	39.7	13.8	6.3
	Paclo	1.19	19.7	38.9	14.9	7.6
4-17-85	Control	1.96	19.5	34.3	21.8	10.2
	Paclo	0.84	14.3	21.3	31.9	15.5

*Data represent the mean of three analyses.

less bound to the other cells than those produced during the Winter dormant period. The decrease of galactose residues may be related to an altered rate of cell wall polysaccharide turnover. The nature of the decrease in galactose residue from cell walls during growth resumption in the Spring cannot yet be established due to the lack of information on apple shoot cell wall structure and metabolism. Paclobutrazol treatment did not affect the level of galactose in the apple shoot (Tables 1 and 2).

On the basis of the present results there are indications of changes in wall composition accompanying shoot extension. Paclobutrazol treatment markedly altered the deposition of certain cell wall carbohydrates. Paclobutrazol decreased cellulose and increased the galacturonic acid, rhamnose and arabinose content in the wall. Several enzymes could be involved in the synthesis and metabolism of cell wall carbohydrates in apple shoots treated with paclobutrazol. Whether the paclobutrazol treatment suppressed wall deposition by lowering the activities of the required synthetic enzymes or by enhancing hydrolase activity warrants further study.

EXPERIMENTAL

Plant materials and treatments. The growing conditions and treatments of the apple trees were as described previously [23]. 'Spartan' apple (*Malus domestica* Borkh) trees on Malling Merton 106 rootstocks planted in 1976 in an orchard at Beltsville, MD, were treated with paclobutrazol via foliage sprays in 1982 and by a trunk painting technique [32] in the spring of 1983. Untreated and paclobutrazol-treated trees were selected for evaluation from three replicates which had been treated as follows: 1982—Paclobutrazol (50 WP) was applied as a foliage spray on May 4, 14 and 25, 1982 at 333 mg/l. with 0.1% Tween-20. 1983—On April 27, 1983, trunks of these trees were painted with 75 g/l. of paclobutrazol [90% technical dissolved in 1 part MeOH plus 1 part surfactant Regulaid (polyoxyethylenepoly propoxypropanol, dihydroxypropane, alkyl oxyethanol)]. The length of the trunk portion painted equalled 6 times the trunk diameter. The trees were grown under normal orchard cultural practices without irrigation [18, 23]. Ten random 1983- and 1984-produced shoots were collected between 8.30 and 9.30 am and measured on 7 Dec., 1984, and on 7 Jan., 4 Feb., 4 Mar., 27 Mar. and 17 Apr. (full bloom), 1985. Wood and bark from these shoots (buds removed) were cut into thin slices, composited, frozen and used for carbohydrate analysis.

Cell wall carbohydrate analysis. Samples for cell wall carbohydrate analysis were homogenized with a Brinkmann Polyton homogenizer (1 min) and extracted sequentially with 20 mM HEPES–NaOH (pH 6.9), $CHCl_3$–MeOH (1:1) and Me_2CO. An α-amylase treatment was included before $CHCl_3$–MeOH extraction to remove starch [33]. The residue was dried in a vacuum desiccator and used for cell wall carbohydrate analysis. The non-cellulosic neutral sugars in the cell wall materials were hydrolysed with 2 M TFA according to the procedure of ref. [34]. The sugars liberated were converted into aldononitrile acetate as described by Lehrfeld [35]. The sugar derivatives were injected for GC separation and quantification. A Hewlett–Packard 5880 GC equipped with an FID and a fused silica capillary column (methylsilicone fluid, 12.5 m × 0.2 mm) was used for separation of sugars. Initial oven temp. was set at 140° and was held isothermal for 3 min followed by a temp. program of 6°/min^{-1} to a final oven temp. of 200°. The final oven temp. was held isothermal for 1.5 min. The injection port and detector temps were set at 250° and 275°, respectively. He was used as a carrier gas and the column head pressure was set at 100 kPa. A 1-μl injection was made, using a split ratio of 30:1. Flow rates were 30 and 400 ml min^{-1} for H_2 and air, respectively. Separated sugars were compared with derivatized sugar standards for qualitative and quantitative determinations. A known amount of myo-inositol was included in all samples as an internal standard.

Cellulose content of cell wall samples was determined using a method similar to that described by Updegraff [36]. Cell wall samples were hydrolysed with 2 N TFA (1 hr, 121°). The insoluble residue was then dissolved in 78% H_2SO_4 and assayed for hexose as described by ref. [36]; glucose was used as the standard.

Galacturonic acid content of cell walls was determined by a Carbazole method [37] using galacturonic acid as a standard.

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