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Short Communication

Effect of Paclobutrazol on Accumulation of Organic Acids and Total Phenols in Apple Wood

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Abstract. Apple trees (*Malus domestica* Borkh "Spartan" grafted on MM 106 rootstock) planted in 1976 in an orchard at Beltsville, Maryland, were treated with paclobutrazol (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol) using a foliage spray in 1982 and by trunk banding in 1983. Paclobutrazol did not inhibit shoot growth in 1983; however, shoot growth was significantly retarded in 1984. Increases in organic acids, including succinic, malic, citric, and quinic, and also in total phenols, occurred in wood produced in 1983 on paclobutrazol-treated trees when growth was not inhibited and in wood produced in 1984 when growth was inhibited. The organic acid content of both paclobutrazol-treated and untreated wood tended to decrease from the winter dormant period through growth resumption in the spring. However, the content of total phenols remained nearly the same throughout this sampling period.

Controlling tree size is a necessary component for developing an efficient, high-density orchard system. Paclobutrazol is effective for inhibiting shoot growth (Quinlan and Richardson 1984, Steffens et al. 1985a,b, Stinchcombe et al. 1984, Williams 1984) and regulating various metabolic processes on apple trees. Treatment with paclobutrazol resulted in a shift of the partitioning of assimilates from the leaves to roots, increased carbohydrates in all parts of apple seedlings, increased chlorophyll, soluble protein and mineral element concentration in leaf tissue, increased root respiration, reduced cell wall polysaccharide, and water loss, and it prevented the accumulation of water-stress-

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induced abscisic acid (Wang and Steffens 1985; Steffens et al. 1985a,b, Wang et al. 1985, 1986a,b, 1987a).

Paclobutrazol treatment of "Spartan" apple trees did not inhibit shoot growth in 1983 but retarded the shoot growth during 1984 (Wang et al. 1986b). The increase in carbohydrates in the wood throughout the winter dormant period and during resumption of growth in the spring induced by the treatment was similar for wood produced in 1983 (when growth was not inhibited) as for wood produced in 1984 (when growth was inhibited). Since organic acids and carbohydrates can be interconverted and organic acids also play an important role in phenolic biosynthesis (Siegelman 1964), this study was initiated to determine whether changes in organic acids and total phenols also occurred from paclobutrazol treatment.

Materials and Methods

Plants treated with paclobutrazol from previous research were used in this study (Wang et al. 1986b). "Spartan" apple trees on MM 106 rootstocks planted in an orchard at Beltsville in 1976 were treated with paclobutrazol via foliage sprays in 1982 and by trunk banding in spring 1983. Paclobutrazol (50 WP) was applied as a foliage spray on May 4, 14, and 25, 1982, at 333 mg/l a.i. with 0.1% Tween-20. In 1983, trunks of these trees were banded on April 27 with 75 g/L of paclobutrazol [90% technical dissolved in 1 part methyl alcohol plus 1 part Regulaid surfactant (polyoxyethylenepolypropoxypropanol, dihydroxypropane, alkyl oxyethanol; Kalo Agricultural Chemicals, Overland Park, Kansas)]. The length of the trunk portion painted was six times the trunk diameter. Three trees each were selected for the paclobutrazol treatment and the control (2 treatments × 3 single tree replicates). The trees were grown under normal orchard cultural practices without irrigation. Ten 1983- and 1984-produced shoots were randomly collected between 8:30 and 9:30 AM for sampling uniformity and measured on December 7, 1984, and on January 7, February 4, March 4, March 27, and April 17 (full bloom), 1985. Wood grown in 1983 was separated from the 1984 wood (buds removed), cut into thin slices, frozen, and used for organic acid (Wang et al. 1987b) and total phenol analysis (Singleton and Rossi 1965). Data were evaluated statistically as a randomized complete-block experiment having a repeated measurement design using analysis of variance.

Results and Discussion

Several organic acids, including succinic, malic, citric, and quinic, occur in apple wood (Tables 1, 2). Other tricarboxylic acid cycle intermediates, namely aconitic, isocitric, fumaric, α-ketogulatric, and oxalacetic, are present in relatively small amounts and consequently were more difficult to detect. The winter dormant wood contained substantial amounts of organic acids. Marked reduction of organic acids in woods was observed during the resumption of growth in the spring (Tables 1, 2). This may be attributed to translocation of reserves into new growth or to respiration loss. Wood produced in 1984 contained a higher concentration of organic acids than 1983 wood.

Table 1. Effect of 1982 and 1983 paclobutrazol (PB) treatment of "Spartan" apple trees on organic acid content of wood produced in 1983.

Sampling date	Treatment	Individual organic acid ($mg \cdot g^{-1}$ FW) Succinic	Malic	Citric	Quinic	Total organic acid $mg \cdot g^{-1}$ FW	Total organic acid $mg \cdot g^{-1}$ DW
Dec 7, 1984	Control	68.9	73.2	50.0	88.7	280.8	466.2
	PB	93.9*	143.4*	60.0	177.4*	474.7*	807.0*
Jan 7, 1985	Control	48.9	66.8	38.0	76.8	230.5	403.4
	PB	73.7*	106.2*	50.0	118.2*	348.1*	696.2*
Feb 4, 1985	Control	63.1	72.6	40.0	43.6	219.3	386.0
	PB	99.6*	105.1*	41.3	100.4*	346.4*	599.3*
Mar 4, 1985	Control	41.5	50.0	38.0	39.0	168.5	298.2
	PB	47.9	51.3	40.0	90.0*	229.2*	412.6*
Mar 27, 1985	Control	19.2	29.1	35.3	33.7	117.3	214.7
	PB	22.2	36.2	39.0	70.9*	168.3*	321.5*
Apr 17, 1985[a]	Control	17.3	26.0	34.0	30.5	107.8	213.4
	PB	21.9	32.8	37.0	65.2*	156.9*	317.0*

[a] Full bloom.
* Significantly different from control at the 5% level using analysis of variance.

Table 2. Effect of 1982 and 1983 paclobutrazol (PB) treatment of "Spartan" apple trees on organic acid content of wood produced in 1984.

Sampling date	Treatment	Individual organic acid ($mg \cdot g^{-1}$ FW) Succinic	Malic	Citric	Quinic	Total organic acid $mg \cdot g^{-1}$ FW	Total organic acid $mg \cdot g^{-1}$ DW
Dec 7, 1984	Control	133.4	244.4	56.6	533.3	967.7	1674.1
	PB	236.7*	292.6*	190.6*	1422.5*	2142.4*	3642.1*
Jan 7, 1985	Control	93.8	240.4	57.6	518.9	910.7	1593.7
	PB	258.9*	285.5*	121.8*	1231.3*	1797.5*	3145.6*
Feb 4, 1985	Control	96.0	206.0	42.4	500.7	845.1	1478.9
	PB	158.3*	271.4*	85.1*	1067.2*	1740.3*	3010.7*
Mar 4, 1985	Control	44.0	115.0	34.0	491.0	684.0	1210.7
	PB	84.5*	204.0*	80.5*	954.0*	1323.0*	2381.4*
Mar 27, 1985	Control	20.7	20.2	12.4	356.4	409.7	749.8
	PB	24.4	77.5*	70.6*	850.7*	1023.2*	1954.3*
Apr 17, 1985[a]	Control	17.2	17.0	10.9	315.5	360.6	714.0
	PB	21.4	65.8*	60.2*	832.7*	980.1*	1890.7*

[a] Full bloom.
* Significantly different from control at the 5% level using analysis of variance.

The organic acid levels in paclobutrazol-treated wood were generally higher than those of untreated wood at all sampling dates, from the winter dormant period through resumption of growth in the spring (Tables 1, 2). The major organic acids that increased consistently throughout the sampling period were quinic and malic acids. Even though paclobutrazol did not inhibit growth in 1983 (Wang et al. 1986b), it greatly increased the total organic acid content of the 1983-produced wood (Tables 1, 2). This indicates that paclobutrazol has an

Table 3. Effect of 1982 and 1983 paclobutrazol (PB) treatment of "Spartan" apple trees on total phenol content of wood produced in 1983 and 1984.

		Total phenols (mg · g^{-1} FW)	
Sampling date	Treatment	1983 wood	1984 wood
Dec 7, 1984	Control	8.8	10.1
	PB	10.8*	12.0*
Jan 7, 1985	Control	9.8	9.5
	PB	11.3*	11.5*
Feb 4, 1985	Control	8.6	8.5
	PB	11.9*	10.3*
Mar 4, 1985	Control	9.0	8.5
	PB	12.4*	9.9
Mar 27, 1985	Control	8.2	9.1
	PB	10.5*	14.5*
Apr 17, 1985[a]	Control	9.1	9.0
	PB	10.3*	11.3*

[a] Full bloom.
* Significantly different from control at the 5% level using analysis of variance.

effect on organic acid metabolism. Paclobutrazol treatments significantly retarded shoot growth in 1984 (32 ± 5.8 vs. 4 ± 0.9 cm for untreated vs. treated shoot) (Wang et al. 1986b). The reduction of 1984 shoot growth may have resulted in a shift of organic acids so that increased amounts were available for storage in both new (1984) and the previous year's (1983) wood. Increases in carbohydrate content in the treated 1983 and 1984 wood have also been reported earlier (Wang et al. 1986b). A high content of quinic acid occurred in paclobutrazol-treated wood (Tables 1, 2). Quinic acid increased more than twofold in treated wood. This increase may be associated with high carbohydrate content which promotes pentose phosphate pathway activity and in turn enhances quinic acid production. It has been suggested that quinic acid plays an important role in aromatic metabolism of plants. When labeled quinate was fed to plants, it was converted to shikimic acid, phenylalanine, and tyrosine (Weinstein et al. 1959). Shikimic acid is an intermediate product in the biosynthesis of phenolic compounds in higher plants (Neish 1964). Quinic acid also is the precursor of chlorogenic acid in potato slices (Levy and Zucker 1960) and leaf tissue of *Pyrus communis* (Lawson 1961). Paclobutrazol-treated wood contained higher total phenols than the nontreated wood (Table 3). The amounts of total phenols in 1983 wood and 1984 wood were comparable, and their contents were not markedly changed from the winter dormant season to the resumption of growth in the spring. Higher levels of total phenols in paclobutrazol-treated wood may be derived from the higher organic acid content. However, Buta et al. (1985) indicated no major difference in the phlorizin level in the new fibrous roots of paclobutrazol-treated apple seedlings compared to the controls during 4 weeks of paclobutrazol treatment. This may be due to differences in plant materials and experimental conditions. Taken together, our data inferred that organic acids were normally utilized for growth and the inhibition of growth by paclobutrazol resulted in the accumulation of organic acids. Further research is needed to determine the effect of paclobutrazol on organic acid biosynthesis and the nature of its primary effect in apple tissues.

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