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Peroxidase and IAA Oxidase Activities and Peroxidase Isoenzymes in the Pericarp of Seeded and Seedless "Redhaven" Peach Fruit

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Abstract. Parthenocarpic peach fruit (*Prunus persica* L. Batsch., cv. Redhaven) were induced with 1-(3-chlorophthalimide)-cyclohexane carboxamide (AC 94377). The activities of soluble, and ionically and covalently bound peroxidase and indole-3-acetic acid (IAA) oxidase in the pericarp of both seeded and parthenocarpic fruit were determined from 21–43 days after anthesis. Seedless fruit grew faster during early stage I and ceased growth earlier than seeded fruit. Total peroxidase and IAA oxidase activities increased with development on both types of fruit, but higher values were found in seedless fruit. The ionic fraction showed the greatest increase for both enzyme activities. Isoperoxidase profile showed new cationic isoenzymes and higher levels of the less anionic isoenzymes in the pericarp of seedless fruit, whereas the seeded fruit contained higher levels of the more acidic isoperoxidases.

Developmental changes in peach fruit during ovary wall growth are well documented (Tukey 1936). Two important questions need to be answered: (1) how does the seed affect the development of ovary wall tissue; and (2) is there a specific role for indole-3-acetic acid (IAA) in this seed development fruit growth relationship?

Destruction of the seed during stages I and II of fruit development induces abscission, while seed abortion during stage III advances maturation and accelerates abscission (Tukey 1936). The effect of the seed can be partially or completely replaced (parthenocarpy induced) in "Redhaven" peach by exogenous application of gibberellin A_3 (GA_3) (Crane 1964, Stembridge and Gambrell 1970) or AC 94377 (Bukovac, unpublished observations), but IAA and napthaleneacetic (NAA) are without effect (Bukovac, unpublished observations). The effectiveness of AC 94377 on development of seedless sour cherry fruit was greater than that of GA_3 and is markedly enhanced by addition of NAA (Bukovac et al. 1985).

Levels of free IAA have been measured in the seeds (Valpuesta et al. 1989) and pericarp (Sánchez-Roldán et al. 1990) of peach fruits. The results may be explained by an export of this hormone from the seed to the surrounding tissue. Peroxidases are present in almost every plant tissue and their levels and isoenzymes vary with developmental events (Thorpe et al. 1978). Even though they have not been definitively involved in specific metabolic pathways, their tissue levels have been negatively correlated to plant growth (Singhal et al. 1979) and IAA levels (Hamdi 1988), and positively to lignin synthesis (Fukuda and Komamine 1982).

The ability to induce parthenocarpic peach fruit offers a unique system to evaluate not only the effect of the seed in the growth of the whole fruit, but also whether or not the peroxidases in the pericarp are involved in this relationship.

Materials and Methods

Plant Material

Mature "Redhaven" peach trees receiving standard horticultural practices at Clarksville Horticultural Experiment Station were used in this study. Parthenocarpic fruit were induced by exogenous application of a 70% ethanolic solution of 5 mM AC 94377 (American Cyanamid, Princeton, NJ, USA) and 0.2 mM NAA containing 0.1% surfactant (Atlox B-1, Kao Corp., Tokyo, Japan). About 50 flowers of uniform development at the balloon stage were selected on three or four branches on each of 10 trees and the remaining flowers removed. The flowers were emasculated and 50 μl of treatment solution was applied in the floral cup surrounding the ovary. The treatment was repeated 1 week later.

A similar treatment without NAA was also applied. Control shoots were selected and processed in a similar manner except flowers were open pollinated and permitted to develop. Samples of about 25 fruit were measured at periodic intervals to develop a fruit growth curve (Fig. 1). All samples were transported to the laboratory, where some fruits were frozen and held at −20°C until used for enzyme activity, and some others were lyophilized and used for lignin content measurement and electrophoretic profiles.

Enzyme Extraction and Measurement

Soluble. Pericarp tissue was excised and macerated in a Sorvall Omnimixer with 0.05 M acetate buffer at pH 4.0 (tissue:buffer ratio, 1:4, wt/vol). The homogenate was filtered through four layers of cheesecloth and the filtrate was incubated overnight at 4°C with polyvinylpyrrolidone (PVP; tissue:PVP ratio, 2:1, wt/vol) before centrifugation for 40 min at 25,000 *g*. The supernatant was used for determination of soluble peroxidase and IAA oxidase activities.

Ionically Bound. Residue remaining in the cheesecloth was extracted overnight at 4°C with 1 M KCl in 0.05 M acetate buffer at pH 4.0. The extract was then filtered using a Büchner funnel and Whatman no. 1 paper. The filtrate was centrifuged as above, dialyzed against 0.025 M Tris buffer at pH 7.0 for 48 h with three changes of buffer and assayed for peroxidase and IAA oxidase activities. This was considered the ionically bound fraction.

Covalently Bound. The residue, after extraction of the ionically bound fraction, was incubated for 5 h at room temperature with 0.2 M phosphate buffer, pH 5.25, containing peroxidase-free cellulase (250 mg/100 ml; Sigma Chemical Co., St. Louis, MO, USA) and pectinase (150 mg/100 ml; ICN Nutritional Biochemicals, Cleveland, OH, USA). After incubation, the mixture was extracted, filtered, centrifuged, and dialyzed as described for the ionically bound fraction.

Peroxidase Activity. Enzyme activity was measured by following absorbance at 460 nm after incubation of the extracts with 0.26 mM *o*-dianisidine, 8.8 mM H_2O_2 in 20 mM phosphate buffer, pH 6.0, at 25°C. A unit represents a one increment increase in absorbance (460 nm) per minute at these assay conditions.

IAA Oxidase Activity. Activity was measured by following the increase in absorbance at 247 nm after incubation of the extracts with 0.6 mM IAA, 0.5 mM $MnCl_2$, 0.1 mM *p*-coumaric acid in 60 mM phosphate buffer, pH 5.25, at 30°C. A unit represents a 1×10^{-3} increment increase in absorbance (247 nm) per minute at these assay conditions.

Electrophoresis

Anionic. The procedure described by Davis (1964) was used with 7.5% polyacrylamide for the separating gel and 4% for the stacking gel. The same amount of protein for all samples was loaded per lane, which corresponded to 0.72 μg for soluble extracts and 0.69 μg for ionically bound extracts. Currents of 1.5 and 3 to 4 mA per lane were applied to the stacking and separating gels, respectively.

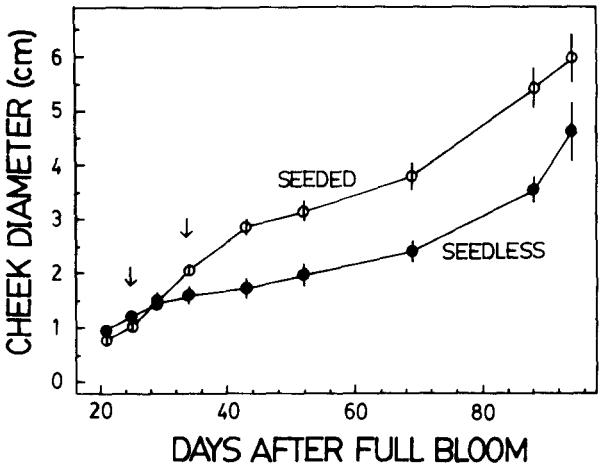

Fig. 1. Growth curves for seeded and seedless (AC 94377 + NAA) "Redhaven" peach fruit from bloom to maturity. Arrows indicate time of sampling (25 and 34 days after full bloom).

Cationic. Cationic separation was achieved using the procedure described by Reisfield et al. (1962), except 7.5% polyacrylamide was used for the separating and 4% for the stacking gel. The amount of protein per lane was identical to the anionic electrophoresis above. Separation was achieved with 2 mA for the stacking and 6 to 7 mA per lane for the separating gel.

Gel Staining. The gels were incubated in the presence of 6 mM *o*-dianisidine and 8.8 mM H_2O_2 in 20 mM phosphate buffer, pH 6.0, for 20–30 min at room temperature (about 23°C).

Protein. The Bradford (1976) procedure was used with bovine serum albumin as standard.

Lignin. The procedure described by Bruce and West (1989) was followed to evaluate lignin content of pericarp tissue, starting from 0.5 g of lyophilized plant material.

Results and Discussion

Application of N-substituted phthalimide to emasculated flowers induced parthenocarpic fruit development with the final size significantly greater for seeded fruits (Fig. 1). Parthenocarpic fruit exhibited a slightly different growth pattern than seeded fruit;

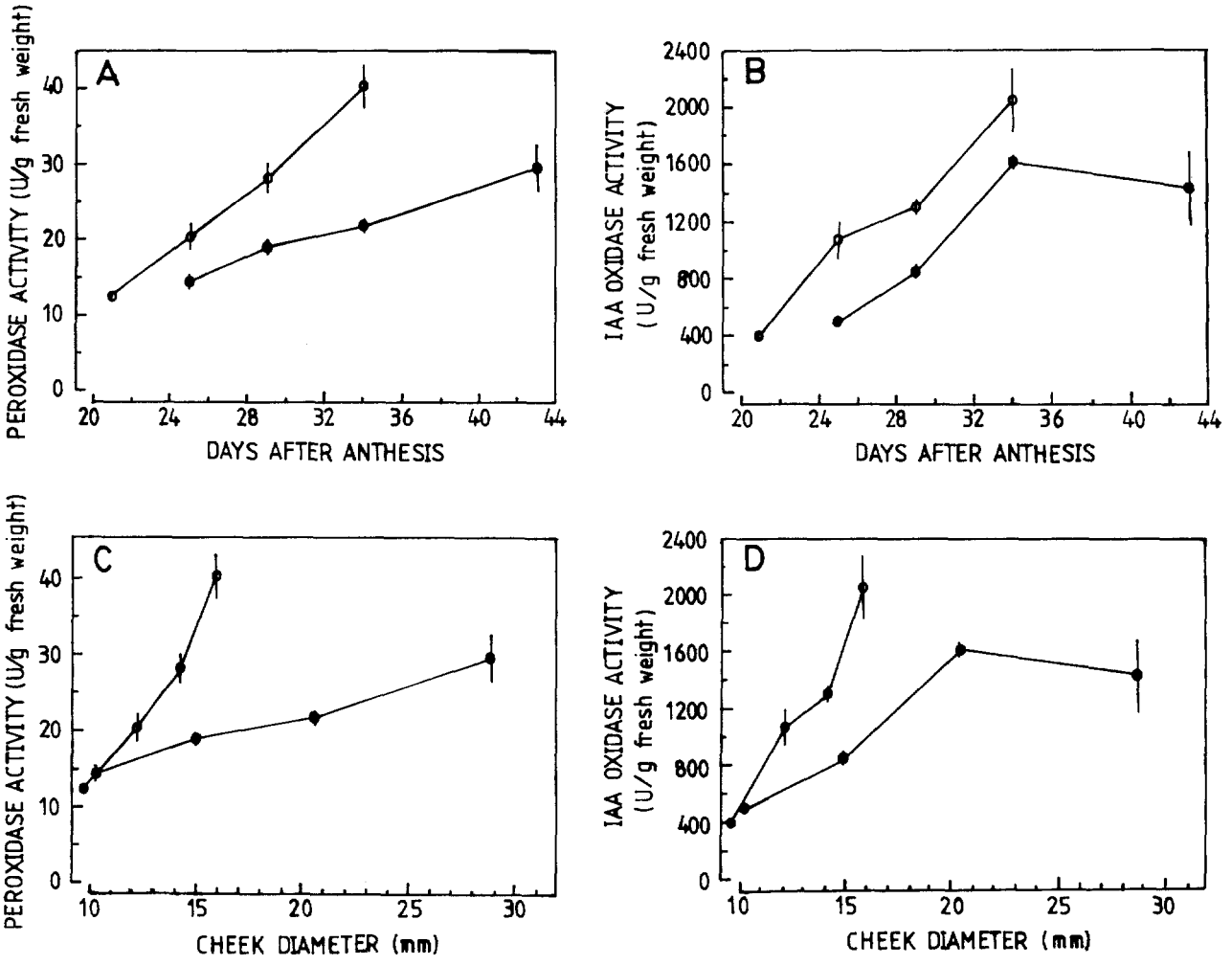

Fig. 2. Peroxidase and IAA oxidase activities in the pericarp of seeded (•) and seedless (○) peach fruit in relation to days after full bloom **(A,B)** and fruit size **(C,D)**.

Table 1. Soluble, ionically, and covalently bound peroxidase activity in the pericarp of seeded and seedless (with and without NAA addition) fruit at 25 and 34 days after full bloom.

		Peroxidase activity (units/g fresh weight)[a]		
Days after full bloom	Treatment	Soluble	Ionically bound	Covalently bound
25	Control	3.3 ± 0.5	5.6 ± 1.1	5.6 ± 0.4
	AC 94377	2.8 ± 0.2	12.6 ± 1.4	5.0 ± 0.7
	AC 94377 + NAA	2.0 ± 0.2	13.7 ± 2.4	4.9 ± 0.8
34	Control	3.1 ± 0.4	14.3 ± 0.7	4.5 ± 0.2
	AC 94377	6.5 ± 0.4	20.5 ± 1.0	13.3 ± 2.1
	AC 94377 + NAA	7.8 ± 0.4	20.0 ± 3.1	12.5 ± 1.1

[a] A unit represents one increment increase in absorbance (460 nm) per minute at assay conditions specified in Materials and Methods. Each value represents the mean (±SE) of three extracts assayed three times each.

Table 2. Soluble, ionically, and covalently bound IAA oxidase activity in the pericarp of seeded and seedless (with and without NAA addition) fruit at 25 and 34 days after full bloom.

Days after full bloom	Treatment	IAA oxidase activity (units/g fresh weight)[a]		
		Soluble	Ionically bound	Covalently bound
25	Control	4.7 ± 1.1	239 ± 40	258 ± 16
	AC 94377	17.5 ± 2.4	1027 ± 167	450 ± 15
	AC 94377 + NAA	5.0 ± 0.7	856 ± 126	212 ± 13
34	Control	34.8 ± 1.4	1190 ± 48	392 ± 2
	AC 94377	96.3 ± 2.8	1136 ± 70	1050 ± 272
	AC 94377 + NAA	135.2 ± 2.9	1111 ± 166	806 ± 132

[a] A unit represents 1×10^{-3} increment increase in absorbance (247 nm) per minute at assay conditions specified in Materials and Methods. Each value represents the mean (±SE) of three extracts assayed three times each.

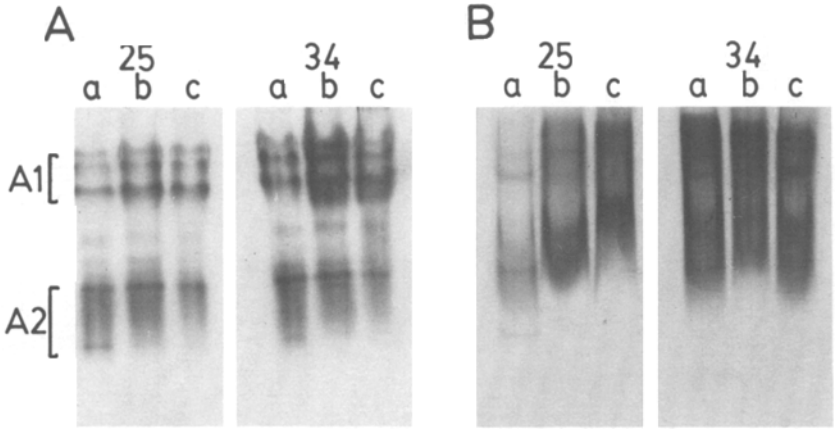

Fig. 3. Anionic electrophoretic profile of the soluble (0.72 μg of protein per lane) **(A)**, and ionically bound (0.69 μg of protein per lane) **(B)** peroxidase fractions from peach pericarp tissue of control (*a*), AC 94377 + NAA (*b*), and AC 94377 (*c*) treated fruit, 25 and 34 days after full bloom.

their initial growth rate was greater, but they grew more slowly during stage II and matured (based on color and firmness) 3–10 days before seeded fruit. In the absence of NAA, the phthalimide also induced parthenocarpic fruit but the percentage of persisting fruit was lower, as it has been previously found in sour cherry (Bukovac et al. 1985).

During the transition from stage I to stage II, both total peroxidase activity and IAA oxidase activity in the pericarp were always higher for seedless (AC 94377 plus NAA treatment) than for seeded fruit (Fig. 2A and B). These relationships were also apparent when the enzyme activities were expressed as a function of fruit size (Fig. 2C and D); enzyme activities in the pericarp of seedless fruit were also higher when compared to seeded fruit of the same size.

Two sampling times were chosen to study the peroxidase and IAA oxidase activities of different fractions and phthalimide treatments (Tables 1 and 2). They represent dates when the pericarp was actively enlarging in both seeded and seedless fruits (25 days after full bloom) and when growth of the seedless fruit had almost ceased, but the seeded fruit was still enlarging (34 days after full bloom). The results in Table 1 show first that there was no specific effect of NAA on total peroxidase activity, since the values were not significantly different for both treatments, with or without NAA. Second, it was also apparent that the highest activities were found in the ionically bound fraction, followed by the covalently bound fraction. Consistent differences between seeded and seedless (with or without NAA) fruits were found only in the ionically bound fraction. Higher IAA degrading capacity occurred in the ionically and covalently bound fractions, whereas the lowest activities were present in the soluble fractions (Table 2). These results indicate that the absence of the seed causes an earlier expression of peroxidases, which results in higher values for both total peroxidase and IAA oxidase activities in seedless fruits. However, since the IAA

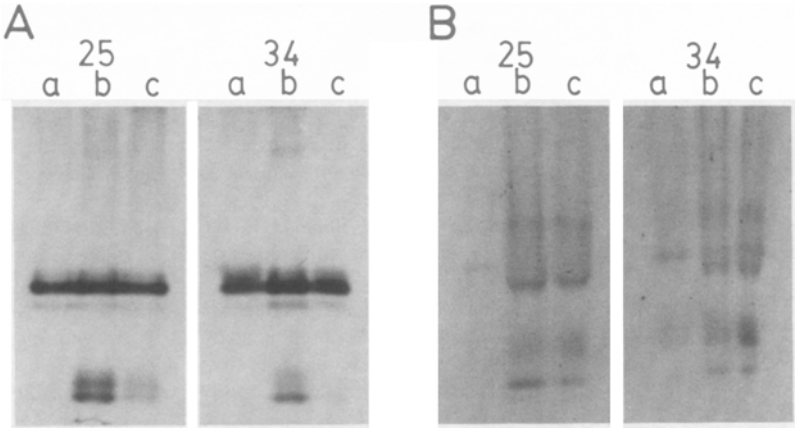

Fig. 4. Cationic electrophoretic profile of soluble (0.72 μg of protein per lane) (**A**), and ionically bound (0.69 μg of protein per lane) (**B**) peroxidase fractions from the pericarp tissue of control (*a*), AC 94377 + NAA (*b*), and AC 94377 (*c*) treated fruit, 25 and 34 days after full bloom.

Table 3. Lignin content in the pericarp of seeded and seedless (with and without NAA addition) peach fruit at 34 and 69 days after full bloom.

	Lignin[a] (280 nm/g lyophilized weight)		
Days after full bloom	Control	AC 94377	AC 94377 + NAA
34	8.8 ± 0.1	8.0 ± 0.2	6.4 ± 0.2
69	60.4 ± 2.4	35.7 ± 2.8	35.4 ± 1.0

[a] Values displayed correspond to the absorbance at 280 nm, of the lignin thioglycolic acid solution per gram of lyophilized pericarp obtained following the procedure indicated in Materials and Methods. Each value represents the mean (+SE) of three extracts determined three times each.

oxidase to peroxidase ratio for all the fractions was not significantly different between seeded and seedless fruits, further studies focused on the peroxidase isoenzymes.

In many plant tissues there is a clear developmental pattern for peroxidases. Thus, recent studies have shown that an altered expression of this activity in tobacco has a great effect on the physiology and the morphology of this plant (Lagrimini et al. 1990). In peaches, it has been reported that the pericarp tissue of the "Merry" cultivar displays a consistent pattern of peroxidase isoenzymes from stage I to stage III fruit growth (Sánchez-Roldán et al. 1990).

Distinct differences were found in the peroxidase isoenzyme profiles of the soluble and ionically bound fractions when an identical amount of protein was loaded per lane (Figs. 3 and 4). Two groups of anionic isoenzymes were distinguished (Fig. 3A and B). The more acidic isoenzymes (A_2) appear more expressed in seeded fruits, whereas the less acidic ones (A_1) showed higher staining in seedless fruits, at both sampling dates. In relation to the cationic isoenzymes, several new bands were apparent in parthenocarpic fruits (Fig. 4A and B) in both soluble and ionically bound fractions at the two sampling dates. Slight differences were found in the staining pattern of the more cationic bands of the soluble extract when the two treatments inducing parthenocarpy were compared.

Some reports assign a main role to the cationic isoenzymes (Church and Galston 1988) in lignin biosynthesis. Our results did not support this relationship (Table 3), since the lignin content of the parthenocarpic fruit was not higher at 34 days after full bloom and was even significantly lower at 69 days after full bloom. The only difference found in the isoenzyme profiles to explain this lignin content difference was the presence of highly acidic anionic isoenzymes (A_2) in the pericarp of seeded fruits (Fig. 3) at 25 and 34 days after full bloom at a time when the lignification process is initiating. This would agree with previous results of Catesson et al. (1986) involving anionic isoenzymes in lignin biosynthesis.

Thus, it can be concluded that parthenocarpic peach fruits can be obtained by using a gibberellin-like compound, AC 94377, and the absence of seed changes the developmental expression pattern of peroxidases in the pericarp. Changes in anionic isoenzymes are only quantitative and they could be related to the different lignin contents of seeded and seedless fruits. New cationic isoperoxidases appear in the pericarp of parthenocarpic fruits whose significance has to be established.

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