of Ca and is an important mineral associated with membrane structure. The concentration of Mg was slightly higher during the 1st part of the 1981–82 sampling period (0.25–0.28 $mg \cdot g^{-1}$; September-November) when contrasted to the latter period (0.18–0.23 $mg \cdot g^{-1}$; December-May). When Mg values of the 1981–82 season were compared to the 1982–83 season values, the only significant difference was noted with the 1 Feb. sampling date; the concentration on that date was elevated in the 1982–83 season.

Because Na was found at very low levels in peel, its concentration range was not reported in Table 1. Over the 9-month maturation period, Na fluctuated between 0.003 and 0.034 $mg \cdot g^{-1}$ fresh peel weight. It was difficult to assess any impact of freezing on peel Na because of its low levels (about 1/100 to 1/1000 the level of K).

Zinc, also not reported in Table 1, showed no significant variation over the 1981–82 and 1982–83 maturation periods. Its concentration (0.001–0.002 $mg \cdot g^{-1}$ fresh peel weight) in peel was negligible.

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Endogenous Plant Growth Substances in Developing Fruit of *Prunus cerasus* XIII. Relationship between Conjugated and Free Abscisic Acid in Pericarp

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Abstract. **The presence of abscisic acid (ABA) in both conjugated and free forms was established by thin-layer and gas-liquid chromatography and by combined gas chromatography-mass spectrometry. The concentration of the free acid was greater than that of conjugated ABA early in fruit development (Stages I and II); however, the conjugated form was more prevalent than the free acid during Stage III. The highest concentration of both the free acid and the conjugated ABA was found during periods of most active fruit enlargement and low concentrations during the retarded phase of pericarp growth. The conjugated form represented a significant and, during Stage III, the major portion of the total ABA present.**

Abscisic acid (ABA) occurs naturally in fruits of numerous species (3, 4, 6, 7, 8, 12, 15). In addition, a water-soluble conjugate, abscisyl-β-D-glucopyranoside, has been isolated from fruit of *Lupinus luteus* (6) and since has been found in other fruits (8, 10, 13). Of the total ABA found in fruit of *Rosa arvensis*, 20% was in the form of the glucosyl abscisate (8). Plant tissues readily convert exogenously supplied ABA to the glycosyl abscisate (8, 16), and there is indication that this conjugate may be hydrolyzed (8). Any assessment of the role of ABA in fruit growth should take into consideration not only the free acid, but also the conjugated fraction. We reported (4), based on biological activity, that inhibitor levels in sour cherry fruit were directly related to growth rate of the fruit. We now report on changes in levels of a hydrolyzable ABA conjugate during fruit development and discuss its relationship to ABA.

Sour cherry (*Prunus cerasus* L.) fruit were collected from 7-year-old trees at weekly intervals from one week after anthesis until fruit maturity. The fruit were frozen immediately in dry ice, transferred to the laboratory, and lyophylized. A fruit growth curve was constructed based on pericarp dry weight. Fruit from collections made on 21 and 28, 35 and 42, and 56 and 70 days after anthesis, selected as being in Stage I, II, and III of fruit development, respectively, were used for analysis. The pericarp (exo-, meso-, and endo-) tissue was removed, ground to pass a 20-mesh screen, and stored at -25°C until extraction.

Procedures employed in extraction, fractionation, thin-layer chromatography (TLC), and wheat coleoptile bioassay of ABA have been described previously (4, 13). The aqueous fraction of the methanol extract, after partitioning against diethyl ether at pH 2.5, was adjusted to pH 11 with NaOH and hydrolyzed for 30 min at 60°C. The solution was cooled, the pH adjusted to 3.0, and the released inhibitor extracted and purified as previously described.

The ABA in the acid-ether fraction and that released on hydrolysis was methylated with diazomethane following TLC and subjected to gas-liquid chromatography (Packard 7300) using a U-column (183 cm × 2 mm) packed with 3% SE 30 on silanized Gas Chrome Q (60-80 mesh). The injector block and detector temperature was 250°C, the column was isothermal at 170°, and the carrier gas (N_2) flow was 42 $ml \cdot min^{-1}$. To quantify ABA, the peak heights were compared with those of standard quantities of both the *cis-trans* and *trans-trans* isomers of MeABA. Conclusive identification of the inhibitor (both the free acid and that released on hydrolysis) as ABA was obtained by combined gas chromatography-mass spectrometry (GC-MS). Procedures employed and equipment used were identical to those described earlier (4).

Two zones of growth inhibition, as indexed by the wheat coleoptile assay, were found after hydrolysis of the aqueous phase and partial purification by TLC. We previously identified (13) *p*-coumaric acid as the inhibitor running at Rf 0.6–0.9. The inhibitory substance running at Rf 0.0–0.2 cochromatographed with synthetic ABA and, after further purification on TLC (benzene:ethyl acetate:formic acid, 70:30:1), was identified by GC-MS as ABA. The inhibitory substance in the acid-ether fraction (free acid)

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Table 1. Concentrations of free and conjugated ABA in cherry pericarp tissue during fruit development.

Stage of fruit development	Days after anthesis	Free ABA			Conjugated ABA			Ratio[z]	Conjugated (%)
		cis	*trans*	Total	*cis*	*trans*	Total		
		(ng · g · dry wt^{-1})							
I	21	157	42	199	66	13	79	2.52	28.4
I	28	108	31	139	58	7	65	2.14	31.9
II	35	109	36	145	19	6	25	5.80	14.7
II	42	69	22	91	55	32	87	1.04	48.9
III	56	104	6	110	175	55	230	0.48	67.6
III	70	69	13	82	165	59	224	0.37	73.2

[z]Ratio of total free ABA to total conjugated ABA.

also was confirmed by TLC, GLC, and GC-MS as being ABA.

The concentration of free ABA in the pericarp decreased from 21 days after anthesis to the lowest level during Stage II (42 days after anthesis), then increased during early Stage III (Table 1). The concentrations of the total conjugated ABA (25 to 87 ng g dry weight^{-1}) were lower than those of free ABA during Stage I and early Stage II, but rose to levels markedly higher than those of free ABA in Stage III (Table 1).

On a per fruit basis, the conjugated ABA varied between 4.7 and 9.0 μg·fruit^{-1} (pericarp) during Stage I and early Stage II, then rose dramatically to 121 and 149 μg fruit^{-1} at 56 and 70 days after anthesis (Stage III), respectively. This increase was closely associated with the increase in pericarp dry weight. In comparison, the free acid increased from 11.5 to 27 μg·fruit^{-1} during Stages I and II and then increased to about 55 μg·fruit^{-1} during Stage III.

The ratio of total free to total conjugated ABA increased from 2 to 2.5 in Stage I, to 5.8 in early Stage II, and the declined to less than 0.5 during Stage III. Thus, the free acid was predominant early and the conjugated form late in fruit development (Table 1). It is noteworthy that, with one exception, 28% to 73% of the total ABA present was found in the conjugated form.

The role of conjugated ABA in fruit growth has not been studied extensively. The marked increase in the concentration of the conjugated form occurred during Stage III, a period of rapid pericarp enlargement, sugar accumulation, and ripening. It is tempting to speculate that the conjugated fraction may be involved in pericarp growth by reducing the pool size of free ABA during periods of rapid fruit enlargement. This reduction appears unlikely, however, for high concentrations of free ABA were present during periods of both rapid (Stages I and III) and retarded (Stage II) fruit development. Based on the relationship between concentrations present and growth rate of the pericarp, neither free nor conjugated ABA seem to control pericarp enlargement in light of our knowledge of ABA as a growth inhibitor (1, 5, 15). On the contrary, highly significant positive corelation was found between free ($r=0.88$), conjugated ($r=0.96$), and total ($r=0.97$) ABA and growth rate of the pericarp. Evidence in other plants points to a role for ABA in directing assimilates to reproductive structures (2, 14). In apple fruit tissue, the ABA level was related to sorbitol uptake, and it was suggested that the capacity of apple tissue to utilize sorbitol was regulated by ABA (2). What role if any the conjuated fraction may play is not known.

Although we did not attempt to identify the nature of the conjugated form, we assume that it is the abscisyl-β-D-glucopyranoside as identified by Koshimizu et al. (6) and Milborrow (8). To what extent the conjugated fraction may serve as a source of free ABA in cherry is not know. Milborrow (8) showed that the cell sap from homogenized tomato shoots hydrolyzed the glucose ester. Zeevaart (16) found that the glucose ester of ABA accumulated in *Xanthium* leaves during water stress. The level of the conjagated ABA remained relatively constant after removal of stress, suggesting little or no conversion back to the free acid or that an equilibrium existed between synthesis and degradation. The increases in free ABA observed in our study obviously did not arise from hydrolysis of the conjugated fraction, for the concentrations of the 2 forms almost paralleled one another with one exception (70 days after anthesis), during fruit development.

Small quantities of *trans-trans* ABA also were found in the pericarp; however, these quantities may have resulted from light-induced isomerization (8, 11), for no special precaution was taken to prevent this.

Levels of the conjugated ABA fraction and their relationship to those of the free acid deserve greater attention in studies of fruit growth. Conjugated ABA has been found in numerous fruit (3, 6, 8, 10, 12) and generally is a significant, if not the major, constituent of the ABA complex. Whether it is merely an inactive storage form (9) or a physiologically meaningful fraction remains to be determined.

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