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Endogenous Plant Growth Substances in Developing Fruit of *Prunus cerasus* L. IV. Extractable Auxin in the Seed and Pericarp[1]

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Abstract. Endogenous acidic and neutral growth-promoting substances were extracted from sour cherry (*Prunus cerasus* L. cv. Montmorency) seed and pericarp tissues, separated by ascending paper chromatography and detected by the *Avena* first internode bioassay. Although correlations were established between the levels of seed auxin and concomitant development of the nucellus and integuments and the development of the endosperm and embryo, no correlation was found between the levels of seed and pericarp auxin or between the level of pericarp auxin and concomitant fruit development.

The relationship between seed development and concomitant fruit growth was initially shown as correlations between seed number and fruit size, and between seed distribution and fruit shape (1, 6, 16). Evidence presented by Dollfus (4), and later by Gustafson (5), showed that developing ovules produced growth substances active in the *Avena* curvature test. These substances, which appeared to be closely related to auxins, were suggested to be responsible for the growth of the fruit. Gustafson (5) established that naturally parthenocarpic fruits contained sufficiently high auxin levels at anthesis to induce ovary swelling in the absence of seeds.

In a comprehensive review, Crane (3) evaluated the role of growth substances in fruit setting and development. Although developing seeds were found to be possible sites of synthesis for auxins, gibberellins, cytokinins, and inhibitors, he concluded that no consistent relationship was apparent between the levels of any of the growth substances and fruit development. However, auxin levels have seldom been determined in both seed and ovary wall and associated tissues of the same fruit (9, 11, 14). In this regard, no relationship has been established between the level of auxin in the seed and that located in the remainder of the fruit, or between the level of auxin in the ovary wall tissues and concomitant fruit development.

Tukey (17) demonstrated a marked dependence of sour cherry fruit growth on the seed by destroying the embryo at different stages of fruit development. Destruction of the seed during early Stage II [as defined by Conners (2)] of fruit growth resulted in an abrupt cessation of ovary wall development. He concluded that the presence of a developing embryo was essential to the development of the fruit.

Because of the marked dependence of fruit growth on seed development and since changes between consecutive seed and fruit growth stages in the sour cherry are relatively abrupt (10), we investigated the relationship between auxin levels in the seed and pericarp and development of the fruit.

Materials and Methods

Fruit collection. Fruits were collected from trees at the Horticultural Research Center, East Lansing, at weekly intervals from anthesis to maturity, immediately frozen in dry ice and lyophilized. The lyophilized fruits from each harvest date were separated into seed and pericarp tissues.

Extraction. After grinding to pass a 20 mesh screen, each tissue from each harvest date was extracted for 24 hr in the dark with cold methanol (90%) at −25°C. The extracts were filtered through glass wool and the resulting residue was washed several times with cold 90% methanol. The filtrate and washings were combined and evaporated to the aqueous phase under reduced pressure (flash evaporator, water bath temp. 30°C). The aqueous phase was adjusted to pH 8.5 and partitioned against peroxide-free ethyl ether to give a basic ethyl ether-soluble fraction. The aqueous phase was then adjusted to pH 3.0 and extracted with ethyl ether to give an acidic ethyl ether-soluble fraction (8). The basic ethyl ether-soluble fraction was evaporated to near dryness and the residue was extracted with hexane and acetonitrile (7) to yield an acetonitrile fraction that contained neutral and basic substances. In the first year (1969), the acetonitrile and acidic ether fractions were combined, thoroughly mixed and then divided into 2 portions. Each portion was further purified by paper chromatography using 2 solvent systems of markedly different polarity. In a second year (1971), the acetonitrile phase was evaporated to near dryness, redissolved in water (pH 6.5) and partitioned against ethyl ether to yield an ether-soluble fraction (7), hereafter referred to as the neutral fraction. Each fraction (acidic and neutral) was independently

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resolved by paper chromatography. All solvents were redistilled before use.

Paper chromatography. Extracted substances were quantitatively streaked on Whatman 3 MM paper that had been previously washed in the developing solvent. Chromatograms were equilibrated over the developing solvent for 12 hr and then developed to a distance of 20 cm using 1 of the following solvent systems; A) isopropanol:water (4:1 v/v), B) hexane:water (9:1 v/v), C) isopropanol:water (9:1 v/v), and D) hexane:water (upper phase). After development, chromatograms were dried in a cool air stream and stored at −25°C, in the dark, in a N atmosphere.

Bioassay. Strips (2 × 4 cm) from developed chromatograms were bioassayed for auxin activity by the *Avena* first internode test (12). Control strips were taken from the area below the origin. In order to standardize the growth response, known concn of indole-3-acetic acid (IAA) were bioassayed in the presence of strips cut from unspotted, but developed, chromatograms. The bioassay response (final section length − initial section length) was expressed as a percentage of the initial length and where applicable, upper and lower 5% fiducial limits were calculated (19) outside of which a treatment must fall in order to be significant.

Results

Histograms depicting the bioassay response to seed and pericarp extracts (1969) exhibited both quantitative and qualitative changes with fruit development (Fig. 1). Although the auxin levels were determined at weekly intervals, we present data only for 21, 28, 42, and 56 days after anthesis, since they are representative of the changes observed. Depending on the sampling date, 1–3 zones of growth promotion were observed on chromatograms of seed and pericarp tissues with solvent system A. A noticeable zone of activity detectable at Rf 0.3–0.6 (21 days after anthesis) was not apparent at later stages of fruit growth, while pronounced activity was noted at Rf 0.5–0.7 later in fruit development. A less conspicuous zone of activity was observed at Rf 0.0–0.1 at all sampling dates except 28 days after anthesis. A third zone of activity, which occurred at Rf 0.8–0.9 at 21 days after anthesis, was replaced by one of inhibition (28 and 42 days after anthesis). The promotive activity was again present at this zone 56 days after anthesis.

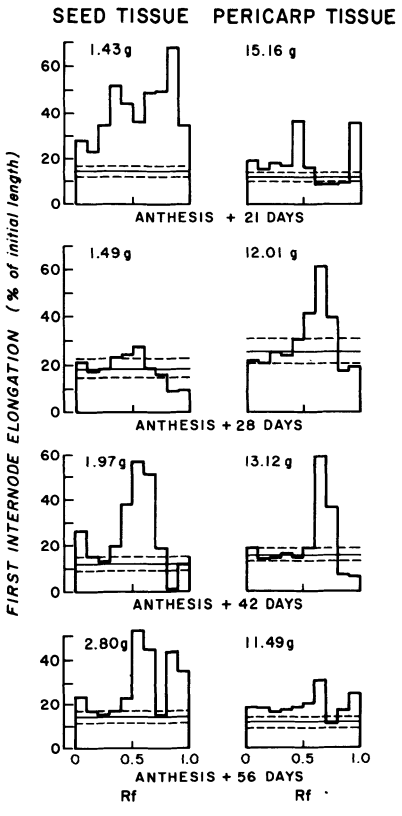

Fig. 1. Representative histograms depicting the *Avena* first internode bioassay response to growth-promoting substances separated by paper chromatography in sour cherry seed and pericarp tissues. Solvent system: isopropanol:water (4:1 v/v). Control value (——). Upper and lower 5% fiducial limits (– – –).

Equal g equivalents of seed and pericarp extracts were also chromatographed in solvent system B. Although fluctuations in growth-promotive activity between harvests were similar to those shown in Fig. 1, poor resolution between the 2 promoters was achieved with this solvent system.

In order to relate detected growth-promotive activity to seed and fruit development, each histogram peak delineated by the upper 5% fiducial limit (Fig. 1) was converted to IAA equivalents by means of a standard curve and summed to give the total auxin level for each harvest date. Since organic solvent residues from extraction and fractionation procedures were found to be inactive, the computed growth response can be ascribed entirely to extracted substances. Detailed studies on the morphological development of fruit from this same population have been reported (10).

Growth-promoting substances in seed tissues (Fig. 2A) increased from initially low levels (7, 14 days after anthesis) to a maximum (21 days after anthesis) and then declined. A second peak of growth-promoting activity was found between 28 and 49 days from anthesis. The lack of agreement between the 2 solvent systems (28 to 49 days after anthesis) can be attributed to the presence of a zone of inhibition on chromatograms developed in solvent A (isopropanol:water). This zone of inhibition was absent from chromatograms developed in solvent B. Subsequently, we have shown that this zone of inhibition was due to an interaction between 2 neutral promoters (7). A third, but smaller, peak of promotive activity was found in seed tissues during the latter stages of fruit growth (49 to 63 days after anthesis).

In contrast to levels found in seed tissue, growth-promoter levels in the pericarp were found to be approximately 2 orders of magnitude lower (Fig. 2B). Promoter levels increased slowly from 21 days after anthesis to the onset of rapid cell enlargement (42 days after anthesis) and then decreased. The sharp increase in promoter levels 63 to 70 days after anthesis (solvent A) was not found for chromatograms developed in solvent B.

In 1971, seed and pericarp extracts were further separated into acidic and neutral fractions. The acidic fraction was chromatographed in solvent system C. A single zone of growth promotion was located

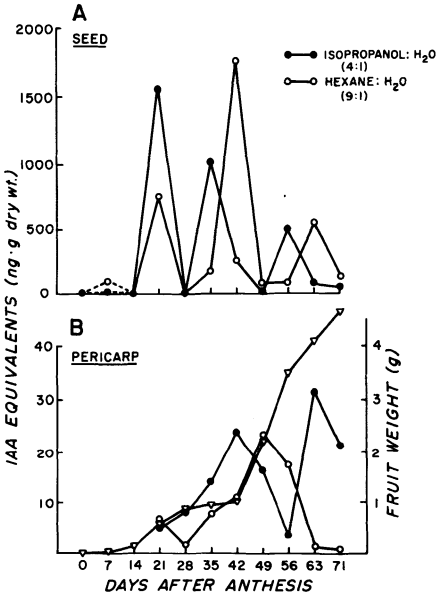

Fig. 2. Changes in levels of total endogenous growth-promoting substances in sour cherry seed and pericarp tissues, expressed as IAA equivalents per g dry wt, in relation to fruit development. IAA equivalents (—○—, —●—). Fruit wt (—▽—).

between Rf 0.5–0.9 that chromatographed with authentic IAA (8). This promoter has been identified as IAA (8). The neutral fractions were chromatographed in solvent system D. Two zones of growth promotion were detected, one at Rf 0.0–0.1 (designated neutral auxin I) and the other at Rf 0.5–0.7 (designated neutral auxin II) (7).

High levels of all 3 auxins were found in seed tissue 21 days after anthesis (Table 1). Subsequently, the level of auxins declined (28, 42, and 56 days after anthesis) although the decline in acidic auxin level was more rapid than for either of the neutral auxins. These trends in auxin level were not found for pericarp tissue (Table 1). For pericarp tissue, the initially high level of acidic auxin (21 days after anthesis) decreased to below the bioassay detection level (28 days after anthesis) and then increased during Stages II and III of growth. A similar trend was found for neutral auxin II although its level declined during growth Stage III. In contrast, neutral auxin I was not detected in any pericarp extract.

Table 1. Comparison of auxin levels in sour cherry seed and pericarp tissues at different stages of fruit development in 1969 and 1971.

Days after anthesis	IAA equivalents (ng·fruit^{-1}) Acidic auxin[z]	Neutral auxin I[y]	Neutral auxin II[x]	Total auxin (1971)	Total auxin (1969) IW[w]	Total auxin (1969) HW[v]
			Seed			
21	11.2	8.6	22.1	41.9	5.6	2.6
28	0.8	7.7	10.4	18.9	0.02	0.02
42	0.7	0.6	10.3	11.6	2.6	18.5
56	0.7	0[u]	6.6	7.3	16.4	3.0
			Pericarp			
21	8.7	0	0.4	9.1	0.2	0.4
28	0	0	0	0	1.0	0.2
42	2.2	0	20.8	23.0	5.0	2.3
56	4.1	0	2.5	6.6	1.8	9.1

[z] Rf 0.5–0.9, Isopropanol:water (9:1); identified as IAA (8).
[y] Rf 0.0–0.1, Hexane:water (upper phase); IAA, Rf 0.0; IAN Rf 0.2.
[x] Rf 0.5–0.7, Hexane:water (upper phase)
[w] Rf 0.0–1.0, Isopropanol:water (4:1), Fig. 2A, 2B
[v] Rf 0.0–1.0, Hexane:water (9:1), Fig. 2A, 2B
[u] 0 denotes no activity detected

Discussion

The total auxin content of the seed (1969) exhibited 3 peaks of activity (Fig. 2A). The first and second peaks correspond with nucellus and integument development and rapid growth of the endosperm and embryo, respectively (10). Similar findings have been reported for other *Prunus* sp. (13, 14, 15, 18). The third peak of activity cannot be correlated with any morphological change in the seed. However, computing auxin content in concn terms (ng·g dry wt^{-1}) invokes a hidden dilution factor of increasing seed wt with successive harvests. When the above data were expressed on a per seed basis (Table 1, 1969), the peaks of activity were shifted from initial to later stages of seed development. This effect was not found in a second season of growth (Table 1, total auxin, 1971). Furthermore, the level of auxin associated with nucellus and integument development was found to be considerably higher than that associated with the endosperm and embryo (Table 1, Ref. 10, and Fig. 1).

The total auxin content of the pericarp (Fig. 2B, Table 1) did not mirror the seasonal fluctuations found in the seed. In both years, the level of pericarp auxin increased to a peak at 42–49 days after anthesis and then declined. The considerable difference between seed and pericarp auxin concn (Fig. 2A, 2B) and the absence of neutral auxin I in pericarp extracts (Table 1) suggest that the rapid decrease of seed auxin (21 to 49 days after anthesis) was not simply due to movement into the pericarp.

No correlation could be established between the level of pericarp auxin (Fig. 2B) and the first period of rapid cell enlargement in the pericarp. Moreover, the period of reduced pericarp cell enlargement (28 to 42 days after anthesis) did not appear to be associated with low auxin levels, since the level of pericarp auxin increased during this time (Fig. 2B, Table 1). The regulation of cell enlargement during this period could be due to one, or more, inhibitors that conceivably would be maintained at a high level until the onset of Stage III of fruit growth.

The onset of rapid cell enlargement in the pericarp (42 to 56 days after anthesis) was marked by a decrease in pericarp auxin concn (Fig. 2B, Table 1). Subsequently, the pericarp auxin level increased just prior to maturity when separated with solvent A but not when solvent system B was used (Fig. 2B). Several investigators have sought a correlation between seed auxin levels and the initiation of this period of rapid cell enlargement in the fruit (3). Since the marked dependence of sour cherry fruit growth on the seed is lost by the end of Stage II (17), any hormonal factors necessary for pericarp cell enlargement must be mobilized from the seed to the pericarp before this time. Although we have demonstrated the presence of auxin in the pericarp, our evidence strongly suggests no correlation exists between the level of this auxin with any stage of fruit development.

One further point needs mention. We have no simple explanation for differences observed between seasons or when using different solvent systems in chromatography. Such difficulties point up the need for an improved approach to the study of the role of growth substances in fruit development.

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