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Hormones in Pear Seeds I. Levels of Gibberellins, Abscisic Acid, Phaseic Acid, Dihydrophaseic Acid, and Two Metabolites of Dihydrophaseic Acid in Immature Seeds of *Pyrus communis* L.[1]

G. C. Martin, F. G. Dennis, Jr.[2], J. MacMillan[3], and P. Gaskin[3]
Department of Pomology, University of California, Davis, CA 95616

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Abstract. **Seeds from 'Bartlett' and 'Winter Nelis' and ovules from seedless 'Bartlett' pears were collected periodically between 25 days after full bloom and harvest. Extracts were analyzed for hormones by combined gas chromatography-mass spectrometry, using the technique of selected ion current monitoring. Levels of 6 gibberellins were low in all samples prior to appreciable embryo growth (25 days after full bloom). Content of gibberellins A_{17}, A_{25}, A_{45} and a presumed 3β-hydroxy gibberellin A_{45} rose dramatically during rapid embryo growth between 65 and 85 days after full bloom, while the gibberellin content of embryoless ovules of 'Bartlett' did not change during this period. Two unidentified gibberellin-like compounds, one isomeric with gibberellin A_{25} and the other corresponding to a hydroxy gibberellin A_{45}, were detected 85 days after full bloom. Abscisic acid content was also maximal between 65 and 106 days, ovules of seedless 'Bartlett' exhibiting considerably higher concentration than seeds of either cultivar. Levels of 5 abscisic acid metabolites varied with seed type and sampling period. Phaseic acid levels remained low in 'Bartlett' seeds and ovules during all developmental stages but increased in 'Winter Nelis' seeds at 122 days. Concentration of *cis, trans*-dihydrophaseic acid, although low, rose as 'Winter Nelis' seeds matured while ovules of seedless 'Bartlett' showed no such increase. Levels of 2 metabolites, tentatively identified as *trans, trans*-dihydrophaseic acid and a hydroxylated derivative of dihydrophaseic acid, varied only slightly with development. A third metabolite, characterized as a keto derivative of dihydrophaseic acid or a hydroxy-derivative of phaseic acid, was present in large quantities in unfertilized ovules during the early period of fruit growth, but increased in seeds only after 65 days. The possible roles of these compounds are discussed in relation to seed, fruit, and flower development.**

In previous papers (1, 13) we reported the presence of 4 gibberellins (GAs) in immature and/or mature pear seeds, together with phaseic acid (PA), 4-dihydrophaseic acid (DPA), and several derivatives of DPA. The purpose of the present investigation was to quantitate these hormones during fruit and seed development.

Materials and Methods

Sampling procedure. 'Bartlett' and 'Winter Nelis' fruits were collected from commercial orchards in the Sacramento River delta. Seedless fruits of 'Bartlett' were obtained from a solid block of this cultivar, seeded 'Bartlett' and 'Winter Nelis' from an orchard where these 2 cultivars were interplanted. 'Bartlett' fruits were sampled at 25, 40, 65, 85, and 106 days (commercial harvest) and 'Winter Nelis' at 25, 40, 65, 85, 122, and 158 days (commercial harvest after full bloom (AFB). Fruits were taken to the laboratory where the seeds were removed from the flesh, frozen immediately on dry ice, and lyophilized. Unfertilized ovules from seedless 'Bartlett' are henceforth referred to as "ovules" to distinguish them from seeds containing embryos.

Extraction and fractionation. Details of procedures for extraction and fractionation have been described elsewhere (13). Briefly, methanol extracts of 20 g seed samples were partitioned sequentially against petroleum ether and ethyl acetate at pH 8.0, then against ethyl acetate at pH 3.0. The residue from the acidic ethyl acetate fraction was dissolved in phosphate buffer and slurried with polyvinylpyrrolidone (PVP). The filtrate was partitioned against petroleum ether, acidified to pH 3, and partitioned against ethyl acetate. This ethyl acetate fraction was evaporated and the residue prepared for combined gas chromatography-mass spectrometry (GC-MS).

GC-MS. Procedures for derivatization and GC have previously been reported (13). The methylated and trimethylsilylated (meTMS) derivatives were chromatographed on a Pye 104 gas chromatograph coupled with an A.E.I. MS 30 mass spectrometer through a silicone membrane separator (for conditions, see 13). The mass spectrometer was programmed to record the intensity of selected ions, representing the base peaks (m/e values) of the derivatives measured as follows: $MeGA_{17}$TMS-207; $MeGA_{25}$ and the isomer of $MeGA_{25}$-284; $MeGA_{45}$TMS, and the presumed Me 3β-hydroxy TMS GA_{45}-207; the MeTMS of unidentified hydroxy GA_{45}-156; MeABA -190; MePA-125; MeDPA TMS, Me hydroxy-DPA TMS, and Me keto-DPA TMS-159. The ion current at each m/e value was plotted by a UV recorder, and peak heights were used as standards. Known quantities of the remaining compounds were not available, hence relative quantitation was based on ion current at the given m/e values.

Results

Seed and ovule growth. Dry wt of 'Bartlett' ovules and seeds and Winter Nelis' seeds did not differ at 25 and 40 days AFB (Fig. 1). Thereafter, little change in weight occured in ovules, while seeds of both cultivars grew rapidly, with 'Winter Nelis' seeds achieving the greater final dry wt (Fig. 1). Embryos which were not visible macroscopically in seeds until 65 days AFB, grew rapidly between 65 and 85 days, slowing thereafter (Fig. 2).

GA content. All seeds and ovules contained GA_{17}, GA_{25}, GA_{45}, and presumed 3β-hydroxy GA_{45} at some stage of development (Fig. 3). Although mass spectra were not obtained, seed extracts contained 2 additional compounds. One detected by monitoring the m/e 284 ion had a retention time (R_T) slightly longer than $MeGA_{25}$ and is presumably the previously detected but unidentified isomer of $meGA_{25}$ (13). The other detected at m/e 156 with a slightly longer R_T than the presumed Me 3β-hydroxy-GA_{45} TMS is probably the previously

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[2]Department of Horticulture, Michigan State University, East Lansing, MI 48824.
[3]School of Chemistry, University of Bristol, Bristol BS8 1TS, England.

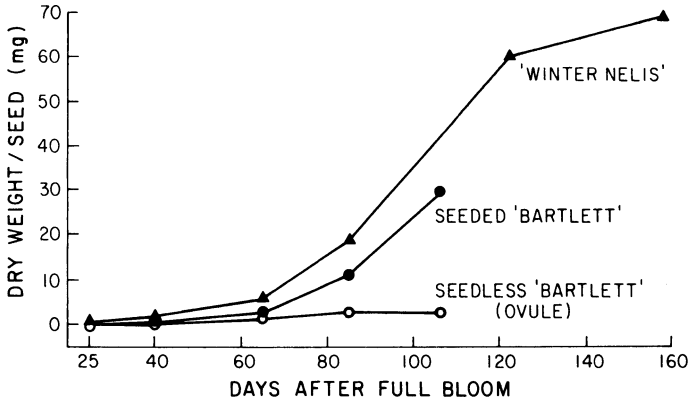

Fig. 1. Cumulative dry wt of seed from 'Bartlett' and 'Winter Nelis' and ovules from parthenocarpic 'Bartlett' pear fruits.

detected isomer of the latter compound (13). Both compounds occurred in 'Bartlett' seeds sampled 85 days AFB, while 'Winter Nelis' seeds contained only the latter.

Levels of all GAs remained low until 65 days AFB, and GA_{17} was the only GA consistently present in early samples. GA content of seeds rose sharply between 65 and 85 days; in contrast, ovules of 'Bartlett' showed small increases during this time. GA_{17}, GA_{45}, and the presumed 3β-hydroxy GA_{45} were prominent in seeds of both cultivars while 'Winter Nelis' seeds contained considerably more of isomers of GA_{25} and 3β-hydroxy GA_{45} than did 'Bartlett' seeds. Levels of all GAs declined subsequently, and were not detected in 'Winter Nelis' seeds by 122 days AFB.

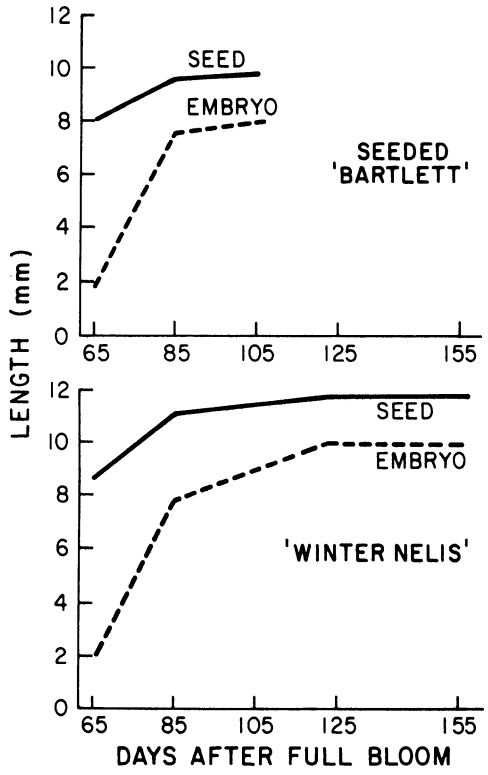

Fig. 2. Cumulative length of seed and embryo from seeded 'Bartlett' and 'Winter Nellis' pear fruits.

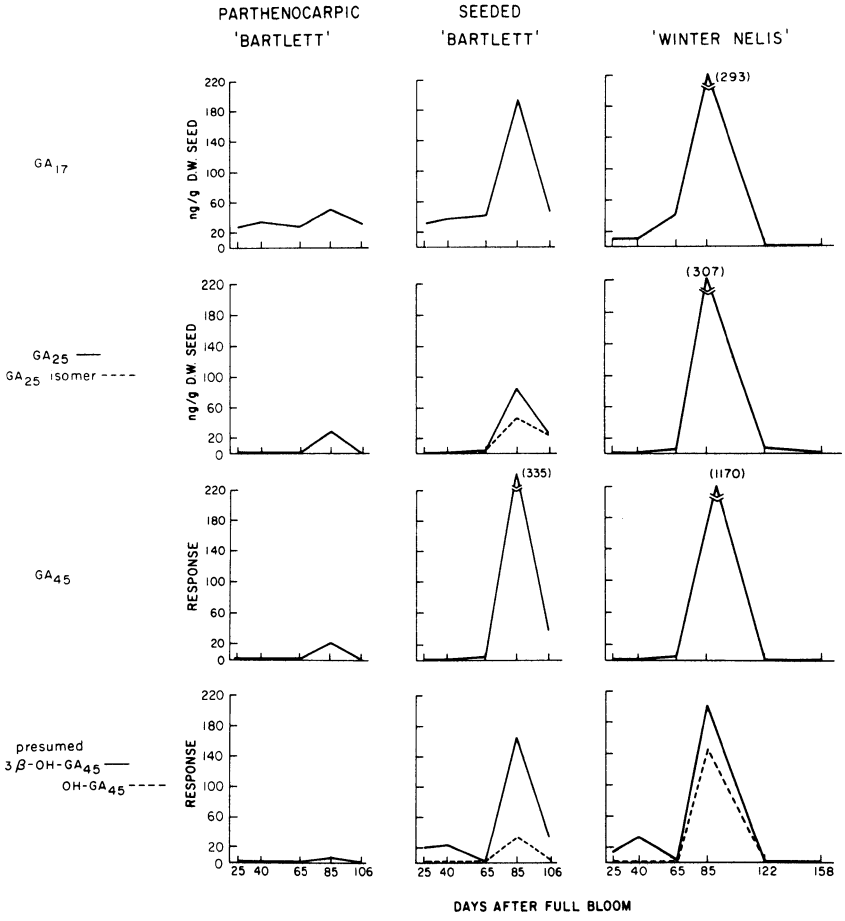

Fig. 3. Absolute levels of GA_{17} and GA_{25} and relative levels of GA_{25} isomer, GA_{45}, presumed 3β-hydroxy GA_{45} and hydroxy GA_{45} in pear seeds and ovules. Response represents the intensity of the ion current at the selected m/e value.

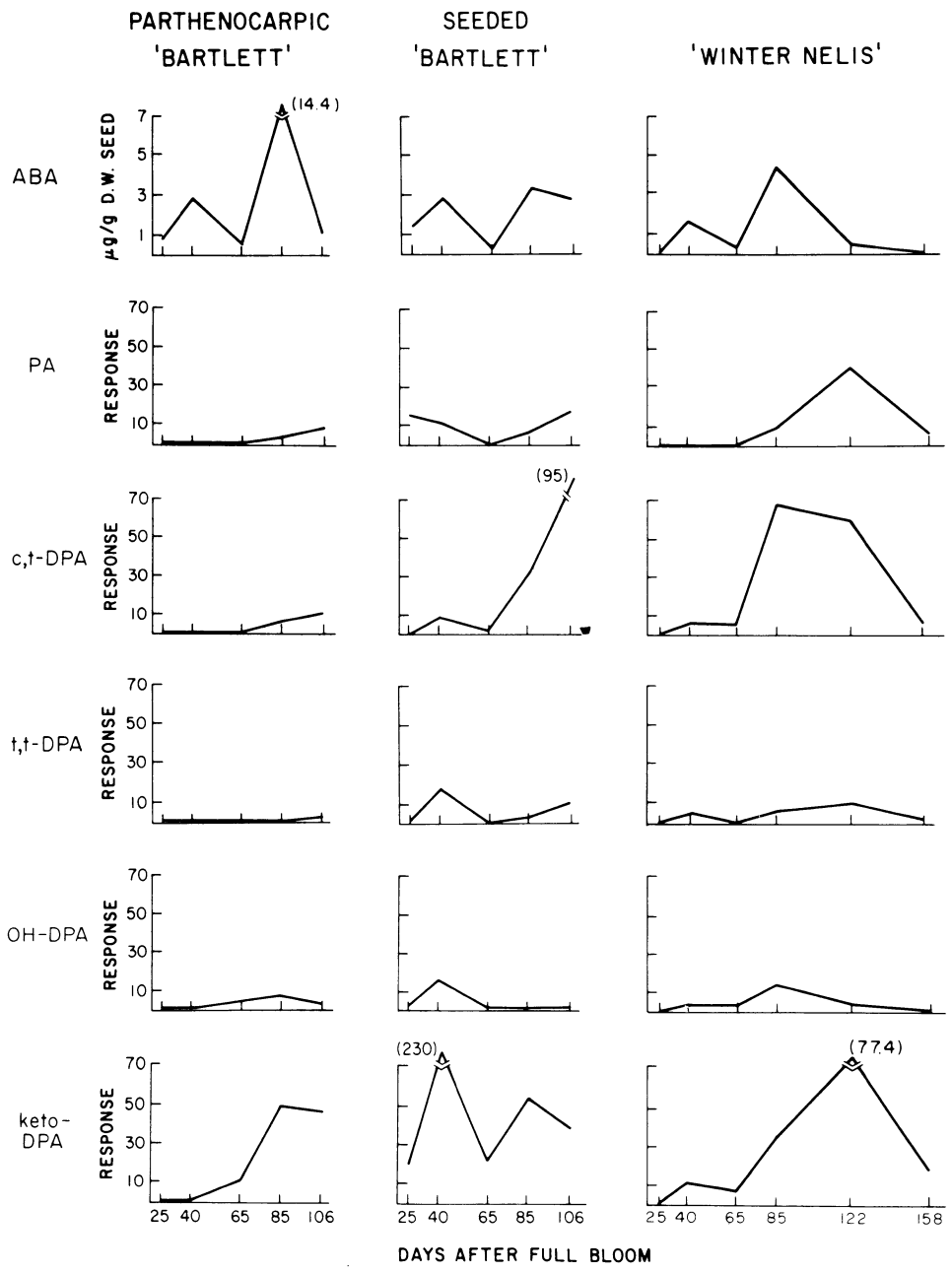

Fig. 4. Absolute levels of ABA and relative levels of PA, c,t-DPA, t,t-DPA, OH-DPA, and keto-DPA in pear seeds and ovules. Response represents the intensity of the ion current at the selected m/e value.

ABA, PA, DPA, and their derivatives. ABA and some ABA metabolites were measurable in seeds and ovules during the season (Fig. 4). Two peaks of ABA content were consistently observed, the first at 40 days, the second, larger, peak at 85 days AFB. Ovules contained much higher levels at 85 days than did seeds. ABA content of 'Bartlett' seeds remained high until harvest, while that of 'Bartlett' ovules and 'Winter Nelis' seeds declined during the same period.

No pronounced changes occurred in concn of PA in 'Bartlett', although a peak was observed in 'Winter Nelis' seeds at 122 days AFB. c,t-DPA rose markedly in seeds from 65 to 85 days, and continued to increase until harvest in 'Bartlett', while declining to the original level in 'Winter Nelis'. A similar pattern was not observed in 'Bartlett' ovules. Peak levels of keto-DPA were noted at 85 days AFB for 'Bartlett' ovules, 40 and 85 days AFB for 'Bartlett' seed and 122 days AFB for 'Winter Nelis' seed. Levels of t,t-DPA and hydroxy-DPA remained low throughout the season.

Discussion

Although data on GA levels were based on only one sample per seed type and time of sampling, seasonal curves for the 3 types of seeds paralleled one another, with maximum concn at 85 days in each case. Ovules from seedless fruits contained consistently lower levels of GAs suggesting that either the embryo or the endosperm was the primary source. No differences in GA content between 'Bartlett' seeds vs. ovules were apparent prior to 85 days, hence the embryo and endosperm probably contribute little to the extractable GA pool in the early phases of fruit development. While the endosperm was not measured, data for apple suggest that endosperm and embryo growth parallel one another (10), and that the endosperm is probably the source of the major portion of GA activity (3).

Of the GAs measured, GA_{17} and GA_{25} are inactive in all bioassays in which they have been tested (15). Our preliminary experiments indicate that GA_{45} is active in both the lettuce hypocotyl (4) and the dwarf rice (14) assays, while the activities of the remaining 3 GAs and/or presumed GAs (3β-hydroxy GA_{45}, hydroxy GA_{45}, and an isomer of GA_{25}) have not been evaluated.

Seeded apple fruits inhibit flower bud formation, while seedless fruits do not (2). GAs produced by the seeds may be responsible for this effect (6, 11, 12). Huet (9) observed similar effects of seed development in 'Williams' ('Bartlett') pear. In California, neither seeded nor seedless 'Bartlett' fruits reduced flower bud formation significantly, while flowering was markedly inhibited by seeded 'Winter Nelis' fruits if not removed before 30 days AFB (7). In our study, only GA_{17} and presumed 3β-hydroxy GA_{45} were apparent in 'Bartlett' and 'Winter Nelis' seeds 25 and 40 days AFB, and levels of both were low relative to those in samples taken at 85 days. Furthermore, cultivar differences were negligible, providing no basis for concluding that seed GAs play a role in flowering. Although 'Winter Nelis' seeds contained consistently higher levels of GAs at 85 days than did 'Bartlett' seeds, flower induction should have occurred much earlier in the season. Gil et al. (5) observed GA-like activity in extracts of pear fruits sampled between 0 and 40 days AFB; however, parthenocarpic 'Bartlett' fruits contained as much or more activity than did seeded 'Winter Nelis' fruits. On the other hand, considerably higher levels of GA-like activity were found in diffusates from pedicels of flowers and fruits of 'Winter Nelis' at 0 and 10 days AFB than in similar diffusates from 'Bartlett' (6). Levels of total extractable GAs may not reflect their rates of production and/or diffusion down the pedicel. Also, the present work did not include samples before 25 days AFB.

Gil et al. (5) did not measure GAs in pear seeds. However, their data for seed levels of an ABA-like inhibitor parallel data obtained in the present study between 65 and 85 days AFB. Thereafter, the curves diverge, bioassay showing either no change ('Bartlett') or an increase ('Winter Nelis'), while GC-MS indicates a decrease in both 'Bartlett' ovules and seeds and 'Winter Nelis' seeds. This discrepancy suggests that inhibitors other than ABA may have been measured in late stages of growth in the earlier work.

The decline in ABA content of maturing 'Winter Nelis' seeds was unexpected as one might expect a rise in ABA content as the seed matures and becomes dormant. In contrast, ABA content of 'Bartlett' seeds decreased only slightly at harvest. 'Bartlett' fruits were harvested at commercial maturity, when the seeds were still white, while mature 'Winter Nelis' fruits contained brown seeds. A similar decline in ABA might have been observed had 'Bartlett' seeds matured to the same degree as 'Winter Nelis'.

The observation that GAs and ABA reach their maximum concn simultaneously suggests that the balance of growth hormones may be more important than absolute levels. Although absolute GA levels could not be determined for lack of standards, the ratio of ABA/GAs was probably considerably lower at 85 days AFB than in earlier phases of seed development. Rapid growth of the embryo could have been a response to this declining ratio.

Several studies have shown that ABA is metabolized to PA, which is rapidly converted to DPA (8, 16, 17, 18, 19). Levels of PA remained low in pear seeds, with one exception ('Winter Nelis', 122 days AFB), and rarely exceeded those of ABA. Concn of t,t-DPA also remained low, which might be expected if this compound had arisen as an artifact from c,t-DPA, as suggested by other workers (19). If an artifact, its concn should not have exceeded that of c,t-DPA, and this was the case with but one exception ('Bartlett' seeds, 40 days AFB). Nothing is presently known of the metabolism of the 2 new metabolites of DPA. The keto derivative of DPA was generally present in much higher quantity than hydroxy-DPA, but no apparent correlation existed between its levels and those of ABA. The high concn of this compound in 'Bartlett' seeds at 40 days relative to the level found in ovules suggests some role in embryo or endosperm development. However, no similar peak was observed in 'Winter Nelis' seeds.

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