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Levels of Extractable Abscisic Acid in the Mesocarp and Seed of Persisting and Abscising Peach Fruit[1]

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Abstract. **Developing fruits of peach (*Prunus persica* (L.) Batsch cv. Andross) were classified as to abscission potential on the basis of fruit growth rate during stage I. Levels of inhibitors, as determined by wheat coleoptile and cress seed assays, were parallel in mesocarp and seed. Extracts from fruits with a high abscission potential contained significantly lower levels of an abscisic acid-like inhibitor than did those with low or intermediate potential. Thus ABA content appears to be positively correlated with the rate of fruit growth, and increases in concentration as the fruit is approaching maturity.**

Chemical thinning of fruits exploits a physiological process represented by the "June" drop. Because the chemical agents magnify a natural response, the thinning effect depends on the natural potential of the fruits to abscise. Further progress in improving chemical thinning depends on the possibility of estimating in advance, using certain biological parameters, this natural ability.

Fruit abscission may be considered the morphogenetic effect of physiological events which become progressively established in the fruit. As all phenomena involving an active morphogenesis, abscission is preceded by an inductive phase in which some chemical message, sent to the abscission zone, induces abscission layer formation (16). Plant hormones are the probable candidates for such a role. Abscisic acid (ABA) has been postulated as having a regulatory function, as it is active in enhancing both fruit and leaf abscission (1, 5, 13).

The purpose of this study was to compare the levels of endogenous plant growth inhibitors in developing fruits with different potential to abscise.

Materials and Methods

Experiments were carried out with 12-year-old 'Andross' peach trees at Ferrara, Italy. Twelve branches, carrying 250 fruit each, were selected from 6 trees in order to follow fruit growth and abscission. Because the peach fruit's natural potential to abscise is strictly dependent on its growth rate during stage I (8, 15, 16), it was possible to discriminate 3 subpopulations of fruit with different abscission potentials. Transverse fruit diameter and abscission were monitored at 6-7 day intervals during the entire period of fruit development. Fruit abscission was expressed as percent of the original number of flowers.

Three samples of fruit from each subpopulation were collected at about weekly intervals throughout stages I, II and III, separated into mesocarp and seed, frozen and freeze dried. Procedures used for extraction and purification followed closely those of Milborrow (9). The 80% aqueous methanol extract was evaporated *in vacuo* at 40°C and the residue was taken up in water. The pH was adjusted to 3.5 and the solution partitioned against ether. The ether fraction was then shaken with saturated $NaHCO_3$ solution. The $NaHCO_3$ fraction was adjusted to pH 7 and partitioned against ether to remove weaker acids, then readjusted to pH 3.5 and again partitioned against ether to yield the more strongly acidic components.

Both acidic ether fractions (seed, mesocarp) were separately chromatographed on thin-layer plates (silica gel G) in hexane: ethyl acetate:formic acid (80:20:1 by volume). Zones corresponding to ABA were scraped off and eluted with ethanol. The ethanol washings were combined, evaporated *in vacuo* at 40°C, and rechromatographed in benzene:ethyl acetate: formic acid (70:30:1 by volume). Eluates were again bioassayed. Racemic abscisic acid was used as standard for inhibitory activity and as a chromatographic reference.

Bioassay. The wheat coleoptile straight growth test as described by Nitsch and Nitsch (10), and cress seed germination bioassay according to Taylor (14) were used to identify inhibitory regions and for quantifying activity.

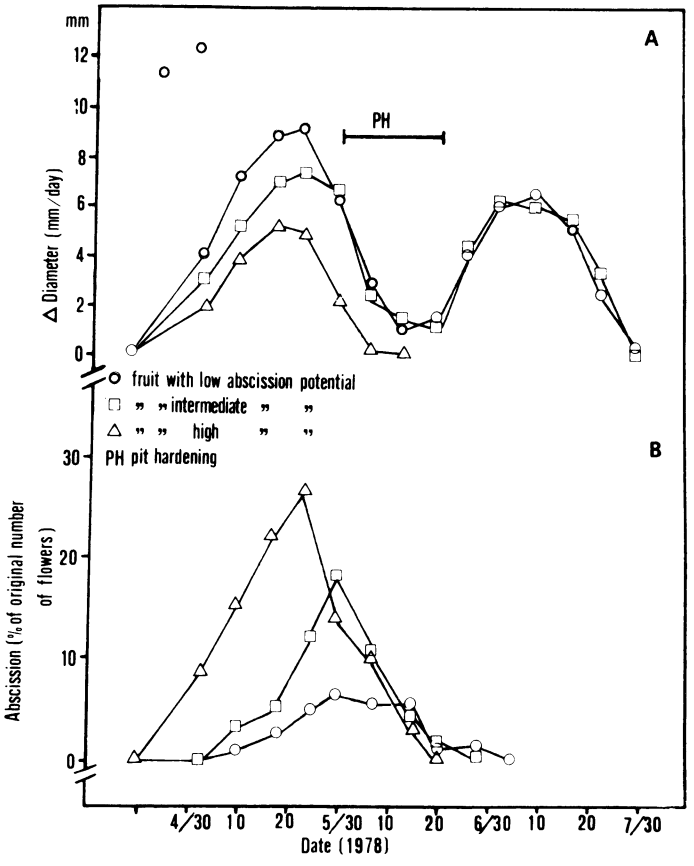

Fig. 1. Growth rate (A) and abscission (B) of 'Andross' peach fruits with different abscission potentials.

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Wheat coleoptile sections, 5.0 mm initial length, were incubated with appropriate aliquots of each fraction in 0.5 ml of phosphate-citrate buffer at pH 5.0 for 24 hr at 25°C. At least 3 replicates of 10 coleoptiles each were used for each TLC zone.

Twenty-five cress seeds were placed on Whatmann No. 1 filter paper in petri dishes in the presence of water (control) or in solutions of the appropriate fractions at 24 to 26°C. Three replications were used. Percent germination was determined after 48 hr, and data expressed as percent of control.

Identification. The identity of the more polar inhibitor was investigated by TLC in several solvent systems. An acidic ether fraction was prepared from an equivalent of 100 mg dry weight mesocarp tissue and chromatographed in hexane: ethyl acetate:formic acid (80:20:1 by volume). The inhibitor running at same Rf as ABA was eluted and rechromatographed in benzene:ethyle acetate:formic acid (70:30:1 by volume).

The inhibitory zone was eluted, and the residue methylated with diazomethane and examined by electron capture gas-liquid chromatography (GLC, Packard 7300) using a 122 cm × 2 mm i.d. column packed with 3% SE 30 on Gas Chrom Q (60-80 mesh). Temperature of the injection block, column and detector were 250°, 170° and 280°C, respectively, and the flow rate of the carrier gas (N_2) was 40 ml min^{-1}.

The identity of the inhibitor was further established by combined gas chromatography-mass spectrometry (GC-MS). The methylated derivative was introduced into an LKB-900 mass spectrometer after fractionation on a 3% SE 30 coated Supelcoport (60-80 mesh) column. The carrier gas was helium (30 ml min^{-1}) and column temperature 180°C. Instrument conditions were: ion source voltage 70 eV, ion source temperature 220° and molecular separator temperature 230°.

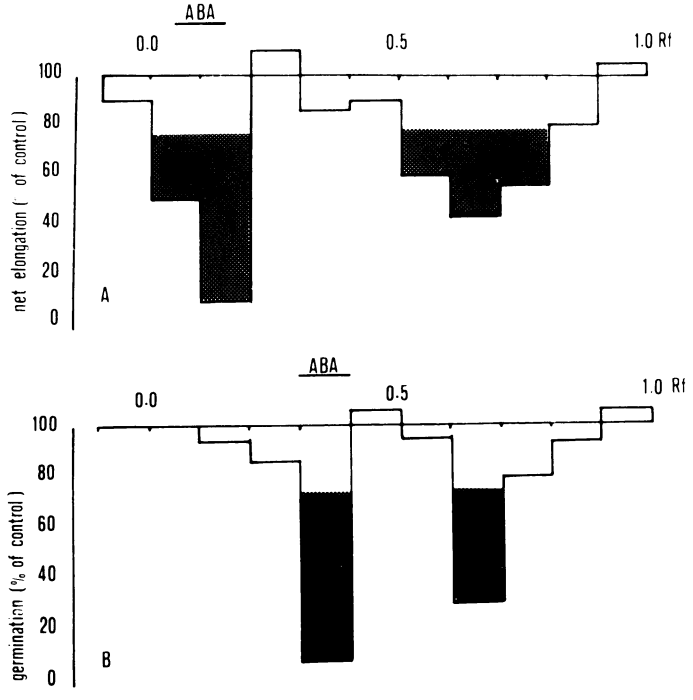

Fig. 2. Histograms illustrating (A) growth response of wheat coleoptile sections and (B) germination of cress seeds in response to eluates from thin layer chromatograms of the acidic ether fraction of a methanol extract of 'Andross' peach mesocarp tissue sampled at pit hardening. (A) TLC in hexane:ethyl-acetate:formic acid (80:20:1 by volume). (B) TLC in benzene:ethyl-acetate:formic acid (70:30:1 by volume). Inhibitory zones Rf 0.0-0.2 and 0.4-0.9 eluted from A and rechromatographed in B. Shaded bars indicate responses below the 5% limit of probability.

Results

Fruit growth and abscission. The growth pattern was distinctive of the stone fruits, with 2 peaks of maximum rate, separated by slow growth during pit hardening (Fig. 1A). The abscission pattern (Fig. 1B) paralleled fruit growth rate during stages I and II, maximum abscission following by 6 days the maximum growth rate.

The data reconfirm that fruit abscission potential can be predicted by growth analysis. Abscission of fruits with low (0.53 mm/day), intermediate (0.80 mm/day) and high (0.98 mm/day) growth rates was 100, 56.5, and 27.3%, respectively. The maximum abscission of fruits with low growth rate preceded by about 6 days the maximum abscission of fruits with high and intermediate growth rates.

Identification of hormones. Bioassays following TLC of the acidic fraction from mesocarp and seed tissues revealed 2 sharp regions of inhibitory activity at Rf 0.1-0.2 and Rf 0.6-0.8, the first corresponding to the Rf of ABA. When the active eluates were rechromatographed in a different solvent system, 2 areas of inhibition of the cress seed germination (Rf 0.4 and 0.7) were apparent, the first again at the Rf of ABA (Fig. 2b). Subsequent data will deal only with this inhibitor. Data for the second will be presented in a forthcoming paper.

When the inhibitor was eluted and rechromatographed in (1) n-butanol:ammonia (5:1 by volume), (b) water, and (c) n-butanol:acetic acid:water (4:5:1 by volume) one region of inhibition occurred at Rf 0.25-0.40, 0.60-0.70, and 0.65-0.75, respectively, identical with those of authentic ABA. GLC trace of the methylated inhibitor contained a peak with a retention

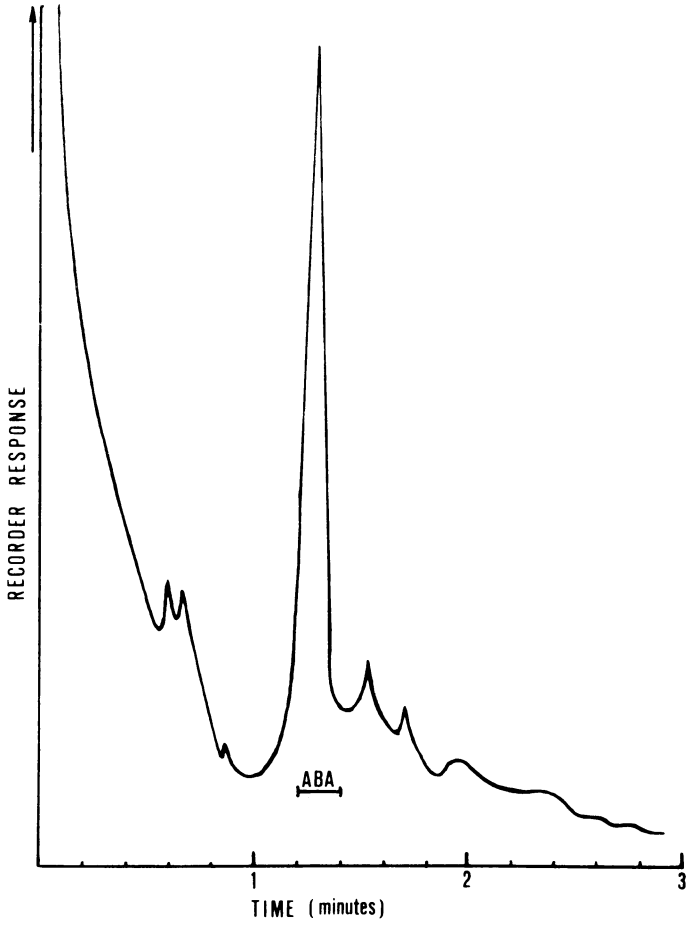

Fig. 3. GLC trace (electron capture detector) of methylated inhibitor containing a peak (retention time 1.24 min.) which co-chromatographed with the *cis-trans* ABA.

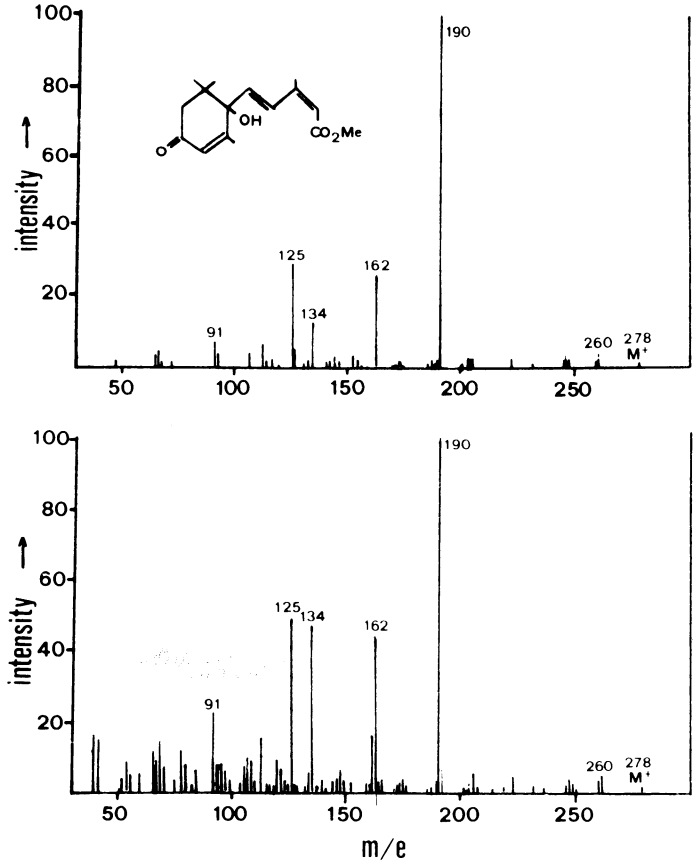

Fig. 4. Mass-spectra, following methylation, of acidic peach inhibitor from the TLC zone corresponding to ABA (top), and of authentic ABA (bottom).

time of 1.25 min which co-chromatographed with the *cis,trans* isomer of synthetic ABA (Fig. 3). Conclusive evidence that the inhibitor was ABA was provided by GC-MS. The mass spectrum (Fig. 4) of the methylated inhibitor was identical with that of the methylated ester of authentic *cis,trans* ABA.

Changes in concentration with growth. Inhibitor levels indexed by inhibition of wheat coleoptile elongation (Fig. 5A) or cress seed germination (Fig. 5B) were parallel. Mesocarp and seed tissues of fruits with a high abscission potential contained significantly lower levels of inhibitors than did tissues of fruits with low or intermediate potential.

In fruits with low or intermediate abscission potential, inhibitor levels increased during stages I and III, with lower levels during stage II. Inhibitor levels appeared to be directly related to fruit growth rate during stages I and II and the onset of stage III. As the fruits approached maturity the concentration reached a maximum and remained there despite the decline in growth rate.

Discussion

Our results confirm the possibility of discriminating within a fruit population, on the basis of the growth rate during stage I, subpopulations with different potentials to abscise. Thus fruits with high potential are recognizable before abscission is evident, allowing analysis of more homogeneous samples. Without this sampling technique a false association between ABA contents and drop would have been observed.

On the contrary, our results indicate that a negative relationship exists between ABA levels and percent abscission. On the other hand, ABA levels seem positively related to the rate of fruit growth, and increase as the fruit approaches maturity. Although ABA is generally considered an inhibitor of growth (7), a synergistic effect has been observed with kinetin in soybean callus growth (3) and with IAA in citrus

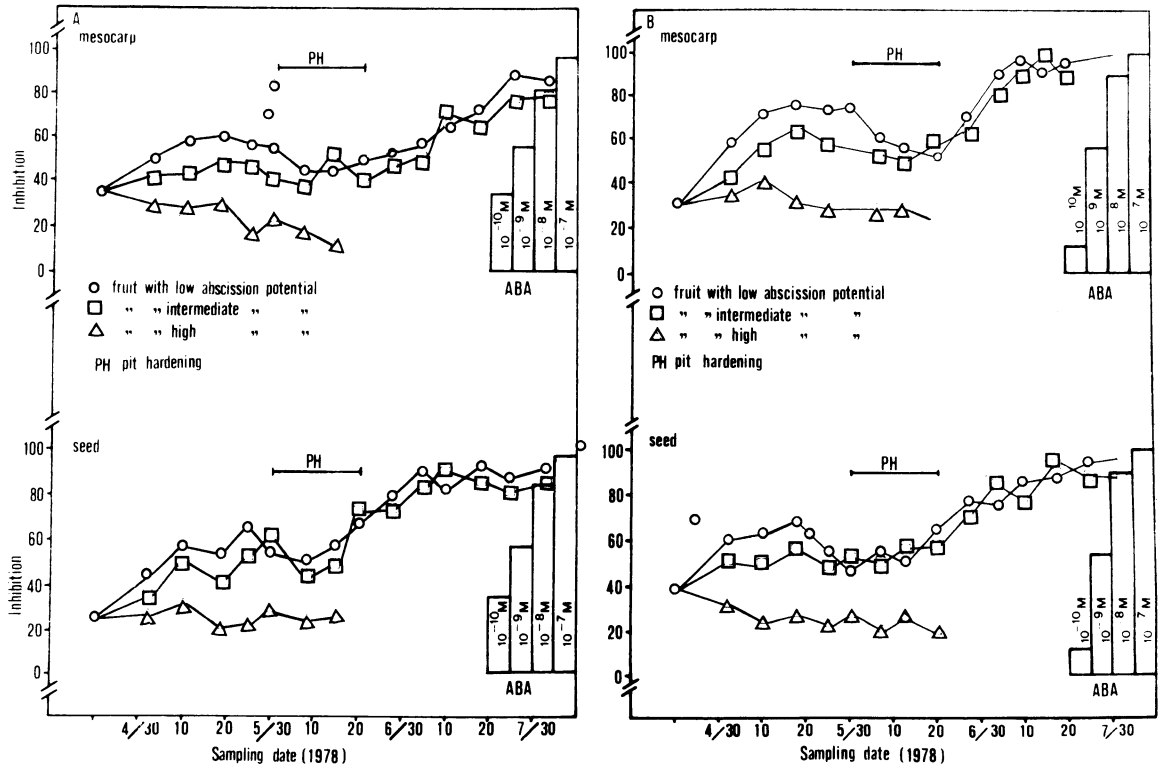

Fig. 5. Levels of ABA-like acidic inhibitor in 'Andross' peach mesocarp and seed tissues during development of fruit with different abscission potentials as measured by bioassay with (A) wheat coleoptile sections or (B) cress seed. An equivalent of 40 mg dry wt of tissue was extracted at each sampling. Responses to ABA standards in each assay are shown by bars at the right. Concentrations are corrected for racemic mixture.

bud cultures (2). A similar association between ABA levels and fruit growth exists in *Prunus cerasus* (6).

The high concentrations of ABA observed as the fruits approached maturity suggest that some association may exist between ABA level and ripening. ABA levels increase during ripening of pears (11), strawberries (12) and grapes (4). In sour cherry, inhibitors increased from the onset of stage III until the ground color changed from green to yellow, but then decreased (6).

Whether or not ABA plays a role in the control of the fruit development and ripening remains to be established; if it has a role, it must be a complex one. However, this role cannot be elucidated by exploring changes in endogenous levels of the hormone, for compartmentation and availability within the cell deserve consideration. In fact ABA might act by restricting, perhaps via modified membrane permeability, a promoter to particular organelles or by making inhibitors available to their sites of action. This could make its level in the tissues as a whole a misleading physiological parameter.

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Comparison of Apple Planting Methods[1]

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Abstract. **The tractor-mounted soil auger, a commonly used machine for simplifying the planting of apple (*Malus domestica* Borkh.) trees, was shown to create a compact hole wall that remains evident over time. Penetrometer measurements of 1- to 3-year-old auger plantings were significantly higher at the interface of bisected tree holes than at either the backfill or undisturbed soil regions. In a comparison of the auger to a backhoe, a commercial tree planter, a ditch trencher, and an auger modified to fracture the sides of the planting hole, shoot length and anchorage measurements of trees planted by the alternative planting methods surpassed those planted by the conventional auger. Trees planted by backhoe or tree planter were most successfully established.**

Basic principles of tree planting have not changed as they have traveled through the texts of pomologists such as Wilkinson (14), Chandler (2), Tukey (11), Teskey and Shoemaker (10), Childers (3) and Westwood (13). The main objective is to dig a hole sufficiently large to accommodate the roots without much bending and to get good root-soil contact.

Although principles have been well established, there is very little numerical data concerning selection of a planting procedure, Stringfellow (7) in 1896, Card (1) in 1898, and Pickering (4) in 1905 conducted the earliest comprehensive studies on methods of planting. Pickering demonstrated that trees could be successfully planted by a number of methods, provided that there was intimate root-soil contact. Card compared the effect of size of hole on tree growth and found very few differences. Stringfellow wrote a book about his success with severing roots and planting with a spud bar. Some years later Van Der Slikke (12) and Preston (5) revived this work, with the aim of exploiting the method for complete mechanical planting. Van Der Slikke reported planting 3 times faster with the Stringfellow method. Preston observed that trees planted without roots and left unpruned did not differ in size from trees that were planted with roots and headed. More recently, Tennes and Burton (8, 9) discovered significant growth increases of peach

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