The Progression of Ethylene Production and Respiration in the Tissues of Ripening 'Fuji' Apple Fruit

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Abstract. 'Fuji' apple (*Malus × domestica* Borkh.) fruits were harvested periodically prior to and during fruit ripening. Ethylene evolution and respiration rates of skin, hypanthial, and carpellary tissue were determined in each fruit. Additionally, whole fruits were used for analyses of internal ethylene concentration, volatile evolution, starch content, flesh firmness, and soluble solids content. Ethylene production was greatest in the carpellary tissue at all sampling dates except the one occurring just before the rise in whole fruit internal ethylene concentration, when production in the skin and carpellary tissue was similar. Respiration was always highest in the skin, in which the climacteric rise was most drastic. Higher ethylene production in the carpellary tissue of pre- and postclimacteric fruit and higher respiration in the skin tissue, including a noticeable climacteric rise, is indicative of a ripening initiation signal originating and/or transduced through the carpels to the rest of the fruit.

Ethylene is a gaseous phytohormone associated with many physiological occurrences in higher plants, including wound response and fruit ripening. The climacteric phase of apple fruit ripening is defined by an exponential increase in fruit respiration (Kidd and West, 1945), and is triggered by a period of autocatalytic ethylene production (Kidd and West, 1932; Wilkinson, 1963). The mechanism by which the autocatalytic production of ethylene during apple fruit ripening is triggered remains undetermined.

The apple fruit is classified as a pome. According to the widely accepted appendicular hypothesis of pome fruit development, a pome fruit is derived from an epigynous floral structure in which the floral tube (hypanthium) portion adnate to the gynoecium expands into the hypanthial (cortical) tissue of the fruit during development (MacArthur and Wetmore, 1939; Pratt, 1988). The tissue surrounding the carpels, but within the core line, is of gynoecial origin and is termed carpellary (pith) tissue (Pratt, 1988). The skin tissue contains a thick, waxy cuticle

covering epidermal and hypodermal layers of cells (Pratt, 1988) (Fig. 1).

Previous investigations show that these tissues not only differ in origin, but also in structure, function, and metabolic activity. Microscopic analyses of the carpellary and hypanthial tissue of 'Cox's Orange Pippin' apple fruit by Leshem et al. (1984) indicated that the former tissue had smaller, more densely packed, cells containing fewer amyloplasts but more fat globules than the latter tissue. However, Bain and Robertson (1951) found that 'Granny Smith' carpellary cells actually have a greater individual cell volume than those in the hypanthial tissue. Lipoygenase activity (Feys et al., 1980; Leshem et al., 1984), superoxide dismutase activity, and total antioxidant content, as determined by specific inhibition of linoleate oxidation (Leshem et al., 1984), are higher in the carpellary tissue than the hypanthial tissue of postclimacteric, poststorage fruit. The content of cell wall pectic substances is proportionally higher in the hypanthial tissue, whereas cellulose and hemicelluloses are more abundant in the carpellary tissue (Massiot et al., 1994). Guadagni et al. (1971) and Knee and Hatfield (1981) provided evidence of greater capacity for synthesis of flavor volatiles in the skin tissue than in the fruit as a whole or the hypanthial tissue alone. Evidence of ethylene synthesis in the placental tissue and seeds early in the ripening process of melon (*Cucumis melo* L.) fruit (Yamamoto et al., 1995) and of early expression of a ripening specific lipoygenase in the locular jelly of tomato (*Lycopersicon esculentum* Mill.) fruit (Kausch and Handa,

1997) suggest that the ripening signal in apple fruit might also originate at or near the seeds.

Our purpose was to determine the extent and ontogenic sequence of respiration and the production of ethylene in these different tissues during ripening of 'Fuji' apple fruit.

Materials and Methods

Randomly selected samples of 'Fuji' fruits were periodically harvested from a commercial orchard located near Vantage, Wash., during the 1997 growing season. Samples were selected only from trees of uniform size and crop load. Following each sampling, subsamples were randomly selected and analyzed. Some fruits from the final sampling date were stored at 20 °C for 25 d before analysis. The fruits used for the wound ethylene assay were obtained directly from an industry warehouse following controlled-atmosphere (CA) storage.

Each fruit from one of the subsamples in the harvest series was separated into skin, hypanthial, and carpellary tissue. The skin tissue (epidermis and subjacent hypodermal cell layers) was removed with a sharp knife. About 1 cm of both the stem and the calyx end was removed and discarded. The hypanthial tissue was separated from the carpellary tissue, and the seeds were removed and discarded. Each of the tissues was sliced into pieces with near equal dimensions to prevent possible error introduced by tissues having different surface areas. Samples were then weighed, placed in open 125 mL Erlenmeyer flasks of uniform volumes for 30 min, and subsequently sealed with large serum stoppers. All flasks remained sealed for 2 h prior to headspace analyses to determine ethylene and carbon dioxide production rates. Ethylene concentration was assayed by removing 1 cm³ of headspace gas from each flask and directly injecting it into a gas chromatograph (Shimadzu G8A; Shimadzu Corp., Kyoto, Japan) equipped with a 3.27-m (0.318 cm ID) stainless steel column packed with Porapak QS (50/80 mesh), and a flame ionization detector. The prepurified N₂ carrier gas flow was adjusted to 30 mL·min⁻¹. Injector/detector and oven temperatures were set at 200 °C and 50 °C, respectively. The headspace CO₂ concentration was analyzed by injecting 0.25 cm³ of headspace gas into a Fisher model 1200 Gas Partitioner (Fisher Scientific, Philadelphia) equipped with a 6.096-m (3.18 mm ID) stainless steel column packed with 80/100 mesh Chromasorb P-AW and a 0.9144-m (4.76 mm ID) aluminum column packed with 60/80 mesh Molecular Sieve 13X connected in series, as well as a thermal conductivity detector. The ultra-pure He₂ carrier gas flow was adjusted to 30 mL·min⁻¹, and the oven temperature was 35 °C. All treatments and analyses were performed sequentially to assure equal time periods between sealing and sampling of the flasks.

A second subsample of whole fruits was used for assay of internal ethylene concentration (IEC), general quality evaluation, and headspace volatile analysis. Internal ethylene

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Fig. 1. (A) Longitudinal and (B) transverse sections of an apple fruit showing (i) the skin and (ii) hypanthial (cortical) tissues, (iii) the core line, and (iv) the carpellary (pith) tissue.

assays were performed by withdrawing a 1-cm³ gas sample from the core-space of a whole apple fruit through a 50-gauge hypodermic needle that had been pushed through the calyx end of the fruit into the core, sealed to the fruit with silicon grease, and fitted with a serum stopper. Gas samples were analyzed as described previously. Flesh firmness was analyzed using a Topping penetrometer (Topping, 1981). Starch content was assayed by staining transverse crosssections of the fruit with iodine (I-KI) and visually rating the color change using a 1–6 scale (1 = 100% of the area stained; 6 = 0%). Soluble solids content was estimated using a Reichert ABBE Mark II refractometer (AO Scientific Instruments, Keene, N.H.). Headspace volatiles of whole fruits were assayed using the gas chromatographic method described by Fellman and Mattheis (1995).

For the wound ethylene analysis, fruits were removed from air storage (0 °C) and kept at 22 °C for 48 h prior to analysis. Each fruit was separated into different tissues as described above and ethylene produced in response to wounding was analyzed after 30 min, as previously described. The serum stoppers were then removed, the headspace purged with ethylene-free air, and the serum stoppers replaced. The sampling and purging process was repeated at 90 and 150 min after tissue separation.

Analysis of variance and mean separations using Fisher's protected LSD analyses were performed on data from each harvest for the tissue separation experiment using the Statview statistics package (SAS Institute, 1998).

Results and Discussion

Wound-induced ethylene has been reported in tissue and organs from many plant species. Boller and Kende (1980) reported an increase in ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) production, and in ACC synthase activity in tomato fruit as a result of wounding. Similar results have been reported in other fruits. Conflicting reports of increased ethylene production as a result of wounding apple tissue have been published by Lieberman and Wang (1982) and Magne and Larher (1995), although Magne and Larher did not begin measuring emissions from excised tissue until 1 h after its preparation. Nevertheless, our study compared different parts of the apple fruit, and the initial burst of ethylene production was observed during the first 30 min following tissue separation. Ethylene evolution remained relatively constant during the 30–90 and 90–150 min periods at $25 \pm 3.6\%$ and $29 \pm 3.6\%$ SE (respectively) of that evolved during the first 30 min. By allowing the samples to remain unsealed for the first 30 min of the procedure, the build-up of initial ethylene within the headspace was negligible. Initial production of ethylene was proportional to later production in all the tissue types.

The increase in ethylene production usually coinciding with the climacteric rise has been reported as slight (Watkins et al., 1993) or even indiscernible (Plotto et al., 1995) in 'Fuji' apple fruit. In this study, IEC appeared to follow a typical, although somewhat subdued, autocatalytic trend that paralleled the

climacteric rise in respiration. Both IEC (Fig. 2) and whole fruit respiration (not shown) increased somewhere between H2 and H3. The increased production of butyl acetate and 2-methylbutyl acetate during this period (Fig. 2), a process governed by ethylene production at the onset of climacteric ripening (Fan et al., 1998), further substantiates this observation.

Ethylene production was highest in the carpellary tissue for most of the sampling dates (Fig. 3A). One exception was H2, where ethylene production by the skin tissue did not differ from that by the carpellary tissue. Although the relative pattern between tissue types remained similar to those on the other sampling dates, no significant difference among the tissues was evident at H4+25d. This was probably due to high levels of variance between individual fruits resulting from slightly differing physiological ages or different maximum rates of ethylene production. This variance was also reflected in the average IEC obtained on that date. Ethylene production in the hypanthial tissue was never different from that in the skin tissue, except at H2, when it was lower than in the other two tissue types.

Respiration rates were in the order skin tissue > carpellary tissue > hypanthial tissue, as demonstrated by CO₂ evolution from each tissue (Fig. 3B). Skin tissue respiration remained low until the onset of the climacteric (between H2 and H3), when it increased rapidly, peaking at H3 and subsequently decreased at the later sampling dates. The ratio of the respiration rates of the hypanthial and carpellary tissue remained similar throughout the sampling period, although the respiration rates of both of these tissues decreased between H1 and H2, increased between H2 and H3 (although the rate increases were not as great as that of the skin tissue), and decreased to a stable level thereafter.

The respiration rate of the whole apple fruit characteristically follows a climacteric pattern (a preclimacteric minimum followed by an increase to a maximum and then a decrease) (Kidd and West, 1945) and ethylene stimulates ripening (Kidd and West, 1932). A large *in vivo* increase in ethylene production coincides with the climacteric in such fruit (Wilkinson, 1963; Reid et al., 1973). Ethylene production in disks of apple skin increases simultaneously with production by the whole fruit during the climacteric (Rhodes et al., 1970). Ethylene production by the isolated tissue was lower than that of the whole fruit.

In this investigation, the elevated rate of ethylene production in the carpellary tissue throughout the sampling period, especially prior to the climacteric, indicates a ripening event initiated within or transduced through this tissue. In addition, although the skin tissue had a significantly higher respiration rate than the other tissues on all sampling dates, it had a lower ethylene production rate ($P \leq 0.05$) than the carpellary tissue in prestorage samples on all sampling dates except H2 and H4+25d. This suggests a "path" by which ethylene produced by the carpellary tissue "signals" an increase in respiration of the skin tissue, while

Fig. 2. Changes in butyl acetate and 2-methylbutyl acetate evolution and IEC over the ripening period. Axis break indicates a change in the ripening environment. Vertical bars represent SE ($n = 7$ for isolated tissue analyses; $n = 10$ for IEC); where no bars appear, SE was smaller than symbol size.

Fig. 3. (A) Changes in tissue specific ethylene evolution and IEC and (B) tissue specific respiration over the ripening period. Axis break indicates a change in the ripening environment. Vertical bars represent SE ($n = 7$ for isolated tissue analyses; $n = 10$ for IEC); where no bars appear, SE was smaller than symbol size. a,b,c = mean separation within sampling dates within sampling dates as determined by Fisher's protected LSD, $P \leq 0.05$.

respiration in the carpellary tissue and ethylene production in the skin tissue rise only nominally. Low rates of ethylene production and respiration within the hypanthial tissue may indicate that it functions as a storage tissue.

Production of flavor volatiles increased during the period between H2 and H3 (Fig. 2). For the most part, flavor volatile production is ethylene dependent in preclimacteric fruit (Fan et al., 1998), increases with the onset of climacteric ripening (Brown et al., 1965), and occurs mainly within the skin tissue (Guadagni et al., 1971). The higher respiration rate in the skin tissue than in other tissues might indicate that volatile synthesis, while dependent on the presence of ethylene, also occurs to a greater extent in cells with a high rate of respiration.

The pattern of starch hydrolysis could provide further evidence of initiation of climacteric ripening in apple fruit. In general, as the fruit ripens, starch hydrolysis proceeds from the core (carpels) toward the skin (Poapst et al., 1959). However, in this investigation, the average starch index value of fruit from H1 was 4.1 (on a 1–6 scale), indicating that the starch hydrolysis front had already proceeded about two-thirds the distance from the core line to the skin around 2 weeks prior to the onset of climacteric ripening. This is far beyond the maturity indicated by the ethylene measurements in the various tissues and by the IEC. Plotto et al. (1995) found that a starch index value of 2.5 (also on a 1–6 scale) coincided with an IEC value just below $1 \mu\text{L}\cdot\text{L}^{-1}$, whereas we recorded an IEC value of $0.31 \mu\text{L}\cdot\text{L}^{-1}$ at H1. This variation could be due to hydrolysis of a greater proportion of amylose (which reacts more intensely with KI solutions than does amylopectin) than of total starch (Fan et al., 1995). Also, if ethylene regulates the hydrolysis of starch, then starch hydrolysis would be dependent upon ethylene produced in the carpellary tissue, since minimal, if any, ethylene is produced in the hypanthial tissue of preclimacteric fruits.

Ethylene production within the carpellary tissue early in the ripening process is suggestive of a seed-controlled mechanism and/or a "tree factor," translocated to the vascular tissue within the carpellary tissue, which would directly or indirectly trigger the onset of climacteric ripening. One argument in favor of a tree factor is the relationship between apple fruit ripening and the fruit's position on the tree. 'Hi Early Delicious' fruits ripen from positions on at the base of the central leader outward and upward in a pattern similar to leaf degreening and senescence (Farhoomand et al., 1977). Furthermore, defoliating and girdling 'Delicious' trees 100 d after bloom hastens the onset of autocatalytic ethylene production by nearly a month, indicating the presence of a phloem-translocated tree factor (Sfakiotakis and Dilley, 1973). Auxin, a plant growth regulator produced in great quantities in apical buds and other active meristems and transported via the phloem to other parts of the plant, can stimulate ethylene production in many fruits (Dilley, 1969). Climacteric

ripening may result from an antagonistic relationship between abscisic acid and one or more gibberellins (abscisic acid as a stimulant and gibberellin as an inhibitor), which are both found in apple seeds (Balboa-Zavala and Dennis, 1977; Steffens et al., 1992). Information correlating seed levels of abscisic acid and gibberellins with fruit ripening is lacking. Direct evidence of a tree factor or seed-borne triggering mechanism must be obtained to facilitate further investigation.

In summary, autocatalytic ethylene production in the carpellary tissue precedes and, for the most part, is greater than that within any other tissue. The onset of autocatalytic ethylene production may be directly or indirectly stimulated by a change in concentration of a hormone or other substance within the vicinity of the carpellary tissue. This signal may be, in turn, transduced, via diffusion of ethylene or some other volatile substance, to the skin where the bulk of the respiration rate increase occurs at the onset of the climacteric phase. This signal may also originate in the vicinity of the carpellary tissue. Future studies into the mechanism of apple fruit climacteric ripening should account for differences in tissue origin.

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