Physiological Changes during Ripening of Raspberry Fruit

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Abstract. Raspberry (*Rubus idaeus* L., 'Heritage') fruit were harvested at six stages of color development to determine the relationship between quality attributes and physiological changes during ripening. Soluble solids concentration and fruit weight increased, whereas titratable acidity decreased during ripening. Fruit darkened and color saturation increased with maturity. Raspberry fruit exhibited a nonclimacteric pattern of respiration, and ethylene (C_2H_4) was detected only after red pigment developed. Respiration and C_2H_4 production of whole fruit were similar to those of drupes. Ethylene-forming enzyme activity commenced in drupes and receptacle tissue from fruit at the yellow and mottled stages, respectively. These data indicate that ripening in raspberry fruit is independent of C_2H_4 production and is nonclimacteric.

Literature on the ripening patterns of small fruits often is unclear as to whether they are climacteric or nonclimacteric. Blueberries (*Vaccinium corymbosum* L.) have shown a respiratory climacteric and increased C_2H_4 production coincident with ripening (Windus et al., 1976). Strawberries (*Fragaria × ananassa* Duch.) did not increase in respiration and C_2H_4 production during ripening (Given et al., 1988). A large increase in C_2H_4 production coincided with the transition to the last stage of color development (black to dull black) in blackberries (*Rubus* spp.), but the respiratory pattern was nonclimacteric (Walsh et al., 1983). Red raspberry fruit have been classified as climacteric (Blanpied, 1972; Jennings, 1988) and nonclimacteric (Kader, 1985) relative to color development. Ethylene emanation and internal C_2H_4 concentrations in raspberry fruit from floricanes increased as fruit color developed (Blanpied, 1972; Burden and Sexton, 1990a, 1990b). Jennings (1988) reported that respiration of floricanes-produced raspberries decreased during ripening. Ethylene also was involved in fruit softening (Robbins et al., 1989a, 1989b) and abscission of raspberry drupes from the receptacle (Burden and Sexton, 1990a, 1990b).

The objective of this research was to determine the relationship between respiration, C_2H_4 production rates, and qualitative changes during ripening of raspberry fruit from primocanes.

Plant material. Fruit from 5-year-old plants of 'Heritage' raspberries were harvested every

other day during 15-30 Sept. 1990. Raspberry plants were grown in a primocane-fruiting hedgerow at the Iowa State Univ. Horticulture Research Station, Ames, and had been sprayed weekly with recommended fungicides (Gleason et al., 1990) on a preventive schedule. Fruit of six color stages were cut with intact pedicels to minimize wounding and were transported to the laboratory. Fruit that were not used immediately were stored at 2°C. Color stages were green, yellow (yellowish drupes), mottled (yellow and red drupes), pink, red, and dark red. No symptoms of fungal decay were observed in these experiments.

Physical and chemical attributes. External color of 30 whole fruit from each color stage was measured by placing the aperture of a tristimulus color analyzer (CR200 chromameter, Minolta, Ramsey, N. J.) against each side of individual fruit. The chromameter was equipped with a strobe head having an 8-mm-diameter aperture (56 mm² measuring area) and was calibrated with a white standard plate ($L^* = 97.7$, $a^* = -0.48$, $B^* = 2.23$). Hunter color (L^* , a^* , b^*) data from the chromameter were used to calculate hue

angle [$\tan^{-1}(b/a)$] and chroma [$(a^2 + b^2)^{1/2}$], as recommended for measurement of color changes in food (Hung, 1990). Hue is a measure of visible color, and chroma is a measure of brightness or intensity of the hue.

Following color determination, drupes were removed from receptacles, weighed, and homogenized in a Waring blender with distilled water (1:1, w/w). The homogenate was filtered through four layers of cheesecloth, diluted 10-fold with distilled water, and titrated to pH 7.0 with 0.1 N NaOH. Acidity was expressed as percent citric acid on a fresh-weight basis (Eaves et al., 1972). Percent soluble solids concentration (SSC) was determined on juice expressed onto a hand-held Reichert 10430 refractometer. Color, SSC, and titratable acidity (TA) were measured on three sampling dates.

Respiration and C_2H_4 determinations. Four replications of four fruit with intact pedicels (20 mm long) were used for each color stage. To prevent water stress, fruit pedicels were placed in vials containing distilled water. Two vials with two fruit per vial were placed in a 250-ml glass jar, sealed for 2 h, and kept at 20°C. Headspace gas composition (CO_2 and C_2H_4) was determined by injecting a 5-ml

Fig. 1. Changes in color, defined as lightness, chroma, and hue (A), soluble solids concentrations (SSC), titratable acidity (TA), and SSC/TA (B) in ripening raspberry fruits. Values are means of 90 fruit, $\pm$ SE. Error bars not visible fall within the symbol.

Fig. 2. Respiration rate and C_2H_4 production rates during the various stages of ripening raspberry fruit. Values represent the mean of 80 fruit, $\pm$ SE.

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Fig. 3. Respiration rate (A) and C₂H₄ production (B) of intact fruit and of drupe and receptacle tissues of raspberry fruit. Values represent the mean of 32 fruit, $\pm$ SE.

sample into a stainless steel column (3.0 $\times$ 780 mm) packed with Chromasorb 60/100 in a Varian gas chromatography equipped with thermal conductivity and flame ionization detectors in series. The procedure was repeated five times, each time on a different day.

To determine the contributions of the drupes and receptacle tissue to respiration and C₂H₄ production, freshly harvested fruit with pedicels were placed in vials of water, held at 2°C overnight, rewarmed to 20°C the next day (2 h to reach ambient temperature), and sealed for headspace analysis as described previ-

ously. After headspace samples were taken from intact fruit, each drupe cap was removed from the receptacle and placed in a separate jar. Pedicels with the receptacles were kept in vials of water. Ethylene and CO₂ were determined as described above. Only fruit of the mottled through dark-red stages of maturity were used for this study because drupes could be removed as an intact cap from these stages. Four replications, each of four fruit per replication, were used for each color stage. The experiment was conducted twice using fruit from separate harvest dates.

EFE activity. Ethylene-forming enzyme (EFE) activity was determined by the method of Hoffman and Yang (1980). Separated fruit drupes and receptacle tissues were quartered to aid penetration of the 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC) solution. Vacuum (78 cm Hg) was applied to drupe and receptacle tissue for 1 to 2 min. Separate drupe and receptacle tissues were sealed for 4 h in 125-ml Erlenmeyer flasks that contained 10 ml of 1 mM ACC. Ethylene was determined in the headspace. Four replications (four fruit per replication) were used for each color stage, and the experiment was conducted twice using fruit from separate harvest dates.

Statistics. Data were subjected to analysis of variance. Differences between sampling dates and replications were not significant ($P < 0.05$); data averaged over sampling dates and replications are presented. Differences between maturity stages were significant ($P < 0.05$).

Quality attributes. Raspberries darkened (decreased L* value) as they ripened (Fig. 1A). Chroma (color saturation) gradually increased as fruit ripened to the pink stage, then decreased at the later ripening stages. Hue decreased as raspberries ripened from the yellow to dark-red stage, indicating a color shift from green to red to blue-red. Similar color values were found by Robbins and Moore (1990) for fruit at the red and dark-red stages. Percent SSC increased, TA decreased, and SSC : TA ratio increased as the fruit matured (Fig. 1B). These changes indicate a continuous maturation and improved

fruit quality into the dark-red stage of ripening. For mottled, pink, and red 'Heritage' fruit, SSC was slightly lower than that reported for 'Meeker' fruit at similar stages (Sjulin et al., 1989).

The declining rate of respiration as fruit ripened from green to dark red suggests that raspberry fruit are nonclimacteric (Fig. 2). No doubling in rate concomitant with color development characteristic of climacteric fruit (Biale and Young, 1981) was observed in raspberry fruit. The respiration rate for red 'Heritage' fruit was lower than that reported for 'Chilcotin', 'Meeker', or 'Willamette' fruit (Robbins et al., 1989a, 1989b). This difference may be due to the alleviation of water stress in harvested 'Heritage' fruit. Ethylene production of intact fruit was low in green and yellow fruit, higher during the transitions to mottled and pink stages, and similar to pink fruit during the transitions to red and dark-red stages (Fig. 2). Burden and Sexton (1990b) detected low levels of ethylene production at the green and yellow stages of ripening in 'Glen Cova' raspberry fruit, but production increased greatly at the mottled and later stages of ripening.

Rates of CO₂ and C₂H₄ production were highest in receptacle tissue and similar for intact fruit and drupes (Fig. 3). The respiration rates of the receptacle tissue from mottled and pink fruits were higher than those of intact fruits. Receptacle tissue from red and dark-red fruits had respiration rates similar to those of intact fruits and drupes (Fig. 3A). The C₂H₄ production rates were low (3 to 8 μ l · kg⁻¹ · h⁻¹) for intact fruit and drupes, but were 40 times higher for detached receptacle tissues (Fig. 3B). Burden and Sexton (1990b) have suggested that C₂H₄ produced in raspberry receptacle tissue accelerates formation of the abscission zone.

Ethylene-forming enzyme activity, which can be limited by the supply of ACC and/or by the stage of ripeness (Cameron et al., 1979), was measurable at the green stage (Fig. 4). Activity was high in yellow fruit and low in mottled fruit and increased during ripening. The effect was more pronounced in receptacle than in drupe tissue.

We have found the respiratory pattern of 'Heritage' raspberry to be similar to that of other nonclimacteric fruits. Therefore, it should be classified as nonclimacteric, as defined by Biale and Young (1981). Ethylene accelerates abscission zone formation (Burden and Sexton, 1990a, 1990b) and promotes softening in raspberries (Robbins et al., 1989b). In our experiment, C₂H₄ production increased after SSC had increased and TA had decreased substantially, indicating a lack of involvement of C₂H₄ in these ripening changes.

From our data, we conclude that many of the changes in raspberry fruit associated with ripening begin at or before the yellow stage and are independent of C₂H₄ production changes. Furthermore, the pattern of CO₂ production is nonclimacteric. Exogenous ethylene has been shown to induce softening and color formation in raspberry (Burden and Sexton, 1990b). The effect of exposure to

Fig. 4. Ethylene-forming enzyme (EFE) activity in raspberry drupes and receptacles as a function of maturity. Values represent the mean of 32 fruit, $\pm$ SE.

exogenous ethylene on respiration needs to be studied to further clarify the role of ethylene in ripening of raspberries.

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