Ripening Physiology in 'Navaho' Thornless Blackberries: Color, Respiration, Ethylene Production, Softening, and Compositional Changes

P. Perkins-Veazie
U.S. Department of Agriculture, Agricultural Research Service, South Central Agricultural Research Laboratory, P.O. Box 159, Lane, OK 74555

J.R. Clark
University of Arkansas, Department of Horticulture, 316 Plant Science Building, Fayetteville, AR 72701

D.J. Huber
University of Florida, Horticultural Sciences Department, P.O. Box 110690, Gainesville, FL 32611-0690

E.A. Baldwin
U.S. Department of Agriculture, Agricultural Research Service, Subtropical Fruit Products Laboratory, P.O. Box 1909, Winter Haven, FL 33883

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ABSTRACT. Fruit were harvested from an erect, thornless blackberry (*Rubus* L. subgenus *Rubus* Watson, 'Navaho' to study ripening changes. Soluble solids content increased between the red (unripe) and dull-black (overripe) stages of ripening while titratable acidity decreased sharply between the mottled and shiny-black ripeness stages. Anthocyanin content increased sharply between the mottled and shiny-black stages. Firmness of drupelet and receptacle tissues decreased between the mottled and shiny-black stages of ripeness. In whole blackberries, total uronic acids decreased, and water soluble uronic acids increased between the green-red and shiny-black ripeness stages. Volatile production paralleled ripening changes, and was highest in dull-black fruit, with alcohols and aldehydes predominating. Respiration of intact fruit maintained in water decreased between the green and red ripeness stages and increased at the mottled (part-black) and black ripeness stages. Ethylene production remained below 10 nmol·kg^{-1}·h^{-1} until the dull-black (overripe) stage of maturity. Free 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC oxidase did not increase in berries until the shiny-black stage, corresponding with the onset of detectable ethylene production. ACC oxidase activity decreased in the drupelet tissue (0.5 to 0.01 µmol·kg^{-1}·h^{-1}) and increased in the receptacle tissue (2 to 3.8 µmol·kg^{-1}·h^{-1}) as fruit changed from red to dull black. These results indicate that ripening in blackberries may be initiated in the receptacle tissue. Ripening in blackberries is likely independent of ethylene, but ethylene may regulate berry detachment from pedicels, thus controlling timing of fruit harvests.

The blackberry (*Rubus* sp.) is an aggregate fruit, composed of drupelets arranged on a column of receptacle tissue. Each drupelet is composed of a pyrene (stony endocarp) surrounded by ovarian-derived tissue. Blackberries have one large abscission zone between the drupelets and pedicel, rather than the individual zones at each drupelet found in raspberries (*R. idaeus* L.) (Burdon and Sexton, 1993). Vascular traces, rather than cortical cells, are ruptured during blackberry abscission (Burdon and Sexton, 1993). Unlike raspberries, where only drupelets remain, after harvest, receptacle blackberry tissue remains tightly bound to drupelets.

Blackberries undergo distinct color changes as they develop and ripen. In erect-caned, thornless cultivars, berries are initially green [10 d postanthesis (PA)], develop partial then full redness (20 to 40 d PA), and develop partial then full blackness (45 to 55 d PA). The latter ripeness stages of shiny and dull black are distinguished by loss of fruit gloss and increased ease of berry separation from the pedicel.

The role of ethylene in blackberry ripening is unclear. Walsh et al. (1983) reported that semierect, thornless tetraploid blackberry cultivars are climacteric, with a rise in ethylene production beginning at the mottled stage of ripeness. In contrast, Lipe (1978) found no increase in ethylene production and characterized 'Brazos', a thorny, erect-caned blackberry, as nonclimacteric. Burdon and Sexton (1993) found that ethylene production depended on cultivar, ranging from no increase during development to a doubling of ethylene production upon color development.

Other bramble fruits, such as boysenberry (*Rubus* sp.) and red raspberry (*R. idaeus* L.), show a loss in total acidity and an increase in water-soluble pectins and sugars with ripening (Given et al., 1986; Mason, 1974). Aroma compounds of European blackberries (*R. laciniata* Willd. 'Thornless Evergreen') were determined in ripe fruit (Georgilopoulos and Gallois, 1987). However, a comprehensive study of ripening changes in a North American blackberry species has not been conducted.

The erect thornless blackberry, 'Navaho', remains firm during storage (Clark and Moore, 1990; Perkins-Veazie et al., 1996). Unlike other erect blackberry cultivars, whose fruit change from shiny black to dull black within 8 to 24 h at 20 °C after harvest, 'Navaho' fruit change color in ≈40 h (P. Perkins-Veazie, unpublished data). The slower ripening of this blackberry cultivar provided a means to separate ripening events of individual berries when held at 20 °C. Therefore, the purpose of this study was to

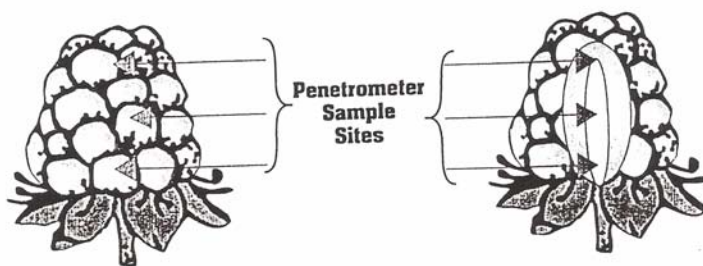J. Amer. Soc. Hort. Sci. 125(3):357–363. 2000.

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Fig. 1. Diagram of blackberries illustrating sites of penetrometer measurements for drupelet (epidermal) (**left**) and receptacle resistance to penetration (**right**). Vascular strands in receptacle were perpendicular to penetrometer measurements.

determine physicochemical changes in 'Navaho' fruit and the temporal relation of ripening events to respiration and ethylene production.

Materials and Methods

PLANT MATERIAL. 'Navaho' blackberry plants grown under standard cultural conditions in Clarksville, Ark., in 1993 and Lane, Okla., in 1996 were used as fruit sources, respectively. Nonterminal fruit, tagged at anthesis, of seven color stages, green ($\approx$14 d PA), green-red ($\approx$20 d PA), red-green ($\approx$30 d PA), red ($\approx$36 d PA), mottled ($\approx$44 d PA), shiny black ($\approx$46 d PA), and dull black ($\approx$48 d PA), were harvested at Clarksville with pedicels attached to prevent abscission zone ethylene production and transported to Lane on ice on two harvest dates in 1993. All stages of berry development were harvested at each harvest date. Fruit were held at 2 °C until used for analysis. All measurements on fresh fruit were made within 3 d of harvest and fruit used for other analyses had pedicels removed and were frozen (–80 °C) within 1 d of harvest. Preliminary experiments indicated that ripening changes in 'Navaho' fruit were negligible when held at 2 °C for 2 weeks. In 1996, secondary blooms were tagged at anthesis and four harvests at 7 d intervals were made. Fruit at the same color stages and days PA as above were transported from plots at Lane to the laboratory within 15 min of harvest and stored as described above.

SEED AND BERRY WEIGHTS. Sixty berries per harvest date per stage of berry development, ranging from 5 to 48 d PA, in 1993 and 1996 were picked without pedicels, weighed, and dried at 60 °C to a constant weight. Individual berries were soaked in acidified water (0.02 mol·L^{-1} HCl), and rinsed until particulate matter was removed. Seeds were dried at 60 °C, counted, and weighed on a per berry basis. Three harvest dates 7 d apart were used each year.

FRUIT COMPOSITION. Measurements for firmness and fruit composition were made on berries harvested in both 1993 and 1996. To measure epidermal firmness, three drupelets attached to a berry were sampled randomly on each of 10 fruit per color stage per harvest date with 10, 30, 50 or 100 g Correx penetrometers (model GDK 10, 30, 50; Wagner Instruments, Greenwich, Conn.), each fitted with a 4.0 $\times$ 0.3-mm-diameter insect pin (Abeles, 1986). The pin was cut and filed to form a blunt surface, held perpendicular to the drupelet surface, and the maximum force required to pierce the skin until juice was visible was measured ($\approx$1 mm). Fruit were then cut longitudinally with a scalpel blade and one half of each fruit used for receptacle firmness determina-

tions. Firmness of the receptacle tissue to $\approx$2 mm depth was measured relative to basal, center, and distal locations of the fruit (Fig. 1). Fruit were held at 20 °C during measurements. Compression measurements were made by placing 20 individual berries shoulder side down on a flat surface and measuring the opposite shoulder with a texture analyzer (model TA-XT2; Texture Technologies Corp.; Scarsdale, N.Y.), equipped with a flat leucite cylinder (3 cm diameter), to a compression depth of 2 mm and at a rate of 1.0 mm^{-1}.

Total anthocyanin content was measured on frozen fruit at each color stage for each of the three harvest dates each year. Fruit (40 g) were homogenized with 90% (v/v) ethanol acidified with 0.12 mol·L^{-1} HCl (Perkins-Veazie et al., 1996), centrifuged at 32,000 g_n for 10 min, and the absorbance of duplicate aliquots of the supernatant measured at 532 nm with a spectophotometer (model UV-160; Shimadzu, Columbia, Md.). Total anthocyanin content was expressed as absorbance per gram fresh weight (FW).

Five grams of frozen fruit at each color stage was homogenized with an equivalent weight of distilled deionized water in a microblender cup (Waring Products, New Hartford, Conn.). The homogenate was centrifuged for 10 min at 32,000 g_n and the supernatant decanted through Miracloth (Calbiochem, San Diego, Calif.). About 0.5 mL of supernatant was used to determine soluble solids content (SSC) with a refractometer (ABBE-3L; Bausch and Lomb, Rochester, N.Y.). Ten milliliters of supernatant was diluted with 90 mL water, pH was determined by electrode, and titratable acidity (TA) (percent citric acid, v/v) was determined by adding 0.1 mol^{-1} NaOH to a final pH of 8.1.

SUGAR COMPOSITION. For sugar analyses, $\approx$75 g of fruit per replicate, with four replications per color stage, was harvested into 50 mL plastic containers on day 0, then frozen immediately at –80 °C. All fruit were then freeze-dried, ground with mortar and pestle, and filtered through plastic mesh to remove seeds. Sugars were extracted from 0.1 g of freeze-dried tissue following methods reported previously (McBee and Maness, 1983; Perkins-Veazie et al., 1999). Sucrose, glucose, and fructose were measured by a high-performance liquid chromatograph (HPLC) (model LC 600; Shimadzu), with a 300 $\times$ 7.8-mm column, (Aminex HPX-87C; Bio-Rad Laboratories, Hercules, Calif.), refractive index detector (model RID-6A; Shimadzu), and internal standards.

TOTAL AND WATER-SOLUBLE URONIC ACIDS. 'Navaho' blackberries at six stages of ripeness were harvested twice in 1996, with 1 week between harvests. Fruit were frozen at –80 °C and transported on dry ice to the University of Florida, Gainesville. Total and water-soluble uronic acids (UA) were determined using the method of Huber (1992).

RESPIRATION, ETHYLENE, ACC OXIDASE, AND ACC. In 1993, pedicels with attached berries were removed from 2 °C storage, placed in vials of water to minimize water stress and one fruit used per 150-mL jar, warmed to 20 °C ($\approx$15 min) and sealed for 0.5 h on the day following harvest in 1993. In 1996, fruit with pedicels were arranged singly in jars but sealed and CO_2 measured within 1 h of harvest for respiration measurements. The CO_2 produced at 20 °C was measured by injecting 1-mL samples of headspace atmosphere into a gas chromatograph (model 14A; Shimadzu), equipped with a thermal conductivity detector, and a 3.1 $\times$ 0.003 m stainless steel column packed with Porapak N 80/100 (Alltech

Associates, Deerfield, Ill.). The temperature of the oven, injector, and detector were 60, 175, and 105 °C, respectively. Fruit were sealed for 24 h in the presence of saturated potassium hydroxide in water after CO_2 determinations to determine ethylene production. One-milliliter samples of headspace gas were injected into a gas chromatograph (model 14A; Shimadzu) equipped with a flame ionization detector (FID) and a 3.1 × 0.003-m stainless steel column packed with 80/100 activated alumina. The temperatures of the oven, injector, and detector were 125, 175, and 150 °C, respectively. A total of 24 individual fruit per color stage were measured, representing two harvests per color stage per year.

Ethylene is evolved from the enzymatic conversion of 1-aminocyclopropane-1-carboxylic acid (ACC). The activity of ACC oxidase was determined by measuring ethylene production in fresh fruit samples incubated in solutions containing ACC. In 1993 and 1996, five individual berries without pedicels from each color stage were cut in half and placed in 10 mL of 1 mmol·L^{-1} ACC and 0.27 mol·L^{-1} (2% w/v) KCl in 25-mL erlenmeyer flasks. A vacuum of 79.8 kPa was drawn on the flasks for 1 min. The flasks were sealed for 15 min and 1 mL headspace samples analyzed for ethylene as described above. To measure ACC oxidase activity in drupelet and receptacle tissue, berries were cut in half from pedicel to tip and receptacles excised quickly from berries using a scalpel. Tissue was placed in 10 mL of 1 mmol·L^{-1} ACC, 1 mmol·L^{-1} (aminooxy) acetic acid, and 0.27 mol·L^{-1} (2% w/v) KCl and headspace samples measured as above.

Four replicates per color stage per harvest date were used to determine the ACC content of blackberries at three harvest dates in 1993 and 1996. Each frozen replicate consisted of four berry halves. ACC was determined in duplicate by the method of Lizada and Yang (1979), with modifications. Free ACC was measured by homogenizing 4 g fruit in 12 mL of 1.6 mol·L^{-1} (95%, v/v) ethanol with a Polytron (model PT 10/35; Brinkman Instruments, Westburg, N.Y.). Solutions were centrifuged 10 min at 32,000 g_n and supernatants held at –80 °C until analysis. Six milliliters extract per sample was taken to dryness, resuspended in 3 mL distilled water, centrifuged at 4000 g_n for 5 min, and the supernatants used for ACC determinations. Extracts were transferred to 12 × 75 mm disposable culture tubes, sealed with serum stoppers, and ethylene generated by a modified method of Hoffman and Yang (1982). To saturate the reaction, the concentration of $HgCl_2$ in tubes was increased to 3 µmol·L^{-1} (Coleman and Hodges, 1991). A 1 mL headspace sample was drawn from each tube and ethylene measured as described above. ACC conversion efficiency was 70% to 85%, using ACC standards.

Conjugated ACC was determined by the method of Spikman (1987). One mL of extract was placed in test tubes with 1 mL of 6 mol·L^{-1} HCl and boiled for 1 h. One milliliter of 6 mol·L^{-1} NaOH was added to neutralize the solution and ethylene generated as described above. Yields of conjugated ACC were 60% to 70%. Total ACC was calculated by adding free and conjugated ACC.

For tissue-specific determinations of ACC, receptacles were excised from drupelet tissue with a scalpel and held in 1.6 mol·L^{-1} ethanol at –80 °C until analysis. Free and total ACC were measured as described above. A total of four replicates per color stage, each consisting of two fruit per replicate, were used each year.

VOLATILE COMPOUNDS. Fruit at the red, shiny-black, and dull-black stages were collected from plantings at Lane in 1996, homogenized and frozen at –80 °C. Homogenates were packed in dry ice and sent to the USDA–ARS Subtropical Fruit Product Laboratory, Winter Haven, Fla. Upon arrival, 2 mL of homogenate was transferred to 6-mL vials, frozen by immersion in liquid nitrogen, and stored at –20 °C until used. Analysis was conducted using a gas chromatograph (model 8500; Perkin-Elmer LLC, Norwalk, Conn.) equipped with a model HS-6 headspace sampler and FID. A polar Durowax column (0.53 mm × 30 m; 1 µm film thickness; J&W Scientific, Folsom, Calif.) was used with a helium carrier gas linear velocity of 81 cm·s^{-1}.
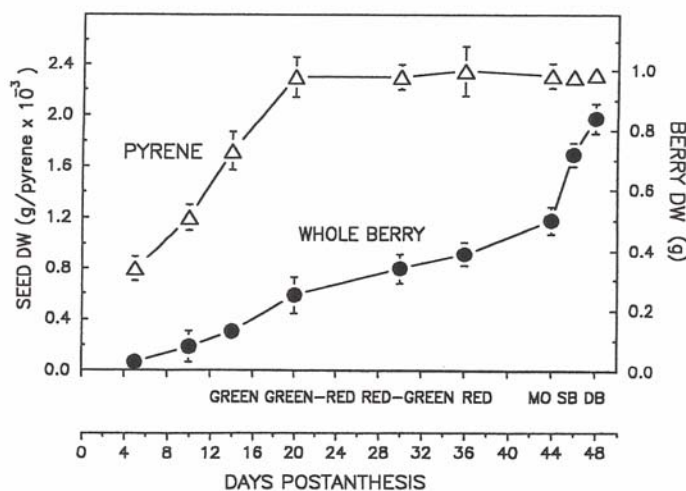
Frozen duplicate samples were thawed quickly under running tap water and equilibrated for 15 min at 80 °C in the headspace sampler before injection. Injection settings of 0.5 min vial pressurization time and 0.2 min injection time were used. Oven temperature was programmed at 40 °C for 6 min and then increased to a final temperature of 180 °C at a rate of 6 °C·min^{-1}. The FID temperature was 250 °C and the amplifier range was set for high sensitivity. Volatile compounds were identified by comparing retention times with those of standards and by enrichment of bland (rotoevaporated at 50 °C) blackberry homogenate with authentic standards.

STATISTICAL ANALYSIS. Means were separated by standard error within color stages. Mean values for volatile compounds were subjected to analysis of variance procedures and separated by LSD, $P \leq 0.05$, using SAS V.5.0, (SAS Institute Inc., Cary, N.C.).

Results

FRUIT COMPOSITIONAL CHANGES. The growth of 'Navaho' blackberry pyrenes was biphasic (Fig. 2) and was complete by the red-green stage. The contribution of pyrenes (average of 50 pyrenes/berry, data not presented) to total berry weight was minimal [<2% of FW and 12% of dry weight (DW) of dull-black

Fig. 2. Berry growth of blackberry including seed or pyrene and whole fruit (drupelets, receptacle, and pyrenes) relative to days post anthesis and ripeness stage. Each value represents the mean ± SE of 60 berries replicated over three harvests dates per year. SE bars are visible when larger than the symbols depicting data points. MO, SB, and DB represent mottled, shiny black, and dull black, respectively.

fruit]. The DW of whole berries (fruit tissue with pyrenes) had a constant growth rate (Fig. 2). Fresh weight followed a growth pattern similar to that of dry (data not presented).

Both compression and resistance to penetration in drupelet and receptacle tissues decreased as blackberries ripened (Fig. 3A). Drupelet and receptacle tissues of red fruit had 30% to 70% and 40% of the puncture resistance of green fruit, respectively. By the dull-black stage, receptacle tissue had only 4% and drupelet tissue only 25% of the resistance to puncture measured at the green stage. In whole fruit, compression decreased linearly from red to black stages and the degree of slope was much sharper than for penetrometer resistance at these ripeness stages (Fig. 3A).

The total UA content of 'Navaho' berries decreased sharply between 14 and 20 d PA (Fig. 3B). Total UA showed a slight decline from the red to shiny-black stages of ripeness, with a corresponding increase in both total and percent water-soluble UA. Total UA increased and water soluble UA decreased in dull-black fruit.

Anthocyanins were first detected in green-red fruit, but the greatest increase occurred between the mottled and shiny-black stages of ripeness (Fig. 4A). Blackberry juice pH was 3.4 in green fruit, decreased to 3.0 in red and mottled fruit, and increased to 3.7 in dull-black fruit (Fig. 4A). Blackberry SSC was ≈4% until the black color stage (Fig. 4B) and then increased from 7% to 10% between the mottled and dull-black stages. TA was high in green fruit (≈3%) and did not decrease appreciably until the shiny-black stage. Between the mottled and shiny-black stages, TA decreased

3-fold, to <1%. The SSC to TA ratio did not increase until the mottled stage.

Total soluble sugar content, mostly glucose and fructose, was 200 µg·g⁻¹ DW in red fruit and increased from 225 to 600 µg·g⁻¹ DW between the mottled and shiny-black stages (Fig. 4C). No significant increase in total sugars occurred between the shiny and dull-black stages. Sucrose was <100 µg·g⁻¹ DW and represented only 0.5% to 1.0% of total sugars. Glucose increased and fructose decreased between the red and mottled ripeness stages.

RESPIRATION, ETHYLENE PRODUCTION, ACC CONTENT AND ACC OXIDASE ACTIVITY. 'Navaho' fruit respiration, measured from intact fruit held with stems in water, was highest in green fruit (Fig. 5A) and decreased between the green-red and red-green stages of ripening. Respiration, measured as CO_2 produced, increased ≈17%, from 3.6 to 4.2 µmol·kg⁻¹·h⁻¹ between the red and shiny-black ripeness stages.

Ethylene production was barely detectable in 'Navaho' fruit until the shiny-black ripeness stage (<10 nmol·kg⁻¹·h⁻¹), and was highest at the dull-black stage (Fig. 5A). Even at the dull-black stage, ethylene production was low, at 44 nmol·kg⁻¹·h⁻¹. ACC oxidase activity increased before ethylene production at the red stage, then increased linearly from the mottled to dull-black stages of ripeness (Fig. 5B).

Total ACC in whole fruit decreased with ripeness, corresponding to an increase in free ACC (Fig. 5C). Free ACC represented
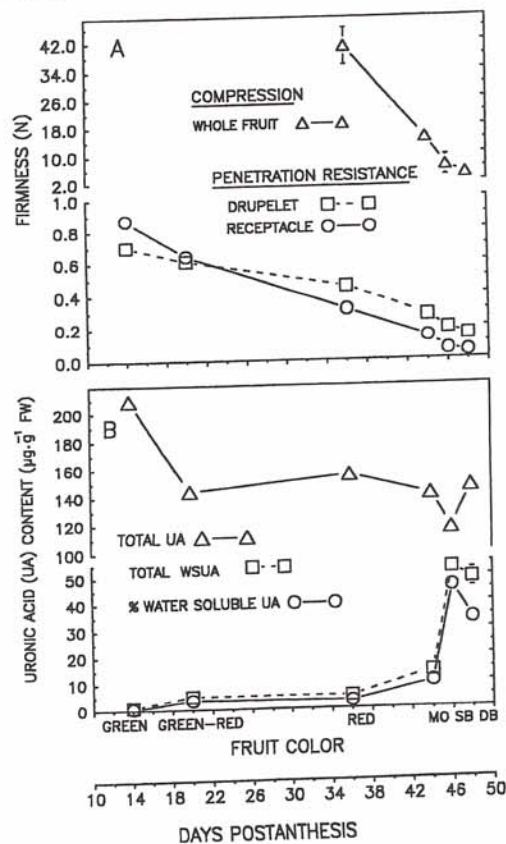

Fig. 3. Changes in (A) blackberry firmness and (B) uronic acid content, measured as compression of whole berry and of penetration resistance of drupelet and receptacle tissues, and of total and water soluble uronic acids. Each value represents the mean ± SE of 10 berries replicated over three harvest dates.
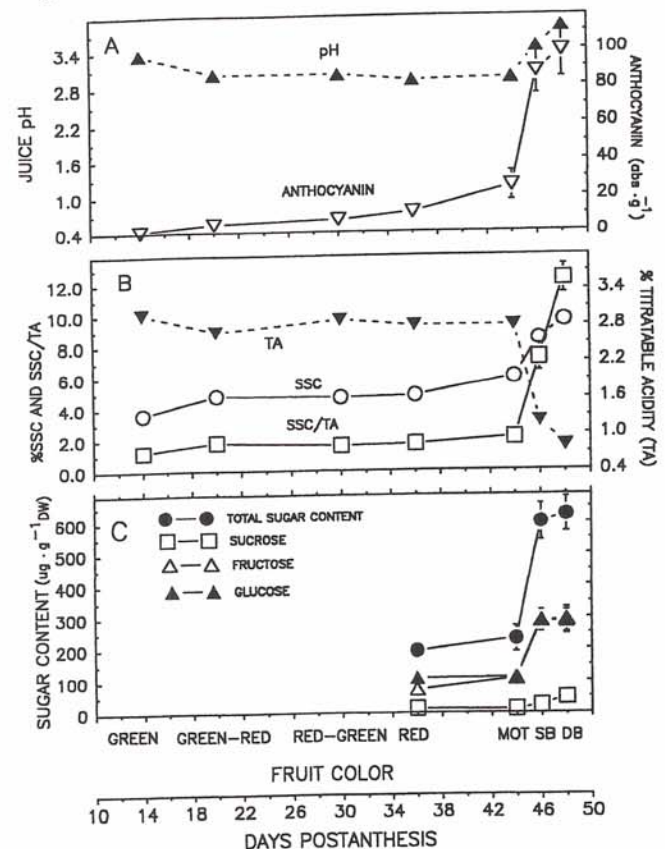

Fig. 4. Physicochemical changes in ripening blackberries, shown as changes in (A) puree pH and anthocyanin content; (B) SSC, TA, and SSC to TA ratio; and (C) sucrose, glucose and fructose content. Each value represents the mean ± SE of 40 g tissue (≈10 berries) duplicates replicated over three harvest dates. SE bars are visible when larger than the symbols depicting data points.
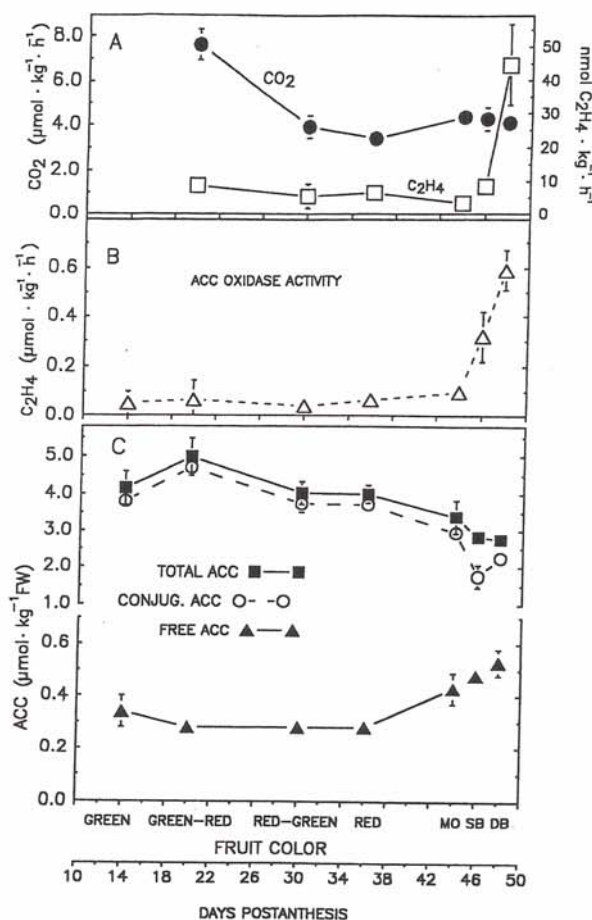
Fig. 5. (A) Respiration and ethylene production, (B) ACC oxidase activity, and (C) ACC content of berries in blackberries harvested at different stages of ripeness. Each value represents the mean ± SE of six berries replicated over two harvest dates per year.
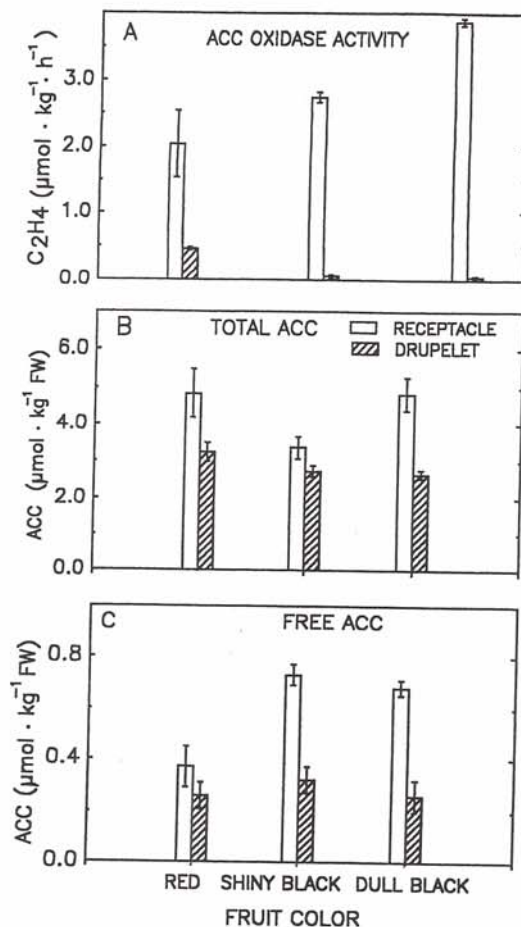

Fig. 6. (A) ACC oxidase activity, (B) total ACC, and (C) free ACC in blackberry receptacle and drupelet tissues at different ripeness stages. Each value represents the mean ± SE of four berries replicated over two harvest dates per year.

only 10% of the total ACC, and increased from ≈0.3 to 0.5 µmol·kg⁻¹ between the green and dull-black stages, while total ACC decreased by ≈50%. Accumulation of free ACC was initiated at the red stage and continued through the dull-black stage. The contribution of free ACC from pyrenes was negligible (0.02 µmol·kg⁻¹) at all stages of ripening (data not presented).

ACC oxidase activity and free and total ACC were higher in receptacle than in drupelet tissues at all fruit maturity stages (Fig. 6). ACC oxidase activity decreased slightly in drupelet tissue and increased greatly in receptacle tissue with ripening (Fig. 6A). ACC oxidase activity in receptacle tissue was at least 10 times higher than that of drupelet tissue at all ripeness stages. Total ACC in drupelet tissue decreased with ripeness and was slightly higher in red receptacle tissue (Fig. 6B). Free ACC increased in receptacle tissue as fruit ripened, but remained constant in drupelet tissue (Fig. 6C). Free and total ACC contents of drupelet tissue were more similar to whole fruit contents than those of receptacle tissue (Figs. 5 and 6).

CHANGES IN VOLATILE PROFILE. Total volatiles increased be-

tween the red and dull-black stages of ripeness, especially after the shiny black stage (Table 1). Most of the volatiles were alcohols, followed by aldehydes. Changes in volatile composition occurred with ripening. In shiny-black and red fruit, 58% of the total volatiles were alcohol-type compounds (primarily ethanol), increasing to 72% in dull-black fruit. Aldehydes, although progressively increasing in amount with ripening, remained at 23% to 30% of the total volatile composition. Primary aldehydes at all ripeness stages were acetaldehyde and cis-3-hexenal (data not presented). Hydrocarbons, ketones, and esters made up a smaller proportion of volatiles in dull-black than in shiny-black or red fruit. Esters and hydrocarbons increased 2- to 6-fold between the red and shiny-black stages and showed little change thereafter.

Discussion

TA changed more than SSC during blackberry ripening. As reported for red raspberry and boysenberry (Given et al., 1986;

Table 1. Classes of volatile compounds found in ripening 'Navaho' blackberries.

Compound	Color stage		
	Red	Shiny black	Dull black
		μL·L⁻¹	
Alcohols	77.06 c	178.75 b	579.14 a[z]
Aldehydes	39.64 c	90.97 b	186.63 a
Hydrocarbons	10.22 b	18.96 a	20.55 a
Esters	1.67 c	18.59 a	7.78 b
Ketones	3.12 a	3.10 a	5.52 a
Linalool oxide	0.45 a	0.72 a	4.56 b
Total	132.17	311.09	804.18

[z]Mean separation within rows by LSD, $P < 0.05$, n = 4.

Mason 1974), TA and SSC to TA ratios were better indicators of fruit maturity in blackberry than SSC alone. The observed decrease in juice pH followed by an increase during the latter ripening stages is similar to that reported in strawberry (*Fragaria ×ananassa* Duch.) and raspberry (Perkins-Veazie and Nonnecke, 1993; Spayd and Morris, 1981). Ozawa et al. (1987) proposed that as *Rubus* fruit mature, pectin fragments released from the cell wall bind with polyphenols, causing reduced astringency and acidity, and increased pH of tissue homogenate.

The anthocyanin content of ripe 'Navaho' fruit was slightly higher than that reported for 'Cherokee', an erect thorny blackberry from the same breeding program (Sapers et al., 1986), but was similar to the anthocyanin content reported for the semierect thornless blackberry cultivar Hull Thornless (Sapers et al., 1986). In our study, the increase in total sugars paralleled the accumulation of anthocyanins. Creasy (1968) reported that anthocyanin accumulation in strawberry leaf disks is directly related to sucrose accumulation. Similarly, Mori and Sakurai (1994) found that anthocyanin accumulation in tissue-cultured strawberry is enhanced by glucose, fructose or sucrose.

Application of ethephon [(2-chloroethyl) phosphonic acid] to blackberries 1 to 3 d before harvest advances maturity, as measured by the yield of black fruit (Sims and Morris, 1982). However, fruit weight and SSC are reduced while pH and black color ratings are higher with ethephon treatment (Lipe, 1980; Sims and Morris, 1982; Takeda and Peterson, 1988). Mottled or red fruit appear to be more sensitive to ethephon than black fruit (Sims and Morris, 1982). Burdon and Sexton (1993) found that partially black fruit show more anthocyanin accumulation than do fully black fruit in response to ethephon treatment. Thus, although ethephon appears to promote anthocyanin production, it has minimal effect on other ripening processes in blackberries. Given (1985) concluded that ethephon promotes anthocyanin development and abscission unrelated to other ripening processes in blackberries.

Berry softening was inversely related to fruit growth in this study. Sapers et al. (1987) reported that semierect black (ripe) or red (unripe) 'Hull Thornless' blackberries are similar in levels of soluble, insoluble, and total pectin. Redgewell et al. (1997a), however, found that polyuronide content and xylose are higher in ripe European blackberry (*R. fructicosus* 'Aurora') fruit compared with unripe fruit. Blackberry cell walls expand, remain cohesive, and exhibit general cell wall dissolution rather than dissolution of the middle lamella (Redgewell et al., 1997b). Redgewell et al. (1997a) concluded that solubilization, rather than depolymerization of pectins, is the major change in cell walls of ripening blackberries. Our results show that water-soluble

uronic acids increased 50% during blackberry ripening, consistent with the findings of Redgewell et al. (1997a).

Ethylene production by 'Navaho' at the dull-black fruit stage was very low compared with that reported for erect thorny and semierect thornless cultivars (Perkins-Veazie et al., 1996; Walsh et al., 1983). Walsh et al. (1983) suggested that blackberries with higher ethylene production have a shorter shelf life. Shelf life in blackberries is defined by fruit firmness and freedom from decay. Burdon and Sexton (1993) reported wide differences in ethylene production among blackberry cultivars originating in the Pacific Northwest and Scotland. Although these authors could not correlate higher ethylene production with softer fruit, cultivars with higher ethylene production abscised more readily.

As reported for other fruit, ACC oxidase appeared to regulate ACC levels in blackberries during the latter stages of ripening (Yang and Hoffman, 1984). Burdon and Sexton (1993) concluded that ACC oxidase activity is the limiting step in ethylene production in blackberries. In 'Navaho' fruit, ACC oxidase activity and free ACC increased (Fig. 3) slightly before ethylene production increased. The very different ACC content and ACC oxidase activities between the receptacle and drupelet tissues in blackberries indicate spatial and temporal separation of ethylene production and/or ethylene sensitivity. The more rapid softening and lower firmness values in receptacle tissue relative to drupelets suggests that ripening was initiated in the receptacle tissue.

The tetraploid 'Navaho' blackberry is a mixture of species, unlike 'Thornless Evergreen', whose volatile composition has been studied by Georgilopoulos and Gallois (1987). They used different analytical methods and found far more esters and ketones, with a corresponding change in percent contribution to total volatiles, than in our study. In 'Thornless Evergreen', 2-heptanol is the most abundant alcohol (43% of alcohols), in contrast to 'Navaho', which had <1% of alcohols as 2-heptanol. These differences may be due either to the different analytical extraction procedures used or to the warm conditions (35/30 °C day/night) to which 'Navaho' fruit were held during ripening.

Anthocyanin synthesis, increased SSC, and decreased TA all occurred before either ethylene or ACC could be detected. It is possible that increased respiration is associated with the onset of ethylene production, or with the formation of abscission zones, but neither the respiratory climacteric nor ethylene appear to be involved in the initiation of blackberry ripening. As berries were held intact on pedicels during transit, and measurements were taken within 1 or 24 h of harvest, a wounding response was not indicated. It is possible that the slight ethylene production occurring earlier in ripening sensitized the fruit, and particularly the abscission zone cells, to ethylene. Alternatively, ripening events in blackberries may be independent of ethylene sensitivity, as has been shown in strawberries (Given et al., 1988), with the late production of ethylene in blackberries involved only in fruit detachment.

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