Exhibit 2A, #26

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Cultivar and Maturity Affect Postharvest Quality of Fruit from Erect Blackberries

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***Abstract.* Fruit at three stages of ripeness were harvested from four erect blackberry (*Rubus* spp.) cultivars, 'Navaho', 'Choctaw', 'Cheyenne', and 'Shawnee', for 2 years to evaluate fresh-market shelf life during 7 days of storage at 2C, 95% relative humidity. Ethylene production was highest from dull black fruit and varied widely among cultivars, ranging from 7.3 to 51.1 pmol·kg^{-1}·s^{-1} for 'Navaho' and 'Choctaw' fruit, respectively. Weight loss ranged from 0.8% ('Shawnee') to 3.3% ('Navaho') after storage. Mottled (50% black) fruit of all cultivars were higher in fruit firmness and titratable acidity and had lower soluble solids and anthocyanin concentrations than fruit at other stages of maturity. Cultivars did not differ in total anthocyanin concentration, but dull black fruit had a higher anthocyanin concentration than shiny black fruit. Dull black 'Choctaw', 'Shawnee', and 'Cheyenne' fruit were softer and had more leakage and decay than shiny black fruit. Both shiny and dull black 'Navaho' fruit had less leakage than fruit of other cultivars. All cultivars at the shiny black stage were considered marketable after 7 days at 2C because fruit were firm with little decay or leakage. However, red discoloration appeared more frequently on shiny black than on dull black fruit. Mottled fruit of erect cultivars should not be harvested, while shiny black fruit of 'Cheyenne', 'Shawnee', and 'Choctaw' might be suited for regional markets. Either shiny black or dull black 'Navaho' fruit could be shipped to distant markets.**

Consumption of fresh blackberries has increased in the past 3 years; 31% of U.S. consumers purchased blackberries from retail stores in 1994 (Pomerantz, 1995). This increase in consumption may have resulted from a 77% increase in commercial blackberry production between 1980 and 1990 (Clark, 1992).

Currently, 65% of total blackberry production east of the Rocky Mountains is of erect-type cultivars (Clark, 1992). Erect-type blackberry production requires less labor since plants can be mechanically pruned and do not need trellising. Local pick-your-own and fresh markets for erect-type blackberries have increased and there is interest in shipping these fruit (Strik, 1992). Blackberry fruit undergo distinct color changes during ripening. Fruit change from green to red then turn black (Burdon and Sexton, 1993). The latter stages of ripeness occur rapidly in erect cultivars grown in warm climates (i.e., 1 to 3 days). These ripeness stages are commercially divided by color into mottled (50% black), shiny black (visible fruit sheen), and dull black (no fruit sheen). Generally, fully black (shiny or dull black) fruit are harvested, based on ease of abscission from the pedicel. For semi-erect blackberry cultivars, Walsh et al. (1983) reported that only dull black blackberries should be harvested for fresh markets due to a higher sugar : acid ratio. To our knowledge, no information on ripeness stage and postharvest quality is available for erect cultivars.

Currently, the recommended storage life for blackberries is 2 to 3 days when held at 0C (Hardenburg et al., 1986; Morris et al., 1981). However, Clark and Moore (1990) found that fruit of some erect cultivars could be held up to 7 days at 5C. The objective of our study was to evaluate the storage quality of erect blackberry cultivars of three maturity stages for fresh-market use.

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Materials and Methods

Fruit were harvested from established blackberry plots grown with standard cultural practices in a hedgerow system in Clarksville, Ark. No fungicides were applied. Fruit at mottled, (50% black; 2 days from dull black), shiny black (1 day from dull black), and dull black ripeness stages from thorny 'Cheyenne', 'Choctaw', 'Shawnee', and thornless 'Navaho' were harvested twice over 4 to 6 weeks in 1992 and 1994, representing field ripeness of ≈30% and 60%, respectively. Each cultivar had a 2- to 3-week harvest period at this location. Two harvests per year per cultivar and color stage were used. Fruit of each ripening stage and cultivar were harvested into 250-ml pulp boxes and held at 7 to 10C during the 4-h transport by car to Lane, Okla.

On the day following harvest, six individual fruit per cultivar were sealed in 150-ml jars and held for 0.5 h at 20C in darkness. To determine CO_2 evolution rates, 1-ml headspace samples were injected into a Shimadzu gas chromatograph (Columbia, Md.) equipped with a thermal conductivity detector and a 3.1 × 0.03-m stainless steel column packed with Poropak N 80/100 (Alltech Assoc., Deerfield, Ill.). To determine ethylene production, the jars were sealed for 18 h in the presence of saturated potassium hydroxide and held at 20C in darkness. One-milliliter headspace samples were injected into a Shimadzu gas chromatograph equipped with a flame ionization detector and a 3.1 × 0.03-m stainless steel column packed with activated alumina.

Fruit for storage were sorted to eliminate leaky, diseased, excessively soft, or overripe fruit. Samples consisted of two pulp boxes per cultivar, color stage, and harvest date, containing 20 fruit per box. Samples were randomized within cultivar and color stage. To reduce weight loss from excessive fan air circulation, pulp boxes were placed in Plexiglas boxes (25 × 12.5 × 7.5 cm) and covered with a Plexiglas lid having two holes (2.3 cm in diameter) 10 cm apart and held for 7 days at 2 ± 0.5C and ≈95% relative humidity. The remaining portion of fruit was frozen for compositional analysis.

Skin firmness was measured on 20 fruit per sample at days 0 and 7 using Correx penetrometers (10-, 30-, 50-, and 100-g gauges; Wagnor Instruments, Greenwich, Conn.) adapted with a #000 (0.3 mm in diameter, 4 mm in length) blunt insect pin (Abeles, 1986). Three measurements were made per fruit—at the base, center, and tip locations—by holding the pin perpendicular to the drupelet surface and inserting the pin ≈1 mm until a drop of liquid was visible.

Following storage, fruit in boxes were weighed and rated individually. Leakage was measured by the presence or absence of a red spot under each fruit on the white paper. Firmness for individual fruit were subjectively rated on a scale of 1 to 9 where 1 = very soft, 5 = moderate firmness, and 9 = firm. The presence or absence of red discoloration and decay on fruit was evaluated.

For compositional analysis, 10 fruit per sample were ground with an equivalent weight of water in a Waring blender, centrifuged, and filtered through cheesecloth to remove debris. Ten milliliters of filtrate was added to 90 ml distilled water and titrated to pH 8.2 with 0.1 N NaOH. Titratable acidity (TA) was calculated as percent citric acid. Soluble solids concentration (SSC) was determined by placing ≈0.5 ml of remaining filtrate on the stage of Abbe-3L refractometer. Anthocyanin was extracted by double extraction of 5 g of fruit per replicate with 20 ml of acidified (0.02 M HCl) 90% ethanol. Absorbance was read at 532 nm on a spectrophotometer (Shimadzu UV160), and data were expressed as absorbance units/gram frozen weight.

The experiment was designed as a split-plot arrangement consisting of four cultivars × three stages of ripeness. To represent an average response for the short (2 to 3 week) harvest season, storage trials were replicated over time using harvest dates and years as replications for each cultivar and ripeness stage, and data were subjected to analysis of variance.

Results and Discussion

Dull black fruit were heavier than fruit from the other color stages (Table 1). Mottled fruit had only 60% of the fresh weight of dull black fruit. 'Shawnee' had the heaviest berries at all color stages. Weight loss among cultivars ranged from 0.8% to 3.3% after 7 days at 2C and was influenced by cultivar and color stage. Dull black 'Shawnee' fruit lost less weight than those of the other cultivars, probably because 'Shawnee' had the heaviest and, presumably, largest berries (7 g vs. 4 to 5 g). 'Navaho' and 'Choctaw' fruit tended to lose more weight than the others at all color stages.

Fruit skin firmness depended on cultivar, ripeness stage, and storage duration (Table 1). 'Choctaw' fruit were softer than those of 'Navaho' or 'Cheyenne' at all color stages. Within cultivars, mottled fruit had the highest skin firmness and dull black the lowest. Following storage, 'Navaho' fruit had higher skin firmness values than the other cultivars, regardless of color stage. Occasionally, skin firmness values were higher after storage. This phenomenon happened most often with 'Navaho' and may have resulted from weight loss and subsequent epidermal cell desiccation, as reported for strawberries (Sistrunk, 1963).

Subjective ratings for firmness differed among cultivars and color stages (Table 1). Mottled fruit were rated firmest, and no cultivar was rated excessively soft. However, 'Choctaw' and 'Shawnee' dull black fruit were soft after storage. Both dull and shiny black fruit of 'Navaho' had high firmness ratings and were never softer than other cultivars. These results were similar to those reported by Moore and Clark (1988), who found that 'Navaho' had better subjective firmness ratings than 'Shawnee' or 'Cheyenne'. Subjective firmness was positively correlated with penetrometer readings ($P \leq 0.001$). Regression analysis failed to yield an r^2 value greater than 0.17 for linear, quadratic, or cubic relationships.

SSC was higher in dull black than in mottled fruit for all cultivars (Table 2). This trend is similar to that reported for semi-erect blackberries (Walsh et al., 1983). Except for 'Navaho', there was little difference in SSC between shiny and dull black fruit among cultivars, and fruit of all ripeness stages increased in SSC during storage. However, only dull or shiny black 'Shawnee' fruit significantly increased in SSC during storage. An increase in SSC was expected, since weight loss concentrates cell sap. However, the increase in SSC in this study exceeded values predicted from weight loss. Increased SSC during storage may be due to a gain in sugars or hydrolysis of cell wall materials. Dull and shiny black 'Navaho' and 'Choctaw' fruit were higher in SSC than 'Shawnee' and 'Cheyenne' before and after storage. Our data for SSC are similar to those reported for black 'Shawnee', 'Choctaw', and 'Navaho' fruit (Clark and Moore, 1990; Moore and Clark, 1988, 1994; Moore et al., 1985).

Table 1. Initial berry weights, skin firmness measurements (resistance to penetrometer), and subjective firmness ratings of four erect blackberry cultivars at three stages of ripeness after 7 days of storage at 2C, 95% relative humidity.[z]

Color stage	Cultivar	Mean[y] berry wt (g)	Wt[x] loss (%)	Skin firmness (N × 10^{-3}), duration of storage 0 days	Skin firmness (N × 10^{-3}), duration of storage 7 days	Subjective[w] firmness
Mottled	Choctaw	3.2 ± 0.2	2.3 ± 0.6	179 ± 4	139 ± 3	8.4 ± 0.1
	Cheyenne	3.3 ± 0.1	1.6 ± 0.4	207 ± 5	209 ± 6	9.0 ± 0.2
	Navaho	3.2 ± 0.1	3.3 ± 0.4	230 ± 5	278 ± 5	8.8 ± 0.2
	Shawnee	4.1 ± 0.3	2.4 ± 0.4	220 ± 7	247 ± 5	8.4 ± 0.2
Shiny black	Choctaw	3.8 ± 0.2	2.6 ± 0.9	138 ± 6	116 ± 7	6.6 ± 0.2
	Cheyenne	4.9 ± 0.3	1.9 ± 0.5	180 ± 10	171 ± 6	8.0 ± 0.1
	Navaho	4.7 ± 0.1	2.4 ± 0.4	168 ± 5	196 ± 4	8.0 ± 0.1
	Shawnee	6.3 ± 0.2	1.7 ± 0.3	145 ± 5	162 ± 1	7.0 ± 0.1
Dull black	Choctaw	5.0 ± 0.1	2.6 ± 0.4	107 ± 5	91 ± 4	5.0 ± 0.1
	Cheyenne	6.0 ± 0.3	1.6 ± 0.4	146 ± 6	137 ± 4	5.8 ± 0.2
	Navaho	5.3 ± 0.2	2.2 ± 0.3	130 ± 6	159 ± 4	7.2 ± 0.1
	Shawnee	7.4 ± 0.3	0.8 ± 0.1	126 ± 6	126 ± 4	5.2 ± 0.1

[z]Significant cultivar × color stage interaction, $P \leq 0.05$.
[x]Means of 52 fruit ± SE.
[y]Means of 15 samples ± SE.
[w]Subjective firmness scale: 9 = firm, 1 = soft; after 7 days storage.

Table 2. Percent soluble solids content (SSC), titratable acidity (TA) (citric acid), and ratio of SSC : TA of fruit from four erect blackberry cultivars of three ripeness stages stored for 7 days at 2C, 95% relative humidity.[z]

Color stage	Cultivar	SSC (%), duration of storage 0 days	SSC (%), duration of storage 7 days	TA (%), duration of storage 0 days	TA (%), duration of storage 7 days	SSC : TA (%), duration of storage 0 days	SSC : TA (%), duration of storage 7 days
Mottled	Choctaw	6.5 ± 0.6	8.4 ± 1.3	2.3 ± 0.1	1.5 ± 0.2	2.8 ± 0.6	5.6 ± 1.3
	Cheyenne	4.7 ± 0.5	5.6 ± 0.7	2.2 ± 0.1	1.8 ± 0.1	2.1 ± 0.5	3.1 ± 0.7
	Navaho	6.0 ± 1.1	8.7 ± 1.1	2.8 ± 0.2	2.2 ± 0.1	2.1 ± 1.1	4.0 ± 1.1
	Shawnee	6.2 ± 0.4	6.4 ± 0.6	2.5 ± 0.2	1.8 ± 0.1	2.5 ± 0.4	3.6 ± 0.6
Shiny black	Choctaw	8.5 ± 0.6	9.4 ± 0.6	1.3 ± 0.1	1.2 ± 0.1	6.5 ± 0.6	7.8 ± 0.6
	Cheyenne	6.8 ± 0.6	7.2 ± 0.3	1.1 ± 0.1	0.9 ± 0.1	6.2 ± 0.6	8.0 ± 0.3
	Navaho	8.4 ± 0.8	8.7 ± 0.5	1.2 ± 0.1	1.6 ± 0.1	7.0 ± 0.8	5.4 ± 0.5
	Shawnee	7.3 ± 0.4	8.5 ± 0.6	1.4 ± 0.1	1.0 ± 0.1	5.2 ± 0.4	8.5 ± 0.6
Dull black	Choctaw	8.7 ± 1.0	10.5 ± 1.3	0.9 ± 0.1	0.8 ± 0.1	9.7 ± 1.0	13.1 ± 1.2
	Cheyenne	6.2 ± 1.0	7.7 ± 0.8	1.0 ± 0.1	0.7 ± 0.1	6.2 ± 1.0	11.0 ± 0.8
	Navaho	9.6 ± 0.8	10.7 ± 0.9	0.8 ± 0.1	1.0 ± 0.1	2.0 ± 0.8	10.7 ± 0.9
	Shawnee	7.2 ± 0.6	8.6 ± 0.6	1.2 ± 0.1	0.9 ± 0.2	6.0 ± 0.5	9.6 ± 0.5

[z]Significant cultivar × color stage interaction, $P \leq 0.05$. Values are means of 60 fruit ± SE.

There were greater changes in TA than in SSC as ripening progressed (Table 2). TA decreased as much as 50% between mottled and shiny black ripeness stages. Decreased TA during blackberry fruit ripening also has been reported for semi-erect cultivars (Walsh et al., 1983). Between shiny and dull black stages, fruit TA decreased 10% to 30%, depending on cultivar. Among cultivars, 'Navaho' fruit had the highest TA at the shiny black and mottled stages. TA declined ≈20% to 30% for all cultivars in mottled fruit and ≈10% to 30% in shiny or dull black fruit during storage for all cultivars except 'Navaho'. Shiny black and dull black 'Navaho' fruit were 20% to 30% higher in TA following storage than initially. About 10% of this increase could be accounted for by concentration of acidity due to weight loss. The high TA and low SSC found in mottled fruit even after storage would make them unacceptable for fresh market.

Anthocyanin concentration differed among color stages but not among cultivars (data not shown). The anthocyanin concentration of dull black fruit declined during 7 days of storage from 114 to 93 absorbance units/gram, that of shiny black and mottled fruit did not change appreciably during storage and were 92 and 28 absorbance units/gram, respectively. The anthocyanin content of these fruit is similar to that reported for the black fruit of 'Black Satin' and 'Hull Thornless' blackberries (Sapers et al., 1986).

The percentage of fruit exhibiting decay after storage ranged from 0% to 40% depending on stage of ripeness and cultivar (Table 3). More dull black than mottled or shiny black fruit was decayed. There were more decayed dull black 'Cheyenne' berries than 'Choctaw' or 'Navaho'. Shiny black 'Choctaw', 'Shawnee', and 'Cheyenne' had 5% to 10% decayed berries. Less than 1% of shiny black

'Navaho' fruit had decay after 7 days of storage, meeting USDA number 1 grade standards (U.S. Dept. of Agriculture, 1928). Clark and Moore (1990) reported that 'Choctaw' and 'Shawnee' fruit were unmarketable after 7 days of storage at 5C because of softness and decay. The better quality for these cultivars found in our study may have been the result of prompt postharvest cooling.

The percentage of berries with leakage far exceeded those with decay (Table 3). 'Navaho' dull and shiny black fruit had much less leakage (30% to 40%) than the other cultivars. The percentage of berries exhibiting red discoloration was higher for shiny black than for dull black fruit and was not an evaluation criterion for mottled fruit (Table 3). Of the shiny black berries, discoloration occurred least often on 'Navaho' fruit. Discoloration of black fruit ("reddening") has been reported for frozen blackberries (Sapers et al., 1986). The causes of discoloration may be due to less mature fruit being harvested, resulting in less total pigment content and a lower pH, or differences in the relative concentrations of various pigments (Mazza and Miniatio, 1993; Morris et al., 1981; Sapers et al., 1986). Our data support the view that blackberry discoloration occurs more often on less ripe fruit (Jennings, 1988). However, our results indicate a strong cultivar influence on expression of this postharvest problem.

At 20C, the respiration rate of blackberry fruit ranged from 1150 to 1480 $nmol \cdot kg^{-1} \cdot s^{-1}$, depending on cultivar and color stage (Table 4). The maximum respiration rate occurred at a different ripeness stage for each cultivar. 'Navaho' fruit had the highest respiration rate at the mottled stage, 'Shawnee' and 'Cheyenne' at the shiny black stage, and 'Choctaw' at the dull black stage. The rate of respiration among the color stages was similar to that reported for 'Chester' and'Hull Thornless' semi-erect blackberries (Walsh et al., 1983).

Ethylene production increased with ripeness stage and differed among cultivars (Table 4). Dull black 'Choctaw' fruit had the highest rate of ethylene production and 'Navaho' the lowest. 'Choctaw' fruit produced more ethylene than other cultivars at mottled and shiny black stages. The trend of increased ethylene production with increased ripeness is similar to that reported by Walsh et al. (1983) for semi-erect blackberries. However, ethylene production from dull black fruit of the erect cultivars in our study was considerably lower than that reported for dull black 'Chester' fruit, the lowest reported ethylene-producing semi-erect cultivar (Walsh et al., 1983).

There was a strong positive correlation between subjective firmness ratings and ethylene content for cultivars at the dull black and mottled color stages ($P \leq 0.001$). Mottled fruit were hard and had little or no ethylene production, while dull black fruit were usually soft and had high ethylene production. 'Choctaw' fruit produced more ethylene and were softer than 'Navaho' fruit. Shelf life is inversely related to ethylene production rates (Walsh et al., 1983). The high percentage of 'Choctaw' and 'Shawnee' berries with leakage after 7 days suggests that ethylene production may be related to this postharvest problem.

Table 3. Percent fruit rated with decay, leakage, or red discoloration from four erect blackberry cultivars of three stages of ripeness after 7 days at 2C, 95% relative humidity.[z]

Color stage	Cultivar	Berries affected (%) Decay	Leakage	Red discoloration
Mottled	Choctaw	1.3 ± 1.3	5.0 ± 5.0	NA
	Cheyenne	5.7 ± 1.8	1.0 ± 1.0	NA
	Navaho	0 ± 0	0.8 ± 0.8	NA
	Shawnee	2.4 ± 1.9	8.0 ± 4.3	NA
Shiny black	Choctaw	5.0 ± 5.0	46.5 ± 23.5	47.5 ± 12.5
	Cheyenne	8.8 ± 3.1	35.0 ± 10.6	41.3 ± 13.3
	Navaho	0.3 ± 0.3	5.5 ± 2.7	18.8 ± 4.4
	Shawnee	5.8 ± 2.2	41.7 ± 10.3	56.3 ± 7.6
Dull black	Choctaw	21.7 ± 4.8	72.2 ± 4.2	23.5 ± 4.0
	Cheyenne	40.6 ± 8.5	89.0 ± 4.0	6.2 ± 3.5
	Navaho	17.3 ± 5.0	40.4 ± 4.6	3.9 ± 1.5
	Shawnee	37.8 ± 14.8	87.0 ± 1.9	30.2 ± 4.2

[z]Significant cultivar × storage day interaction, $P \leq 0.05$. Values represent means of 225 fruit ± SE. NA = not applicable.

Table 4. Ethylene production and respiration rate of four erect blackberry cultivars from three stages of ripeness held at 20C for 1 day after harvest.[z]

Color stage	Cultivar	Ethylene production ($pmol \cdot kg^{-1} \cdot s^{-1}$)	Respiration rate ($nmol \cdot kg^{-1} \cdot s^{-1}$)
Mottled	Choctaw	5.5 ± 1.0	1167 ± 52
	Cheyenne	NA	1148 ± 46
	Navaho	0.6 ± 0.1	1357 ± 25
	Shawnee	1.8 ± 1.0	1227 ± 32
Shiny black	Choctaw	21.9 ± 2.0	1174 ± 35
	Cheyenne	3.6 ± 1.0	1468 ± 59
	Navaho	3.6 ± 1.0	1242 ± 24
	Shawnee	12.8 ± 4.0	1287 ± 37
Dull black	Choctaw	51.1 ± 5.0	1479 ± 40
	Cheyenne	21.9 ± 2.0	1390 ± 42
	Navaho	7.3 ± 2.0	1155 ± 31
	Shawnee	29.2 ± 3.0	1226 ± 32

[z]Significant cultivar × color stage × storage day interaction ($P \leq 0.05$). Values represent means of 59 fruit ± SE. NA = fruit not available for analysis.

For all cultivars, mottled fruit did not darken appreciably during storage. In 1992, none of the mottled fruit changed to black, while in 1994, 50% to 80% of mottled 'Navaho' and 'Shawnee' fruit from the last harvest became darker, possibly due to higher day and night temperatures (mean 35/20C) near that harvest date than in 1992. The SSC and anthocyanin levels were very low in mottled fruit and TA very high relative to other color stages, even after storage. Therefore, using mottled fruit for processing or fresh-market use is not recommended.

Walsh et al. (1983) suggested that only dull black blackberries of semi-erect cultivars should be used for fresh market use due to higher SSC and lower TA. Data from our study indicate that the appropriate stage of ripeness for harvest depends greatly on the cultivar. Although 'Choctaw', 'Cheyenne', and 'Shawnee' fruit did not differ appreciably in SSC or TA between the shiny black and dull black stages, dull black fruit had excessive leakage and decay after 7 days of storage. Therefore, fruit of these cultivars destined for fresh markets should be harvested at the shiny black stage. Since SSC was higher and TA lower in dull black 'Navaho' fruit compared to shiny black fruit, and dull black fruit were firm following storage, dull black 'Navaho' might be suitable for fresh markets.

Dull and shiny black 'Navaho' fruit had much less softening, decay, leakage, and discoloration than 'Choctaw', 'Cheyenne', or 'Shawnee'. Therefore, 'Navaho' fruit would be most suited to shipping to distant markets. More research needs to be done to determine the maximum shelf life and commercial acceptance of these blackberry cultivars.

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