Resistance to *Botrytis cinerea* and Quality Characteristics during Storage of Raspberry Genotypes

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Abstract. Raspberries are a delicate, high-value crop with an extremely short shelf life exacerbated by postharvest decay caused by *Botrytis cinerea* Pers. European red raspberry (*Rubus idaeus* L.) is the most widely grown variety. Yellow (*R. idaeus* L.), black (*R. occidentalis* L.), and purple raspberries (*R. × neglectus* Peck. or *R. occidentalis* × *idaeus* hybrids) are available mainly at local markets and U-pick farms. To compare the postharvest quality of the raspberry color groups, pesticide-free fruit from cultivars and breeding selections of red, yellow, purple, and black raspberries were examined for oxygen radical absorbance capacity (ORAC), phenolics, anthocyanins, soluble solids, titratable acids, pH, color, firmness, decay and juice leakage rates, ethylene evolution, and respiration. There were significant correlations between decay rate and physiochemical properties. Both decay and leakage rates were correlated with weather conditions before harvest, but each color group responded differently to different weather factors. There were no correlations among changes in color, firmness, decay, or juice leakage rates. All the other color groups were less acidic than the familiar red raspberry. Yellow raspberries had the worst decay rates but the best leakage rates. Black and purple raspberries, with the highest phenolics and anthocyanins and the lowest ethylene evolution rates, resisted decay the longest but bled soonest.

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Raspberries (*Rubus* sp.) are the third most popular berry in the United States (Geisler, 2012) and a growing specialty crop for both the wholesale industry and smaller, local markets, and U-pick. Postharvest susceptibility to gray mold (*Botrytis cinerea*) drastically reduces the shelf life of this delicate fruit. The wholesale commercial industry has optimized a preharvest fungicide application program to complement storing them in a controlled atmosphere regime and maintaining cold chain management to achieve maximum shelf life (Robbins and Fellman, 1993). Producers marketing fruit locally are far more vulnerable to losing yields resulting from gray mold because they are less likely to pursue aggressive fungicide regimes to control the fungus preharvest as a result of the costs associated with chemicals and their application. Additionally, retail producers may not have the cold storage equipment necessary to increase postharvest shelf life.

Three species of raspberries are commonly grown for local markets: the European red raspberry (*R. idaeus* L.), which is also available in yellow, the eastern black raspberry (*R. occidentalis* L.), and an interspecific hybrid between the two (*R. × neglectus* Peck.) that yields purple berries. In the United States, the primary production areas for red and black raspberries are California (wholesale) and the Pacific Northwest (processing). Research has not been published comparing the shelf life of these different species and colors of raspberries.

Fruit physiology, firmness, color, and ethylene biology for red raspberries have been studied in their relationship to postharvest quality and disease resistance. The length of time in storage affects concentrations of compounds contributing to flavor, appearance, and nutritive value of red raspberries (Kruger et al., 2011). Soluble solids increase in storage, which causes the fruit to taste sweeter, whereas titratable acids decrease in storage making the berries taste more dull and flat (Paliyath and Murr, 2008). Increases in total anthocyanins and total phenolics cause undesirable darkening during postharvest storage (Robbins and Fellman, 1993). Phenolics have been targeted for their antimicrobial effects on plant pathogens and their health-related benefits in humans (Nohynek et al., 2006). Antioxidant capacity, which is strongly correlated with anthocyanin and phenolic content, increases during storage (Kruger et al., 2011). Berry cultivars with higher firmness values at harvest tend to maintain their relative firmness in storage and are also less susceptible to juice leakage during postharvest storage.

Red raspberries behave as an intermediate between climacteric and non-climacteric fruit in that they demonstrate an ethylene evolution peak analogous to a climacteric fruit with no concomitant rise in respiration rate (Iannetta et al., 1999; Perkins-Veazie and Nonnecke, 1992; Robbins and Fellman, 1993). The expression level of 1-aminocyclopropane-1-carboxylate synthase, the regulatory enzyme in the ethylene biosynthetic pathway, indicates that red raspberries are a non-climacteric fruit resulting from lack of gene expression in the early ripening stages and only at very low levels in later ripening stages (Zheng and Hrazdina, 2010). Similar work during this study has been carried out to determine if black, yellow, or purple raspberries liberate ethylene and CO₂ levels that mirror what has been observed in red fruit.

The biology of *B. cinerea* on *R. idaeus* has been well studied (Jarvis, 1962). Primary inoculum comes from conidia that arise from sclerotia that overwinter on dead or decaying material such as leftover floricanes infected with the fungus. In this tissue, the pathogen lives as a saprophyte. In the spring, during cool, moist weather, the sclerotia germinate to form conidiophores containing conidia. Conidia are then spread by wind and splashing water and they germinate in the stigmatic fluid on landing on the floral surface. The hyphae grow intercellularly within unripe

fruit and remain dormant. Once the fruit begins to ripen, the fungus resumes its growth and decay symptoms appear, both in the field and postharvest. Inoculum concentration increases as the first crop ripens and remains high through the rest of the floricanes and primocane fruiting season (Jarvis, 1962). Control is achieved primarily with fungicides and field maintenance to reduce overwintering sources and inoculum levels. Removing dead leaves and canes, keeping weed pressure down, and pruning to promote an open canopy that allows for quick drying and ample air movement are effective cultural practices to reduce gray mold inoculum levels (Jarvis, 1962).

There is an abundant amount of information regarding red raspberry production, *B. cinerea* life cycle and control, and ethylene levels in red raspberries. However, very little research has been conducted on postharvest physiology of black, yellow, or purple raspberries. As production of alternate raspberry species increases for wholesale and direct markets, more information is needed about postharvest physiology and gray mold resistance. The main goal of this research was to compare the postharvest quality of different colored raspberries that were harvested from floricanes under direct-market conditions of the Mid-Atlantic area with minimal pesticide inputs.

Materials and Methods

Field layout. Thirty genotypes of raspberries were randomly assigned to plots within two replicate blocks at the Agricultural Research Center in Beltsville, MD, in August of 2007. Each plot of raspberries contained five plants. Plants were 1 m apart within the plot and 3 m between each plot. Rows were 3 m apart. The 30 cultivars planted consisted of both named varieties and breeding selections. Released varieties were purchased from Indiana Berry and Plant Co. (Plymouth, IN), Nourse Farms Inc. (Whatley, MA), and Hartmann's Plant Co. (Lacota, MI) over the course of 2006 and 2007. Breeding selections, coded A to C, were obtained from private breeder

Peter Tallman, Cornell University (Courtney Weber), The University of Maryland (Harry Swartz), and North Carolina State University (Gina Fernandez). Seventeen genotypes with sufficient yields were selected for use in the postharvest experiments (Table 1). Cultivars and selections were grouped based on the berry color under which they would be marketed. There are three genotypes included in this project that are notably different from the others in their color group. 'Kiwigold' is not a complete anthocyanin mutant and thus is referred to as an amber type (Weber et al., 2005). 'Mandarin' is 25% *R. parvifolius*, being the result of the cross (*R. parvifolius* × 'Taylor') × 'Newburgh' (USDA, 2011). 'Royalty' is 75% red, whereas the other purple genotypes are 50% red (Weber et al., 2005). Ammonium nitrate fertilizer (34N-0P-0K) was broadcast annually at the rate of 11 kg nitrogen per hectare (60 lbs per acre) before leaves emerged in spring and between the floricanes and primocane fruit harvests. Sulfate of potash fertilizer (0N-0P-50K) and borax were broadcast with the spring nitrogen application at rates indicated by the winter soil test for potassium and boron. Weed management included Surflan (Oryzalin; Southern Agricultural Insecticides, Inc., Palmetto, FL) as a pre-emergent herbicide, paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) as a post-emergent herbicide at the base of the plants in the spring, and hand-weeding in the row. Trickle irrigation was used to supplement rainfall. No insecticides or fungicides were used. Plots were individually trellised in 2010 using a rebar V-trellis (Vanden Heuvel et al., 2000) to accommodate the different growth habits. All plots were pruned for both floricanes and primocane fruit production; pruning was done once in midsummer to remove spent floricanes and to train the primocanes. Daily maximum and minimum temperature; maximum and minimum relative humidity; and average radiation and total rainfall were recorded during the fruiting period using a weather station located ≈300 m from the field. Maximum/minimum temperature and humidity were more informative than using the averages.

Sampling techniques. Floricanes fruiting began in early June and continued for 1 month. Primocanes began fruiting in early July and ended the first week of September. Raspberries were harvested twice a week throughout the season in both years. When harvesting for the shelf life tests, firmness and color determinations, and ethylene and respiration tests, care was taken to reduce the introduction of inoculum between plots by wearing gloves and surface sterilization of gloves with an ethyl alcohol hand sanitizer. Raspberries were harvested when they were fully ripe, meaning they detached from their receptacles easily. Berries that were overripe or that showed signs of fungal infection were not harvested. For the storage life tests, berries were harvested into 12-well Corning® Costar® plates (Corning Inc., Corning, NY) lined with filter paper. Plates were closed, field-cooled briefly in an insulated portable cooler containing chipped ice, and moved to their treatment area within 1 h of harvest. For the firmness and color determination tests, and the ethylene and respiration tests, berries were harvested and treated similarly but were harvested into labeled clamshells. After berries were harvested for these tests, all remaining fully ripe berries from the plots were harvested for the physiochemical analyses. Berries were harvested into labeled polypropylene bags (Package Concept Inc., Salinas, CA) and stored on ice in an insulated portable cooler. Within 1 h, the berries were weighed and the air within the bags was exchanged with nitrogen gas as the bags were sealed using an impulse sealer (Model PFS-F450; Kingstar Group, Wenzhou Zhejiang, China). The bags were then frozen at -60 °C. Berries from the same plot were bulked across harvest dates for physiochemical analyses because of the amount of berries required.

Physiochemical analysis: antioxidant capacity for lipophilic and hydrophilic (oxygen radical absorbance capacity) assay. Five grams of raspberry fruit were extracted twice with 10 mL of hexane and then centrifuged at 7500 × g for 20 min at 4 °C. The hexane extracts were combined and designated as the lipophilic fraction. From the original tube, residual hexane was evaporated, and the hydrophilic residue was extracted with 2 × 10 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v). After adding solvent, the tube was vortexed for 30 s and then sonicated for 5 min at 37 °C. The tube was left at room temperature for 10 min and occasionally shaken. The tube was centrifuged at 7500 g for 15 min. The supernatant was removed and the total volume was brought to 25 mL with acetone/water/acetic acid (70:29.5:0.5, v/v/v). Any further dilution of the hydrophilic fraction was made up with phosphate buffer for the antioxidant assay.

The lipophilic combined hexane fractions from above were dried using a Buchler Evaporomix (Haake Buchler Instruments, Fort Lee, NJ) in a 30 °C water bath. The dried hexane extract was dissolved in 250 µL of acetone and then diluted with 750 µL of a 7% RMCD solution (random methylated β-cyclodextrin,

Table 1. Berry color and fruiting season for 17 raspberry (*Rubus* sp.) genotypes evaluated for postharvest quality and storage life in 2010 and 2011 in Beltsville, MD.

Genotype	Berry color	Fruiting season
Caroline	Red	Floricanes, primocane
Heritage	Red	Floricanes, primocane
Mandarin	Red	Floricanes, primocane
Prelude	Red	Floricanes, primocane
Spinefree Willamette	Red	Floricanes
Anne	Yellow	Floricanes, primocane
Kiwigold	Yellow, amber	Floricanes, primocane
Selection A	Purple	Floricanes
Royalty	Purple	Floricanes
Selection B	Purple	Floricanes, primocane
Bristol	Black	Floricanes
Earlisweet	Black	Floricanes
Explorer	Black	Floricanes, primocane
Huron	Black	Floricanes
Jewel	Black	Floricanes
Munger	Black	Floricanes
Selection C	Black	Floricanes

50% acetone/50% water, v/v) for the antioxidant assay. Any further dilution was conducted with the same solution. The 7% RMCD solution was used as a blank and to dissolve the Trolox standards for the lipophilic antioxidant assay.

The oxygen radical absorbance capacity assay for hydrophilic and lipophilic antioxidant assay was carried out according to Huang et al. (2002) using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system. A microplate fluorescence reader (FL800; Bio-Tek Instruments, Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples (Huang et al., 2002). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using a regression equation between Trolox concentration and the net AUC and were expressed as micromole Trolox equivalents per gram fresh weight (Huang et al., 2002; Prior et al., 2003).

Total phenolic and anthocyanin content. Using a Polytron homogenizer (Brinkman Instruments, Westbury, NY), 5 g of raspberries was extracted twice with 15 mL of 80% acetone + 0.2% formic acid. Those samples were then centrifuged at 4 °C for 20 min. The extracts were combined and then used for total anthocyanin and phenolic measurements.

Total phenolic content in the fruit extract was measured with Folin-Ciocalteu reagent. Solid-phase extraction procedures were used to remove water-soluble compounds from the extracts initially, because the Folin-Ciocalteu reagent is affected by them. Five milliliters from these extracts were concentrated to 1 mL using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 30 °C. The concentrated samples were dissolved in 4 mL of acidified water (3% formic acid) and then passed through a Sep-Pak C18 cartridge (Waters Corp., Milford, MA), which was previously activated with methanol followed by water and 3% aqueous formic acid. The interfering substances, sugars, ascorbic acid, organic acids, and non-phenolic organic substances, which react with Folin-Ciocalteu, passed through the Sep-Pak C18 column. Anthocyanins and other phenolics were retained by the column and then recovered with 5.0 mL of acidified methanol containing 3% formic acid. Total phenolics were then determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1977) using gallic acid as the standard. Results were expressed as milligrams gallic acid equivalent, in the raspberry extract, per 100 g fresh weight.

Total anthocyanin content was measured using the pH differential method (Cheng and Breen, 1991). Absorbance was measured with a spectrophotometer (ultraviolet-160; Shimadzu Scientific Instruments, Columbia, MD) at 510 nm and 700 nm in pH buffers at

1.0 and 4.5 using $A = [(A_{510} - A_{700}) \text{ pH } 1.0 / (A_{510} - A_{700}) \text{ pH } 4.5]$ with a molar extinction coefficient of cyanidin 3-glucoside (26,900 L/cm-mol) for raspberry fruit extracts. Results were expressed as milligrams of cyanidin 3-glucoside equivalent, in the raspberry fruit extract, per 100 g fresh weight.

Soluble solids, titratable acidity, and pH. Raspberry juice was extracted from 400-g samples manually using cheesecloth. Using a digital refractometer, soluble solids of the fruit were determined at 20 °C (PR-101; Spectrum Technologies, Plainfield, IL). Titratable acidity was measured by first diluting each 5-mL aliquot of raspberry juice to 10 mL with distilled water and then titrating to pH 8.2 using 0.1 N NaOH using the Mettler DL12 autotitrator and method from Mettler-Toledo, Inc. (Hagerstown, MD). Titratable acidity was expressed as percent of citric acid equivalents. The pH was determined with a Denver pH meter (Bohemia, NY).

Color and firmness determinations. Twelve berries for each plot were harvested into Costar® plates (Corning Inc.) on select days. Six berries were analyzed for color and then firmness on the day of harvest. Then six berries were analyzed for color and then firmness after 6 d in cold storage (5 °C). Color was measured at two points on each berry using a portable L^*a^*b colorimeter Chroma-Meter CR 400 (Minolta, Ramsey, NJ). The colorimeter was calibrated using a white reflective plate ($L^* = 97.93$, $a^* = -0.34$, $b^* = 2.27$, standard illumination C). The color measure after storage was subtracted from the color measure before storage to understand how storage affects berry color for the four color groups. Hue and chroma were calculated for the L^* , a^* , and b^* values. Firmness measurements were taken on those same six berries, calyx side down, using the TA-25 tip on a TA XT-plus Texture Analyzer (Stable Micro Systems, Godalming, U.K.). This test is destructive, so these berries were discarded after analysis.

Shelf life tests. After harvest, one plate for each plot was placed in a walk-in cooler at 5 °C. This temperature was chosen to mimic the cold-chain management conditions of direct-market producers. Plates were stacked on trays and then loosely sealed with plastic liners. The number of berries leaking juice (bleeding) and number of berries showing signs of fungal infection were recorded every other day for 2 weeks after each harvest. A berry was considered to be leaking if any spotting was seen on the filter paper when looking at the bottom of the Costar plate. A berry was considered infected if mycelia could be seen without the aid of a dissecting microscope. Straight-line equations between the two measurements that most closely bracketed 25% decay or juice leakage were calculated for each Costar® plate (Corning Incorporated, Lowell, MA). The threshold of 25% was chosen to reflect a number that would be easily visible to a consumer, beyond the grower's ability to repack to remove contamination, and to protect plates from being immediately discarded that contained

a single infected berry that might not reflect the true storability of the cultivar or selection.

Identification of Botrytis cinerea using Koch's postulates and gene sequencing. *Botrytis cinerea* was isolated from symptomatic raspberries and maintained on potato dextrose agar (PDA). The isolate was first identified as *B. cinerea* by cultural morphology. Conidia were harvested by washing plates containing the sporulating fungus with 2 to 3 mL of sterilized distilled water containing Tween 20 surfactant (1 μ L Tween 20/1 mL water). The conidial suspension was quantified using a hemacytometer and adjusted to $10^4 \cdot \text{mL}^{-1}$ concentration with sterile distilled water. Raspberries were surface-sterilized with 70% ethanol and allowed to air dry in a laminar flow hood before being inoculated. A 10- μ L volume of a $10^4 \cdot \text{mL}^{-1}$ conidial spore suspension of *B. cinerea* was pipetted on to individual sterilized raspberries using sterile techniques (Celik et al., 2009). The inoculated raspberries were placed at 25 °C and, once mycelial growth was visualized, the fungus was cultured from the infected fruit onto PDA plates as well as 50-mL potato dextrose broth (PDB) liquid cultures that were used to extract genomic DNA from the mycelia. Fungal identification of the gray mold isolate to complete Koch's postulates was carried out by visually identifying the cultural characteristics (i.e., conidia, cultural morphology, etc.) and by subsequently sequencing the ITS1 (intertranscribed ribosomal RNA spacer) locus.

The nucleotide sequence for the ITS1 locus was obtained from *B. cinerea* GenBank accession GU395993.1. The following gene-specific primers were designed to amplify the ITS1 locus using conventional polymerase chain reaction (PCR): forward—5' GAT GCC CGA AAG GGT AGA 3'; reverse—5' ATA TAG TAC TCA GAC GAC ATT 3'. Mycelial mats were grown in PDB liquid shake cultures at 150 rpm in Innova 4230 refrigerated incubator shaker incubators (New Brunswick Sci, Edison, NJ) at 25 °C. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) following the manufacturer's protocol. The quantity and quality of the genomic DNA were assessed using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). The genomic DNA product was diluted to $1.8 \text{ ng} \cdot \mu\text{L}^{-1}$ using standard procedures according to Sambrook and Russell (2001). The reaction components included 10 μ L of 5 $\times$ TaqFlexi buffer (Promega), 3 μ L 25 mM MgCl_2 , 1 μ L 10 mM dNTP mix, 1 μ L GoTaq Flexi DNA polymerase (Promega), 1 μ L of each 100 μ M primer, 1 μ L of template DNA, and water for a total reaction volume of 50 μ L. The PCR cycling parameters were as follows: initial denaturation for 3 min at 95 °C; 30 cycles of 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. PCR products were loaded on a 1% TBE (Tris-Borate-EDTA) agarose gel containing ethidium

bromide and electrophoretically separated at 100 V for 50 min (Sambrook and Russell, 2001). Products were visualized using an Alpha imager mini ultraviolet light box (Model M-26E; Alpha Innotech Corporation) and sized with 1 Kb Plus DNA ladder (Invitrogen, Grand Island, NY). The PCR products were purified using QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA). The purified products were sequenced by Macrogen Inc. (Rockville, MD) using the Sanger method and the gene-specific primers that were used to generate the PCR amplicon. Similarities to other sequences in GenBank were determined using the National Center for Biotechnology Information's nucleotide BLAST default settings.

Ethylene and respiration measurements using gas chromatography. To explore the ethylene evolution and respiration rates for the four color groups of berries, several genotypes at select harvests with maximum yields during the primocane fruiting season were chosen for evaluation. Ethylene evolution rates and respiration rates were measured on the following primocane-fruiting raspberries: three red *R. idaeus* genotypes, one yellow *R. idaeus* genotype, one *R. ×neglectus* genotype, and one *R. occidentalis* genotype. Berries were harvested underripe and allowed to ripen over 7 d during the test. Raspberries collected for gas chromatographic analysis were harvested slightly under ripe, which meant that they still have a “plastic” appearance and had to be pulled firmly from the receptacle. The berries were then weighed to 25 g and then surface-sterilized with 70% ethanol. The fruit were placed in sterilized 236.59-mL containers and labeled by plot number. The jars were cooled to 5 °C and the first measurements were taken ≈6 h after harvest (Day 1). Carbon dioxide and ethylene evolution rates were measured by sealing the jars for 1 h and then sampling 25 mL of the headspace. Carbon dioxide was measured using a gas chromatograph (GC; Model GC-3BT; Shimadzu, Kyoto, Japan) fitted with Porapak Q and molecular sieve 5A columns (2 m × 3 mm) and a thermal conductivity detector. Ethylene was measured with a GC (Model AGC-211; Carle, Tulsa, OK) fitted with an alumina column (2 m × 3 mm) and a flame ionization detector. Measurements were taken every 24 h until the point where further data were unusable as a result of decay.

Statistical analysis. To determine the effects of berry color on days to 25% decay and days to 25% juice leakage and physiochemical variables, an analysis of variance

(ANOVA) was conducted using the Mixed procedure (SAS, Version 9.2; SAS Institute Inc., Cary, NC). Data were analyzed on a plot by harvest basis to maximize power. The model statement was $y = \text{color, harvest date, color by harvest date}$. Because plots were measured multiple times over the season, the repeated statement was invoked. Year and replication were considered random. Mean separations were carried out using the Tukey statement option when the treatment effect was statistically significant ($P < 0.05$). Regression analysis was used to determine the effect of harvest day on juice leakage and decay incidence.

To determine the effect of storage on color, an ANOVA was conducted using the mixed procedure (Proc Mixed) for two harvests in 2010. Each change in color determinant was analyzed separately on a plot-by-harvest basis. Color on the day of harvest and color 6 d after storage were used.

To determine the effect of storage on firmness, an ANOVA was conducted on the difference in firmness using the Mixed procedure (Proc Mixed) for select harvests on a plot-by-harvest basis. Firmness on the day of harvest and firmness after 6 d in storage were used.

To determine how weather-related measures affected leakage and decay, the correlation procedure (Proc Corr) was used with the means of days to 25% decay and days to 25% juice leakage for each harvest, maximum humidity, minimum humidity, maximum temperature, minimum temperature, average radiation, total precipitation, for the days preceding each harvest, separated by fruiting season. Each color group was analyzed separately on a plot-by-harvest basis.

To determine if there were any underlying dimensions that could explain the interrelationships among the variables, multivariate analysis was done with exploratory factor analysis using the factor procedure (Proc Factor). The variables included were days to 25% leakage, days to 25% decay, pH, titratable acidity, anthocyanin content, phenolic content, and both lipophilic and hydrophilic ORAC content. Because the physiochemical variables were available only as plot means across the floricane season each year, days to 25% leakage and days to 25% decay were also averaged across harvests for this analysis, which was done on a plot-by-year basis. To better understand the relationship between the variables that clustered together in the factor analysis, the correlation procedure (Proc Corr) was done using that same data set.

Results

Physiochemical properties. Physiochemical analysis was performed on floricane-harvested fruit for 2 harvest years. Lipophilic ORAC varied significantly between color groups ($P < 0.0001$) as did hydrophilic ORAC ($P = 0.0001$). In both instances, black raspberries and purple raspberries had a significantly higher concentration than either red or yellow raspberries (Table 2). As expected, there were significant differences in anthocyanins ($P < 0.0001$) and phenolics ($P < 0.0001$) between the berry colors. In both cases, black raspberries had significantly higher amounts followed by purple, red, and, finally, yellow raspberries. The higher levels of ORAC, total phenolics, and total anthocyanins were consistent with consumer association of black raspberries and potential health benefits compared with red raspberries.

The ratio of soluble solids to titratable acids, a critical value affecting flavor perception, differed significantly between berry color groups ($P = 0.0404$) with black raspberries having the highest ratio. The differences in the ratio of soluble solids to titratable acids between color types was influenced more by titratable acids than by soluble solids, because the amount of soluble solids was similar among the different berry colors. Red raspberries had the highest level of titratable acids followed by yellow, purple, and black. There were no differences in pH among the color types; titratable acids and pH were slightly correlated ($r = 0.448$, $P = 0.027$). Because fruit samples were bulked across harvest dates within year, any effect of harvest date or effect of weather on the physiological properties measured for the different color types could not be determined.

Color analysis. For two select harvests in 2010, color was measured on the day of harvest. Differences between the raspberry fruit colors were statistically significant for L^* and hue angle values ($P < 0.0001$), and the effects were consistent for both harvest dates. Red raspberries were represented by ‘Heritage’, yellow raspberries were represented by the amber-type ‘Kiwigold’, purple raspberries were represented by Selection A, Selection B, ‘Royalty’, and black raspberries were represented by ‘Bristol’, ‘Huron’, ‘Jewel’, ‘Munger’. On the day of harvest, the colorimeter L^* values confirmed what is visually observable on the white–black scale: black raspberries were darker than purple and red, which were darker than yellow fruit as shown

Table 2. Physiochemical variables for four different colored, field-grown floricane-harvested raspberries averaged for 2010 and 2011.

Berry color	L-ORAC (μmol trolox equivalent/g fresh wt)	H-ORAC (μmol Trolox equivalent/g fresh wt)	pH	TA (percent citric acid equivalent)	SSC	SSC:TA	Anthocyanins (μg·g ⁻¹ fresh wt cyan-3-gluc equivalent)	Phenolics (mg·kg ⁻¹ fresh wt gallic acid equivalent)
Red	1.15 b ²	29.29 b	3.38	1.48 a	10.31	6.85 b	46.24 bc	261.48 b
Yellow	1.00 b	26.03 b	3.41	1.45 ab	10.97	7.815 ab	1.26 c	200.26 c
Purple	1.29 a	32.05 a	3.37	1.32 b	9.74	7.73 ab	95.71 b	280.40 b
Black	1.39 a	35.55 a	3.34	1.26 bc	10.80	8.895 a	325.60 a	379.68 a

²Any means within a column not followed by the same letter are significantly different at $P \leq 0.05$ by the Tukey test.

L-ORAC = lipophilic oxygen radical absorbance capacity; H-ORAC = hydrophilic oxygen radical absorbance capacity; TA = titratable acidity; SSC = soluble solids content.

in Table 3. Hue angle values also confirmed what is observable; the four raspberries were different colored with 'Kiwigold' measuring in the yellow-orange range, 'Heritage' measuring in the red-orange range, the purple raspberries were on the red side of purple, whereas black raspberries were closer to purple although still on the red side of purple. The outcomes from this portion of the research were expected and provide important foundational data for analyzing the changes in different colored raspberry fruits during storage.

Color was measured again after 6 d of cold storage (5 °C) to determine the effect of storage for marketing purposes. Analysis of variance showed that purple and black raspberries darkened in storage, as indicated by significantly lower L^* values, but that yellow and red raspberries did not darken. Although hue angle at harvest and hue angle after storage were significantly different between the colors, the differences between the two were not significant within color. Changes in

L^* values were not statistically significant. Changes in hue value were significant ($P = 0.001$) with yellow and purple types changing the most. 'Kiwigold' turned more orange in storage, which is expected as an amber type.

Juice leakage assessments. All 17 genotypes were included in this analysis. The time to 25% leakage varied significantly by fruit color ($P < 0.0001$) and harvest date ($P = 0.0003$). The interaction between fruit color and harvest date also was significant ($P = 0.0009$). Overall, the average time to 25% leakage was shortest for the black and purple raspberries, which could present a marketing problem, because it was ≈ 5 d. Time to 25% leakage for red raspberries (8.6 d) was approximately half as many days for the yellow raspberries (15.7 d) although they are the same species. This considerable difference did not appear to be the result of the yellow juice being more difficult to see than red. However, even if it were, what the consumer sees is what matters when making the decision to purchase or reject the product on the shelf.

When the interaction between harvest day and juice leakage was considered, yellow raspberries were quite variable in the first year of the study as shown in Figure 2. In the same year, red raspberries resisted leakage better than in the second year. This could be the result of different genotypes representing a color being harvested at different times in the season. Harvest day had a positive r value (0.35993) in 2010 and a negative r value in 2011 (-0.09323); this indicates that berries were leaking less quickly later in the 2010 season, whereas berries were leaking more quickly later in the 2011 season.

To study the effects of weather variables on postharvest leaking, weather data and days to 25% juice leakage from the florican season were correlated. Both red and black raspberries reached 25% leakage sooner if the period before harvest was rainy and overcast, and neither color type was affected by daily air temperatures (Table 4). Red raspberries also reached 25% leakage sooner if the maximum humidity, reached during the night, was high the day before harvest. The effect of humidity on black raspberry resistance to juice leakage seemed to differ depending on whether the measure of humidity was the maximum humidity (nighttime) or the minimum humidity (daytime). The days to 25% leakage for black raspberries were greater when the daytime minimum humidity for the multiday period before harvest was low, and the overnight maximum humidity the day before harvest was high. In other words, black raspberries resisted juice leakage better when harvested after dry days as long as the humidity the night before harvest was high. Yellow raspberries were most affected by average radiation the day before harvest, in that, if the day before harvest had a low average radiation and was more overcast, the days to 25% leakage were shorter, but if the day before harvest was sunny, the days to 25% leakage were longer. Purple raspberries were most affected by maximum daily temperatures the day before harvest, because cooler days with lower maximum temperatures resulted in shorter times before leakage was evident, and days with high maximum temperatures resulted in longer times before 25% leakage. There was no single environmental factor that affected the number of days to 25% juice leakage for all the color groups.

Firmness measurements. Berry firmness at harvest was unaffected by harvest date but differed significantly between the berry color types ($P = 0.0009$). Red raspberries were

Table 3. L^* and hue values for four different colored, field-grown florican-harvested raspberries averaged for two selected harvest dates in 2010.

Berry color	L^*			Hue		
	At harvest	After storage	Change in	At harvest	After storage	Change in
Yellow	55.5 a ^c	52.3 a	3.2	24.0 d	35.4 c	-11.4 a
Red	34.6 b	32.7 b	1.8	64.1 c	66.0 b	-1.9 b
Purple	30.2 b	26.0 c	4.2	74.9 b	79.1 a	-4.2 ab
Black	25.0 c	20.5 d	4.5	82.1 a	78.8 a	3.2 b

^aAny means within a column not followed by the same letter are significantly different at $P \leq 0.05$ by the Tukey test.

Fig. 1. Mean juice leakage and decay incidence values for four different colored, field-grown, florican-harvested raspberries stored at 5 °C for 2010 and 2011. Means followed by the same letter were not significantly different using the Tukey mean separation method ($P = 0.05$). a–d and a'–d' represent two different mean separations.

Table 4. Correlations between juice leakage and weather variables for florican-harvested raspberries stored at 5 °C in both 2010 and 2011.

Berry color	Year	One day before harvest	One day before harvest	One day before harvest	One day before harvest	Three days before harvest	One day before harvest	Three days before harvest
		Maximum temp (°C)	Maximum humidity	Minimum humidity	Avg radiation	Avg radiation	Precipitation	Precipitation
Red	-0.38**	0.07	-0.15*	-0.02	0.29*	0.04	-0.38**	-0.25*
Yellow	0.1	-0.18	-0.09	-0.15	0.37*	0.05	0.1	0.12
Purple	-0.23	0.31*	-0.04	0.07	0.17	0.1	-0.25	-0.12
Black	-0.08	0.24	0.12***	-0.23**	0.20**	0.18*	-0.19*	-0.30***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 2. Mean juice leakage values for four different colored field-grown, floricane-harvested raspberries stored at 5 °C over multiple harvest days for 2010 and 2011 (SE = 0.75).

represented by 'Heritage', yellow raspberries were represented by 'Kiwigold', purple raspberries were represented by Selection A, 'Royalty', Selection B, and black raspberries were represented by 'Bristol', 'Huron', 'Jewel', 'Munger'. An ANOVA showed that black raspberries were significantly firmer than purple raspberries with yellow and red raspberries intermediate between the black and purple and not significantly different from either. Although all the berries seemed to soften in harvest, the firmness for each color group after harvest was not significantly different from the firmness at harvest as shown in Figure 3. These trends were consistent across the harvest dates selected for analysis.

Decay analyses. The dominant decay species was *B. cinerea*, which was confirmed visually and with a 100% match of the ITS1 region using BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990). All 17 genotypes were included in this analysis. The time to reach 25% decay varied significantly between color groups ($P < 0.0015$) and harvest dates ($P < 0.0001$), whereas the interaction between color groups and harvest day was not significant as shown in Figure 4. Overall, the average time to 25% decay was longest for black raspberries, which was significantly longer than either red raspberries or yellow raspberries as shown in Figure 1. Purple raspberries were intermediate, with respect to decay, between black raspberries and red raspberries. Berries tended to decay more quickly in 2010 than in 2011, although the difference was not significant as shown in Figure 1. Berries also tended to decay more quickly from later harvest days ($r = -0.16203$).

Although purple, red, and yellow raspberries reached 25% decay sooner when harvested after humid periods, red raspberries decayed sooner when harvested after hot days with humid nights, and yellow raspberries reached 25% decay sooner when harvested after overcast and cool humid days, especially if the night before harvest also was humid (Table 5). Only high nighttime humidity in the multiday period before harvest hastened the decay of purple raspberries. The rate at which black raspberries reached 25%

Fig. 3. Mean firmness values (N) for four different colored, field-grown floricane-harvested raspberries both at harvest and after 6 d in cold storage (5 °C) for selected harvests in 2010. Means followed by the same letter were not significantly different using the Tukey mean separation method ($P = 0.05$). Harvest and storage represent separate mean separation tests.

Fig. 4. Mean raspberry postharvest decay incidence values for harvest days in 2010 and 2011 and averaged across four fruit color groupings of field-grown, floricane-harvested raspberries stored at 5 °C (SE = 0.8). Julian Day 162 corresponds to 10 June; Julian Day 183 corresponds to 1 July.

decay was unaffected by the weather parameters measured.

To determine if any of the physiochemical traits measured affected the rate of decay, a factor analysis was performed. Physiochemical variables (ORAC, total phenolics, total anthocyanins, soluble solids, titratable acids,

and pH) and the postharvest disease incidence averaged over the floricane season were included for both years. The first two factors explained 69% of the variance. Number of days to 25% decay, total anthocyanin, total phenolics, and both hydrophilic and lipophilic antioxidant capacity cluster closely

together as shown in Figure 5. The other fruit quality measurements, soluble solids content (SSC), titratable acids (TA), ratio of SSC to TA, and pH, did not cluster together. The number of days to 25% juice leakage did not cluster with days to 25% decay or any of the other variables. Correlation analyses support that rate of leakage and rate of decay are not related (Table 6). Total phenolics has the greatest positive correlation with decay incidence ($r = 0.72$, $P < 0.0001$); as phenolics increase, the time for the berries to reach 25% rot also increases. This is in complete agree-

ment with the levels of phenolics (Table 2) and the level of decay for the different color groups as shown in Figure 1.

Ethylene and CO₂ liberation. Ethylene evolution and respiration rates were measured for several genotypes for select harvests during the primocane fruiting season in an effort to explore the difference between the color groups. Red raspberries were represented by ‘Caroline,’ ‘Heritage,’ and ‘Prelude’; ‘Kiwigold’ represents yellow, Selection B represents purple, and ‘Explorer’ represents black raspberries. Data were similar for all

repetitions of the test so only two representative graphs are shown. Ethylene evolution rates decreased from the first measurement to the second one, likely as a result of a wound response after harvest coupled with equilibration to 5 °C. Both red and yellow cultivars showed the ethylene response of climacteric fruit as shown in Figure 6A. ‘Explorer’, the black raspberry genotype, did not show the typical ethylene response of a climacteric fruit. Selection B, the purple genotype, did not show the characteristic ethylene curve of a climacteric fruit when measured unripe,

Table 5. Correlations between decay incidence and weather variables for floricane-harvested raspberries stored at 5 °C in both 2010 and 2011.

Berry color	One d before harvest Maximum temperature	Three d before harvest Maximum temperature	One d before harvest Maximum humidity	Three d before harvest Maximum humidity	Three d before harvest Minimum humidity	Three d before harvest Avg radiation
Red	-0.25*	-0.29*	-0.24	-0.24*	-0.05	-0.03
Yellow	0.28	0.33*	-0.41*	-0.07	-0.34*	0.55**
Purple	0.07	-0.23	0.13	-0.30*	0.1	-0.02
Black	0.13	-0.08	0.12	-0.09	0.03	0.01

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 5. Factor analysis plot of response variables for 2010 and 2011 on all raspberry color groups on a plot x year basis.

although ethylene evolution rates began to increase by Day 7 in storage. Respiration rates declined during the first days after harvest in all color groups as shown in Figure 6B. No rise in respiration rate, which would be typical of a climacteric fruit, was detected in any of these cultivars. Therefore, none of the genotypes responded in the way expected for a climacteric fruit: with a rise in both ethylene production and respiration rate.

Discussion

As consumers are being encouraged to eat a more colorful diet and small growers are capitalizing on novelty fruit and vegetable crops, different colored raspberries are of

greater interest. Increasing the diversity of raspberry colors in the market has potential to benefit both consumers and producers, who will need to know how fruit of the other color groups compare with red raspberries with regard to the many postharvest qualities. In comparing the four commonly grown colors of raspberry, several important conclusions can be drawn from this study. The mechanism controlling decay and juice leakage are distinct and mediated by both biotic and abiotic factors. The colors that performed well for one area are opposite the ones that did well in the other. Firmness, although important on its own, was expected to track closely with either leakage or decay resistance and this was not observed.

Red raspberries are the most commonly consumed raspberry, and therefore the most information is known about how this color fruit performs postharvest (Perkins-Weazie and Nonnecke, 1992; Robbins and Fellman, 1993). Red raspberries, in comparison with the other three colors analyzed during this study, had the highest TA and the lowest ratio of soluble solids to TA, which is the tart raspberry flavor consumers expect. They have lower anthocyanin and phenolic content levels than black or purple raspberries. They were intermediate in firmness at harvest and tend to leak juice postharvest. Red raspberries, harvested after overcast rainy days and a humid night, leaked more. Red fruit had higher levels of rot, especially when harvested after hot, humid days, and they liberated the highest levels of ethylene among the berry colors examined.

Yellow raspberries, as expected, had the lowest levels of anthocyanins and phenolics. Their TA was lower than red raspberries but their ratio of soluble solids to TA was the second highest. This bodes well for consumer acceptance because this measure is an important indicator of flavor (Wang et al., 1997). Represented by two genotypes in this study,

Table 6. Correlations between disease incidence and physiochemical variables that clustered together.

	Total anthocyanins	Total phenolics	H-ORAC	L-ORAC
Days to 25% decay	0.527**	0.72484***	0.631***	0.623***
Total anthocyanins	—	0.828***	0.646***	0.581***
Total phenolics	—	—	0.883***	0.85305***
H-ORAC	—	—	—	0.94935***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

H-ORAC = hydrophilic oxygen radical absorbance capacity; L-ORAC = lipophilic oxygen radical absorbance capacity.

Fig. 6. Ethylene evolution rates (A) and respiration rates (B) of underripe primocane-fruited genotypes measured for 7 d in 2011.

yellow raspberries resist juice leakage the best, although they tend to leak juice more quickly when harvested on overcast days. Yellow raspberries were among the most firm at harvest but were very susceptible to gray mold, again particularly after being harvested on overcast, cool, humid days. Future varieties will likely have greater storability. Their ethylene and CO₂ evolution rates were similar to red raspberries.

Black raspberries, mainly harvested for processing, are an increasing presence in local, fresh markets. With their high anthocyanin and phenolic content, they are very attractive to consumers seeking novel colors of fruit for their perceived health benefits. The flavor is less tart than red raspberries, because they have the lowest TA and highest (best) ratio of soluble solids to TA of all the color groups. Black raspberries resist leakage the least of all of the colors, particularly after rainy, humid, overcast days. This will make their move to the wholesale, fresh market challenging. On the other hand, they were most firm at harvest and the most decay-resistant, which appears to be unaffected by weather conditions before harvest. Black raspberries had the lowest ethylene and CO₂ evolution rates, which may indicate why they were the most resistant to gray mold.

Purple raspberries, the hybrid between red and black raspberries, are popular primarily in the northeastern United States. They had the third highest anthocyanin and phenolic content, and their flavor was intermediate between black and yellow raspberries. Similar to black raspberries, their ability to resist juice leakage was poor, and cool weather tended to exacerbate this. Although among the least firm at harvest, they resisted decay similar to black raspberries. Humid days before harvest lowered their decay resistance. Their ethylene and CO₂ evolution rates were intermediate between red raspberries and black raspberries, which also corresponded with their ability to resist decay. Phenolic content coupled with low ethylene evolution rates may help explain the low susceptibility of decay for purple raspberries despite a lack of firmness at harvest and rapid juice leakage postharvest. High total phenolic content has been shown to have antifungal effects in other plant species (Vio-Michaelis et al., 2012) and a study to determine if total phenolics actually cause the slowed rate of decay may be appropriate.

Data from this study support the claim that raspberries are not a climacteric fruit, in the classical sense, because they did not have a corresponding CO₂ peak coinciding with ethylene liberation. However, we have shown

for the first time that significant differences between ethylene rates and decay incidence coincide; the berries that produced the highest ethylene rates rotted the most quickly. It is unclear if initial and subsequent ethylene was liberated by the fungus or produced by the plant or both. Because it has been shown that ethylene is a ripening hormone that promotes senescence and that *B. cinerea* has the ability to sense and respond to exogenous ethylene, it is logical to assume that ethylene is involved in the host-parasite interaction (Chague et al., 2006; Owino and Ezura, 2008). Our findings have great impact because they open the door for potential disease mitigation strategies that center around lowering ethylene emission rates on berries to reduce decay. Plant breeders can also use this information to screen raspberry germplasm to look for low ethylene-liberating berries to use as material for generating more decay-resistant fruit.

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