Use of Combinations of Commercially Relevant O₂ and CO₂ Partial Pressures to Evaluate the Sensitivity of Nine Highbush Blueberry Fruit Cultivars to Controlled Atmospheres

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Additional index words. *Vaccinium corymbosum*, quality, decay, firmness, controlled atmosphere, discoloration

Abstract. We tested the impact of storage atmospheres in which the CO₂ and O₂ percentages sum to 21% on highbush blueberry (*Vaccinium corymbosum* L.) fruit condition and quality. The CO₂ and O₂ combinations, in percent composition, were 19%/2%, 18%/3%, 16.5%/4.5%, 15%/6%, 13.5%/7.5%, 12%/9%, 6%/15%, and 0%/21% for CO₂/O₂, respectively. Nine blueberry cultivars were evaluated (Duke, Toro, Brigitta, Ozarkblue, Nelson, Liberty, Elliott, Legacy, and Jersey) after 8 weeks of controlled atmosphere (CA) storage at 0 °C. Surface mold, berry decay, skin reddening (associated with fruit pulp browning), fruit firmness, pulp discoloration, and the content of ethanol and acetaldehyde were assessed. Fruit firmness, skin reddening, and decay declined and the proportion of fruit with severe internal discoloration tended to increase as CO₂ concentrations increased. Ethanol and acetaldehyde accumulation was minimal, indicating fermentation was not induced by the atmospheric conditions applied. Cultivar effects were far more pronounced than atmosphere effects. Some cultivars such as Duke, Toro, Brigitta, Liberty, and Legacy appear to be well suited to extended CA storage, whereas other cultivars such as Elliott stored moderately well, and Ozarkblue, Nelson, and Jersey stored poorly. The data indicate that responses to high levels of CO₂, while O₂ is maintained at its maximum level practicable, can, in a cultivar-dependent manner, include significant negative effects on quality while achieving the desired suppression of decay.

Blueberry (*Vaccinium corymbosum* L.) fruit benefit from elevated CO₂ levels, through its impact on suppressing fungal decay, but not from low O₂ levels (Blasing, 1993; Ceponis and Cappellini, 1979, 1983, 1985; Schotsmans et al., 2007; Smittle and Miller, 1988). Unlike climacteric fruit, which typically are harvested mature, but not ripe, and require ethylene to drive ripening processes (Asif et al., 2009; Bapat et al., 2010; Pathak et al., 2003), blueberry fruit are ripe at the time of harvest and do not depend on ethylene for ripening (DeLong et al., 2003). Thus, low O₂, which is used to suppress ethylene synthesis and response

in climacteric fruit storage (Banks, 1985; Elyatem et al., 1994), is not expected to benefit blueberry storability (Beaudry, 1999). The atmosphere for blueberry fruit in CA storage and in modified atmosphere (MA) packaging is usually one in which the CO₂ level is of primary importance and O₂ is maintained at a level sufficient to prevent fermentation.

Commercially, CA storage of blueberry fruit is commonly used during extended sea shipments and less commonly used in land-based storage facilities. It is recommended that the CO₂ partial pressure be maintained between 8 and 15 kPa and O₂ levels are maintained above 2 to 4 kPa (Ceponis and Cappellini, 1979, 1983, 1985). However, the concentration of CO₂ necessary for decay control is often close to the level of product tolerance (Kader, 1995). Importantly, most, if not all, fruits or vegetables possesses a low O₂ threshold, below which fermentation damage is liable to occur, and a threshold to high CO₂ levels, above which injury resulting from fermentation and/or acute toxicity is

likely (Beaudry, 1999; Thomas, 1925). Sensitivity of plant tissues to both gases is a function of cultivar, temperature, and duration of exposure (Pesis, 2005) and off-flavor development can occur with either low O₂ or high CO₂ (Couey and Wells, 1970; El-Kazzaz et al., 1983; Harris and Harvey, 1973). An interaction between O₂ and CO₂ on fermentation has been demonstrated for blueberry; as the partial pressure of CO₂ increases, the tolerance of ‘Bluecrop’ fruit to low O₂ declines (Beaudry, 1993).

As CO₂ levels in CA and MA rise, they necessarily displace both nitrogen and oxygen. In perforated packages, the proportions of O₂ and CO₂ sum to ≈21% in most circumstances as long as the plant material has a respiratory quotient (RQ) near unity (Beaudry, 1999; Cameron et al., 1994, 1995). This is the result of the near-equal diffusion rates of O₂ and CO₂ through pores. CA storages can behave somewhat similarly to perforated MA packages in this regard. If the intent of the storage operator is to maximize the CO₂ level for any given O₂ level (to suppress decay) and to maximize the O₂ level for any given CO₂ level (to avoid fermentation), then the only two gasses used for atmosphere modification are CO₂ and air, the latter being used as a source of O₂. Initially, as CO₂ is added to achieve its target level, it displaces the air in the storage environment and reduces O₂. Thereafter, the fruit in the environment produce CO₂ and deplete O₂ according to the ratio of their RQ (usually close to 1:1). As a consequence, CO₂ will accumulate until it exceeds the actionable threshold of the controller mechanism. Controllers then purge CO₂ with air, rather than N₂, to maintain O₂ levels. As purging displaces CO₂ to reach target CO₂ levels, that same fraction of all gasses, including O₂ and N₂, is also displaced from the storage environment. In that the atmosphere used to purge the storage is air, the portion of the gases purged is replaced with air containing one part O₂ and approximately four parts N₂. This process is repeated each time CO₂ partial pressure reaches actionable levels, maintaining a relatively constant CO₂ level but eroding the O₂ partial pressure until a steady state is reached [i.e., the oxygen molecules lost resulting from respiration and those lost in the effluent gas stream as CO₂ is purged equal the number of O₂ molecules gained through the inlet gas (air) stream used for purging]. This relationship can be expressed as a mass-balance equation, which can then be used to predict the steady-state O₂ level by iterative calculation. After each purge event, the proportion of O₂ in a chamber of unchanging volume can be expressed as:

$$O_2 = O_2 \text{ at end of } CO_2 \text{ accumulation} \\ \text{period} - \text{percent of } O_2 \text{ displaced due} \\ \text{to purging} + \text{proportion of } O_2 \text{ gained} \\ \text{due to purging.}$$

where:

Received for publication 21 Apr. 2010. Accepted for publication 9 June 2010.

We acknowledge the financial support of the Michigan Agricultural Experiment Station and the University of Jordan.

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O_2 at end of CO_2 accumulation period =
 $[Pre-purge\ O_2 - (1/RQ) \times (CO_2$
threshold- CO_2 target)]

Percent of O_2 displaced due to purging =
fraction of atmosphere purged $\times O_2$ at
end of CO_2 accumulation period

Proportion of O_2 gained due to purging =
fraction of atmosphere purged $\times$ percent
 O_2 in purge air

Fraction of atmosphere purged = $(CO_2$
threshold- CO_2 target)/ CO_2 threshold

and where O_2 and CO_2 are expressed in percent and RQ is the respiratory quotient.

After several iterations of CO_2 accumulation and purging, the equation demonstrates that the sum of partial pressures of O_2 and CO_2 converges on $\approx 21\%$, which is ≈ 21 kPa (1.013 kPa = 0.01 atm) (Fig. 1).

For blueberry fruit, effective CO_2 levels have generally been reported to be in the range of 8 to 20 kPa. Ceperis and Cappellini (1979, 1983, 1985) and Schotsmans et al. (2007) reported that a CO_2 partial pressure in the range of 8 to 15 kPa is effective in suppressing decay and preserving fresh blueberries stored for several weeks at low temperature.

The aim of this study was to investigate the sensitivity of nine blueberry cultivars (Duke, Toro, Brigitta, Ozarkblue, Nelson, Liberty, Elliott, Legacy, and Jersey) to combinations of O_2 and CO_2 that are commercially attainable in CA storage and perforated MA packages when fruit are to be stored for relatively extreme durations. The 8-week storage duration simulated the time needed for transoceanic container shipments plus distribution and retail sales periods.

Materials and Methods

Full-blue fruit from nine blueberry (*Vaccinium corymbosum* L.) cultivars were hand-

harvested from trial plots at the Michigan Blueberry Growers Association near Grand Haven, MI on 9 July ('Duke'), 15 July ('Toro'), 22 July ('Brigitta'), 29 July ('Ozarkblue' and 'Nelson'), 3 Aug. ('Liberty'), and 5 Aug. ('Elliott', 'Legacy', and 'Jersey') of 2009. Except for 'Liberty', fruit from each cultivar were from the first harvest, which took place after 30% to 50% of the fruit on the bush were full-blue. 'Liberty' fruit were from the second harvest, which followed the removal of the first harvest fruit by 10 d. After each harvest, fruit were transported to Michigan State University in insulated containers and transferred that afternoon to a cold storeroom at $0^\circ C$. The next morning, fruit of each cultivar was segregated into 32 lots, discarding fruit with obvious defects. The weight of each lot was adjusted to ≈ 200 g and the fruit placed into $12 \times 12 \times 5$ -cm (length, width, and height, respectively) ventilated plastic clamshell containers. The number of fruit in each clamshell ranged from 70 to 120, depending on fruit size. For each cultivar, the clamshells were randomly assigned to eight treatments with four replicate clamshells per treatment in a randomized complete block design.

Initial fruit firmness was determined on 10 fruit randomly selected from each of the four replicates. Fruit firmness was determined as resistance to deformation using a durometer (Type 00; Shore Instruments, Jamaica, NY) fitted with a 2.4-mm diameter hemispherical probe and imparting a force of 0.49 N (50 g-force) per millimeter deformation. The maximum displacement of the probe was 2 mm. Durometer readings of 25, 50, 75, and 100 are equivalent to a force of 0.25, 0.49, 0.75, and 0.98 N and 1.5, 1.0, 0.5, and 0 mm of deformation, respectively. At harvest, the durometer was applied for ≈ 6 s to take a firmness measurement. After storage, the durometer was applied for 3 s to minimize the potential for puncturing the fruit skin.

After sorting and segregation into lots, the fruit were placed into aluminum-walled CA

chambers (Storage Control Systems, Sparta, MI) measuring $143 \times 71 \times 96$ cm (length, width, and height, respectively) and held at $0^\circ C$ for 8 weeks. Chamber atmospheres were regulated with an automated atmosphere control system (ICA 61 Laboratory System; International Controlled Atmosphere Ltd., Paddock Wood, UK) and managed so that flow rates for CO_2 , N_2 , and air were similar for all chambers. CO_2 and O_2 proportions were constrained to sum to 21% using the following combinations: 19%/2%, 18%/3%, 16.5%/4.5%, 15%/6%, 13.5%/7.5%, 12%/9%, 6%/15%, and 0%/21% for CO_2/O_2 . At the altitude of the research site, roughly 270 m above sea level, 1% of atmospheric pressure equals 0.98 kPa. Target atmospheres were established within 24 h of fruit placement into the CA chambers. The relative humidity of the chambers was not measured.

Post-storage quality evaluations were carried out 4 to 6 h after removal of the fruit from the storage environment to allow the fruit to warm to room temperature. Weight loss was calculated for each clamshell by subtracting weight on removal from storage from initial weight. Fruit were removed from the clamshells and five fruit were removed for determination of ethanol and acetaldehyde content (see subsequently). Ten additional fruit, free from obvious defects, were selected for firmness measurements, and 50 of the remaining fruit were used to assess external and internal quality. Firmness was determined as described previously. External quality assessments included the incidence of skin reddening and visible surface mold. Skin reddening refers to the discoloration of the fruit skin from blue to a brownish red color indicative of dead and discolored epidermal and hypodermal cells. Determination of surface mold was based on the presence of obvious fungal mycelia on the surface of the fruit. No attempt was made to determine the identity of the fungal organism(s).

Internal quality was assessed by measuring the incidence and intensity of pulp (mesocarp and carpel) discoloration. Berries were cut in half using a stainless steel knife and fruit were assigned to quality categories based on the severity of pulp darkening resulting from water soaking and/or pigment bleeding (Beaudry et al., 1998). Fruit quality classes were 1 (less than 25% internal discoloration, white-green pulp; acceptable), 2 (between 25% and 50% internal discoloration, white-green to light pink pulp; edible but not acceptable), 3 (50% to 75% discoloration with some portion of white-green to light pink flesh; inedible), and 4 (75% to 100% discoloration with little to no white-green to light pink flesh; inedible). The number and percentage of fruit in each category for each replicate was determined. Determination of the incidence of berry decay was based on the presence of surface mold and/or liquefaction of the interior of the fruit with or without obvious shrivel.

Ethanol and acetaldehyde levels were determined according to the method of Pesis and Avissar (1990), based on the partition ratio between air and water (Harger et al.,

Fig. 1. Percent of atmosphere for O_2 , CO_2 , and the sum of O_2 and CO_2 in controlled atmosphere (CA) chambers for which the atmosphere control strategy is to maintain elevated CO_2 and using air as a purge gas. In this example, the target concentration for CO_2 is 12%, the actionable threshold to instigate a purge event is 13.5% CO_2 , and O_2 is not actively controlled. Initial conditions are depicted as resulting from an initial addition of CO_2 to obtain the target CO_2 concentration. Dashed line represents the sum of O_2 and CO_2 at equilibrium.

1950), with minor modifications. For each replicate, five fruit were halved and macerated to a puree in a 22-mL clear glass vial using a Teflon-coated pestle. The vial was sealed with a valved septum (Mininert valve; Supelco, Bellefonte, PA) and placed within a water bath maintained at 25 °C. After a 10-min equilibration, a 1-mL headspace sample was removed from the vials using a plastic syringe and injected in the inlet septum of a gas chromatograph (Carle AGC Series 100; Hach Co., Loveland, CO) fitted with a 2-m long, 2-mm i.d. stainless steel column packed with Chromosorb OV-103, 60/80 mesh (Altech Associates Inc., Deerfield, IL) and equipped with a flame ionization detector. The gas chromatograph was run isothermally at 140 °C and the flow rate of the carrier gas (helium) and combustion gases (H₂ and O₂) were ≈50, 50, and 200 mL·min⁻¹, respectively. Ethanol and acetaldehyde concentrations in the sample headspace were calculated using standards of 0.1% aqueous solutions of ethanol and acetaldehyde in identical vials to those used for samples and held in the same water bath. At 25 °C, a 0.1% solution of ethanol and acetaldehyde yield headspace concentrations of 120 and 203 μL·L⁻¹, respectively.

Analysis of variance was performed using commercially available software (SAS 9.1, Cary, NC) using the PROC MIXED procedure. Atmosphere and cultivar were considered fixed effects and Tukey's test was used for multiple comparison analysis. An arcsine square root transformation was performed on all percentage data before statistical analysis. Non-transformed data are presented.

Results and Discussion

The rate of weight loss differed almost threefold between cultivars and ranged from 0.6% to 2.3% over the 8 weeks of the trial (Table 1), but no shrivel was observed on any

fruit. The cultivar Jersey experienced 50% more weight loss than the next two nearest cultivars Toro and Legacy. This may reflect differences in cuticle permeance to water vapor, differences in the stem scar morphology, and/or the surface-to-volume ratios. In fact, 'Jersey' has the largest stem scar of all of the varieties evaluated, supporting the role of the stem scar in moisture loss during storage (Hancock et al., 2001; Hancock, personal observation).

Atmosphere affected moisture loss more than cultivar, yielding a 13-fold difference between the 0%/21% treatment (0.25% weight loss) and the 19%/2% treatment (3.3% weight loss). The greater weight loss for the highest CO₂/lowO₂ treatment may stem largely from physical causes. An elevated CO₂ and low O₂ environment requires more frequent and aggressive atmosphere modification than other treatments, so the greater flux of dry gas through the CA chambers likely contributed to the extreme in water loss. Although a physiological impact on moisture loss may have occurred because high levels of CO₂ can stress blueberry fruit (Beaudry, 1993), a linkage is not obvious. Moisture loss in some cultivars resulting from the atmosphere treatment was more marked than in others. 'Jersey', for instance, lost over 6% of its weight in the high CO₂ 19%/2% treatment, whereas in this atmosphere, 'Elliott', 'Toro', and 'Legacy' each lost ≈4% of their weight; the other cultivars lost ≈2.5% of their original weight.

Initially, the firmest fruit was 'Duke' and the softest cultivar was Jersey (Table 1; Fig. 2). After storage, 'Jersey' fruit were markedly softer than all others, although 'Nelson', 'Ozarkblue', and 'Elliott' were quite soft as well. The relatively low initial firmness of the 'Jersey' fruit and the excessive firmness loss during storage might be partially indicative of a somewhat more advanced stage of maturity. It seems probable that the extreme loss in

firmness of 'Jersey' also was at least partly a result of the high moisture loss (Table 1). The cultivar effect on firmness is consistent with previous publications. 'Jersey' is known to store poorly and several of those cultivars with the highest firmness after storage, Brigitta, Toro, Liberty, and Legacy, have been demonstrated to store well (Hancock et al., 2008).

The effect of atmosphere on fruit firmness was not as pronounced as the effect of cultivar, but fruit firmness was negatively affected by an increasing ratio of CO₂/O₂ with the treatment of 19%/2% having the greatest negative impact on firmness (Table 1). Schotsmans et al. (2007) found a similar result for rabbiteye blueberries (*Vaccinium ashei* Reade) with an atmosphere of 15 kPa CO₂ and 2.5 kPa O₂ leading to a loss in firmness relative to air storage in as little as 4 weeks' storage for Centurion and Maru cultivars. Cranberry (*Vaccinium macrocarpon* Aiton) fruit undergo firmness loss at CO₂ partial pressures of 15 kPa or more at O₂ levels ranging from 2 to 70 kPa or under a partial pressure of 0 kPa O₂ without added CO₂, but firmness is not diminished by low O₂ (2 kPa) relative to air storage (Gunes et al., 2002). Interestingly, high CO₂ is known to have a positive influence on the firmness of strawberry (*Fragaria × ananassa* Duch.) fruit (Harker et al., 2000; Pérez and Sanz, 2001). The mechanism of the impact of CO₂ on fruit firmness is hypothesized to be related to a reduction in the apoplastic pH and its impact on the interaction of cell wall constituents (Harker et al., 2000). It is not clear why this mechanism would behave one way in strawberry but the opposite in *Vaccinium* species.

The firmness response to atmosphere differed between cultivars with seven of the nine cultivars exhibiting lower firmness for 19% CO₂ treatment than for the air treatment (Fig.

Table 1. Cultivar (C) and atmospheric treatment (T) effects on quality characteristics of highbush blueberry fruit held in cold storage at 0 °C for 8 weeks.^a

Cultivar (C)	Wt loss (%)	Firmness (durometer units)		Red berries	Surface mold (%)	Berry decay (%)	Internal discoloration class				EtOH (μL·L ⁻¹)	Acet. (μL·L ⁻¹)
		Pre-storage	Post-storage				1 (%)	2 (%)	3 (%)	4 (%)		
Duke	1.07	64.2	57.3	3.0	1.08	4.8	13.6	38.9	22.3	25.1	ND	ND
Toro	1.47	59.5	60.3	2.1	2.07	4.4	15.1	48.4	25.6	10.7	3.49	0.27
Brigitta	0.75	54.7	59.3	1.5	0.26	5.2	1.2	49.7	31.9	17.0	3.11	0.53
Ozarkblue	0.66	56.3	44.9	7.4	2.50	7.7	0.8	26.9	43.9	28.3	0.02	0.01
Nelson	0.67	56.2	41.7	3.6	0.00	5.1	0.0	7.5	16.8	75.6	0.07	0.01
Liberty	0.89	56.3	61.0	0.9	0.06	3.0	0.0	45.4	48.5	6.0	0.00	0.01
Elliott	1.34	51.6	47.8	0.7	0.06	14.2	0.0	35.6	38.6	25.7	0.01	0.01
Legacy	1.54	54.8	61.3	0.6	0.14	3.4	0.0	33.8	51.3	14.7	0.03	0.01
Jersey	2.34	46.3	26.4	0.4	0.75	15.7	0.0	13.7	33.8	52.4	0.01	0.01
Main effects (C)	*	*	*	*	*	*	*	*	*	*	*	NS
Treatment (T)												
19% CO ₂ + 2% O ₂	3.37	NA	45.0	0.6	0.11	5.8	4.1	27.3	35.1	33.4	0.38	0.53
18% CO ₂ + 3% O ₂	1.31	NA	50.3	0.2	0.06	5.4	3.3	29.9	36.4	30.2	0.38	0.02
16.5% CO ₂ + 4.5% O ₂	1.20	NA	49.0	0.6	0.23	5.8	4.4	27.6	34.9	32.9	1.17	0.01
15% CO ₂ + 6% O ₂	0.94	NA	50.6	0.4	0.11	6.7	3.4	31.4	35.2	29.8	0.97	0.01
13.5% CO ₂ + 7.5% O ₂	1.12	NA	51.8	1.0	0.33	6.0	4.2	30.2	36.1	29.4	1.32	0.01
12% CO ₂ + 9% O ₂	1.13	NA	51.9	0.9	0.11	6.8	3.3	35.0	35.2	26.3	0.84	0.01
6% CO ₂ + 15% O ₂	1.10	NA	55.5	3.2	0.30	7.2	3.0	41.5	34.8	20.5	0.86	0.27
0% CO ₂ + 21% O ₂	0.25	NA	54.9	11.2	4.90	12.6	1.5	43.7	30.0	24.5	0.82	0.01
Main effects (T)	*		*	*	*	*	NS	*	*	*	*	NS
Interaction (C × T)	*		*	*	*	NS	NS	*	*	*	*	*

^aClasses for internal discoloration are: 1 (less than 25% internal discoloration), 2 (between 25% and 50% internal discoloration), 3 (50% to 75% discoloration; inedible), and 4 (75% to 100% discoloration; inedible). Statistically significant interactions or main effects within columns are indicated by asterisks ($P \leq 0.05$). Non-significant interactions or effects are indicated by NS; ND indicates measurement was not determined; NA indicates non-applicable.

2). Conversely, the firmness of 'Duke' and 'Ozarkblue' fruit was lower for air-stored fruit than for fruit exposed to any of the other atmospheres. The low firmness readings were the result of the durometer probe occasionally penetrating the skin of the berry fruit for this treatment. Approximately 10% to 15% of readings for air-stored 'Duke' and 'Ozarkblue' fruit were below 15, symptomatic of puncturing during the firmness measurement (data not shown), despite the average firmness for the air treatment being 45 and 40, respectively. This apparent weakening of the skin and/or cuticle of these two cultivars during air storage was not associated with higher rates of moisture loss. The relationship between storage atmosphere and skin and cuticle properties is, to our knowledge, unexplored in blueberry.

'Ozarkblue' had a markedly greater incidence of reddening than all other cultivars, followed, in order of declining severity of response, by 'Nelson', 'Duke', and 'Toro' (Table 1). Reddening was almost negligible in the remaining cultivars. Where found, reddening was most severe among those fruit held in air storage, averaging over 11% of the fruit affected. A 6%/15% atmosphere resulted in $\approx 3.5\%$ reddened fruit, with 1%, or less, in higher CO₂/O₂ ratio treatments. The decrease in reddening with increasing CO₂ and decreasing O₂ can probably be ascribed to the impact of the CO₂ rather than O₂ given that the most marked decline in the disorder took place as O₂ dropped from 21 to 15 kPa. O₂ levels in this range are not considered to confer marked physiological responses (Beaudry, 1999). The interaction between cultivar and atmosphere results from the high incidence of reddening in the air treatments of 'Duke', 'Toro', 'Brigitta', 'Ozarkblue', and 'Nelson', averaging 7%, 16%, 10%, 46%, and 11%, respectively, with close to 0% for the other cultivars (data not shown).

Cultivar and atmosphere impacted the internal quality of the fruit as measured by the distribution of fruit having internal discoloration classes of 1, 2, 3, or 4. Cultivar had a statistically significant effect on each quality class. 'Jersey' and 'Nelson' had a greater fraction of fruit in the more severe categories 3 and 4 (summing to 85% and 93%, respectively), whereas 'Duke', 'Toro', 'Brigitta', and 'Liberty' had lower fractions in the more severe categories (47%, 36%, 49%, and 54%, respectively), and 'Ozarkblue', 'Elliott', and 'Legacy' had intermediate fractions in the more severe categories (72%, 64%, and 66%, respectively) (Fig. 3). The relatively poor condition of 'Jersey' and 'Nelson' fruit at the end of the storage period is consistent with previous evaluations of the storability of these two cultivars (Hancock et al., 2008). Similarly, the better maintenance of internal coloration by 'Brigitta' and 'Toro' is also consistent with previous findings (Hancock et al., 2008). The storability of the remaining cultivars appears to be somewhat more variable from year to year, but the current results are generally in agreement with those published by Hancock et al. (2008) with 'Legacy', 'Liberty', and 'Elliott' storing moderately well to very well depending

Fig. 2. Effects of cultivar and storage atmosphere on the firmness of highbush blueberry fruit as determined using a Type 00 durometer after 8 weeks storage at 0 °C. Each data point represents the average of 40 fruit; vertical bars represent 1 SD.

Fig. 3. Effects of cultivar and storage atmosphere on the incidence of severe (greater than 50%) internal discoloration of highbush blueberry fruit after 8 weeks storage at 0 °C. Each data point represents the average of 40 fruit; vertical bars represent 1 SD.

on the season. There was a significant negative correlation between the percentage of fruit experiencing severe internal discoloration and fruit firmness ($P < 0.001$, $R^2 = 0.42$).

The impact of atmosphere on internal quality was not as marked as cultivar, affecting only the fractions of fruit classed as 2 and 4 (Table 1). Relatively, a greater portion of the variability of the response is explained by significant interactions for classes 2, 3, and 4. 'Brigitta', 'Ozarkblue', 'Nelson', and 'Liberty' exhibited an increase in the fraction of fruit in the more severe classes 3 and 4 as CO₂ levels increased and O₂ levels declined, but the internal condition of the remaining cultivars was not influenced by atmosphere. The data suggest that some cultivars may respond to more extreme CO₂ atmospheres by

loss of cellular integrity leading to darkening and discoloration of the pulp, whereas others are more resistant to the influence of atmosphere. The response of 'Liberty' and 'Brigitta' to the range of atmospheres is instructive; both cultivars had among the lowest levels of severe internal discoloration for the 6%/15% and 12%/9% treatments and nearly twofold higher severely discolored fruit in the atmospheres having the highest CO₂ partial pressures. 'Toro', on the other hand, exhibited low levels of severe discoloration for all atmospheres.

Surface mold incidence was extremely low, averaging less than 1%. However, cultivar and atmosphere both had effects and the interaction was significant. 'Duke', 'Toro', and 'Ozarkblue' had a markedly higher incidence

of surface mold than the other six cultivars. For the atmosphere treatments, the incidence of surface mold in the air-storage treatment (0%/21%) was $\approx 4\%$, roughly 10 times higher than for the various MAs. All the CO₂-enriched atmospheres suppressed surface mold similarly such that the atmosphere combination containing 6 kPa CO₂ was as effective as the more extreme CO₂ treatments. The interaction was the result of uneven responses of the cultivars to the atmosphere treatments because 'Toro', 'Ozarkblue', and 'Jersey' were essentially decay-free except for the air-stored fruit, which averaged 16%, 19%, and 6%, respectively. 'Duke' had between 0.5% and 2% surface mold and there was no relationship to atmosphere. The other cultivars had little surface mold even in the air storage treatment (data not shown).

The extent of berry decay was relatively low compared with previously published reports (Ceponis and Cappellini, 1979, 1983, 1985; Hancock et al., 2008) given the long storage duration of the current study (Table 1). The average decay incidence of the nine cultivars ranged from $\approx 3\%$ to 16% with 'Elliott' and 'Jersey' exhibiting two- to threefold higher decay rates than the other cultivars and 'Liberty' having the lowest rate of decay. The degree of decay declined as the ratio of CO₂ to O₂ increased (Fig. 4). CO₂, but not O₂, applied at similar partial pressures to those used in this study have been demonstrated to suppress decay (Ceponis and Cappellini, 1979, 1983, 1985; Schotsmans et al., 2007; Smittle and Miller, 1988). The effect of atmosphere was therefore the result of the CO₂ partial pressure rather than changes in the ratio of the two gases per se.

Ethanol and acetaldehyde concentrations were extremely low throughout the study (Table 1). The highest individual reading for both compounds was less than 10 $\mu\text{L}\cdot\text{L}^{-1}$, which means the maximum concentrations of

ethanol and acetaldehyde in the fruit were less than 0.01% and 0.005%, respectively. If fermentation is actively engaged, then it would be expected that concentrations of these fermentation products in the berry pulp would be in the tenths-of-a-percent range (Beaudry, 1993; Beaudry et al., 1993; Toivonen and DeEll, 2001). Inasmuch as this was not the case, the data are likely not indicative of fermentation as a result of the atmospheric treatments, suggesting that none of the treatments were sufficiently stressful to modify respiratory metabolism. This is consistent with the data from Beaudry (1993) on 'Bluecrop' fruit, which fermented at CO₂ partial pressures above 40 kPa when the O₂ partial pressure was below 4 kPa. Similarly, Cameron et al. (1995) found no evidence of CO₂ levels as high as 16 kPa altering either the apparent Km for oxygen or the maximal rate of respiration at temperatures between 0 and 20 °C.

The data indicate that important differences exist between these blueberry cultivars in their capacity to store well in air or in MAs. Responses to high levels of CO₂, while O₂ is maintained at its maximum level practicable, can include significant moisture loss, softening, and external and internal discoloration in addition to the well-documented and desirable suppression of decay. Some cultivars such as Duke, Toro, Brigitta, Liberty, and Legacy appear to be well suited to extended storage. 'Toro' seemed to respond well to the range of atmosphere combinations used. This variety underwent little softening even at relatively high CO₂ partial pressures and had the lowest overall level of internal discoloration. On the other hand, 'Liberty' had extremely low levels of berry decay and skin reddening, especially under the influence of atmospheres with elevated CO₂, and, when the CO₂ levels were between 6 and 12 kPa, had among the lowest incidence of severe internal discoloration and the high-

est firmness levels. Collectively, the data suggest that CA storage can improve storability of highbush blueberry fruit relative to air storage, but very high levels of CO₂ are to be avoided to prevent softening or internal discoloration in susceptible cultivars. When the intent is to establish an atmosphere enriched in CO₂ to suppress decay while maximizing the O₂ level, the data suggest a CO₂ partial pressure near 12 kPa is broadly useful for blueberry and near optimal for specific cultivars.

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Fig. 4. Effects of cultivar and storage atmosphere on the incidence of berry decay of highbush blueberry fruit after 8 weeks storage at 0 °C. Each data point represents the average of 40 fruit; vertical bars represent 1 SD.

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