Effect of hexanal vapor to control postharvest decay and extend shelf-life of highbush blueberry fruit during controlled atmosphere storage

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Postharvest disease control has become more challenging due to the limited number of registered fungicides, fungicide resistance, consumers’ desire for reduced fungicide residues and demand for blemish-free, high-quality product. The interest in the use of environmental alternatives to prevent fungal growth has markedly increased. Many biologically active volatile compounds, including hexanal, a natural plant volatile with antifungal properties, have been reported to reduce postharvest diseases. In this study, highbush blueberry fruit (Vaccinium corymbosum ‘Duke’, ‘Brigitta’ and ‘Burlington’) were treated with hexanal vapor at 900 µL L⁻¹ for 24 h either once immediately before storage or repeated after 1 and 2 wk of CA storage (10–12 kPa O₂ and 12–15 kPa CO₂) at 0.5°C for up to 15 wk. Fruit removed from storage after 3, 5, 7, 9, 12 and 15 wk were evaluated following 1 or 7 d at 10°C. Decayed fruit were significantly reduced by 50–70% in treated fruit compared with the control. A 17% reduction of split Duke fruit was also found in hexanal treated fruit after 9 wk CA storage followed by 7 d at 10°C. Marketable fruit in all three cultivars was 20–40% greater in hexanal treatments after 12 wk of storage as compared with controls. Fruit firmness increased during storage in Burlington. No significant changes in weight loss were found. These results indicate that postharvest application of hexanal vapor can reduce fruit decay, maintain fruit quality and extend storage life. It has potential as an alternative fungicide to reduce postharvest decay in highbush blueberry fruit.

Key words: Postharvest, fungi, decay control, quality

Fruits and vegetables are perishable, and their storage life and quality are affected by both physiological and environmental factors, such as temperature, humidity and controlled atmosphere (CA) gas composition. Despite the use of sophisticated modern postharvest storage facilities and techniques, postharvest loss due to fungal decay continues to be a significant problem resulting in substantial quality loss of many stored fruits and vegetables. Postharvest disease control is challenging due to the limited number of registered fungicides,
fungicide resistance, consumer objections to fungicide residues and demand for blemish-free, high-quality product. Alternatives to chemical fungicides have been sought to reduce losses resulting from postharvest decay. The popularity of blueberry fruit (*Vaccinium* spp.) is increasing in North America, and its production has rapidly expanded throughout the world, due to its nutritional value and market potentials. Blueberry fruit are highly perishable and are considered to be non-climacteric fruit, due to the lack of an associated burst of respiration and ethylene production associated with ripening (Sargent et al. 2006). The storage life of fruit is mainly limited by fruit decay, weight loss and flavor loss during postharvest storage (Day et al. 1990; Nunes et al. 2004). The market life of blueberry fruit during storage varies depending on cultivar, pre-harvest environment and postharvest conditions (Hancock et al. 2008). In general, storage life of blueberry is about 2–3 wk at 0°C for cold storage and 4–7 wk under CA conditions with 10–12 kPa O₂ and 15 kPa CO₂ (Forney et al. 1998; Harb and Streif 2004, 2006; Hancock et al. 2008). Unlike pome fruit, the benefit of CA is due to its high CO₂ concentration (12–15 kPa), which retards fungal decay, rather than to low oxygen to minimizing physiological activities (Forney 2009). Among the CA recommendations, O₂ (10–15 kPa) has been widely accepted for both highbush and rabbiteye blueberries (Harb and Streif 2004, 2006; Schotsmans et al. 2007; Hancock et al. 2008). In order to maintain fruit quality during postharvest handling, any additional strategies aimed at inhibiting decay should be considered a high priority.

Alternatives to chemical control of postharvest decay on highbush blueberry such as modified atmosphere packaging (MAP) (Beaudry et al. 1998; Rosenfeld and Meberg 1999), UV radiation (Perkins-Veazie et al. 2008), heat (Fan et al. 2008), ozone (Song et al. 2003) as well as electron beam irradiation (Moreno et al. 2008) have shown potential, but all have limitations to their effectiveness.

As an alternative to fungicide treatment, many biologically active plant volatiles such as acetalddehyde (Stadelbacher and Prasad 1974; Avisssar and Pesis 1991), acetic acid (Sholberg et al. 2000), (E)-2-hexenal (Fallik et al. 1998; Archbold et al. 1999), hexanal (Song et al. 1996, 2007; Gardini et al. 1997), and methyl jasmonate (Wang and Bula 2003) have shown potential to inhibit the growth of postharvest microbials and reduce postharvest diseases. Among them, hexanal vapor was reported to inhibit fungal growth and enhance aroma biosynthesis in apple slices and whole apple fruit (Song et al. 1996, Lanciotti et al. 1999, Fan et al. 2006). Effectiveness of hexanal vapor to inhibit spore germination of *Penicillium expansum* (Link) was also demonstrated by Fan et al. (2006). The effect of hexanal on spore germination of *Botrytis cinerea*, *Monilinia fructicola* and *P. expansum* as well as mycelial growth of *Sclerotinia sclerotiorum*, *Alternaria alternata* and *Colletotrichum gloeosporioides* was further investigated (Song et al. 2007). The effectiveness of hexanal is dependent on hexanal concentration, treatment duration and the sensitivity of fungal pathogens to hexanal vapor. Hexanal, a natural plant volatile, has been widely used as a food flavoring agent and is generally recognized as safe (Newberne et al. 2000). Since the storage life of highbush blueberry may be limited by fungal decay caused by *B. cinerea*, *Colletotrichum* spp. or other pathogens (Caruso and Ramsdell 1995), hexanal fumigation to control blueberry decay could maintain fruit quality and reduce fruit decay, which would improve fruit storage life. To date, there has been no use of hexanal vapor to control postharvest decay in highbush blueberry fruit.

The objectives of this study were to investigate the potential of hexanal vapor treatment to control postharvest decay and enhance quality of blueberry fruit by quantifying the effect of hexanal treatments on product quality including decay, fruit splitting, marketable fruit, weight loss and firmness over 15 wk of CA storage.

**MATERIALS AND METHODS**

**Blueberry Fruit**

Highbush blueberry fruit (*Vaccinium corymbosum* 'Duke', 'Brigitta' and 'Burlington') were obtained from Nova Agri Inc., Centreville, NS, in 2005. Fruit were harvested at commercial ripeness, pre-cooled and packed in clamshells at Nova Agri Inc. and then delivered to the Atlantic Food and Horticulture Research Centre within 24 h. Upon arrival the clamshells (ca. 450 g of fruit) were labeled and weighed. Two separate harvests were conducted to serve as experimental replicates.

**Hexanal Vapor Treatment**

The hexanal treatments were carried out in air-tight 134-L stainless steel chambers. The treatment chamber was fitted with a small heating plate that was capable of reaching 130°C within a few minutes. A known volume of liquid hexanal was placed on the heating plate and vaporized to produce the target hexanal concentration of 900 μL L⁻¹ in the treatment chamber (Song et al. 2007). In each chamber, approximately 27 kg of fruit were treated with hexanal vapour. Treatments consisted of: (1) non-treated fruit (control); (2) single dose 900 μL L⁻¹ hexanal (Single), (3) initial dose of 900 μL L⁻¹ hexanal followed by another 900 μL L⁻¹ dose after 1 wk of CA storage (Double), (4) initial dose of 900 μL L⁻¹ hexanal followed by a second 900 μL L⁻¹ dose after 1 wk of CA storage and a third 900 μL L⁻¹ dose after 2 wk of CA storage (Triple). Fruit were held in chamber for 24 h at 0.5°C for each dose. The hexanal concentration in the chamber was measured and confirmed by gas chromatograph during the treatment period using a gas chromatography (GC) as described by Song et al. (2007). Hexanal concentrations were measured by taking 1.0 mL gas samples using a gas tight syringe (Hamilton no. 1810) and stainless-steel needle with a Mininert gas-tight sampling valve (Alltech...
clamshells of fruit (ca. 350-400 g) were removed and the chamber was opened and the fruit was transferred to a similar stainless steel chamber for storage. All 4 treatments were stored under CA (10-12 kPa CO₂ + 12-15 kPa O₂) (Forney 2009) at 0.5°C using a CA control system (Oxystat 2002, David Bishop Instrument, Heathfield, E. Sussex, UK) to monitor and maintain CA conditions.

Fruit Quality Evaluation
Fruit samples were assessed for quality after 3, 5, 7, 9, 12 and 15 wk of storage. At each removal two pint clamshells of fruit (ca. 350-400 g) were removed and one assessed after 1 d and the other after 7 d of being held at 10°C in air. CA conditions were re-established within 1 h after fruit removals by flushing chamber with N₂ and CO₂. Fruit were evaluated for weigh loss, and then sorted into four categories: (1) marketable — unblemished fruit; (2) shriveled — any fruit with visible outer skin wrinkling; (3) split — any fruit with a visible fracture in its outer skin, and (4) decay — any fruit with visible mould growth. Fruit numbers in each category were calculated as a percentage of the total number of berries. Fruit firmness was measured on a Firm-Tech 2 Firmness Tester (Bioworks Inc., Wamego, KS). Samples of 25 marketable fruit were loaded onto the turntable of the tester and the average firmness was determined as Newtons per millimeter of deformation.

Statistical Analysis
The study was conducted using a split-split plot design. The control and three hexanal treatments were completely randomized in the main plot. The subplots were the three cultivars, and the sub-subplots were the removal weeks and sample days. The experiment was replicated across the two harvests during the season. All values except for firmness were subjected to square root transformation prior to statistical analysis to normalize distribution. Data were analyzed using the analysis of variance (ANOVA) directive and standard error (SEM) option of Genstat (2008). Linear and quadratic differences across removal weeks were evaluated using a polynomial contrast.

RESULTS
Headspace Hexanal Concentration In Fumigation Chambers
Hexanal was fully vaporized using a heat plate to reach the targeted concentration, which was confirmed by headspace analysis at 0.5 h after fumigation. After 6 h, however, 217, 230 and 309 µL L⁻¹ hexanal concentrations were found in chambers containing Duke, Brigitta and Burlington fruit, respectively. After 24 h, only 150 µL L⁻¹ was found in the Burlington chamber, while trace amount of hexanal was found in chambers containing the other two cultivars.

Effect of Hexanal Vapor on Decay and Splitting
The effect of hexanal vapor on decay and split fruit of three blueberry cultivars was investigated. Less decay was found in all fruit treated with hexanal than in the control following CA storage. In general, a 3-5% reduction was seen immediately after 9 wk of storage. After 12 wk, fruit treated with the triple treatments of hexanal had 6.5, 5.2 and 4.3% less decay than controls in Duke, Brigitta and Burlington, respectively, when evaluated 1 d after removal (Fig. 1A, 1C and 1E), and 4, 16 and 7% less after 7 d at 10°C (Fig. 1B, 1D and 1F). After 15 wk of CA storage, the double and triple treatments of hexanal provided the best reduction in decay and it was evident in all cultivars. The triple hexanal treatments resulted in 74, 11 and 34% less decay than controls in Duke, Brigitta and Burlington, respectively, when evaluated 1 d after removal (Fig. 1A, 1C and 1E), and 26, 66 and 32% less after 7 d at 10°C (Fig. 1B, 1D and 1F).

Percent of split fruit was also reduced by hexanal treatment compared with control fruit after 9-12 wk of storage. At 12 wk, 17, 5 and 4% less split fruit were seen 1 d after removal from storage in Duke, Brigitta, and Burlington, respectively (Fig. 2A-F). After 7 d at 10°C, 4% and 11% less splitting occurred in Brigitta and Burlington fruit, respectively (Fig. 2D and 2F), however, in Duke there was a 7% increase in splitting compared with controls. After 15 wk of storage, no split fruit were found due to the high decay rate in Duke and Brigitta fruit. Hexanal treatment reduced decay and prolonged the storage life of blueberry fruit.

Effect of Hexanal Vapor on Marketable Blueberry Fruit
Depending on the cultivar and time of evaluation, overall percent marketable fruit was 15 to 55% higher in hexanal-treated fruit than in the control after 9 or 12 wk of storage. After 9 wk of CA storage and 7 d at 10°C, the triple-dose hexanal treated fruit had 47, 17 and 15% more marketable fruit in Duke, Brigitta and Burlington, respectively, than controls (Fig. 3A, 3C and 3E), which translated into 87, 79 and 83% total marketable fruit. On day 1, after 12 wk of storage, fruit treated with triple doses of hexanal had 23, 18 and 7% more marketable fruit in Duke, Brigitta and Burlington, respectively, than controls (Fig. 3A, 3C and 3E) and 18, 32 and 19% more marketable fruit after 7 d at 10°C (Fig. 3B, 3D and 3F). After 15 wk of storage, no marketable fruit were found in controls of both Duke and Brigitta as well as the single-dose treated Brigitta.
Fig. 1. Percent of decayed Duke, Brigitta and Burlington fruit following CA storage (10–12 kPa O₂ + 12–15 kPa CO₂) at 0.5°C after fumigation with hexanal. Treatments consisted of: (1) non-treated fruit (control); (2) single dose 900 μL L⁻¹ hexanal (S), (3) initial dose of 900 μL L⁻¹ hexanal followed by another 900 μL L⁻¹ dose after 1 wk of CA storage (Double), (4) initial dose of 900 μL L⁻¹ hexanal followed by a second and third 900 μL L⁻¹ dose after 1 and 2 wk of CA storage (Triple) for 24 h at 0.5°C. Evaluations were conducted at (A) 1 d and (B) 7 d after removal from storage and holding at 10°C. The vertical bar represents 2 x the standard error for comparison of means. Note that the y axis reflects the square root transformation.

**Effect of Hexanal Treatment on Blueberry Fruit Firmness**

Firmness is another important fruit quality criterion. In Duke, firmness of control and single-dose hexanal treated fruit decreased from 2.1 to 1.2 N mm⁻¹ during 15 wk of storage. The double- or triple-dose hexanal treatment reduced loss of firmness and maintained it at 1.5–1.6 g mm⁻¹ after 15 wk of storage (Fig. 4A and 4B). No significant difference of firmness was found in Brigitta. Interestingly, average firmness in Burlington increased significantly from 1.6–1.7 to 2.4 N mm⁻¹. There was no significant difference in firmness among treatments in Burlington at day 1. However, firmness of fruit treated with hexanal was greater after 9, 12 and 15 wk of CA storage when fruit were evaluated after 7 d at 10°C (Fig. 4E and 4F).

**DISCUSSION**

It is well known that many infections of blueberry fruit occur during the growing season prior to harvest with symptoms not developing until during storage. Therefore, the ability to control latent infections in the postharvest environment is crucial (Caruso and Ramsdell 1995). In blueberry, little is known about infection, pathogen populations and dynamic changes in disease incidence during fruit production. However, the effects of pathogen inoculum, surface wetness and the stem scar were found to influence postharvest fungal infection (Cline 1996; Elenfeldt et al. 2006). It is generally believed that highbush blueberry fruit are typically infected during the green stage of development. Spores that germinate on infected fruit, form appressoria and then become dormant until the fruit ripens (Daykin and Milholland 1984). In this study, hexanal vapor treatment prior to CA storage inhibited fruit decay during prolonged storage periods with decay reductions of up to 70%. Marketable fruit was 15–55% greater in Duke, Brigitta and Burlington due to reduction in decay and splitting. The effect of hexanal treatment on decay seemed to be most obvious after 9 wk of storage and varied among cultivars. Overall, less decay was found in hexanal treated Burlington fruit, because Burlington is a late harvest cultivar and generally has less decay. This study also demonstrated that hexanal treatment can not completely control fruit decay even with a multi-treatment approach. It can be postulated that some fungal spores causing decay during storage may be inside the fruit, beyond the reach of the hexanal treatment. It is also possible that secondary contamination of spores may cause further decay of fruit, which cannot be distinguished by our evaluation...
of decay. We postulate that incidence of split fruit may be an early sign of decay and fruit breakdown. Reduced decay and split fruit resulted in higher amounts of marketable fruit.

Despite the significant control of decay and splitting, and improvement of marketable fruit, hexanal treatment did not completely control fruit decay. In vitro, a treatment of 900 µL L$^{-1}$ for 12 to 24 h was effective to control spore germination or mycelial growth of many fungi (Song et al. 2007). They suggested that the maximum treatment effect can be achieved when the product of hexanal exposure time (h) and concentration (µL L$^{-1}$) is >10 800. In this study with blueberry fruit, the headspace concentration of hexanal vapor was monitored during fumigation. It was found that the hexanal concentration decreased from the initial concentration of 900 µL L$^{-1}$ to 150 µL L$^{-1}$ or less during the 24 h fumigation period. Similar result of an 84% decrease of hexanal vapour concentration after a 24 h fumigation of Jonagold apples at 15°C were reported by Sholberg and Randall (2007). The explanation for the decrease of hexanal concentration may be due to fruit metabolism of hexanal and/or adsorption of the hexanal onto chamber walls, packaging materials or fruit surfaces. This could reduce the effectiveness of the hexanal treatment. Utama et al. (2002) noted that adsorption of volatiles onto culture media used as a supporting surface for microorganisms during exposure may alter the head space concentration. To achieve optimal treatment effects, it is important to establish an effective volatile concentration and exposure duration combination. Apparently, maintaining hexanal concentration during the treatment period may be a limiting factor for maximizing hexanal effectiveness. To overcome this problem, weekly treatments with hexanal during the first 2 wk of storage were included in this study, which resulted in more effective disease control and a significant improvement in marketable fruit when compared with the control and the single-dose treatment. To increase efficacy, new treatment regimes need to be developed that maintain hexanal concentration around fruit and packaging materials during the entire treatment period.

Recently, the relationship between natural volatile production from both whole and extracted fruit and decay resistance against anthracnose rot in 10 blueberry cultivars was investigated (Polashock et al. 2007). Despite the significant difference in volatile production, no relationship was found. Since only relative volatile production at day 0 was reported, it is difficult to compare these results with other publications and this study in terms of the effectiveness of aldehydes to reduce...
Fig. 3. Percent of marketable Duke, Brigitta and Burlington fruit following CA storage (10–12 kPa O₂ + 12–15 kPa CO₂) at 0.5°C after fumigation with hexanal. Treatments consisted of: (1) non-treated fruit (control); (2) single dose 900 µL L⁻¹ hexanal (S), (3) initial dose of 900 µL L⁻¹ hexanal followed by another 900 µL L⁻¹ dose after 1 wk of CA storage (Double), (4) initial dose of 900 µL L⁻¹ hexanal followed by a second and third 900 µL L⁻¹ dose after 1 and 2 wk of CA storage (Triple) for 24 h at 0.5°C. Evaluations were conducted at (A) 1 d and (B) 7 d after removal from storage and holding at 10°C. The vertical bar represents 2 x the standard error for comparison of means. Note that the y axis reflects the square root transformation.

Decay. In addition to volatile quantization in both fruit and extracts, localization of volatile production such as at the inoculation site may be crucial for our understanding of the resistance caused by natural volatiles. Unlike the microbial study using Petri plates, the metabolism of hexanal by blueberry fruit may play an important role, not only in altering hexanal headspace concentration, but also affecting blueberry fruit flavor. It is well known that hexanal can be metabolized by many fruits to generate “fruity” flavor compounds, and therefore enhance fruit flavor (Song et al. 1996). The presence of aldehyde dehydrogenase and alcohol acetyl-CoA transferase is responsible for the conversion of hexanal to hexanol and hexyl acetate (Song et al. 1996). Despite the lack of information about the above mentioned enzymes in blueberry fruit, small amounts of hexyl acetate, hexyl hexanoate and 2-methylbutyl hexanoate were identified as aroma volatiles in the headspace of Coville blueberry fruit (Song et al. 2003). Based on this result, it can be postulated that blueberry fruit have physiological mechanisms to convert hexanal to hexanol, hexyl acetate and other corresponding volatiles. Currently, research in volatile biosynthesis of blueberry with hexanal treatment and formal sensory evaluation of fruit following hexanal treatment are being conducted in our laboratory and results will be reported.

Firmness is an important quality indicator for high-bush blueberry fruit. During storage, no significant change in firmness was found in Duke and Brigitta, while an increase of firmness was found in Burlington. This confirms previous reports of firmness increases in Burlington fruit during storage (Forney et al. 1998). Significant difference in fruit firmness was found between treatment and control in Burlington after 9, 12 and 15 wk of CA storage when fruit were evaluated 7 d after removal from CA storage. The reason for an increase of firmness in Burlington fruit is still unknown, although thickening of cell walls has been observed during storage of Burlington fruit (Allan-Wojtas et al. 2001). Their study showed that fruit cell structure and water potential may vary among cultivars and result in differences in storage life span. Treatment of C₆-aldehydes such as (E)-2-hexenal and (Z)-3-hexenal on Arabidopsis leaves indicated that C₆ aldehydes made Arabidopsis resistant to the fungal pathogen, such as B. cinerea. Volatile treatments induced lignification, accumulation of the phytoalexin, camalexin, and pathogen resistance gene (PR-3) (Kishimoto et al. 2006).
Fig. 4. Firmness of Duke, Brigitta and Burlington fruit following CA storage (10-12 kPa O₂+12-15 kPa CO₂) at 0.5°C after fumigation with hexanal. Treatments consisted of: (1) non-treated fruit (control); (2) single dose 900 µL L⁻¹ hexanal (S), (3) initial dose of 900 µL L⁻¹ hexanal followed by another 900 µL L⁻¹ dose after 1 wk of CA storage (Double), (4) initial dose of 900 µL L⁻¹ hexanal followed by a second and third 900 µL L⁻¹ dose after 1 and 2 wk of CA storage (Triple) for 24 h at 0.5°C. Evaluations were conducted at (A) 1 d and (B) 7 d after removal from storage and holding at 10°C. The vertical bar represents 2 x the standard error for comparison of means.

Apple fruit exposed to 900 µL L⁻¹ (40 µmol L⁻¹) hexanal vapor for 48 h showed signs of phytotoxicity expressed as surface browning (Fan et al. 2006). However, we did not observe any sign of phytotoxicity or damage to blueberry fruit at the concentration of 900 µL L⁻¹ for 24 h. The tolerance of blueberry fruit to higher hexanal vapour requires further study.

The results of this study indicate that fumigation with hexanal vapor has potential as an alternative fungicide to control postharvest decay in blueberries. The volatility of hexanal and its capability to easily penetrate stacked commodities allow its application for fumigation of products in cold storage rooms. Hexanal treatment may also be incorporated into postharvest procedures prior to storage, because rapid cooling following harvest has been recommended for most fresh products (Kader 2002). Using natural products with anti-fungal properties as fumigants is a very attractive alternative to synthetic fungicide applications. If the growth of *B. cinerea* or other fungi can be halted or reduced, the storage life of blueberries can be extended. Data collected in this study and previous studies demonstrate that fumigation with hexanal in combination with CA storage reduces decay and splitting, improved marketable fruit, and extended the storage life of fresh highbush blueberries. Further research is needed to investigate biological mechanisms of the antimicrobial effect of hexanal and to assess the development of its commercial application on selected fruits and vegetables.

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