Canadian Journal of Plant Science
 Revue canadienne de phytotechnie

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Journal:	<i>Canadian Journal of Plant Science</i>
Manuscript ID	CJPS-2017-0365.R1
Manuscript Type:	Article
Date Submitted by the Author:	14-Feb-2018
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Keywords:	Phospholipase D, shelf-life, blue honeysuckle, fruit drop, <i>Lonicera caerulea</i>

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Performance of five haskap (*Lonicera caerulea* L.) cultivars and the effect of hexanal on postharvest quality

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Growers are challenged to provide premium, ripe haskap fruit to market while limiting the negative attributes associated with overripening. Hexanal is an inhibitor of phospholipase D, an enzyme involved in membrane degradation, and has shown promise for extending the longevity of fruit. This study investigated the performance of a hexanal-based preharvest spray for enhancing the quality and shelf life of haskap fruit, an emerging crop in Canada. At two locations in 2015, 2016, and 2017, five cultivars were sprayed with a control or a 0.02% hexanal formulation at two to three weeks preharvest. Fruit was stored at 4 °C and assessed for quality at successive times. Although inconsistent, results suggest a hexanal spray may impart a small benefit to the postharvest quality of haskap fruit. Secondary objectives included describing important agronomic characteristics of haskap and assessing the potential of a hexanal preharvest spray for enhancing fruit retention. Hexanal did not reduce fruit drop and had no effect on quality at harvest. Fruit contained high levels of soluble solids and titratable acids, were very

dark in colour, and did not degrade in quality as rapidly as softer fruit crops such as strawberry or raspberry.

Key words: Phospholipase D, shelf life, blue honeysuckle, fruit drop, *Lonicera caerulea*

Abbreviations: Commission Internationale de l'Eclairage (CIE), control (CTL), DPH (number of days stored postharvest), Enhanced Freshness Formulation (EFF), phospholipase D (PLD), soluble solids concentration (SSC), titratable acidity (TA)

Fruit ripening is an irreversible developmental process that involves numerous biochemical and physiological changes that result in a fruit with favourable organoleptic properties (Giovannoni 2001). In addition to the development of pigments, sugars, acids, and aromatic volatiles, the softening of fruit tissues is an important aspect of the ripening process. This decline in tissue structure, which is driven by the deterioration of cell walls and cell membranes, results in the loss of compartmentalization of ions and metabolites and ultimately homeostasis (Paliyath et al. 2008). Cell wall disassembly occurs through the increased action of enzymes such as cellulase and pectinase (Negi and Handa 2008) whereas membrane degradation is initiated by the enzyme phospholipase D (PLD); a key enzyme in the lipid catabolic pathway (Paliyath and Droillard 1992).

Haskap (*Lonicera caerulea* L.), also known as the blue honeysuckle, is an early flowering fruit species with several important horticultural subspecies native to the northern boreal forests of North America, Europe, and Asia. Haskap fruit are dark blue to purple berries with variable shapes that range from oval to long and thin. The fruit are formed from pale yellow flowers that develop in couplets in early spring before leaf emergence. Haskap are generally cultivated in

northern regions due to their exceptional cold hardiness. Some reports suggest that haskap flowers can tolerate temperatures as low as -8°C and whole plants as low as -40°C (Hummer et al. 2012; Ochmian et al. 2012). The nutritional value and potential health benefits of consuming haskap fruit have been well documented (Palíková et al. 2009; Rupasinghe et al. 2012; Wu et al. 2015; Caprioli et al. 2016) and are the subject of a critical review by Celli et al. (2014). Haskap fruit are rich in a variety of bioactive compounds, particularly phenolics, anthocyanins, and vitamin C (Wojdyło et al. 2013; Zhao et al. 2015). Due to their superior cold hardiness, high nutritional content, and potential health benefits, haskap production has gained increasing popularity in Canada and the northern U.S. in recent years (Hummer et al. 2012; Burns 2015; Herrick 2016).

Emerging crops like haskap that appeal to market preferences for nutritious, local food can be highly profitable. Unfortunately, similar to many other high value fruit crops, haskap plants can have significant levels of fruit drop prior to harvest, thereby reducing economic benefit. Also, growers of these crops are challenged by a limited amount of information regarding appropriate postharvest handling and storage procedures. The highly perishable nature of fresh fruit necessitates rapid transport or sophisticated means of regulating postharvest metabolism, both of which can be economically difficult for the success of emerging fruit crops.

There has been increasing interest into extending the longevity of horticultural produce through the inhibition of PLD, an enzyme involved in fruit deterioration (Paliyath and Subramanian 2008; Sharma et al. 2010; Cheema et al. 2014; Gill et al. 2016; El Kayal et al. 2017a, 2017b; Kumar et al. 2018). PLD activity is strongly inhibited by hexanal, a naturally occurring, volatile, C_6 aldehyde (Paliyath et al. 1999; Paliyath et al. 2003; Tiwari and Paliyath 2011). Hexanal is the active ingredient of the 'Enhanced Freshness Formulation' (EFF; Paliyath

et al. 2003; Paliyath and Murr 2007) that is used to inhibit the catabolic reactions that are controlled by PLD. Preharvest spray application of EFF can extend shelf life of several commercially important fruit crops such as nectarine (Kumar et al. 2018), tomato (Cheema et al. 2014), guava (Gill et al. 2016), sweet cherry (Sharma et al. 2010) and strawberry (El Kayal et al. 2017b) through the inhibition of PLD. In addition to enhancing shelf life, preharvest sprays of EFF were shown to increase fruit retention of mango (Anusuya et al. 2016) and raspberry (El Kayal et al. 2017a).

Prolonged tissue softening during postharvest storage increases the susceptibility of fruit to pathogens, leading to reduced quality and shelf life (Goulao and Oliveira 2008). As such, fruit growers are challenged to deliver ripe fruit with favorable characteristics to market while inhibiting or delaying the ripening process enough to avoid over softening and decay that is associated with overripe fruit (Matas et al. 2009). The use of an EFF may hold promise to enhance the shelf life of haskap fruit and reduce fruit drop with applications of a preharvest spray. For this study, we used an EFF containing hexanal to spray five popular haskap cultivars at the degreening stage of fruit development. The primary objective of this investigation was to assess the performance of a preharvest EFF application for enhancing the shelf life and reducing fruit drop of haskap fruit. A secondary objective was the characterization of several important horticultural traits of five haskap cultivars that are of interest to consumers and growers.

MATERIALS AND METHODS

Field Trials

Plantings of haskap (*Lonicera caerulea* L.) at the University of Guelph's Elora Research (43°38'N, 80°24'W) and Simcoe Research Stations (42°51'N, 80°15'W) served as the study

locations for assessing the effects of a preharvest EFF spray on postharvest fruit quality. The haskap plantings consisted of five commercially available cultivars (Borealis, Czech No.17, Indigo Gem, Indigo Treat, and Tundra) that were planted in 2010. At the Elora location in 2016 and 2017, two plots in a separate planting of Borealis were used to assess fruit drop in response to a preharvest EFF spray. Management practices for these two whole plots were similar to those for the main planting at the Elora location. For all haskap plantings, plants were spaced 1.2 m apart, with 2.5 m between rows and were protected with bird netting from start of berry development until harvest.

In 2015 and 2017, haskap fruit were also collected from a similarly aged planting of haskap at the University of Guelph's Simcoe Research Station. The management practices at this Simcoe location were similar to those described for Elora, with the main differences being the addition of straw mulch and the use of naturally occurring pollinators. In 2016, the Simcoe trial was abandoned because of extensive damage by birds. In 2017, due to a delayed harvest and extensive damage due to spotted wing drosophila (*Drosophila suzukii*), Czech No.17 and Borealis were excluded from the harvest in Simcoe.

Hexanal Treatments

Each year haskap plants were sprayed three times with the EFF, at weekly intervals starting from the degreening stage. A stock solution of EFF was prepared according to protocols described by Misran et al. (2015). Just before application, a 0.02% (v/v) EFF was prepared by diluting a stock solution with water (1:50 v:v) and thoroughly mixing by agitation. A control treatment was prepared from a similar stock solution containing the same ingredients but without hexanal. Using a pressurized hand-held sprayer, treatment solutions were applied in the morning to the

developing fruit within each subplot to the point of dripping. The two treatments were applied starting at the degreening stage of haskap fruit development and then every five days.

Experimental Design

The main haskap plantings at the Elora and Simcoe locations utilized a randomized split-plot complete block design with repeated measures (measurements performed throughout postharvest storage) and four blocks. Cultivar served as the whole plot factor to which the EFF subplot treatments were assigned. Each whole plot was approximately 3.75 m in length. Four plants composed the whole plots and one of two treatments were assigned to each experimental unit (a subplot consisting of two adjacent plants). Quality parameters were repeatedly measured at successive times during postharvest storage at 4 °C. Physicochemical assessments were performed at a later date with fruit that was frozen after various durations of postharvest storage.

At the Elora location, a 2008 planting of Borealis was used for monitoring fruit drop in response to a control or EFF preharvest spray. Two non-adjacent whole plots were assigned either a control or EFF treatment that was applied at the same time as treatment applications for the main 2010 haskap planting. A 1.5 m × 12 m fabric row cover was placed under both sides of the rows for collection of fallen fruit. At approximately three day intervals, plants were gently shaken and the fallen fruit on top of the fabric row cover were collected and counted. The percentage of total fruit dropped during the study period was assessed for each plant until all fruit had fallen. The percentage of dropped fruit was determined by dividing the cumulative number of dropped fruit at each measurement date by the total number of fruit dropped throughout the entire study period.

Yield, Average Fruit Weight, and Postharvest Shelf Life

Ripe, undamaged fruit were assessed for the number and total weight of fruit. Average fruit weight was determined by measuring the weight of 20 berries. The weight of fruit was summed across harvest dates to generate the total yield per treatment subplot. Postharvest shelf life was assessed by measuring the percent of marketable fruit (100% at harvest), determined by assessing a small sample (≈ 70 fruit) from each treatment subplot. Fruit were considered unmarketable if they possessed any visual signs of bruising, tissue softening, or decay. Firmness measurements were attempted with a digital penetrometer equipped with a 2 mm diameter flat probe (FHT200; Extech Instruments, Nashua, NH). However, data could not be collected due to the small size and softness of the fruit. Both average fruit weight and shelf life were assessed at three to seven day intervals postharvest. Days stored is the number of days between harvest date and each measurement date. Yield per plant and postharvest shelf life assessments were only conducted for the Elora trial.

Soluble Solids Concentration, pH, and Total Titratable Acids

A sample of 10 to 20 fruit from each replication was homogenized and passed through cheese cloth to extract juice for measurements of soluble solids concentration (SSC), pH, and total titratable acidity (TA). Using several drops of juice from each replication, SSC was measured using a handheld digital refractometer (PAL-1; ATAGO Co., Ltd., Tokyo, Japan) and expressed as percent soluble solids (%). Using extracted juice, pH was determined with a benchtop pH meter (sympHony B10P; VWR International, Radnor, PA). Total TA of haskap fruit was assessed for the Elora trial only. Ten millilitres of filtered juice were titrated with 0.1 M NaOH to a pH of 8.1 according to general guidelines on objective tests (The Organisation for Economic

Co-operation and Development 2009). Using calculations outlined in Sadler and Murphy (2010), TA was expressed as percent (w/v) citric acid equivalent.

Colour

The colour of haskap fruit was assessed for the Elora trial in 2015 only. The skin colour of three fruit from each block were measured in the $L^*a^*b^*$ colour space according to guidelines developed by the Commission Internationale de l'Eclairage (CIE; 2004). Fruit colour was measured with a chroma meter (CR-10 Chroma Meter; Konica Minolta, Inc., Tokyo, Japan) relative to the standard illuminant D65 over a white background with an 8 mm measuring port aperture. The a^* and b^* values from the CIE $L^*a^*b^*$ colour space were transformed to derive colour measurements in the CIE L^*C^*h colour space using the following equations:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (\text{Eq. 1})$$

$$h = \arctan(b^*/a^*) \quad (\text{Eq. 2})$$

where C^* is chroma, the relative chromatic intensity of a colour and h is hue angle, a value between 0° and 360° that represents a saturated colour within the CIE L^*C^*h colour space.

Quadrant compensation for the calculation of hue angle was performed according to the procedure described in McLellan et al. (1994). Colour was measured on two sides of each fruit and the average of the two measurements was determined.

Statistical Analyses

Data analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Unless stated otherwise, each location and year were evaluated separately and individual harvest dates were considered as separate trials of the same experiment. The average of subsample observations within each experimental unit was used for all of the statistical analyses. An analysis of variance was conducted for each dependent variable using the generalized linear mixed model procedure (Proc Glimmix) in SAS. This procedure was used to identify significant sources of variation for the fixed effects of treatment, number of days stored (shelf life examination only), and the interactions between these fixed effects. The normality assumptions for an analysis of variance were assessed and the Satterthwaite approximation for the denominator degrees of freedom was used to account for heterogeneous variances. Days stored was designated as a repeated within-subject effect with a spatial power covariance structure. Block and block $\times$ treatment were designated as random effects with a compound symmetric covariance structure. Post-hoc least squares means comparisons were performed with a Tukey-Kramer test ($P \leq 0.05$).

RESULTS

Yield and Yield Components

Elora Trial

Traits that were measured at harvest exhibited some moderate differences between cultivars. There were no significant differences in yield among cultivars and the average yield over the three years and among the five haskap cultivars was 117 g per plant (Table 1). In general, the average weight of haskap fruit in the Elora trials ranged from 350 to 730 mg depending on the particular cultivar (Table 1). Czech No.17 had a significantly lower average fruit weight than the other cultivars in the trial and also had the lowest yield per plant (Table 1). The majority of the

means comparisons for colour measurements of haskap fruit did not identify any large differences between cultivars with respect to lightness, chroma, or hue angle (Table 1). There was some moderate variation between cultivars for chroma and hue angle but lightness was relatively stable among the five haskap cultivars (Table 1). Of the five cultivars assessed for colour at harvest, Czech No.17 fruit had the greatest lightness, chroma, and hue angle values, which indicates this cultivar has a moderately increased colour intensity in comparison to the other four haskap cultivars (Table 1). Overall, the five haskap cultivars in this experiment developed fruit with very dark blue to purple colouration.

SSC, pH, and titratable acidity measurements for haskap cultivars at the Elora location had few differences. Averaged over cultivars, haskap fruit at harvest had a SSC of 13.8, 16.9, and 17.1% Brix for 2015, 2016, and 2017 respectively (Table 2). Limited differences were observed between cultivars for pH measurements performed at harvest. The greatest pH variability occurred in 2016 and there were small but significant differences between cultivars for the pH of haskap fruit in this year (Table 2). In 2015 and 2017, the pH of haskap fruit had a narrow range between 3.0 and 3.2 with Borealis and Czech No.17 having slightly lower pH than the other three cultivars (Table 2). In regard to TA, there was some moderate variation among the cultivars for the level of titratable acids. Over all three years, Borealis exhibited increased TA in comparison to the other cultivars in the trial (Table 2). Alternatively, throughout this study the fruit of Indigo Gem had reduced levels of TA in comparison to the other cultivars in the trial (Table 2).

Simcoe Trial

The harvest dates of haskap cultivars in Simcoe, from the earliest to the latest maturing cultivars, varied by 21 days in 2015 and 9 days in 2017. The cultivars can be divided into three maturity groups: early- (Indigo Gem and Indigo Treat), mid- (Czech No.17 and Tundra) and late-season (Borealis) (Table 3). The traits measured at harvest for the Simcoe trial exhibited the same moderate variability among cultivars that was observed for the Elora trial. Similar to the Elora trials, in 2015 the Czech No.17 cultivar had a significantly lower average fruit weight (450 mg) in comparison to the other cultivars, which all ranged in weight from 840 to 882 mg (Table 3). In comparison to 2015, the average fruit weights of Indigo Gem and Indigo Treat in 2017 were reduced by an average of 149 mg whereas Tundra maintained a similar average fruit weight between the two seasons (Table 3). The SSC of haskap grown in Simcoe was relatively stable across cultivars and seasons and ranged 12.7 to 16.1% (Table 3). Indigo Gem attained the highest SSC in both 2015 and 2017 with observations of 15.4 and 16.8% respectively (Table 3). Excluding Borealis and Czech No.17, the ranking of cultivars for SSC was the same between the 2015 and 2017 growing seasons at Simcoe (Table 3). Measurements of pH from the Simcoe trial were comparable to those attained in the Elora trial and contained several small statistical differences between cultivars (Table 3). The average pH value of Borealis in 2015 was 3.0 and, similar to the performance at Elora, was the lowest observed among the five cultivars (Table 3).

Effect of Hexanal on Shelf life

Elora Trial

The average weight of a haskap fruit decreased during postharvest storage (Table 4). Comparisons of means did not identify any significant differences between the EFF and control treatments for the response of average fruit weight, pH, colour, SSC, or total TA during

postharvest storage (data not shown). The percentage of marketable fruit during postharvest storage was significantly influenced by the number of days stored postharvest (Table 5). The rate at which fruit marketability decreased varied between growing seasons. For example, in 2015 and 2016, 60 to 70% of haskap fruit remained marketable after 7 days of postharvest storage (Table 5). However, in 2017, only an average of 30% of the fruit within a sample were marketable after 7 days of postharvest storage (Table 5).

The proportion of marketable fruit during postharvest storage was affected by cultivar and harvest date but was not consistently improved with the application of an EFF. For the first harvest of 2015, the EFF treatments on Indigo Gem resulted in a significantly greater percentage of marketable fruit after 11 days of storage (Table 5). For the second harvest of 2016, at three days of storage, Borealis and Indigo Gem had a greater percentage of marketable fruit due to EFF than with the control treatment (Table 5). In 2017, Czech No.17 was the only cultivar that had a significant treatment by days stored interaction effect for the percentage of marketable fruit. However, means comparisons of the percent marketable fruit at each measurement date did not reveal significant differences between the two treatments (Table 5).

Simcoe Trial

There was a decline in average fruit weight in response to postharvest storage for the haskap cultivars at the Simcoe location (Table 6). In 2015, average fruit weight significantly decreased during postharvest storage but there were no significant differences between EFF and control treatments during storage (Table 6). Haskap fruit lost an average of 23 to 76 mg, or 3 to 12%, after two weeks of cold storage (Table 6). At Simcoe, the application of an EFF preharvest spray had no effect on postharvest weight loss, pH, or SSC of haskap fruit at harvest or during

postharvest storage at 4 °C. At harvest and throughout postharvest storage the pH and SSC content of haskap fruit were in the range of 3.0–3.6 and 13–16%, respectively (Table 6).

Effect of Hexanal on Fruit Retention

In general, the majority of fruit dropped from the haskap plants in 2016 and 2017 occurred within a twenty day period starting near the beginning of harvest. In 2016, the number of dropped fruit increased with observation date following a linear model whereas a non-linear relationship between fruit drop and observation date was observed for 2017 (Figure 1). For both 2016 and 2017, the control and EFF treatments had similar rates of fruit drop during the study period (Figure 1). In 2016, at 4 and 18 days after the start of monitoring fruit retention, there were significant differences between the two treatments for the proportion of total dropped fruit within study period (Figure 1). For these two observation dates, EFF treated plants dropped a significantly greater proportion of fruit than the control plants (Figure 1). In 2017 there were no significant differences between the two treatments for the number of dropped fruit (Figure 1).

DISCUSSION

Traits

Several characteristics of haskap that have importance to producers and consumers of haskap fruit in Canada were measured. Due in part to the high nutritional content, tolerance to cold temperatures, and growing consumer demand for local, sustainable food sources, haskap production and popularity are increasing in Canada (Bors et al. 2012; Burns 2015; Herrick 2016). To date, data on the performance and quality measurements are lacking for eastern Canada.

Average fruit weight, a key yield component, significantly differed between cultivars at the Elora and Simcoe locations. For both locations within our study, the average fruit weight of Czech No.17 was approximately half that of the other four cultivars. Alternatively, Indigo Gem and Borealis had the highest fruit weight among the haskap cultivars examined at these locations in 2015. The average fruit weight of haskap cultivars in this study was considerably lower than previously published reports on haskap fruit weight that range from 1000 to 2000 mg (Skupień et al. 2009; Malodobry et al. 2010; Miyashita and Hoshino 2010; Ochmian et al. 2012). New releases, such as ‘Boreal Blizzard’ from the University of Saskatchewan haskap breeding program, have much larger fruit that average 2800 mg (Bors 2017).

In addition to varietal variation, environmental differences are a likely source for the differences in average fruit weight between this experiment and other published reports of haskap fruit weight. On average, haskap fruit weight was approximately 100 mg greater in Simcoe than in Elora, suggesting growing conditions in Simcoe were more favourable for haskap production than the conditions experienced in Elora. The Simcoe location is approximately 80 km south of Elora and had warmer temperatures (with a mulch), improved irrigation and better weed management over the Elora trial. Taken together, these differences in growing conditions are probable causes for the increased fruit weight observed at Simcoe in comparison to Elora.

Yield per plant is a characteristic that is of great importance to growers. In comparison to other published accounts of haskap yield, the yield per plant was relatively low for the five cultivars assessed in this study. The highest yielding cultivar within our study, Indigo Treat, produced an average of 155 g of marketable fruit per plant. The highest yield observed in this study was significantly less than the yield of haskap reported by other studies (Plekhanova 2000; Malodobry et al. 2010; Miyashita and Hoshino 2010; Ochmian et al. 2012; Wojdyło et al. 2013),

where haskap yield ranged between 0.5 and 1.5 kg per plant. Many of the locations for these studies (e.g. Japan, Poland, and Russia) are known to have more temperate climates than southern Ontario, suggesting environmental variation may be limiting the yield performance of haskap. For example, mid-winter warm spells that are common to southern Ontario can cause premature bud break and result in damage to susceptible tissues, thereby reducing yield.

In addition to low average fruit weight, the removal of bird netting when applying treatments and harvesting fruit imposed physical stress to the plants and may have caused fruit to drop reducing yield per plant further. At the outset of experimentation, because of several field management limitations, the yield per plant at Elora was not expected to attain levels reported by commercial growers. However, the drastically reduced yield per plant for this study in comparison to reports from more temperate regions may indicate growing conditions are less than ideal for haskap production in southern Ontario. As evidence for this, the average plant height for the cultivars in this study was 86 cm, which is 30 to 60% lower than the mature plant height reported in cultivar descriptions provided by Canadian commercial nurseries (Neil Vanderkruk Holdings Inc. 2012; Prairie Plant Systems Inc. 2015).

Although haskap fruit are noted for their deep blue colour and high pigmentation, there have been very few published accounts of analytical measurements of fruit colour (Ochmian et al. 2012; Yamamoto et al. 2014; Lee et al. 2015). These research groups assessed fruit colour in the CIE $L^*a^*b^*$ colour space at several stages of ripening and provided the opportunity to make comparisons to fruit from our experiment. Using a formula for quantifying colour differences, CIEDE2000 (CIE 2004), an exploratory analysis of colour differences between our observations and values reported in the literature was performed. The CIEDE2000 formula generates ΔE_{00} , a value that represents the distance between two colour values in the CIE $L^*a^*b^*$ colour space.

ΔE_{00} can be any value between 0 (no difference in colour) and 100 (colours are exact opposites). In comparison to colour data reported by Ochmian et al. (2012), haskap fruit within our study were most similar in colour to 'Wojtek' and 'Brazowa' cultivars at the ripe stage of fruit development (ΔE_{00} = 12–15). Lee et al. (2015) assessed the colour of freeze-dried fruit harvested from a Korean haskap cultivar at seven stages of ripening. For this study, fruit at the seventh stage of ripening were the most similar in colour to the measurements of fruit colour within our study (ΔE_{00} = 16). Furthermore, colour measurements of mature fruit from a Japanese haskap variety by Yamamoto et al. (2014) were very similar to the colour measurements observed within this study (ΔE_{00} = 2.5). Small colour differences between this study and these previously published examinations of haskap fruit colour may be attributable to a combination of differences in the stages of ripening, specific cultivars being assessed, and locations that were used for experimentation.

Soluble solids concentration is an important quality trait measured in many economically important fruit species. In general, SSC measurements within our study ranged between 13 and 18% which were similar to values reported in the literature (Malodobry et al. 2010; Ochmian et al. 2012; Wojdyło et al. 2013). Two of these studies both examined the SSC of the cultivar 'Duet' and reported SSC values of 10.4 to 11.5% and 15.8% (Malodobry et al. 2010; Wojdyło et al. 2013). These studies were conducted at three different locations in Poland (northwest, central, and southern Poland) where the season is expected to have slightly milder winters and cooler summers in comparison to southern Ontario. Within our study, we observed variability between growing seasons for SSC at Elora and Simcoe. For both locations, SSC was marginally lower for 2015 than for 2016 or 2017. The low SSC for Elora and Simcoe in 2015 may be associated with these two locations having lower average June temperatures in 2015 than in 2016 or 2017. There

was variability in SSC between the Elora and Simcoe locations but no distinct patterns of variation could be identified. In comparison to other commercially important fruit species, the SSC of haskap fruit is in the higher end of the range of typical SSC measurements (Sadler and Murphy 2010).

Although both pH and total TA are associated with acidity, total TA is a better predictor of the impact of an acid on flavour than pH (Sadler and Murphy 2010). Among the five cultivars examined in this study, Borealis had the greatest percent citric acid equivalent for 2015, 2016 and 2017, with respective values of 3.0, 1.9 and 2.8% citric acid equivalent. The total TA of haskap fruit in this experiment and other studies were highly variable and may be related to regional and genetic differences. Malodobry et al. (2010) reported 1.8 to 3.1 g citric acid 100 g⁻¹ and Wojdyło et al. (2013) observed an average of 1.9% citric acid, both fairly similar to TA values reported in this study. In contrast, two studies conducted in northwest Poland identified a total TA content that was greater than observations in our study. Skupień et al. (2009) documented a total TA content of 2.3 to 2.8 g citric acid 100 g⁻¹ and Ochmian et al. (2012) noted 2.7 to 3.4 g citric acid 100 g⁻¹, both greater than many observations within this experiment. Differences between this study and previously published examinations of TA content in haskap fruit may be attributable to environmental differences or the examination of different haskap cultivars that are of Russian origin. In comparison to other commercially important fruits at maturity, the acid profile of haskap in this research is most similar to that of raspberries (Sadler and Murphy 2010).

The pH of haskap fruit in the current study was relatively stable and ranged from 3.0 to 3.5, supporting previous results of haskap pH (Malodobry et al. 2010; Wojdyło et al. 2013). At both Elora and Simcoe locations, Borealis fruit had a significantly lower pH than many of the

cultivars. Using comparisons of pH values for 2015, 2016, and 2017 respectfully, Borealis was 1.4, 1.3, and 1.1 times more acidic than the average of the four other cultivars in this study. Similarly, for each year of this study the total TA of Borealis was the greatest observed among the five haskap cultivars. Averaged across 2015, 2016, and 2017, Borealis had approximately 0.84% greater citric acid equivalents than the average TA of the other four cultivars.

Both assessments of acidity, pH and total TA content, for Borealis indicate the presence of increased amounts of food acids and possibly increased sour flavours for this cultivar. However, since the tartness of an acid is reduced by sugars, considering acid content alone does not offer a clear indication of sourness (Sadler and Murphy 2010). In addition, the details surrounding the chemistry of sour compounds, the interaction of these compounds with taste receptor cells, and the neurological responses that mediate sour taste perception in humans are still being investigated (Neta et al. 2007). Current understanding of sour taste perception suggests that sour flavours are primarily influenced by the concentration of free hydrogen ions and the total molar concentration of organic acid species with protonated carboxyl groups (Neta et al. 2007). As such, it is difficult to ascertain whether the elevated levels of food acids present in the fruit of Borealis contribute to increased bitter flavours.

Effect of Hexanal on Shelf life

The performance of a preharvest hexanal-containing spray on the postharvest quality and shelf life of five commercially available haskap cultivars was quantified through assessments of marketability and physicochemical traits during postharvest storage. The ability of hexanal-based formulations to inhibit PLD activity, reduce membrane degradation, and enhance postharvest shelf life has been demonstrated for both climacteric and non-climacteric fruit (Paliyath et al.

2003; Paliyath and Subramanian 2008). Hexanal-based formulations have been used successfully as preharvest sprays to increase postharvest quality of sweet cherry (Sharma et al. 2010), strawberry (El Kayal et al. 2017b), nectarine (Kumar et al. 2018), tomato (Cheema et al. 2014), guava (Gill et al. 2016), and mango (Anusuya et al. 2016). These studies were in a wide range of geographic locations, examined a variety of fruit species, and demonstrate the potential of hexanal to enhance postharvest quality and shelf life for many economically important fruit species. This is the first examination of the performance of a preharvest hexanal-based spray for enhancing postharvest quality and shelf life of haskap fruit.

For several combinations of cultivar, year and harvest date, samples of EFF treated fruit had a significantly longer shelf life when compared with the control. Aside from these few occasions where EFF successfully enhanced postharvest quality, the majority of comparisons between control and EFF were not significantly different. Since this marginal improvement to postharvest shelf life requires multiple pre-harvest sprays, EFF is not likely an economical method for enhancing shelf life and quality of haskap fruit.

One of the problems with haskap fruit relative to the EFF treatment is that it is difficult to determine the proper stage for picking. The fruit attain a very dark colour early in the ripening process, making identification of the optimum harvest stage difficult. This leads to the harvest of fruit at various stages of ripeness and difficulty assessing the effectiveness of an EFF preharvest spray. In 2017, haskap fruit degraded rapidly with just 20 to 40% of the fruit remaining marketable for fresh consumption after 4 days of postharvest storage. Since fruit with higher moisture content generally have poorer storage quality (Thomson 2014), the rapid degradation of fruit in 2017 may be due to a heavy rainfall that preceded harvest. Overall, results for this experiment suggest haskap fruit are resilient to postharvest loss of quality and have the potential

for a relatively long shelf life in comparison to other soft fruit such as raspberries and strawberries.

Fruit Retention

Fruit drop is a horticultural trait that is important to fruit growers for maximizing harvestable yield. Both EFF and control treated haskap plants dropped fruit at similar rates. In mango, a preharvest spray of EFF significantly enhanced the retention of fruit due to inhibition of RNA and protein synthesis in the abscission zone, thereby delaying the onset of abscission and extending the retention of fruit in trees (Anusuya et al. 2016). The EFF was also shown to enhance fruit retention of raspberry by strengthening the attachment of the berry to the receptacle through the increased presence of rigid epidermal hairs (El Kayal et al. 2017a). Although a preharvest EFF spray was favourable for fruit retention of mango and raspberry, it did not enhance the retention of haskap fruit and appears to have had no effect on the formation of the abscission layer. This would have benefitted crop management because the dark colouration of haskap fruit in the early stages of fruit development often leads to the harvest of under ripe fruit with astringent flavours. To avoid this problem, the harvest may be delayed in order to allow fruit to develop the desired sweetness required for fresh consumption. However, this delay contributes to harvest losses caused by fruit drop.

Conclusions

The yield and average fruit weight of the haskap cultivars grown in Ontario were significantly lower than previously published reports from other regions in Canada (Bors et al. 2012). The average fruit weight in Ontario was less than one gram and fruit yield was generally

100 – 150 grams per plant. Quality traits of haskap fruit were relatively uniform across cultivars, locations, and years and can be summarized as high soluble solids concentration, a high concentration of titratable acids and dark blue colouration. The performance of an EFF preharvest spray did not reduce fruit drop and provided only a small and inconsistent benefit to postharvest quality of haskap fruit. The EFF preharvest spray had no detrimental effects on quality traits at harvest.

Acknowledgements

This project was undertaken with the financial support of Canada's International Development Research Centre (IDRC), www.idrc.ca, and the Government of Canada, provided through Global Affairs Canada (GAC), www.international.gc.ca and Ontario Ministry of Agriculture, Food and Rural Affairs.

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Table 1. Yield, average fruit weight and colour of five haskap cultivars grown in Elora.

Cultivar	Yield ^a (g plant ⁻¹)	Average fruit weight (mg)	Fruit colour		
			<i>L</i> *	<i>C</i> *	<i>h</i>
Borealis	102a	803a	23.7b	3.0ab	292.9ab
Indigo Gem	133a	729ab	22.5b	2.7b	285.4ab
Indigo Treat	155a	631b	23.8b	3.0ab	282.1b
Tundra	104a	701ab	24.1b	3.0b	283.1ab
Czech No.17	91a	384c	25.5a	3.6a	296.2a
<i>P</i> value ^c	0.023	<0.001	0.169	0.280	0.093

Note: Within each column, least squares means followed by the same letter are not different based on a Tukey-Kramer test at the $P < 0.05$ level.

^a Yield and average fruit weight data were pooled over treatments (control and Enhanced Freshness Formulation), multiple harvests and over years (2015–2017).

^b *P* values denote the level of significance for the cultivar main effect.

Table 2. Soluble solids concentration (SSC), pH, and titratable acidity (TA) of five haskap cultivars grown in Elora in 2015, 2016, and 2017.

Cultivar	SSC (%)	pH	TA (% citric acid equivalent)
2015			
Borealis	14.7a	3.0b	3.0a
Indigo Gem	14.5a	3.2a	1.5b
Indigo Treat	13.1a	3.2a	1.7b
Tundra	12.4a	3.1ab	1.7b
Czech No.17	14.2a	3.1a	1.6b
P value ^a	0.054	0.003	<0.001
2016			
Borealis	13.8d	3.2d	1.9a
Indigo Gem	19.9a	3.5a	1.2d
Indigo Treat	15.4cd	3.4b	1.6c
Tundra	17.9b	3.3c	1.8ab
Czech No.17	16.7bc	3.1d	1.6bc
P value	<0.001	<0.001	0.002
2017			
Borealis	16.1bc	3.1b	2.8a
Indigo Gem	17.4b	3.2a	1.6c
Indigo Treat	15.2c	3.2a	2.0b
Tundra	17.4b	3.2a	2.1b

Czech No.17	19.2 ^a	3.0 ^b	2.3 ^b
<i>P</i> value	<0.001	<0.001	<0.001

Note: Within each year and trait, least squares means followed by the same letter are not statistically different according to a Tukey-Kramer test ($P \leq 0.05$). Data were pooled over multiple harvests and treatments (control and Enhanced Freshness Formulation) to calculate least squares means.

^a *P* values denote the level of significance for the cultivar main effect.

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Table 3. Average fruit weight, soluble solids concentration (SSC), and pH of five haskap cultivars grown in Simcoe in 2015 and 2017.

		First	Average fruit		
		harvest	weight	SSC	
	Cultivar	date	(mg)	(%)	pH
2015	Borealis	July 17	882a	12.7b	3.0a
	Indigo Gem	June 26	848a	15.4a	3.2a
	Indigo Treat	June 26	841a	14.4ab	3.3a
	Tundra	July 10	841a	14.3ab	3.2a
	Czech No.17	July 10	450b	13.6ab	3.4a
	<i>P</i> value ^a		0.001	0.058	0.380
2017	Indigo Gem	June 27	674a	16.8a	3.3a
	Indigo Treat	June 27	718ab	16.4a	3.4b
	Tundra	July 6	858b	14.1b	3.3a
	<i>P</i> value		0.042	0.002	0.019

Note: For each trait, data were pooled over multiple harvests and treatments (control and Enhanced Freshness Formulation) to calculate least squares means. Within each year, least squares means followed by the same letter are not statistically different according to a Tukey-Kramer test ($P \leq 0.05$).

^a *P* values denote the level of significance for the cultivar main effect.

Table 4. Average fruit weight of five haskap cultivars grown in Elora in 2015, 2016, and 2017 and stored at 4 °C.

Days stored postharvest	Average fruit weight (mg)				
	Borealis ^a	Indigo Gem	Indigo Treat	Tundra	Czech No.17
2015 Harvest 1					
0	814a	828a	744a	792a	425a
4	795a	709b	707ab	793a	371ab
7	729a	759ab	708ab	782a	358ab
11	763a	681b	632b	727a	320b
<i>P</i> value ^b	0.274	0.003	0.032	0.240	0.050
2015 Harvest 2					
0	690a	713a	674a	774a	385a
5	— ^c	683ab	614ab	818a	378ab
12	505a	594b	510b	693a	308b
<i>P</i> value	0.111	0.038	0.052	0.070	0.030
2016 Harvest 1					
0	—	635a	582a	—	—
9	—	656a	610a	—	—
13	—	601a	—	—	—
16	—	600a	555a	—	—
<i>P</i> value	—	0.131	0.055	—	—
2016 Harvest 2					

0	749a	555a	495a	492a	344b
3	684ab	537ab	490a	483a	387a
8	690ab	498ab	489a	468a	351b
15	656b	471b	485a	455a	361b
<i>P</i> value	0.014	0.026	0.961	0.070	<0.001

2017 Harvest 1

0	996a	799a	706a	741a	408a
4	980a	781ab	681b	721b	402ab
7	941b	757bc	672c	710c	394ab
10	920bc	745c	653d	693d	385b
12	909c	733c	647e	683e	391ab
14	–	–	–	672f	386b
<i>P</i> value	<0.001	<0.001	<0.001	<0.001	0.003

Note: For each harvest date, least squares means followed by the same letter within each cultivar are not statistically different according to a Tukey-Kramer test ($P \leq 0.05$).

^a Cultivars were evaluated separately for each year and harvest.

^b *P* values denote the level of significance for the days stored main effect.

^c Missing data.

Table 5. Percent marketable fruit of five haskap cultivars grown in Elora and subjected to either a control (CTL) or Enhanced Freshness Formulation (EFF) preharvest spray in 2015, 2016, and 2017.

Percent marketable fruit										
(%)										
	Borealis ^b		Indigo Gem		Indigo Treat		Tundra		Czech No.17	
DPH ^a	CTL	EFF	CTL	EFF	CTL	EFF	CTL	EFF	CTL	EFF
2015 Harvest 1										
0	100	100	100	100	100	100	100	100	100	100
4	84	78	86	94	93	93	95	98	87	88
7	76	75	86	93	97	100	94	94	91	80
11	49	43	15 [*]	53 [*]	46	61	60	79	92 [*]	71 [*]
P value ^c	0.585		<0.001		0.479		0.150		0.011	
2015 Harvest 2										
0	— ^d	—	100	100	100	100	100	100 ^a	100	100
5	—	—	86 [*]	96 [*]	93	96	90 [*]	99 [*]	98	97
P value			0.013		0.456		0.017		0.667	
2016 Harvest 1										
0	—	—	100	100	100	100	100	100	—	—
6	—	—	83	82	84	84	59	91	—	—
9	—	—	66	63	64	70	64	78	—	—
13	—	—	66	64	69	63	—	—	—	—
P value			0.957		0.044		0.285			

2016 Harvest 2										
0	100	100	100	100	100	100	100	100	100	100
3	72 [*]	83 [*]	70 [*]	77 [*]	74	73	74	86	88	82
8	59 [*]	65 [*]	62	64	57	61	59 [*]	78 [*]	69	69
15	53 [*]	59 [*]	53	55	53	54	53	62	61	63
<i>P</i> value	0.001		0.038		0.402		0.030		0.362	
2017 Harvest 1										
0	100	100	100	100	100	100	100	100	100	100
4	25	41	—	—	41	44	64	61	54	63
7	12	19	32	26	22	24	44	38	42	51
10	1	4	12	15	12	19	29	25	23	29
12	—	—	7	11	6	8	25	22	21	14
14	—	—	—	—	—	—	13	12	1	10
<i>P</i> value	0.149		0.063		0.887		0.977		0.008	

Note: Least squares means followed by an asterisk indicate a significant difference between control and Enhance Freshness Formulation treatments at the same storage time according to a Tukey-Kramer test ($P \leq 0.05$).

^a DPH = number of days stored postharvest.

^b Cultivars were evaluated separately for each year and harvest.

^c *P* values denote the level of significance for the interaction between the treatment and days stored main effects.

^d Missing data.

Table 6. Postharvest measurements of average fruit weight, pH, and soluble solids content (SSC) for haskap grown in 2015 in Simcoe.

Days stored	Average fruit weight		SSC
	(mg)	pH	(%)
<i>Harvest 1</i>			
0	844a	3.2a	14.9a
7	847a	3.3a	14.7a
14	821b	3.4ab	14.5a
21	805c	3.5bc	13.9a
28	746d	3.6c	16.1a
35	732e	— ^a	—
<i>P</i> value ^b	0.014	<0.001	0.373
<i>Harvest 2</i>			
0	645a	3.3ab	13.9a
7	636a	3.3b	13.6a
14	569b	3.5a	14.0a
21	573b	—	15.9b
<i>P</i> value	0.993	0.028	0.234
<i>Harvest 3</i>			
0	882a	3.0a	12.7a
7	826b	3.2b	13.2a
14	813c	3.0ab	13.3a
<i>P</i> value	0.945	0.038	0.310

Note: Least squares means followed by the same letter within each harvest are not statistically different according to a Tukey-Kramer test ($P \leq 0.05$). Within each harvest, data were pooled over harvested cultivars and treatments (control and Enhanced Freshness Formulation) treatments to calculate least squares means.

^a Missing data.

^b P values denote the level of significance for the days stored main effect.

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Fig. 1. The progression of fruit drop for control (CTL) and Enhanced Freshness Formulation (EFF) treated haskap plants grown in 2016 and 2017. (*) denote a significant difference between CTRL and EFF treatments for a particular observation date according to a Tukey-Kramer test ($P \leq 0.05$).

For Review Only

Fig. 1. The progression of fruit drop for control (CTL) and Enhanced Freshness Formulation (EFF) treated haskap plants grown in 2016 and 2017. (*) denote a significant difference between CTRL and EFF treatments for a particular observation date according to a Tukey-Kramer test ($P \leq 0.05$).

134x76mm (300 x 300 DPI)