Estudio de los factores fisio-morfológicos del fruto de arándano (*Vaccinium corymbosum* L.) que predisponen al ablandamiento y deshidratación en postcosecha

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TESIS DOCTORAL

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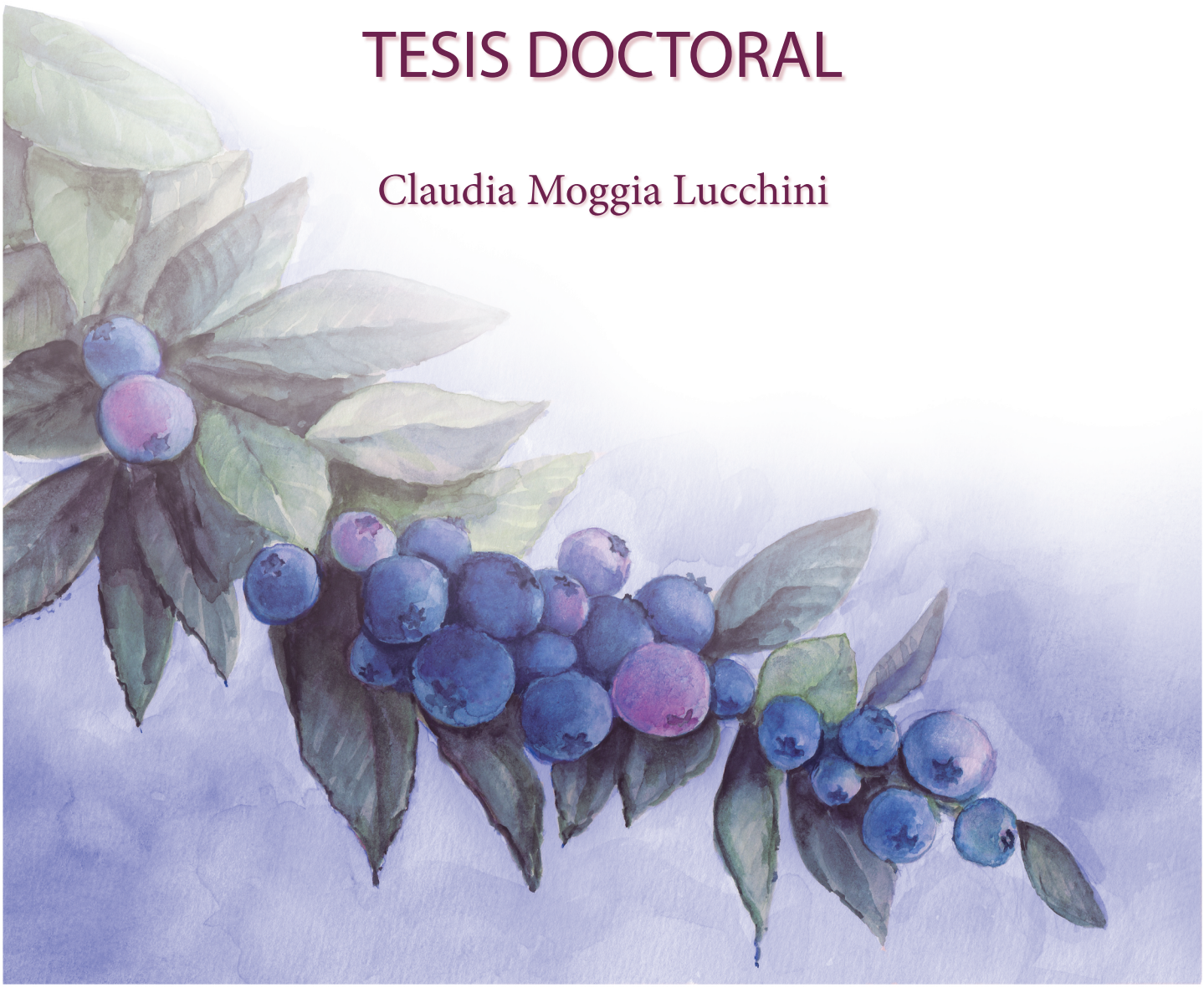

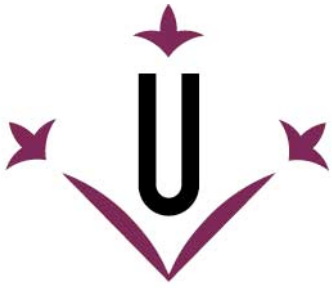

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Claudia Moggia Lucchini

Memoria presentada para optar al grado de Doctor
por la Universidad de Lleida
Programa de Doctorado en Ciencia y Tecnología Agraria y Alimentaria

Directores de Tesis

Dr. Jordi Graell Sarlé **Dra. Isabel Lara Ayala**

Lleida, 2017

La presente memoria de Tesis Doctoral que lleva por título **"Estudio de los factores fisio-morfológicos del fruto de arándano (*Vaccinium Corymbosum* L.) que predisponen al ablandamiento y deshidratación en postcosecha"** es presentada por **Claudia Moggia Lucchini** para poder optar al grado de Doctora por la Universidad de Lleida (España) y ha sido realizada bajo la dirección del Dr. **Jordi Graell Sarlé** y de la Dra**. Isabel Lara Ayala**. Ambos autorizan la presentación de la memoria de Tesis ya que reúne las condiciones necesarias para su defensa.

Jordi Graell Sarlé
Director de Tesis

Isabel Lara Ayala
Directora de Tesis

Claudia Moggia Lucchini
Doctoranda

Lleida, septiembre de 2017

La Tesis Doctoral se ha desarrollado en Chile, en el marco de la línea de investigación de “Postcosecha de Frutales Menores” del Centro de Mejoramiento y Fenómica Vegetal, Facultad de Ciencias Agrarias de la Universidad de Talca, Chile; con el patrocinio de “Fondo Proyectos de Investigación” y “Núcleo Científico Multidisciplinario” de la Universidad de Talca. En España este trabajo ha sido realizado bajo la tutela del Programa de Beca Doctoral de la Fundación Carolina, y el “Programa de Doctorado en Ciencia y Tecnología Agraria y Alimentaria” de la Universidad de Lleida.

Los trabajos experimentales se han realizado en Chile en el Laboratorio de Postcosecha y cámaras frigoríficas del Centro de Mejoramiento y Fenómica Vegetal, Facultad de Ciencias Agrarias de la Universidad de Talca, Laboratorio de Química de Productos Naturales perteneciente al Instituto de Química de Recursos Naturales de la Universidad de Talca, Chile y en los Laboratorios de Bioquímica del Departamento de Química y de los Servicios Científico-Técnico de la Universidad de Lleida, España.

Dedicatoria

A mi hermosa familia, por su constante amor y
apoyo para enfrentar esta etapa.
Sus consejos, paciencia, y motivación
me han permitido llegar al final de este proceso.

A Ricardo, el amor de mi vida,
toda mi gratitud y admiración por
estar siempre presente.

A mis maravillosos hijos
Daniela, Javiera y Joaquín, que son
mi mayor alegría.

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RESUMEN

El arándano azul (*Vaccinium corymbosum*) se ha convertido en la segunda especie de frutos del bosque en importancia en producción a nivel mundial, después de la fresa. Es un fruto altamente perecedero cuyo consumo es, mayoritariamente, en estado fresco. Países como Chile, que exportan principalmente por vía marítima, tienen la necesidad de asegurar una larga duración en postcosecha (30-50 días) y una excelente calidad al consumo. Los arándanos maduran en un período de tres a cuatro semanas, por lo que requieren múltiples recolecciones, cada 4 a 10 días. Debido a que el índice de cosecha se basa en la coloración externa del fruto, dentro de cada recolección existirán frutos de apariencia similar, pero en diferente madurez fisiológica. Esto explicaría las variadas situaciones de rechazo que ocurren en los procesos de exportación. Los principales defectos de arándanos chilenos en destino corresponden a problemas de ablandamiento y deshidratación, que pueden llegar a representar entre 10 y 45 % de los defectos totales.

El objetivo general de esta Tesis fue caracterizar y comprender, para los cultivares 'Duke' y 'Brigitta', la influencia del estado de madurez del fruto a la cosecha, las características de la cutícula y la posición del fruto dentro de la planta, como factores de variabilidad asociados al ablandamiento y deshidratación de arándanos en postcosecha. Adicionalmente, para un grupo de híbridos provenientes de un programa de mejoramiento genético, se analizó el rol de la cicatriz pedicelar sobre las mismas variables. La fruta de todos los ensayos se obtuvo de fincas ubicadas en la zona centro-sur de Chile, entre las campañas 2011-2012 y 2015-2016.

Los principales resultados para ambos cultivares corroboraron la alta variabilidad de los frutos en cuanto a su firmeza en cosecha (entre 5 y 50% de fruta muy blanda); esto ocurrió entre campañas, entre cosechas de una misma campaña y entre estados de madurez dentro de una misma cosecha. El factor más importante de variabilidad fue el estado de madurez, por falta de indicadores para discriminar, objetivamente, entre frutos maduros y sobremaduros. Frutos cosechados al llegar al 100% de coloración azul mostraron un potencial de almacenamiento diferente respecto a los cosechados sobremaduros. El efecto fue más marcado en frutos del cultivar 'Duke', con mayor tasa de ablandamiento y deshidratación en almacenaje.

Otro factor que explicó parte de las diferencias en almacenaje entre los cultivares fue el nivel de triterpenoides en la cutícula de la fruta en cosecha. El contenido de ácido ursólico se correlacionó positivamente con la pérdida de peso y con el ablandamiento de los frutos después de almacenaje; el contenido de ácido oleanólico se correlacionó negativamente con el ablandamiento postcosecha. La incidencia de ablandamiento y deshidratación disminuyó con el uso de coberturas plásticas, al aportar éstas una barrera a la difusión del vapor de agua.

El ablandamiento y el desarrollo de pardeamiento interno, producto de daños mecánicos, dependieron fuertemente de la firmeza de los frutos en cosecha, resultando los frutos blandos (< 1.6 N) y los frutos firmes (1.8-2.0 N) aquéllos con mayor y menor daño, respectivamente.

El efecto de la ubicación de la fruta en la planta sobre la firmeza en cosecha fue menos marcado que el debido a las diferencias entre estados de madurez. Cuando hubo diferencias, cosechas provenientes del lado Oriente tuvieron mayor proporción de fruta blanda que las del lado Poniente. La mayor proporción de frutos blandos en cosecha ocurrió en huertos y campañas con temperaturas máximas extremas (>27-32 °C) durante la última fase de maduración de los frutos, o bien en huertos y campañas en los que junto con temperaturas elevadas se dieron eventos de precipitaciones al inicio de la temporada de crecimiento y durante el período de maduración de los frutos.

Según el cultivar, la cicatriz pedicelar cubrió entre 0.19 y 0.74 % de la superficie total del fruto y fue responsable de un 40 % de la pérdida de peso a 20 °C. La influencia de la cicatriz sobre la pérdida de agua de los frutos fue mayor a temperaturas bajas, con una relación entre pérdida por cutícula/pérdida por cicatriz de 1.2 para fruta almacenada a 0 °C y de 9 para fruta almacenada a 20 °C.

RESUM

El nabiu blau (*Vaccinium corymbosum*) s'ha convertit en la segona espècie de fruits del bosc en importància en producció a nivell mundial, després de la maduixa. És un fruit altament perible el consum del qual és majoritàriament en estat fresc. Països com Xile, que exporten principalment per via marítima, tenen la necessitat d'assegurar una llarga durada en postcollita (30-50 dies) i una excel·lent qualitat del producte al consum. Els nabius maduren en un període de tres a quatre setmanes, per la qual cosa requereixen múltiples recol·leccions, cada 4 a 10 dies. A causa que l'índex de collita es basa en la coloració externa del fruit, dins de cada recol·lecció existiran fruits d'aparença similar, però diferent maduresa fisiològica. Això explicaria les variades situacions de rebuig que ocorren en els processos d'exportació. Els principals defectes de nabius xilens en destinació corresponen a l'estovament i la deshidratació, arribant a representar entre 10 i 45 % dels defectes totals.

L'objectiu general de la Tesi va ser caracteritzar i comprendre, pels cvs. 'Duke' i 'Brigitta', la influència de l'estat de maduresa del fruit a la collita, les característiques de la cutícula i la posició del fruit dins de la planta, com a factors de variabilitat associats a l'estovament i deshidratació de nabius en postcollita. Addicionalment, per a un grup d'híbrids provinents d'un programa de millorament genètic, es va analitzar el rol de la cicatriu pedicelar sobre les mateixes variables. La fruita de tots els assajos es va obtenir de finques situades a la zona centre-sud de Xile, entre les campanyes 2011-2012 i 2015-2016.

Els principals resultats per a tots dos cvs. van corroborar una alta variabilitat dels fruits quant a la seva fermesa en collita (entre 5 i 50% de fruita molt tova); això va ocórrer entre campanyes, entre collites d'una mateixa campanya i entre estats de maduresa dins d'una mateixa collita. El factor més important de variabilitat va ser l'estat de maduresa, per falta d'indicadors per discriminar, objectivament, fruits madurs d'aquells sobremadurs. Fruits que van ser collits separadament amb 100% de coloració blava o sobremadurs van demostrar un potencial diferencial d'emmagatzematge. L'efecte va ser més marcat en fruits del cv. 'Duke', amb major taxa d'estovament i deshidratació en l'emmagatzematge.

Un altre factor que explica diferències en l'emmagatzematge entre els cvs. és el nivell de triterpenoids en la cutícula de la fruita en collita. El contingut d'àcid ursòlic es va correlacionar positivament amb la pèrdua de pes i amb l'estovament dels fruits després de magatzematge; el contingut d'àcid oleanòlic es va correlacionar negativament amb l'estovament postcollita. Addicionalment, l'estovament i la deshidratació es van veure disminuïts per la incorporació de cobertures plàstiques, en aportar aquestes una barrera a la difusió del vapor d'aigua.

L'estovament i el desenvolupament d'enfosquiment intern, producte de danys mecànics, van dependre fortament de la fermesa dels fruits en collita, resultant els fruits tous (< 1.6 N) i els fruits ferms (1.8-2.0 N) aquells amb major i menor dany, respectivament.

L'efecte sobre la fermesa en collita, per ubicació de la fruita en la planta, va ser menys marcat que les diferències entre estats de maduresa. Quan va haver-hi diferències, collites provinents del costat Orient van tenir major proporció de fruita tova que les del costat Ponent. La major proporció de fruits tous en collita va ocórrer, en horts i campanyes amb temperatures màximes extremes (>27-32 °C) durant l'última fase de maduració dels fruits o en horts i campanyes en els quals juntament amb temperatures elevades es van presentar esdeveniments de precipitacions a l'inici de la temporada de creixement i durant el període de maduració dels fruits.

La cicatriu pedicelar va cobrir entre 0.19 i 0.74 % de la superfície total del fruit i va ser responsable d'un 40 % de la pèrdua de pes, a 20 °C. La influència de la cicatriu en la pèrdua d'aigua dels fruits va ser major a baixa temperatura, amb una relació entre pèrdua per cutícula/pèrdua per cicatriu d'1.2 per a fruita emmagatzemada a 0 °C vs. 9 per a fruita emmagatzemada a 20 °C.

SUMMARY

Blueberry (*Vaccinium corymbosum*) production and consumption has grown significantly on a worldwide basis, these fruit having become the second most important soft fruit species, after strawberries. Blueberries are highly perishable and are intended mainly for fresh consumption. Exporting countries such as Chile, who send their fruit mainly by boat, have the need to ensure a long postharvest period (30-50 days) as well as an excellent quality of produce upon arrival to final markets. Blueberries ripen over a three- to four-week period, and so multiple harvests, every 4 to 10 days, are required. Because harvest index is based on external fruit color, it is likely that within each harvest fruit with similar external appearance, but actually at different physiological maturity will be collected together. This would explain the various rejection situations that occur at the end of the exporting process. The main defects of Chilean blueberries at destination markets are fruit softening and dehydration, which may account for 10 to 45 % of total defects.

The main objective of this Thesis was to characterize and understand, for cultivars 'Duke' and 'Brigitta', the influence of maturity stage at harvest, cuticle characteristics and position of the fruit within the plant as variability factors associated to softening and weight loss during postharvest. Additionally, the role of the stem scar was also assessed on the same variables for a group of hybrids coming from a blueberry-breeding-program. Fruit from all trials were obtained from orchards located in the central-south part of Chile, between 2011-2012 and 2015-2016 seasons.

For both cultivars, main results corroborated high variability in fruit firmness at harvest (5 to 50 % of very soft fruit) which occurred between seasons, between harvests of a single season, and between maturity stages within a single harvest. Maturity stage was the main factor associated to fruit variability, due to the lack of an objective means for discriminating between ripe and overripe fruit. Thus, berries harvested when attaining 100 % blue color displayed different storage potential than those picked overripe. This effect was more evident for 'Duke' berries, which showed higher softening rate and greater water loss during storage.

A second factor explaining differences between cultivars during storage was the triterpenoid fraction in fruit cuticle at harvest. The amount of ursolic acid was positively correlated with weight loss and softening after storage; oleanolic acid content was negatively correlated with softening after storage. Additionally, fruit softening and dehydration were decreased by the use of plastic bags, which created a barrier against water vapor diffusion within the packaging.

Softening and internal browning, resulting from mechanical damages, were highly dependent on fruit firmness at harvest. Thus, soft- (< 1.6 N) and firm-fruit (1.8-2.0 N) exhibited the highest and lowest damage, respectively.

The effect of fruit position within the plant on fruit firmness was less evident than differences due to maturity stages. When differences were found, berries harvested from the East side of the plant contained higher proportion of soft fruit than those obtained from the West side. The greatest proportion of soft fruit at harvest occurred in orchards and seasons with extreme maximum temperatures (>27-32 °C) during the last phase of fruit ripening, or in orchards and seasons where, along with high temperatures, precipitations were present both at the beginning of fruit growth and during fruit maturation.

The stem scar covered between 0.19 and 0.74 % of the fruit surface area and was responsible of 40 % of weight loss at 20 °C. The influence of stem scar on fruit water loss was higher at lower temperature, showing rates of loss through cuticle/loss through scar of 1.2 and 9 for fruit stored at 0 and 20 °C, respectively.

ABREVIATURAS

AC: Atmósfera controlada

AM: Atmósfera modificada

ANOVA: Analysis of Variance

AT: Acidez titulable

BIRD: Blueberry Impact Recording Device

C*: Chroma

Cap.: Capítulo

CONICYT: Comisión Nacional de Investigación Científica y Tecnológica

CPPU: N-(2 chloro-4-pyridyl)-N'-phenylurea

cv.: Cultivar

DAFB: Days After Full Bloom

D K-S: Kolmogorov-Smirnov test

E: East

EP: Ethylene Production

FONDECYT: Fondo Nacional de Desarrollo Científico y Tecnológico

FONDEF: Fondo de Fomento al Desarrollo Científico y Tecnológico

GC: Gas Chromatograph

GC-MS: Gas Chromatography – Mass Spectrometry

h: horas

h°: Hue Angle

HR: Humedad Relativa

IB: Internal Browning

IQF: Individual Quick Frozen

L*: Luminosidad

LDPE: Low Density Polyethylene

MAP: Modified Atmosphere Packaging

N: Norte, North

n: number

ORAC: Oxygen Radical Absorbance Capacity

PD: Pore Difussivity (difusividad a través del poro)

P_{H2O}: water permeance (permeabilidad al agua)

PI: Pardeamiento Interno

r: Correlation Coefficient

r^2, R^2: Coefficient of Determination

RH: Relative Humidity

RR: Respiration Rate

Rt: Retention time

S: Sur, South

SS: sólidos solubles

SSC: soluble solid contents

TA: Tritatable acidity

TLC: Thin Layer Chromatography

TSS: Total Soluble solids

vs.: *versus*

VPD: Vapor Pressure Deficit

Y: year

W: West

ÍNDICE

1. INTRODUCCIÓN

1.1. El sector del arándano: importancia y retos

La mayor parte de la producción mundial de arándano azul proviene de cultivares derivados de *Vaccinium corymbosum L.* (arbusto alto), *V. ashei Reade* (ojo de conejo; syn. *V. virgatum Ait.*) y *V. angustifolium Ait.* (arbusto bajo). Dentro del género *Vaccinium*, que incluye aproximadamente 400 especies, el arándano azul abarca entre 10 y 26 de ellas, según su clasificación taxonómica (Ballington, 1990). Las plantaciones comerciales se componen principalmente de arándanos de arbusto alto y ojo de conejo, en tanto los arándanos de arbusto bajo se manejan de forma semi-domesticada. Los arándanos de arbusto alto, que representan la mayor superficie de cultivo comercial a nivel mundial, se subdividen, de acuerdo a su requerimiento de frío invernal, en tipos del norte y del sur (Retamales y Hancock, 2012).

La mayor conciencia de los consumidores sobre el valor nutricional de los alimentos, junto a la elevada y reconocida actividad antioxidante de los arándanos (Manganaris et al., 2013), que ha demostrado ser benéfica para la salud (Giusti y Jing, 2007), ha incidido en el incremento significativo de su cultivo y consumo en las últimas décadas (Lobos y Hancock, 2015), pasando la producción mundial de 342.000 ton en 2012, a 588.0002 ton en 2015, con un 90 % generado entre USA, Chile y Canadá (ODEPA, 2016).

Los arándanos se clasifican como “frutos blandos”, junto con fresas, frambuesas y moras. Cerca de ⅔ de la producción mundial de arándanos se comercializa en estado fresco siendo Chile el primer país exportador de esta fruta, con el 30.9 % de la producción mundial (Retamales y Hancock, 2012). Diversos autores coinciden que una aceptable calidad del fruto se puede mantener a lo largo de 2 a 4 semanas bajo un almacenaje a 0 ºC y 90-95 % de humedad relativa (Kader, 2002; Perkins-Veazie, 2004); por otra parte, para períodos de almacenaje más prolongados se requeriría del uso adicional de atmósfera modificada (Moggia et al., 2014) o controlada (Ehlenfeldt, 2002).

Debido a su ubicación geográfica, Chile posee la ventaja comercial de proveer fruta fresca al hemisferio norte en contra-estación. Sin embargo, con el fin de reducir costos, y basado en investigaciones que demostraron la factibilidad del envío por barco (Beaudry et al., 1998), actualmente cerca de un 80 % del volumen se exporta vía marítima, con lo cual la fruta puede tardar, dependiendo del mercado, entre 20 y 50 días en llegar al consumidor final. La alta perecibilidad de esta fruta, junto al extenso período que transcurre entre cosecha y destino, hace que

la calidad al arribo sea una de las características de mayor relevancia para asegurar los retornos económicos que aseguren la viabilidad de la industria.

Los arándanos son susceptibles de sufrir pudriciones, alteraciones fisiológicas, daños mecánicos, deshidratación, ablandamiento y pérdida de peso. La calidad con que arriba la fruta a los mercados de destino depende de sus características a cosecha, así como de la manipulación durante y después de la cosecha (Forney, 2009). El ablandamiento del fruto es uno de los factores más limitantes para la comercialización de arándanos (Vicente et al., 2007) y uno de los atributos más críticos que influye en su aceptación por parte de los consumidores (NeSmith et al., 2002). De acuerdo con la industria, los principales defectos encontrados en arándanos chilenos en destino corresponden a ablandamiento y deshidratación, los que según la temporada pueden representar entre 10-45 % y 10-25 % de los defectos totales, respectivamente (Chilean Blueberry Committee, 2015).

La presente Tesis doctoral se centró en estudiar las características fisiológicas, morfológicas y bioquímicas de los frutos de arándano que influyen en su ablandamiento y deshidratación en postcosecha. Se pretendió pues alcanzar nuevos conocimientos científicos a la vez que demostrar su utilidad práctica para los diversos agentes de la cadena sectorial. Especial énfasis se dio a comprender la variabilidad de la fruta en función del estado de madurez, las características de la cutícula y la cicatriz, así como la posición de la fruta en la planta en el momento de cosecha.

1.2. **El fruto del arándano**

1.2.1**.** Características físicas y cambios durante el desarrollo

El fruto del arándano es una baya de forma esferoide oblata (de 7 a 15 mm de diámetro ecuatorial) cubierto de ceras (aspecto comúnmente denominado “bloom”). El color de la epidermis puede variar desde azul claro hasta prácticamente negro (Gough, 1994), siendo uno de los atributos más importantes de calidad. Los frutos de arándano exhiben un patrón de crecimiento doble sigmoideo (Mainland y Eck, 1968; Edwards et al., 1970; Coombe, 1976), distinguiéndose tres fases: el estado I, que se caracteriza por una rápida división celular y aumento de peso seco (Birkhold et al., 1992**;** Cano-Medrano y Darnell, 1997), el cual se extiende por 25 a 35 días, dependiendo del cultivar y las condiciones ambientales. En la fase II hay poco crecimiento del fruto, en favor de un activo desarrollo de las semillas (Edwards et al., 1970); este periodo dura 30 a 50 días en función del

cultivar, el ambiente y el número de semillas viables (Darnell, 2006). El fruto comienza a madurar durante el estado III, que se caracteriza por un rápido aumento en volumen producto de la elongación celular (Galletta 1975; Gough 1994). Es en este lapso de tiempo donde el fruto desarrolla la coloración de la piel, el ablandamiento de la pulpa, así como el incremento en contenido de azúcares y disminución de ácidos orgánicos. Esta etapa final se extiende por lo general entre 16 y 26 días y representa el mayor aumento en tamaño (Gough, 1994).

El proceso de maduración de los frutos fue descrito por Shutak et al. (1980), quien definió seis estados de desarrollo, basados en el color de la epidermis (Cuadro 1). Así, los frutos son extremadamente firmes en el estado verde inmaduro (VI), se ablandan apreciablemente al virar a verde-rosa (VR) y continúan perdiendo firmeza a una tasa menor hasta el momento de cosecha: estados azul (A) y maduro (AA) (Ballinger et al., 1973). Dado el prolongado período de floración, que puede durar de 3 a 4 semanas (Retamales y Hancock, 2012) y el tiempo que demora el desarrollo de los frutos en un racimo, de 42 a 92 días (Darnell, 2006), es esperable que los frutos se desarrollen de forma variable bajo condiciones medioambientales diferentes durante la temporada (Gough, 1994; Lobos et al., 2014). Por ejemplo, flores tempranas cuajan frutos que son más susceptibles de experimentar menores temperaturas iniciales comparados con aquéllos que cuajan al final del período de floración. Esto coincide con una maduración secuencial de los frutos dentro de la planta (Fig. 1), requiriéndose varias cosechas durante la temporada de producción .Por ello, en un momento determinado se cosecharán frutos visualmente similares (estados A y AA) pero que, al poseer una edad fisiológica diferente, presentarían un diferente comportamiento en postcosecha.

Cuadro 1. Descripción de estados de madurez en base a coloración del fruto de arándano.

Grados de Coloración	**Descripción**
Verde inmaduro (VI)	Firme, completamente verde oscuro
Verde maduro (VM)	Algo blando, verde claro
Verde-rosa (VR)	Alguna coloración rosa en el cáliz
Azul-rosa (AR)	Mayormente azul, alguna coloración rosa en extremo pedicelar
Azul (A)	Casi completamente azul, anillo rosa en torno al pedicelo
Maduro (AA)	Completamente azul

Fuente: Shutak et al. (1980).

Figura 1. Maduración de los frutos en forma secuencial en un racimo
Fuente: Michigan State University, Extension Center
http://msue.anr.msu.edu/topic/blueberries/growing_blueberries/growth_stages_table

Se identifica el inicio de la maduración con la aparición del primer color rosa (VR), cuyo avance se acompaña de un aumento en el contenido de azúcares y una disminución de la acidez titulable. Los azúcares pueden variar desde 7 % en fruta VM hasta 15 % en fruta AA, dependiendo del cultivar, siendo glucosa y fructosa los más abundantes en arándanos maduros, y con una menor proporción de sacarosa (Kader y Metche, 1993). Los ácidos orgánicos que predominan en los frutos de arándano son el cítrico, quínico, málico y clorogénico. El ácido cítrico, que es el de mayor abundancia (Kushman y Ballinger, 1975), representa entre 77 y 87 % de los ácidos totales según estado de madurez (Forney et al., 2012) y decrece en forma importante durante la maduración de la fruta, produciendo una disminución de la acidez titulable. En este sentido, Rodarte et al. (2008) reportaron una reducción de la acidez de hasta un 78 % entre los estados de fruto VM y A, y demostraron que existe una gran diferencia entre variedades para la relación sólidos solubles/acidez (en la fecha de la primera cosecha comercial).

El cambio de coloración de la epidermis asociado a la madurez se atribuye principalmente a la disminución de clorofila y posterior síntesis de antocianinas, localizadas en las células epidermales e hipodermales, las que son sintetizadas progresivamente desde el cáliz hacia el extremo pedicelar de la fruta (Ballinger et al., 1970; Ballinger et al., 1972). Con cosechas secuenciales, las antocianinas aumentan, en tanto los flavonoides y ácidos hidroxicinámicos decrecen desde el estado VI al A (Rodarte et al., 2008, Forney at al., 2012). Adicionalmente, se ha visto que las antocianinas continúan produciéndose después de cosecha, con incrementos de hasta un 55 % al cabo de 21 días a 5 °C (Mitcham, 2007); esto implicaría que frutos cosechados antes de alcanzar el 100 % de coloración azul, puedan desarrollar dicha coloración en almacenaje posterior (El-Agamy, 1982).

De acuerdo a su patrón respiratorio durante la maduración, los frutos se clasifican en climatéricos y no climatéricos (Biale y Young, 1981; Arpaia et al., 1982). Los frutos climatéricos experimentan un incremento en la respiración y una fase autocatalítica de producción de etileno (Rhodes, 1970) coincidente con la maduración, que declina cuando el fruto se aproxima a la senescencia, siendo su intensidad y duración muy variable entre las diferentes especies frutícolas. Esta alza climatérica va acompañada de importantes cambios: los frutos se ablandan, el contenido de azúcares aumenta, el contenido de almidón y ácidos decrecen, desaparece la clorofila, aumentan los pigmentos y se desarrollan compuestos volátiles específicos (Biale y Young, 1981).

A diferencia de otras especies, el arándano azul presenta un alza en respiración y etileno en la etapa media de su maduración, que coincide con el viraje de VR a AR (Windus et al., 1976; El-Agamy et al., 1982); por lo tanto, su cosecha se realiza en un estado postclimatérico, y los frutos no mejoran en calidad durante su manejo posterior a la recolección y almacenaje (NeSmith et al., 2005; MacLean y NeSmith, 2011). Su tasa respiratoria es considerada moderadamente alta, con valores entre 2 a 10 mg CO_2 kg^{-1} h^{-1} a 0 ºC (Hardenburg et al., 1986), en tanto su producción de etileno es baja, fluctuando de 0.1 a 1.0 µl kg^{-1} h^{-1} a 20 ºC (Kader, 2002).

1.2.2. Características de los frutos al momento de cosecha

La calidad de la fruta se define como un conjunto de características de aspectos físicos, químicos, organolépticos y nutricionales que reflejan el juicio de los consumidores basado en su percepción (Kader, 1999). La calidad de los arándanos no puede mejorarse después de la cosecha, por lo cual su recolección debe realizarse con una madurez óptima que le permita soportar los procesos de embalaje, almacenaje y transporte hasta alcanzar el consumidor final.

- *COLOR DE PIEL*: Los frutos se consideran listos para cosechar cuando se tornan completamente azules, lo que dependiendo del cultivar puede ocurrir entre 60 y 120 días después de plena floración (Gough, 1994). El color es un importante factor de calidad en estos frutos, y por el momento es el único indicador de cosecha utilizado a nivel comercial. La expresión del color está determinada por las antocianinas producidas en la epidermis al inicio del proceso de maduración, así como por la presencia de una capa cerosa (pruina, que aporta un típico "bloom"), que varía en composición y cantidad. Mediciones objetivas de color se pueden realizar con Colorímetro reportando los valores en el sistema CIE L*, a*, b* (L* para luminosidad; a* para matices del verde al rojo; b* para

matices del amarillo al azul), con los cuales se calculan el "hue angle" (h°), como $\tan^{-1}(b/a)$, y el "Chroma" (C*) que representa $(a^{*2} + b^{*2})^{½}$ (McGuire,1992; León et al., 2006). En un estudio con diez cultivares de arbusto alto y dos cultivares ojo de conejo, Saftner et al. (2008) encontraron que los valores de b* estaban positivamente correlacionados con la percepción sensorial (r = 0.48) y negativamente asociados con la aceptación por apariencia (r = -0.41). Adicionalmente, la presencia de "bloom" que influye en la percepción visual del color, es una característica importante relacionada con la vida postcosecha del fruto, ya que previene su deshidratación; la disminución o ausencia de "bloom" se asocia a una manipulación severa (Sapers et al., 1984).

- *FIRMEZA:* representa una característica física muy importante de los frutos que es frecuentemente utilizada como una medida de calidad (Timm et al., 1996), dado que la fruta firme será más apetecible y resistente a los procesos de cosecha y posterior transporte (Hanson et al., 1993). Los frutos, que son muy firmes en el estado verde inmaduro, experimentan el mayor ablandamiento en su cambio hacia la coloración rojiza, y luego la pérdida de firmeza se hace menos intensa hacia la maduración final (Ballinger et al., 1973) El ablandamiento en arándanos se ha asociado a la degradación enzimática de componentes de pared celular; la solubilización y despolimerización de hemicelulosa se ha visto incrementada entre los estados inmaduro y 100 % de coloración azul, en tanto la solubilización de pectinas se ha detectado desde el estado inmaduro hasta 75 % de coloración azul (Proctor y Peng, 1989). Esto concuerda con otros estudios que muestran menores cambios en firmeza desde el estado 75 % azul hasta el sobremaduro (Ballinger et al., 1973; Proctor y Miesle, 1991) e indicaría que los procesos parecen estar casi terminados al momento de la cosecha (Vicente et al., 2007; Angeletti et al., 2010). Dado lo anterior, habría otros factores, que no han sido debidamente estudiados, involucrados en el posterior ablandamiento que ocurre durante la postcosecha. Uno de estos factores, que será descrito más adelante, puede ser la cutícula, que ha demostrado poseer una preponderante influencia en la calidad postcosecha de algunos frutos (Lara et al., 2014).

Una pequeña diferencia en firmeza entre cultivares puede ser muy importante durante la vida postcosecha, en especial para fruta producida en el hemisferio sur que requiere de un período más prolongado para alcanzar a los consumidores finales situados generalmente en países del hemisferio norte (Beaudry et al., 1998). Diversos autores han reportado diferencias en firmeza para cultivares

de arbusto alto (Ehlenfeldt, 2002; Saftner et al., 2008); sin embargo, las variaciones parecen estar más afectadas por cambios en estado de madurez del fruto dentro de un mismo cultivar (Beaudry et al., 1998; Lobos et al., 2014) y pueden ser reducidas con adecuados manejos de cosecha y postcosecha (Ballinger et al. 1973; Ehlenfeldt, 2002). Dado que la firmeza es uno de los atributos más críticos que influyen en la aceptación por parte del consumidor, es esencial disminuir la variabilidad de la fruta en torno a este parámetro, para lograr un producto de alta calidad (NeSmith et al., 2002). No obstante, existe escasa información sobre la relevancia de la firmeza en cosecha en el comportamiento postcosecha de los distintos cultivares.

A nivel instrumental, la firmeza en arándanos se puede medir como la fuerza necesaria para alcanzar una cierta deformación. El instrumento Firmtech II® (Fig. 2) es un equipo estacionario, fácil de operar y que permite mediciones rápidas y confiables, por lo cual es considerado el instrumento estándar "de facto" para la medición de firmeza en arándanos., sin embargo, su uso no está masificado a nivel de industria, siendo empleado mayoritariamente, a nivel de investigación. Este equipo mide firmeza por medio de la compresión de frutos individuales, registrando la fuerza requerida para deformar el fruto en 1 mm (g mm^{-1}), considerando diferentes umbrales de fuerza (Li et al., 2011).

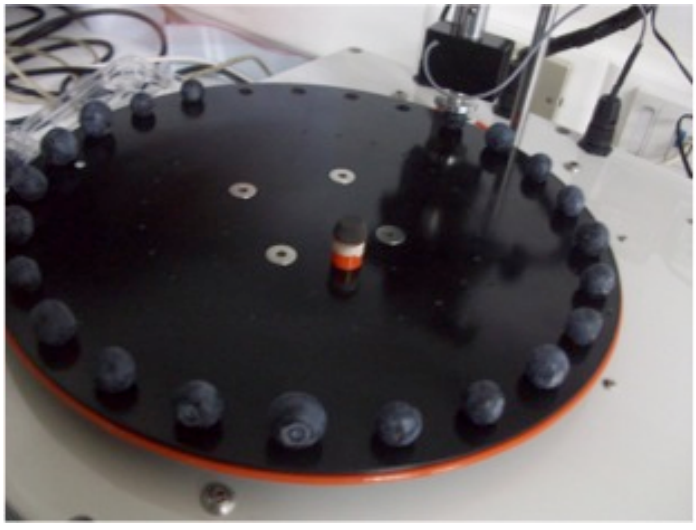

Figura 2. Detalle del instrumento FirmTech II®, utilizado para medir firmeza en arándanos.

Ehlenfeldt y Martin (2002) evaluaron durante tres años la firmeza de 87 cultivares de arándanos, encontrando importantes diferencias entre ellos. La firmeza promedio de todos los cultivares fue de

136.1 g mm^{-1}; para el caso de 'Duke' los valores a cosecha fluctuaron entre 173.0 y 140 g mm^{-1}, en tanto para 'Brigitta' lo hicieron entre 188.0 y 145.9 g mm^{-1}.

- *SÓLIDOS SOLUBLES y ACIDEZ:* una característica que destaca en los frutos de las especies del género Vaccinium es su sabor, dado por el balance entre azúcares (medidos como sólidos solubles, SS) y ácidos orgánicos (medidos como acidez titulable, AT). Beaudry (1992) sugirió los siguientes indicadores óptimos a cosecha: > 10 % SS, 0.3–1.3 % de AT, un pH entre 2.25 y 4.25, y una relación SS/AT entre 10 y 33. Saftner et al. (2008) estudiando las características instrumentales y sensoriales de 12 cultivares, encontraron hasta un 2.6 % de diferencia en SS, presentando los cultivares 'Bluegold' (13.2 %) y 'Lateblue' (10.6 %) los más altos y bajos niveles, respectivamente. En cuanto a AT, los cultivares 'Elliott' y 'Lateblue' exhibieron 2 a 4 veces mayor acidez (> 1.22%) que el resto de los cultivares, y consecuentemente las menores relaciones para SS/AT (< 9.0), impactando ello de forma importante en la percepción sensorial. Por su parte, Lobos et al. (2014) encontraron asociaciones significativas entre SS/AT y la textura de la fruta, su sabor, su firmeza y la proporción de bayas sanas después de almacenaje. Rodarte et al. (2008) hallaron valores de SS/AT que fluctuaron entre 18 y 44.2, dependiendo del cultivar y del momento de cosecha. Desde la perspectiva de potencialidad de almacenaje, Galletta et al. (1971) establecieron tres categorías basadas en el ratio SS/AT: buen potencial de almacenaje con valores de ratio < 18; potencial intermedio para ratios de 18-32; y muy mal potencial para ratios > 32.

1.2.3. Características de la cutícula

La cutícula es una membrana extracelular, que cubre todos los órganos aéreos no lignificados de una planta, a saber flores, hojas, tallos y frutos (Domínguez et al., 2011). Está compuesta por una matriz de cutina (polímero de poliésteres insolubles, formados principalmente por ácidos grasos C16 y C18), embebida en un complejo de ceras intra- y epi-cuticulares, y una fracción menor de compuestos fenólicos (Fig. 3) (Jetter et al., 2000). A pesar de ser una capa muy fina, comparada con la pared celular, es un importante componente de estabilización estructural para los tejidos primarios de la epidermis, influyendo en la calidad postcosecha del fruto en tres aspectos importantes: permeabilidad al agua y la consecuente deshidratación del tejido, susceptibilidad a infecciones, y susceptibilidad a desórdenes fisiológicos (Lara et al., 2014). Adicionalmente, se

atribuye a la cutícula influencia sobre la firmeza y otras características físicas relacionadas con deformación o fracturas (López-Casado et al., 2007).

Las ceras cuticulares se componen de una mezcla de compuestos alifáticos (*n*-alcanos, ácidos alcanoicos, alcanoles, aldehídos, y alquil ésteres), y no-alifáticos (triterpenoides y derivados de esterol) (Kunst y Samuels, 2009). De acuerdo con Lara et al. (2014) los principales triterpenoides de muchas especies frutales son los ácidos ursólico y oleanólico, mientras en otros frutos predominan los triterpenoides como las amirinas (tomate, pimiento, naranja, pera asiática). Se ha reportado lupeol en pera (Cho et al.,2013), cítricos (Lara et al., 2015), tomate, uva, pimiento, berenjena y pomelo (Szakiel et al., 2012).

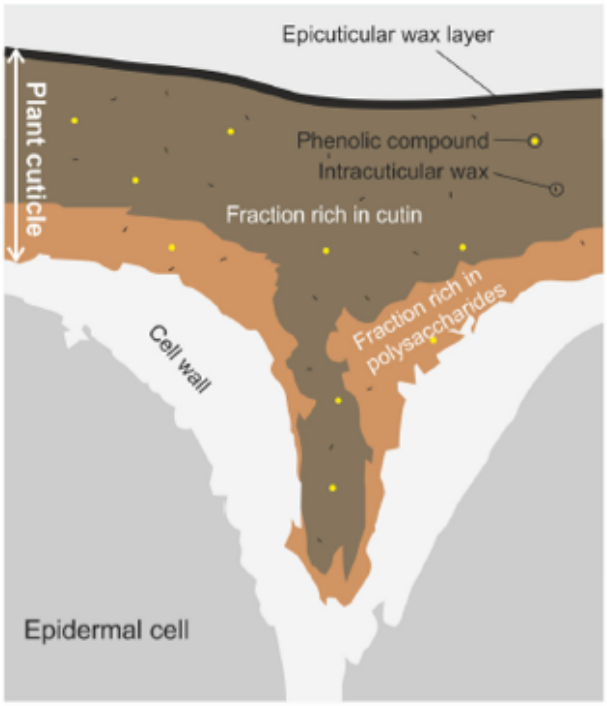

Figura 3. Diagrama de una sección transversal de cutícula.
Fuente: Heredia-Guerrero et al. (2014).

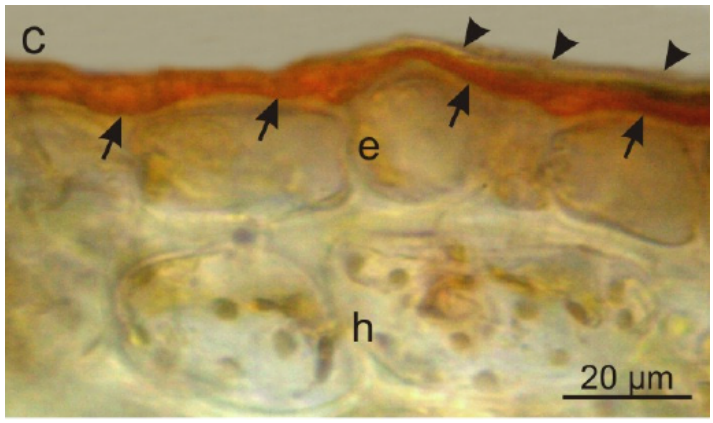

Figura 4. Superficie de la epidermis de un fruto; las flechas indican la cutícula teñida con Sudan III y una capa de cera epicuticular incolora sobre ésta (cabezas de flechas); (**e**) epidermis; (**h**) hipodermis. Fuente: Konarska (2015).

Durante el desarrollo del fruto hay un aumento progresivo en el grosor de la capa de cera epicuticular y la cutícula, además de aumentar el grosor de la epidermis y células hipodermales (Fig. 4) (Konarska, 2015). La barrera que suponen las ceras cuticulares es vista como relativamente impermeable a los gases y al vapor de agua; existe un grupo de ceras cristalinas (principalmente *n*-alcanos) que cubren y se embeben en una matriz de material amorfo (principalmente triterpenoides). Se considera que la difusión de agua ocurre mayormente en la fracción amorfa, en tanto la fracción cristalina prevendría la translocación del agua (Vogg et al., 2004).

En relación a la función de estos compuestos, estudios en tomate (Leide et al., 2011), pimiento (Parsons et al., 2012), cereza (Belge et al., 2014a), y melocotón (Belge et al., 2014b) han demostrado una asociación positiva entre la pérdida de peso y la relación entre *n*-alcanos y triterpenoides. Dentro de las bayas comestibles del género *Vaccinium*, la información disponible se refiere a "cranberry" (*Vaccinium macrocarpon*), como una importante fuente de ácidos ursólico y oleanólico (Crouteau y Fagerson, 1971; Szakiel et al., 2012); en tanto Kondo et al. (2010) detectaron los mismos compuestos en arándanos de arbusto bajo (*Vaccinium angustifolium*). Hasta la fecha no existen reportes de estos compuestos en frutos de *Vaccinium corymbosum.*

1.2.4. Características de la cicatriz pedicelar

La cicatriz pedicelar en el arándano se corresponde con la zona donde el pedicelo se separa del fruto al momento de la cosecha. En frutos para consumo fresco se prefiere aquellos cultivares con una cicatriz pequeña ya que ésta constituye una importante vía de pérdida de humedad (Albrigo et al., 1980, Ehlenfeldt, 2002), y es también considerada como una de las principales vías de ingreso

de agentes causantes de pudriciones (Cappellini y Ceponis, 1977). Su diámetro varía entre cultivares y especies, reportándose valores de 0.7 a 1.2 mm en arándanos ojo de conejo y entre 1.3 y 2.2 mm para los de arbusto alto (Perkins-Veazie et al., 1995; Konarska, 2015). Parra et al. (2007) reportan datos similares para cultivares de arbusto alto (1.5 a 2.2 mm) pero mayores para ojo de conejo, variando entre 1.6 y 2.0 mm. Adicionalmente, en el cv. 'Bluecrop' después de desprenderse el fruto del pedicelo aparecieron grietas alrededor de la cicatriz que contribuirían a la pérdida de agua y la entrada de patógenos (Konarska, 2015).

Si bien la selección de cultivares a nivel comercial privilegia aquellos que producen frutos grandes con cicatriz pequeña, no hay un consenso al respecto; Galleta y Ballington (1996) reportaron que los mayores tamaños de cicatriz se asociaban a frutos más grandes. Sin embargo, Parra et al. (2007), evaluando cultivares comerciales e híbridos obtenidos de polinización abierta, encontraron en 'Bonita', 'Reveille' y 'Premier' frutos de tamaño mediano y grande con cicatrices medianas o pequeñas, en tanto para 'Georgia Gem', 'Snowflake' y 'Marimba', cuyos frutos son pequeños, las cicatrices eran relativamente grandes. Adicionalmente, estos autores reportaron que la relación entre tamaño de la cicatriz y tamaño del fruto para un número importante de líneas de híbridos varió entre temporadas.

1.3. **Postcosecha del arándano**

1.3.1. Condiciones de almacenaje

1.3.1.1. Almacenaje refrigerado: Temperatura y Humedad Relativa

La temperatura es el factor ambiental más influyente en la velocidad de deterioro de los productos frescos (Kader, 2002); una aplicación adecuada de baja temperatura junto a una alta humedad relativa (HR) son claves para mantener la calidad y extender la vida de almacenaje del producto (Wills et al., 2007).

Los arándanos se pueden almacenar con una calidad aceptable durante 2 a 4 semanas a 0 °C y 90-95 % HR, procurando idealmente un enfriamiento rápido hasta esa temperatura (Kader, 2003; Perkins-Veazie, 2004). Niveles mayores de temperatura aceleran el metabolismo del fruto, aumentan la pérdida de agua e incrementan la probabilidad de infección por patógenos (Paniagua et al., 2014). Sin embargo, a nivel comercial el enfriamiento rápido es generalmente difícil de conseguir, debido a retrasos entre cosecha y almacenaje o a una inadecuada o insuficiente

infraestructura frigorífica (Sargent et al., 2006). Dado lo anterior, es común que frutas recién cosechadas lleguen a las empresas de selección y embalaje y sean sometidas a un enfriamiento intermedio a una temperatura de 10 °C, previo a ser procesadas y enfriadas posteriormente a la temperatura óptima de 0 °C después del embalaje (Paniagua et al., 2013). Diversos estudios previos han investigado, para arándanos ojo de conejo y de arbusto alto, el efecto sobre la calidad de la fruta, de 2 a 72 h de retraso con temperaturas entre 20 °C y 32 °C (Ceponis y Cappellini, 1983; Tetteh et al., 2004); por otra parte, Jackson et al. (1999) estudiaron el efecto de retrasos a 12 °C en arándanos de arbusto bajo. Sólo recientemente Paniagua et al. (2013) evaluaron condiciones más cercanas al proceso comercial, sometiendo frutos a 2 y 6 h de retraso a 20 °C (para simular el retraso entre cosecha y el enfriamiento a 10 °C) seguido por 4, 8 y 20 h a 10 °C (simulando el retraso entre procesamiento y enfriado final). De este ensayo se concluyó que para fruta almacenada por 3 semanas a 0 °C, retrasos de hasta 6 h a 20 °C y 8 h a 10 °C no resultaron en una reducción de calidad en comparación al tratamiento de 2 h a 20 °C y 4 h a 10 °C, que están en el rango de las condiciones reales alcanzables normalmente por la industria.

Junto al manejo de la temperatura, una alta HR es esencial para reducir el déficit de vapor de agua entre el producto y el ambiente, causante de las pérdidas de peso y los síntomas por deshidratación en los frutos. Se suelen recomendar valores de 90-95 % como rango óptimo para el almacenaje de arándanos (Kader, 2002). Sin embargo, estas condiciones de alta HR, si bien son óptimas para la conservación de la fruta, también son favorecedoras del crecimiento de varios patógenos que provocan diversas podredumbres en postcosecha (Snowdon, 1990; Thompson , 2002).

1.3.1.2. Atmósfera Controlada y Atmósfera Modificada

El almacenaje en atmósfera controlada (AC) o en atmósfera modificada (AM), que consisten en la modificación de la composición gaseosa en el ambiente alrededor del producto, de manera que difiera de la composición típica del aire (78.08 % N_2, 20.95 % O_2 y 0.03 % CO_2), es una tecnología ampliamente utilizada para extender la vida de almacenaje de muchas especies hortofrutícolas, y que debe ser considerada como un complemento al manejo de temperatura y HR (Kader, 2002). Por lo general, dichas tecnologías implican la reducción de la concentración de O_2 y/o el aumento de la concentración de CO_2, hasta unos niveles óptimos para la conservación de los productos, los cuáles difieren según la especie y cultivar, y además están influenciados por diversos factores agronómicos

y climáticos de cada zona. AC y AM difieren en el grado de control de la composición de los gases, siendo AC una tecnología que implica un control más preciso y exacto de las concentraciones de O_2 y CO_2, y que suele aplicarse a escala mayor (por ejemplo, en cámaras frigorífica de almacenaje), mientras que en AM el control de gases es más variable y flexible, y se aplica generalmente a una escala menor (por ejemplo, en envases, contenedores y pallets para transporte).

Los arándanos se benefician de niveles elevados de CO_2 (Ehlenfeldt, 2002), principalmente por su impacto en la supresión del desarrollo de pudriciones, pero no hay un efecto claro del bajo nivel de O_2 (Ceponis y Cappellini, 1983; Smittle y Miller, 1988; Schotsmans et al., 2007). La atmósfera ideal para conservar arándanos en AC y AM es aquella en que los altos niveles de CO_2 son de primera importancia, manteniéndose el O_2 a niveles suficientes para prevenir fermentación en los tejidos del fruto (Alsmairat et al., 2011). En general, se recomienda que la presión parcial de CO_2 se mantenga entre 8 y 15 kPa y la de O_2 entre 2 a 4 kPa (Ceponis y Cappellini 1983, 1985). Sin embargo, la concentración de CO_2 necesaria para el control de pudriciones está por lo general muy cercana a los niveles de tolerancia del producto (Alsmairat et al., 2011). En arándanos 'Bluecrop' se ha demostrado una interacción entre niveles de O_2 y CO_2 con procesos de fermentación en el fruto, dado que a medida que el CO_2 se incrementa, la tolerancia de la fruta al O_2 decrece (Beaudry, 1993).

Alsmairat et al. (2010) probaron AC en diferentes combinaciones de O_2/CO_2 (cuyos porcentajes sumaban 21 %) en 8 cultivares de arándanos, almacenados por 8 semanas a 0 ºC. Los cultivares difirieron en sus respuestas, pero en algunos de ellos la firmeza de la fruta y la incidencia de pudriciones disminuyeron, en tanto la proporción de frutos con decoloración interna severa aumentó al incrementarse las concentraciones de CO_2, evidenciando que la supresión de la pudriciones puede incluir aspectos negativos en la conservación y calidad de la fruta.

En el caso de AM, también se ha reportado que esta técnica es capaz de extender la vida postcosecha de los arándanos, resultando en menores pérdidas de calidad del fruto, siendo apta y recomendable para envíos marítimos distantes (Beaudry, 1992). A diferencia de los niveles de O_2 y CO_2 utilizados en AC, la aplicación de AM con bolsas microperforadas resulta en la obtención de menores valores de CO_2; Moggia et al. (2014) encontraron que para el cv. ‘Brigitta’ el uso de AM durante 45 días a 0 ºC, resultó en un menor porcentaje de fruta deshidratada y una mayor retención

de firmeza, comparado con fruta control. Sin embargo, el film utilizado tuvo un escaso efecto en la composición de los gases dentro de la bolsa (18 % O_2 y 2 % CO_2), lo que indicaría que la retención de humedad fue el principal efecto positivo del uso de AM. De forma similar, Bounous et al. (1997) encontraron una reducción del 10 y 25 % en las pérdidas de peso de frutos 'Darrow' y 'Coville', respectivamente, después de 6 semanas de almacenaje a 1 °C en condiciones de AM (10 % CO_2), comparado con fruta control mantenida en condiciones de atmósfera normal.

1.3.2. Alteraciones y defectos

1.3.2.1. Ablandamiento

Como se ha mencionado anteriormente en la sección 1.2.2, el ablandamiento en arándanos, que se estima por medidas instrumentales a través de la pérdida de firmeza, se concentra principalmente entre los estados de fruto verde y fruto completamente maduro. Hacia el momento de cosecha, los cambios a nivel de pared celular, que llevan al desmontaje estructural, parecen haberse completado (Vicente et al., 2007; Angeletti et al., 2010). La firmeza a cosecha puede variar en forma importante entre cultivares, pero también entre estados de madurez del fruto dentro de un mismo cultivar (Beaudry, 1992; Lobos et al., 2014). Adicionalmente, los arándanos se ablandan durante el manejo de la cadena postcosecha, principalmente por manejos inadecuados en cuanto a la temperatura (Ehlenfeldt y Martin, 2002; Tetteh et al., 2004; Ne Smith et al., 2005). No obstante, también se han reportado aumentos en la firmeza durante el almacenaje; así Miller et al. (1993) observaron un aumento en la percepción sensorial de firmeza después de 3 semanas a 1 °C, cuando la pérdida de peso fue menor a 1 %; en tanto valores de 4 a 5% coincidieron con fruta percibida como más blanda. De forma similar, Forney et al. (1999) reportaron aumentos de firmeza con 1 y 2 % de pérdida de peso después de 3 a 9 semanas de almacenaje a 3 °C *vs.* una tendencia al ablandamiento cuando los valores fluctuaron entre 4 y 14 %. Finalmente, estudios más recientes, utilizando niveles controlados de humedad relativa (por medio de modificaciones en el flujo de aire), han confirmado el rol de la pérdida de agua como una causa mayor de los cambios de firmeza durante el almacenaje de arándanos (Paniagua et al., 2013).

1.3.2.2. Deshidratación y pérdida de peso

El mayor componente de los productos hortofrutícolas frescos es el agua (Wills et al., 2007) y su pérdida a través de la transpiración es una causa importante de deterioro que resulta no sólo en

pérdidas cuantitativas directas (menor peso vendible), sino también en pérdidas cualitativas (apariencia, textura) y de valor nutricional (Kader, 2002; Wills et al, 2007).

El intercambio gaseoso en un fruto, que incide en la pérdida de agua del mismo, puede ocurrir desde el producto a la atmósfera por cuatro vías: la zona de la cicatriz (donde se desprende el pedicelo), los estomas/lenticelas, el cáliz y la cutícula (Ben-Yehoshua y Rodov, 2002; Díaz-Pérez, 1998). Los frutos de tomate (*Solanum lycopersicum*) poseen una gruesa cutícula cerosa sin poros (Wilson y Sterling, 1976; Das y Barringer, 1999; Thompson, 2002), por lo que el mayor intercambio gaseoso se produce a través de la cicatriz (Yang y Shewfelt, 1999). En berenjenas el cáliz del fruto es el responsable de al menos el 60 % de la pérdida de agua por transpiración (Díaz-Pérez, 1998). Los arándanos poseen una cutícula sin estomas (Gough, 1994), por lo que la pérdida de agua ocurre estrictamente a través de la cicatriz y la cutícula.

La tasa de pérdida de agua varía en forma importante entre cultivares de arándano y sería uno de los principales factores que contribuye al ablandamiento durante el almacenaje refrigerado (Paniagua et al., 2013). En esta especie, el límite para la pérdida de peso es de 5 a 8 %, puesto que a valores superiores la fruta pierde su valor comercial por exceso de deshidratación (Forney et al., 1999; Sanford et al., 1991). Esto concuerda con los límites de tolerancia aceptados usualmente por parte de la industria: un máximo de 5 a 7 % de pérdida de peso en un período de 3 semanas (Paniagua et al., 2014). Rivera et al. (2013) reportaron 2.1 y 3.5 % de pérdida de peso en fruta paletizada de los cultivares ‘Brigitta’ y ‘O´Neal’ después de 45 días a 0 °C. En un ensayo donde se usó atmósfera modificada (AM) para el almacenaje de ‘Brigitta’, se observó una disminución del porcentaje de fruta con síntomas de deshidratación y un menor ablandamiento respecto del control sin AM (Moggia et al., 2014); el uso de AM tuvo poco efecto en la composición de gases, mostrando que la retención de firmeza fue el principal efecto de este tratamiento.

Se considera que la naturaleza hidrofóbica de la cutícula confiere a los frutos una barrera efectiva para la pérdida de agua hacia el ambiente (Martin y Rose, 2014; Lara et al., 2014). Sin embargo, más que la cantidad total de ceras, parece ser que su composición y estructura afectan en mayor medida la permeabilidad al agua (Riederer y Schreiber, 2001). Leide et al. (2011) encontraron que las ceras cuticulares de un cultivar mutante de tomate, altamente susceptible a la pérdida de agua, prácticamente carecía de *n*-alcanos y aldehídos, y presentaba un elevado porcentaje de

triterpenoides y derivados de esterol, a diferencia de tomates silvestres. Belge et al. (2014a) reportaron relaciones entre *n*-alcanos y triterpenoides de 0.18 y 0.33 en cutículas de cerezas cv. 'Celeste' y 'Somerset' para valores de pérdidas de peso de 15.8 y 7.2 % después de dos semanas de almacenaje refrigerado, respectivamente. Resultados similares se encontraron en melocotones 'October Sun' y 'Jesca', donde relaciones de 0.31 y 0.65 se asociaron con valores de 5.6 y 3.9 % de pérdida de peso, a los 5 días después de cosecha (Belge et al., 2014b).

1.3.2.3. Daños mecánicos (pardeamiento interno)

Los arándanos son especialmente susceptibles a los daños mecánicos, que pueden ocurrir durante todas las etapas de manipulación y uso de maquinaria en cosecha y postcosecha (Gil, 2004), resultando en pardeamiento interno (PI) y en pérdida de firmeza de las bayas dañadas, lo que reduce su calidad y vida postcosecha (Xu et al., 2015). Además, como consecuencia adicional de los daños mecánicos se produce una pérdida de humedad, estimulación de la síntesis de etileno, aceleración de la respiración y una mayor predisposición a la infección de patógenos (Studman, 1999; Martínez-Romero et al., 2003). Generalmente, los daños de tipo PI se manifiestan en la pulpa de los frutos en forma de áreas pardas (Fig. 5) que resultan de la ruptura de los tejidos y la consecuente oxidación de compuestos fenólicos (Studman, 1999; Opara y Pathare, 2014).

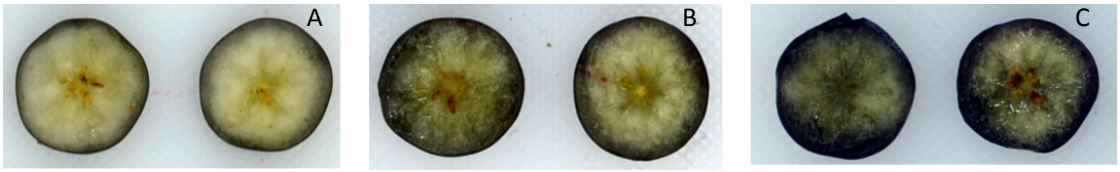

Figura 5. Comparación del corte transversal de frutos de arándano, donde se observa la pulpa sana (A) y diferentes grados de pardeamiento interno (B y C).
Fuente: elaboración propia

La resistencia de los frutos de arándano al desarrollo de PI se evalúa, tal como en otras especies, simulando daño por impacto al lanzarlos desde diferentes alturas y hacerlos colisionar sobre superficies de diversas características; el daño es estimado en base a una escala de severidad del área de la pulpa afectada (al hacer cortes transversales del fruto), después de un período de almacenaje refrigerado posterior al tratamiento (Brown et al., 1996; Yu et al., 2014). Brown et al. (1996) reportaron que bayas arrojadas desde 15 cm de altura sobre una superficie rígida resultaron en un 76 % de frutos dañados (todos en la categoría leve); desde 30 cm de caída se produjo un 100 % de frutos dañados (50 % leve y 50 % moderado), y desde 60 cm de altura el 100 % de la fruta se

clasificó en daño moderado-severo. Las mismas caídas sobre una superficie acolchada resultaron en frutos con daño sólo desde 60 cm de altura (20 % de daño leve).

El desarrollo de PI es variable dependiendo del cultivar; así, Yu et al. (2014) confirmaron que genotipos clasificados como blandos (‘Scintilla’) son más susceptibles a daño, desarrollando un 76% de PI con caídas desde 120 cm de altura sobre una superficie de plástico rígido, en comparación a genotipos firmes (‘Farthing,’ ‘Sweetcrisp,’ y la selección FL 05-528) que resultaron con un 31-68 % de incidencia de PI bajo la misma condición de caída.

En la actualidad la cosecha de arándonos para comercialización en fresco se realiza mayoritariamente en forma manual; sin embargo, dada la menor disponibilidad y mayor costo de la mano de obra en los últimos años, los productores se están viendo forzados a invertir en la mecanización de esta operación (Takeda et al., 2008; Xu et al., 2015). Ello conlleva el riesgo de aumentar el daño por impacto y posterior desarrollo de PI. En un estudio realizado en frutos del cultivar ‘Bluecrop’ se encontró que el 77 % de los arándanos cosechados manualmente no presentaban, o tenían daños leves de PI, en comparación a frutos cosechados en forma mecánica, en donde sólo el 22 % pertenecía a la categoría de síntomas leves (Brown et al., 1996). Adicionalmente, en estos ensayos se comprobó que frutos sin daño o con daño leve perdieron entre un 20 y 30 % de su firmeza original después de almacenaje refrigerado (10 días a 4 °C), mientras que en aquellos frutos dañados la pérdida de firmeza fue de 60 a 65 %, con respecto a los valores en cosecha. Adicionalmente, se ha observado que la fruta mantenida durante 24 horas a temperatura ambiente manifiesta pérdidas de firmeza de 3-8 % en comparación a fruta almacenada inmediatamente en frío (NeSmith et al., 2002).

Con el fin de relacionar los efectos de los daños mecánicos con el desarrollo de pardeamiento interno, se ha desarrollado, de forma similar a la instrumentación utilizada en frutos de gran tamaño, una pequeña esfera denominada BIRD (“blueberry impact-recording device”) que permite determinar el momento e intensidad de impacto al ser lanzado desde diferentes alturas (Yu et al., 2011 y 2014). Éste es un dispositivo de gran utilidad ya que es altamente probable que los arándanos también se dañen durante su paso por las líneas de selección y embalaje. Xu et al. (2015) estudiaron 11 líneas comerciales haciendo pasar el sensor BIRD por el flujo normal de la línea, desde la descarga de la fruta hasta el llenado de envases comerciales (“clamshells”), encontrando

que todas ellas diferían en sus combinaciones y alineaciones de componentes, generando diferentes puntos para un potencial daño por impacto. En general todos los impactos ocurrieron en los puntos de transferencia de las líneas, donde las alturas promedian 35-36 cm. Adicionalmente, los impactos de mayor intensidad se registraron en la etapa final del proceso, cuando el sensor de impactos caía hacia la tolva donde se acopian los frutos previo al llenado de los "clamshells".

1.3.2.4. Pudriciones en postcosecha

El desarrollo de pudriciones en postcosecha es el factor más importante que limita la vida de estantería de los arándanos frescos, siendo la cicatriz pedicelar, el principal lugar de infección (Ceponis y Cappellini 1983; Ehlenfeldt, 2002; Mehra et al., 2013). Las pudriciones más comunes son 'moho o pudrición gris' (*Botrytis cinerea*), 'alternariosis' (*Alternaria* spp.) y antracnosis (*Colletotrichum* spp.). Otros hongos que también suelen aparecer en postcosecha causando pudriciones son especies de *Aspergillus, Cladosporium, Fusarium, Penicillium y Rhizopus* (Ceponis y Cappellini, 1978; Tournas y Katsoudas, 2005; Barrau et al., 2006).

El manejo de las pudriciones de postcosecha comienza con la aplicación de fungicidas en precosecha (Milholland y Jones, 1972), sin embargo, estos tratamientos no siempre son efectivos, dado que puede haber contaminación con esporas de hongos durante la cosecha y el posterior procesamiento de la fruta (Mehra et al. 2013). Por otra parte es imprescindible contar con un adecuado manejo de temperatura en postcosecha (lo que puede reducir la incidencia, si bien no inhibirá el desarrollo) privilegiando un enfriamiento rápido (Ballinger et al., 1973; Ceponis y Cappellini, 1978). Al respecto, Sanford et al. (1991) indican que ya en 1959, Woodruff y Dewey describían los eventos del deterioro en arándanos de arbusto alto concluyendo que éste es principalmente fisiológico, con la infección y crecimiento de hongos ocurriendo de forma ventajosa en tejidos debilitados, y que estos cambios podían ser ralentizados con la reducción de la temperatura de almacenaje.

Adicionalmente al manejo de baja temperatura, el uso de AC o AM con elevados niveles de CO_2 ha demostrado tener impacto en la supresión del desarrollo de pudriciones (Ceponis y Cappellini, 1983; Alsmairat et al., 2011); sin embargo, tal como se ha comentado anteriormente, los niveles de

gases que son efectivos para este fin pueden ocasionar, dependiendo del cultivar, problemas adicionales de calidad como un mayor ablandamiento de pulpa (Alsmairat et al., 2011).

1.4. Efecto del microclima dentro de la planta

El arándano de arbusto alto es una especie frutal cuyo ambiente natural es el sotobosque caducifolio y por lo tanto se presume tolerante a la sombra (Vander Kloet, 1988). Sin embargo, a nivel comercial es cultivado en diferentes áreas geográficas que presentan temperaturas y radiación solar significativamente superiores a las de su origen, y por lo tanto adversas para su normal crecimiento (Hancock y Siefker, 1982; Van der Kloet, 1988; Luby, 1991; Retamales y Hancock, 2012). En general, en presencia de altas temperaturas (pasado el mediodía), el lado de la planta que recibe menor flujo radiativo (correspondiente al lado menos soleado) podría reducir los efectos negativos de un exceso de radiación (foto inhibición) y, mediante el incremento de la conductancia estomática, experimentar un menor gradiente de déficit de presión de vapor. Así, se reduciría la temperatura de las hojas, incrementándose la tasa de asimilación de CO_2 (Dale, 1992; Syvertsen et al., 2003). y con ello se favorecería la acumulación de carbohidratos en torno a la fruta de dicho lado. En ese sentido, Prange y DeEll (1977) ya indicaron que cuando las condiciones microclimáticas son adecuadas, mejora la calidad de la fruta en postcosecha; sin embargo, tales condiciones no han sido claramente determinadas para esta especie.

Como en toda especie vegetal, el agua juega un rol fundamental en su desarrollo y en el caso del arándano su manejo podría ser aún más crítico. El sistema radical, que carece de pelos radicales, no es capaz de abastecer lo suficientemente rápido con agua la parte aérea, cuando la demanda atmosférica es elevada (Gough, 1994), afectando directa (contenido de agua) e indirectamente (temperatura) a la condición de la planta y sus frutos. Trehane (2004) indica que las hojas a pleno sol podrían alcanzar entre 10 y 15 °C más que la temperatura ambiente, siendo 30 ºC el límite donde se comenzarían a registrar daños por exceso térmico a nivel foliar.

Si bien se considera que un racimo totalmente expuesto a la luz solar se encuentra creciendo bajo una condición mejorada para la calidad del fruto (Smart, 1985), el consiguiente aumento de temperatura y el exceso de irradiación solar de la fruta podría tener un efecto negativo en su metabolismo (Bergqvist et al., 2001), afectando indirectamente la estructura celular y otros componentes que determinan la textura (Vicente et al., 2007). Temperaturas superiores a 32 °C

durante la maduración pueden originar frutos más pequeños, blandos y con mayor susceptibilidad a la pérdida de ceras mediante el roce provocado con las hojas o por la manipulación en cosecha (Mainland, 1989). Por otro lado las altas temperaturas también pueden reducir la producción de antocianinas a nivel de fruto (Prange y DeEll, 1997) y por ende su sensibilidad a las condiciones de irradiación solar.

En huertos establecidos en orientación norte-sur, no es extraño encontrar que, pasado el mediodía, frutos ubicados en el lado poniente de la planta alcancen 7-10 °C más que los del lado oriente (Lobos y Moggia, datos no publicados). Al respecto, en experiencias realizadas en uvas 'Emperor', los racimos expuestos a temperaturas mayores de 37 °C, inhibieron la acumulación de azúcares (Kliewer, 1977). Otros estudios en *Vitis vinifera*, en condiciones similares a las de Chile, indican que frutos ubicados en el lado de la planta que permanece sombreada durante la tarde mantienen niveles más altos de acidez (Bergqvist et al., 2001). Al estudiar el uso de mallas sombreadoras en arándanos, Lobos et al. (2014) demostraron que la disminución de la radiación se vio acompañada de reducciones en la temperatura foliar, lo que se tradujo en aumentos del peso del fruto, contenido de agua de los frutos, AT y firmeza, pero con disminuciones del contenido de SS a cosecha. Factores ambientales, como temperatura y luz, que influyen en el microclima de la planta podrían entonces tener un efecto diferenciador en la calidad de fruto, sobre todo en su textura (Sams, 1999). La variabilidad de la fruta observada en los mercados de destino podría en parte estar asociada al microclima dentro de la planta; sin embargo, también habría una contribución importante de las condiciones medioambientales durante la temporada. Esto sería debido al largo periodo de floración y al tiempo total del desarrollo de los frutos en cada racimo, que incidiría en que los frutos se desarrollen bajo condiciones macro y micromabientales diferentes durante la temporada, dando origen a individuos con distintas características fisiológicas y por lo tanto con un potencial de almacenaje variable, tal como fue detallado anteriormente en las secciones 1.2.1. y 1.2.2.

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2. ESTUDIOS PREVIOS A LA TESIS

Los estudios que dan origen a la presente Tesis se basan en los resultados obtenidos los últimos años por el grupo de investigación en fisiología y postcosecha de frutales menores, pertenecientes al Centro de Mejoramiento Genético y Fenómica Vegetal de la Facultad de Ciencias Agrarias de la Universidad de Talca, producto de un proyecto FONDEF regional (2010-2013, con la participación del Estado, la Universidad y la Empresa Privada), cuyo objetivo fue establecer la importancia relativa de diversos factores, en cada fase del proceso de exportación de arándanos en fresco (precosecha, cosecha y postcosecha), con el fin de aumentar la calidad en destino y reducir la variabilidad dentro y entre partidas de fruta. Se estudiaron diversos factores: manejos de precosecha (poda de invierno, aumento de calibre por aplicación de reguladores de crecimiento), cosecha (fecha e índices de cosecha, composición nutricional, madurez, tipo y hora de cosecha), y postcosecha (sistemas de almacenaje, evaluación de daño mecánico). Los principales impactos de las prácticas estudiadas se obtuvieron para las variables de calibre, firmeza, contenido de nutracéuticos y % de frutos sanos exportables. Los resultados más relevantes se pueden resumir de la siguiente manera:

- El calibre del frutos a cosecha se aumentó hasta un 30 % por la aplicación de citoquininas (N-(2-chloro-4-pyridyl)-N'-phenylurea, CPPU) y hasta un 10 % al cosechar frutos con 100 % de coloración azul *vs.* 75 % de coloración azul.
- En cuanto a firmeza, fruta recolectada en la mañana fue un 5 % más firme que la cosechada en la tarde; en tanto, cosechar en 100% azul *vs.* 75% azul, redujo en un 3 % la firmeza de la fruta.
- En postcosecha, la firmeza se afectó positivamente por los factores: aplicación de CPPU (fruta fue hasta 15 % más firme), cosechar en la mañana *vs.* la tarde (5 % más firme) y el uso de bolsa de atmósfera modificada (MAP) *vs.* control sin bolsa (22 % más firme).
- El contenido de compuestos nutracéuticos (medidos como Fenoles y ORAC), aumentó en cosecha en un 40% al pasar los frutos de 75 a 100 % de coloración azul. En postcosecha, dicho incremento sólo fue de un 2 %.
- Cosechar en la mañana aumentó en 20 % la proporción de frutos sanos al final de almacenaje, respecto de una cosecha en la tarde y la cosecha semi-automática, con máquina vibradora no alteró los niveles de frutos sanos.
- El uso de bolsas MAP aumentó en un 28 % los frutos sanos, respecto de no usar bolsa, principalmente por una menor deshidratación y mayor firmeza de la fruta.

Dichos resultados fueron presentados y publicados en diversos congresos y revistas científicas:

i. **"Real world evaluation of packaging strategies and SO_2 fumigation for shipment of blueberry fruit from Chile to North America"**. Beaudry, R.M., Moggia, C.; Lobos, G.A., Retamales, J.B., Bravo, C. 2013**.** HortScience 48(9) Supplement, 2013 ASHS Annual Conference. S138-S139 (Abstract)

Abstract

Blueberry (*Vaccinium corymbossum* L. cv Brigitta) fruit were harvested at commercial maturity (> 80 % blue) from two different farms in the Maule region of Chile on January 9 and 10 in 2013 and packaged over the next two days using several different packaging strategies that included perforated and nonperforated films and fumigation with SO_2. Fruit from farm 1 were placed into: 125 g polystyrene clamshells sealed in low density polyethylene (LDPE) packages (VF101) containing a total of 1.6 kg fruit; 340 g clamshells (one dry pint) in LDPE packages (VF202) containing 4.1 kg fruit; loose fruit in LDPE packages (VF101) containing 1.6 kg fruit; and 125 and 340 g clamshells without packages. Fruit from farm 2 were placed into 125 g clamshells in LDPE packages (VF101) containing a total of 1.6 kg fruit; 340 g clamshells in LDPE packages (VF102) containing 4.1 kg fruit; 340 g clamshells in LDPE packages (VF103) containing 6.1 kg fruit; and 125 and 340 g clamshells without packages. Packages had 0 or 2 perforations for fruit from both farms and a portion of the fruit from farm 1 were fumigated with SO_2 for approximately 10 min prior to packaging. Fruit entered the refrigerated distribution chain for shipment to the U.S. upon packaging, were shipped by sea container from Chile approximately 11 days after packing, and arrived in the U.S. 28 days later. Transport through the U.S. distribution chain required an additional 8 days. Thus, fruit arrived in Michigan 40 days after harvest and were evaluated after an additional 2 days at 2 °C to simulate regional distribution. Fruit were evaluated at that time and after an additional 3 days at 12 °C to simulate retail holding. Assessments included fruit firmness, degree of internal discoloration, decay, and shrivel, and visual sensory analysis. Perforated packages had higher O_2 (14.6 vs 8.9%, respectively) and lower CO_2 (4.8 vs 6.6%, respectively) than nonperforated packages. The greatest effect on storability was the source of the fruit; fruit from farm 2 were in much poorer condition than those from farm 1. The retail holding period significantly reduced the quality of the fruit, especially for farm 2. Packaging tended to improve quality over

nonpackaged berries. Perforated LDPE packages performed similarly to nonperforated packages. Smaller clamshell containers yielded the highest quality fruit. Fumigation with SO_2 suppressed decay and improved visual appearance.

ii. **"Repeated applications of CPPU on highbush blueberry cv. Duke increase yield and enhance fruit quality at harvest and during postharvest"**. Retamales, J.B., Lobos, G.A., Romero, S., Godoy, R., Moggia, C. 2014. Chilean Journal of Agricultural Research. 74: 157-161.

Abstract

CPPU applications can increase blueberry (Vaccinium corymbosum L.) yield and fruit size, but their impact on postharvest is unknown. We studied repeated CPPU applications effects on yield and quality (harvest, postharvest), over 2 yr on mature 'Duke' plants in South-Central Chile. The first year, 5 or 10 ml L-1 CPPU was applied at 3, 10, and/or 17 d after full bloom (DAFB) plus a non-sprayed control. The second year, 5 or 10 ml L-1 CPPU were sprayed 10 and 17 DAFB plus a control. The first year, only 10 ml L-1 CPPU sprayed 3+17 DAFB increased yield (32.5% > control). Ten ml L-1 CPPU applied 10 or 3+17 DAFB had highest fruit diameter. Ten ml L-1 CPPU at 17 DAFB or at 3+10+17 DAFB had highest soluble solids. Overall, 10 ml L-1 CPPU applied 3+17 DAFB, was the best treatment for year one, since it increased fruit yield and diameter, while soluble solids and postharvest weight loss were similar to control. The second year, 10 ml L-1 CPPU reduced fruit coloration (blue color coverage index: BCCI) and soluble solids, but not firmness at harvest. This rate increased berry weight (24.2%) and fruit wax (59% > wax coverage index: WCI) at harvest. Harvest and postharvest WCI increased consistently as CPPU rate increased. CPPU reduced fruit rotting (15% at 45+5 evaluation). During storage, CPPU-treated-fruit had a slower decrease in firmness (30.5% < control at 30+1), but no difference at 30+5. CPPU-treated-fruit usually had higher post harvest soluble solids. Ten ml L-1 CPPU retarded color evolution at harvest and at 30+1, but not at 30+5, 40+1 or 40+5.

iii. **"Modified atmosphere packaging in blueberries: effect of harvest time and moment of bag sealing"**. Moggia, C., Lobos, G. A., Retamales J.B. 2014. Acta Horticulturae, 1017: 153–158

Abstract

The use of modified atmosphere in the packaging of fresh blueberries (*Vaccinium corymbosum* L. 'Brigitta') was studied as a function of harvest time and moment of bag sealing. In trial 1, four treatments were established considering harvest time (morning: AM or afternoon: PM), and moment of bag sealing: before or after cooling at 0°C. In trial 2, the packaging system was compared to a control treatment, without bag, for AM- and PM-harvested fruit. After harvest fruit were placed under shading and subjected to 6 h delay before sorting. Berries were stored for 30 and 45 d at 0°C and evaluated after 1 and 3 d at 18°C (30+1, 30+3, 45+1, 45+3). Evaluations included: %O2 and %CO2 evolution within the bags, Berry firmness (g/mm) and fruit quality (% sound, rotten, dehydrated or mechanically damaged fruit). Results show that the use of bags increased the proportion of sound fruit (80-90%) with regards to controls (<60%), especially in the evaluations at 30+3, 45+1 and 45+3. Bagging increased the % of sound fruit in the AM *vs.* PM; however, there was no effect of time of bag sealing. The main effect of bagging was a lower proportion of dehydrated fruit; which amounted to 4-10% in bag treatments *vs.* 20-30% for control fruit. Additionally bagging retained fruit firmness, both for AM and PM-harvested fruit. Since bagging had little effect on gas composition, its effect must be studied further. These results evidence a high potential for the use of modified atmosphere packaging for boat shipping of blueberries for distant markets

iv. **"Effect of mechanized (automotive or shaker) *vs.* hand harvest on postharvest fruit quality of blueberries (Vaccinium corymbosum L.)"**. Lobos, G. A., Moggia, C., Retamales J.B., Sánchez, C. 2014. Acta Horticulturae 1017: 135-140.

Abstract

This research was carried out during the 2010/11 growing season to establish the effect of different types and times of harvest on the quality of fresh blueberries. The 6 treatments consisted of 3 harvest types (hand, automotive or shaker) and two harvest times (morning: 9-11 AM; afternoon: 3-5 PM). Ten-year-old 'Brigitta' and 'O'Neal' plants, from a

commercial planting in Linares (35°52' South and 71°37' West) were used. Measurements were made of: harvest duration, weight of fruit picked, and proportion, in weight, of fruit in the categories: fresh, discarded, IQF (individual quick frozen) and pre-size. Firmness was measured at harvest, and after 60 d at 0°C plus 1 d at 18°C (60+1). Mechanical damage was measured after 60+3 d. Highest fruit firmness, independent of cultivar and harvest type, was for AM pickings. Firmness for both measuring dates and cultivars was lowest for automotive; while in 'O'Neal', hand and shaker had equivalent firmness, in 'Brigitta' shaker-harvested-fruit had intermediate firmness. Mechanical damage was greater for fruit picked with automotive equipment, and also in AM pickings. Fruit picked by hand and with shaker had similar mechanical damage in 'Brigitta', but not in 'O'Neal', where shaking caused greater damage. After fruit sorting, 'Brigitta' had a greater proportion of fruit suitable for the fresh market. Averaging both cultivars, the proportion of fruit for the fresh market was 71.9, 76, and 82.9%, for automotive, shaker and hand harvest, respectively. These results indicate a positive potential for harvesting with shakers, but its effects on different cultivars and the cost/benefit ratio need to be studied.

v. **"Blueberry production in Chile: Current status and future developments"**. Retamales, J.B., Palma, M.J., Morales, Y.A., Lobos, G.A., Moggia, C. Mena, C.A. 2014.. Bras. Frutic., Jaboticabal-SP, 36: 58-67.

Abstract

Chile has become a major actor in the blueberry industry as the most important supplier of off-season fresh fruit for the northern hemisphere. Blueberry exports passed from US$ 30 million (around 4,000 tons) in 2000 to US$ 380 million (94,000 tons) in 2011. The characteristics of the major blueberry growing regions (North, Central, South-central and South) are presented in terms of acreage, varieties, management practices, extension of the harvest season, and soil and climatic conditions. Most fruit is from highbush varieties, picked by hand and exported fresh by boat to United States. Largest proportion of fruit is exported from mid December to late January, which coincides with lowest prices. The south-central región (latitudes 34º50´ to 38º15´ S) was in 2007 the most important one with 5,075 ha (51.1% of area planted). Among the challenges for the Chilean blueberry industry in the near future are: 1. Lower profitability due to lower rates of currency exchange and higher costs, 2. Greater scarcity and higher cost of labor, 3. Need for higher productivity and

sustainable production practices, 4. Fruit of high and consistent quality, and 5. Greater investment in research. As a case study the article presents three approaches that can help identify areas with low availability of labor and improve its efficiency. The article shows the use of geomatic tools to establish labor availability, application of growth regulators to reduce crop load, increase fruit size and improve harvest efficiency, and the use of shakers to harvest fresh fruit for long distance markets. More research is needed to improve yields, reduce costs and give greater economical and ecological sustainabilityto the Chilean blueberry industry

3. OBJETIVOS

1. Objetivo general

El objetivo general de la presente Tesis fue caracterizar y comprender la influencia del estado de madurez del fruto a la cosecha, las características de la cutícula, el rol de la cicatriz y la posición del fruto dentro de la planta, como factores de variabilidad asociados al ablandamiento y deshidratación de arándanos en postcosecha.

2. Objetivos específicos

- Estudiar los cambios en calidad y madurez durante el desarrollo de frutos de arándano cvs. 'Duke' y 'Brigitta', desde el estado verde hasta cosecha comercial
- Estudiar el comportamiento en postcosecha de frutos cvs. 'Duke' y 'Brigitta' cosechados en tres estados de madurez (75% coloración azul, 100% coloración azul y sobremaduro) y mantenidos durante 45 días en almacenaje refrigerado.
- Determinar la relación entre parámetros de calidad en cosecha y la composición de triterpenoides de la cutícula, para los cvs. 'Duke' y 'Brigitta' cosechados en tres estados de madurez (75% coloración azul, 100% coloración azul y sobremaduro) y mantenidos durante 45 días en almacenaje refrigerado.
- Determinar para los cvs. 'Duke' y 'Brigitta' el efecto de diferentes niveles de firmeza en cosecha y el daño por impacto, sobre el ablandamiento y desarrollo de pardeamiento interno durante almacenaje refrigerado.
- Determinar, para los cvs. 'Duke' y 'Brigitta' el efecto del estado de madurez en cosecha (frutos maduros vs. sobremaduros) y el rol de la posición de la fruta en la planta (oriente *vs.* poniente) sobre la firmeza durante postcosecha.
- Evaluar, para tres líneas de híbridos, provenientes de un programa de mejoramiento genético, las características morfométricas del fruto y la cicatriz y su efecto diferencial sobre la permeabilidad al agua, la deshidratación y el ablandamiento de los frutos.

4. PLAN DE TRABAJO Y MATERIAL

4.1 Cronograma del desarrollo de la Tesis Doctoral

Durante campañas consecutivas, desde 2011-2012 hasta 2015-2016, se realizaron diversas experiencias en las que se llevaron a cabo las siguientes actividades:

- Muestreos de precosecha: seguimiento cada 2-3 días de los frutos desde el estado verde inmaduro hasta el momento de cosecha .

- Establecimiento de estados de madurez*:* marcaje de frutos en estado 75% coloración azul y seguimiento diario para definir los estados 100% azul y 100% sobremaduro.

- Cosechas: en la mayoría de los ensayos se contempló la cosecha de frutos con 75% de coloración azul, 100% de coloración azul y 100% de coloración azul-sobremaduros.

- Almacenamiento: toda la fruta se almacenó en condiciones de refrigeración convencional a 0 °C y 85-88 % de humedad relativa.

- Evaluaciones: dependiendo del ensayo la fruta se evaluó cada 7 días, por un máximo de 35 a 45 días, o directamente el día final del almacenamiento. Las evaluaciones se hicieron el día que la fruta fue retirada de las cámaras más 1 día adicional de "shelf-life" a 18 °C.

En la Figura 6 se detallan las distintas actividades desarrolladas a lo largo de la realización de la Tesis Doctoral. Se incluyen también dos temporadas de ensayos previos que se llevaron a cabo antes iniciarse la Tesis propiamente dicha.

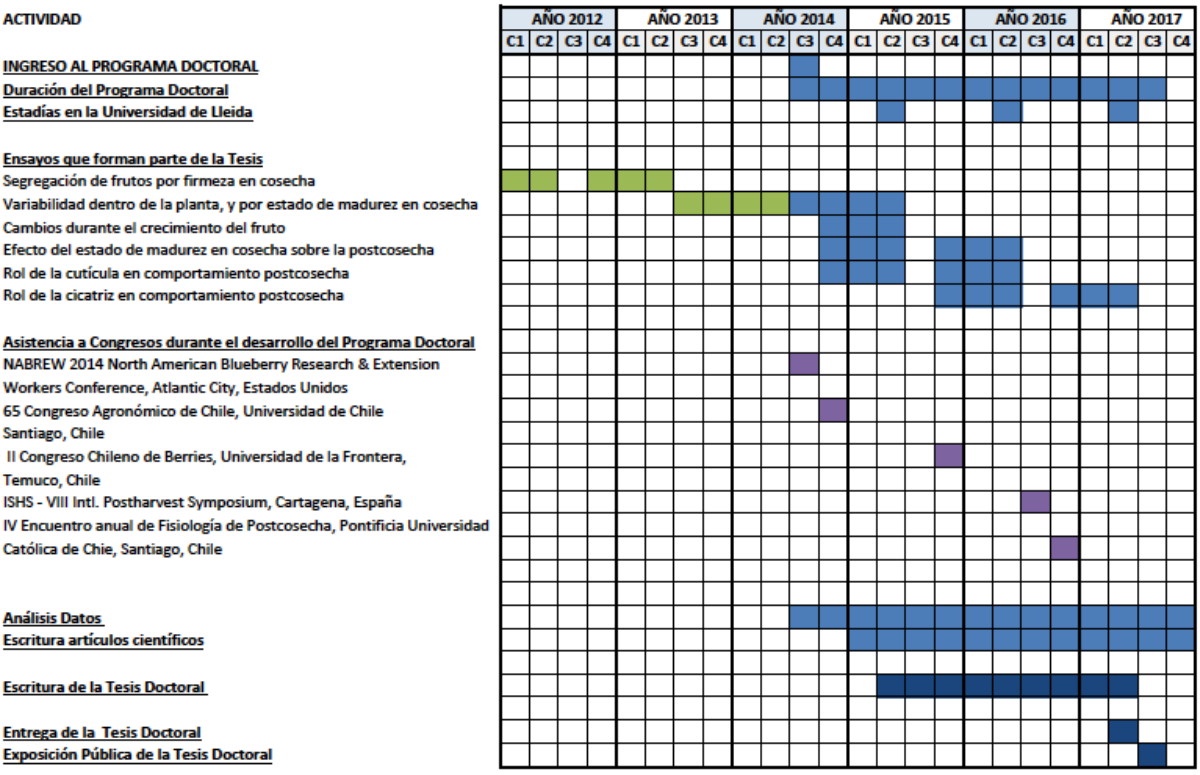

Figura 6. Diagrama de Gantt de las actividades realizadas a lo largo de la Tesis Doctoral.

A continuación se detallan los ensayos, indicando la campaña en la que fueron realizados:

- Variabilidad de la fruta: efectos de la segregación por firmeza en cosecha y los daños por impacto sobre el ablandamiento y el desarrollo de pardeamiento interno en almacenaje en cvs. 'Duke' y 'Brigitta' (Campañas 2011-2012 y 2012-2013) .

- Cambios durante el desarrollo del fruto, madurez en cosecha y comportamiento en almacenaje de cvs. 'Duke' y 'Brigitta' (Campaña 2013-2014)

- Características de la cutícula y el estado de madurez en cosecha, que influyen en la deshidratación y el ablandamiento en almacenaje de arándanos cvs. 'Duke' y 'Brigitta' (Campaña 2014-2015 y 2015-2016).

- Variabilidad de los frutos (cvs. ‘Duke’ y ‘Brigitta’) según el estado de madurez en cosecha y la posición en la planta, en función de cambios en los índices de madurez y evolución del ablandamiento y deshidratación en almacenaje (Campañas 2013-2014 y 2014-2015) .

- Rol de la cicatriz en el ablandamiento y deshidratación en almacenaje de arándanos provenientes de plantas de tres líneas de mejoramiento genético (Campaña 2015/2016).

4.2 Material

En todos lo ensayos se utilizaron frutos de los cultivares ‘Duke’ y ‘Brigitta’, siendo dos de las principales variedades a nivel nacional, así como las de mayor importancia en las regiones del Maule y del Bío-Bío (Ciren, CORFO, 2014). ‘Duke’ es un cultivar de producción temprana, liberado en 1986 (New Jersey/USDA); sus frutos son firmes, de tamaño medio, con una cicatriz pequeña y de sabor débil (Retamales y Hancock, 2012). ‘Brigitta’ de procedencia Australiana (1980), es un cultivar de media estación, que produce frutos grandes, de buena coloración azul, excelente sabor y una prolongada temporada de maduración (Retamales y Hancock, 2012). En la Figura 7 se puede observar los distintos estados de madurez de los frutos que fueron objeto de estudio en los ensayos experimentales.

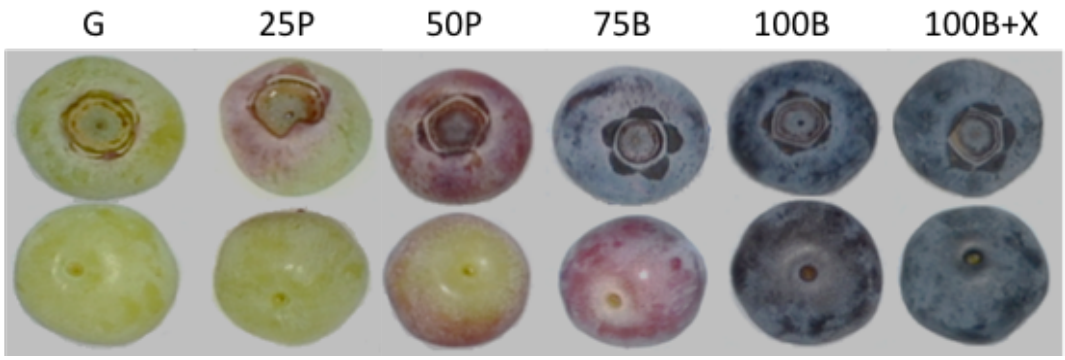

Figura 7. Detalle de los estados de madurez utilizados en los ensayos. **G**: fruto verde, **25P**: 25 % coloración rosa, **50P**: 50 % coloración rosa, **75B**: 75 % coloración azul; **100B**: 100% coloración azul cosechada con un máximo de 2 días; **100B+X**: 100 % coloración azul, cosechada 5 a 7 dias después de 100B)

Los ensayos se realizaron con fruta proveniente de fincas ubicadas en dos regiones de la zona centro-sur de Chile, (Cuadro 3, Fig. 8).

Cuadro 3. Características de los huertos utilizados para los ensayos.

Región	Localidad	Ubicación geográfica	Cultivar	Fecha de plantación
Maule	Río Claro	35° 15' S; 71° 14' W	'Duke' 'Brigitta'	2004 2005
Maule	Panguilemo	35° 22' S; 71° 35' W	Híbridos programa mejoramiento genético	2009
Maule	Longaví	36° 00' S; 71° 35' W	'Duke'	2012
Bío-Bío	Santa Bárbara	37° 29' S; 72° 19' W	'Brigitta'	2008

Figura 8. Ubicación geográfica de Chile. Destacadas en rojo las Regiones de Maule y del Bío-Bío.

La Región del Maule se caracteriza por un clima templado de tipo mediterráneo, con una estación seca de cuatro a seis meses y una pluviometría anual desde 700 mm en los valles, hasta 2140 mm en la cordillera. La temperatura media en la región es de 19 °C, con extremas de 30 °C, gran cantidad de días soleados y gran cantidad de horas de luz durante el período de verano; en invierno las temperaturas mínimas medias son de 7 °C.

En la parte norte de la Región del Bío-Bío, que limita con la Región del Maule, también predomina el clima templado mediterráneo con estación seca de 4 a 5 meses. Se caracteriza por presentar precipitaciones anuales que superan los 1.000 mm, para luego, a partir de diciembre y hasta marzo, producirse una disminución de las lluvias, llegando a registrar sólo 40 mm mensuales.

Tanto para las determinación de las condiciones iniciales, como para la simulación del daño por impacto, toda la fruta fue recepcionada en el Laboratorio de Postcosecha del Centro de Mejoramiento y Fenómica Vegetal de la Universidad de Talca. Una vez establecidos los tratamientos, la fruta fue almacenada en cámaras frigoríficas (0 °C, 80-85 % HR), ubicadas en las dependencias de la Facultad de Ciencias Agrarias de la Universidad de Talca, Chile.

Las determinaciones físico-químicas se realizaron en el Laboratorio de Postcosecha del Centro de Mejoramiento y Fenómica Vegetal de la Universidad de Talca, Chile; Laboratorio de Química de Productos Naturales perteneciente al Instituto de Química de Recursos Naturales, Universidad de Talca, Chile y en los Laboratorios de Bioquímica del Departamento de Química y de los Servicios Científico-Técnico de la Universidad de Lleida, España.

4.3 Determinaciones analíticas

A continuación se resumen las diferentes determinaciones analíticas que se realizaron en los ensayos, cuyo detalle metodológico se encuentra referenciado en cada una de las publicaciones presentadas en la sección de Resultados.

- Peso del fruto (g)
- Superficie del fruto y la cicatriz (cm^2)
- Diámetro ecuatorial y polar de fruto y cicatriz (mm)
- Firmeza (N)

- Sólidos Solubles (%)
- Acidez titulable (% ácido cítrico)
- Tasa respiratoria (μg CO_2 kg^{-1} s^{-1})
- Producción de etileno (ng kg^{-1} s^{-1})
- Contenido de ceras cuticulares (g m^{-2})
- Análisis de cutícula (contenido de triterpenoides, g m^{-2})
- Permeabilidad al agua de la cutícula y de la cicatriz (μmol m^{-2} s^{-1} kPa^{-1})
- Difusividad de poro (cicatriz) (g d^{-1} mm^{-1} kPa^{-1})
- Pérdida de peso (% d^{-1}; g d^{-1})
- Ablandamiento (% de pérdida de firmeza respecto de firmeza inicial)
- Pardeamiento interno (Escala visual de 0 a 4)
- Índice de Deshidratación (Escala visual de 1 a 3)

5. RESULTADOS

5.1. Changes in quality and maturity of ‘Duke’ and ‘Brigitta’ blueberries during fruit development: postharvest implications.

Abstract

Fresh Chilean blueberries take in average 20-50 days to reach overseas markets, so a better knowledge of their postharvest behavior would help maintaining their quality for longer periods. Quality of highbush blueberries (*Vaccinium corymbosum* L., cv. 'Duke' and 'Brigitta') was assessed at six stages based on color: 100% green (100G), 75% green+25% pink (25P), 50% green+50% pink (50P), 25% green+75% pink-blue (75B), 90-100% blue (100B), 100% blue+5 extra days on plant (100B+5). Also, 75P, 100B and 100B+5 fruit were evaluated after storage at 2 °C for 45 days + 1d at 18 °C. Over berry maturation from 100G to 100B, color developed steadily while firmness and TA decreased. Respiration and ethylene production rates peaked early, at 25P and 50P, respectively, and were higher for 'Duke' than for 'Brigitta'. After harvest, cultivar- and maturity-related differences were found. 'Brigitta' fruit retained higher firmness and had lower weight loss than 'Duke'. In general, 100B+5 fruit were over-ripe and showed low quality. Additional physiological, morphological and biochemical studies for a wider range of cultivars will be needed.

1. Introduction

On a worldwide basis, blueberry (*Vaccinium corymbosum* L.) cultivation has grown significantly over the last two decades (Brazelton, 2009). Blueberries are climacteric fruit, which exhibit a raise on CO_2 and ethylene evolution during the middle stage of ripening (Windus et al., 1976; El-Agamy et al., 1982), so they are harvested at a post-climacteric stage, and will not improve in quality during handling and storage (NeSmith et al., 2002; MacLean and NeSmith, 2011). Blueberry fruit are extremely firm when green; soften substantially as they turn to red stages, but only slightly thereafter (Ballinger et al., 1973). The soluble solids content ranges from 7% in a green berry to about 15% in a fully ripe berry (Shutak et al., 1980), whereas fruit harvested as soon as they turn blue contain about 12% (Gough, 1984). Berries are considered ready to pick when they turn 100% blue, but since they do not ripen uniformly on a cluster (Gough, 1984; Lobos et al., 2014) growers usually wait for blue fruit to accumulate in the bushes between harvests. Whether fruit can be left on the bush without negatively impacting storage life is a mainly important question (Retamales and Hancock, 2012), since by this management, fruit with same color but different physiological maturity are harvested. Given that fresh Chilean blueberries are exported mainly by boat (Retamales et al., 2014) and fruit may take up to 50 days to reach final consumers, this heterogeneity may increase with longer storage periods affecting quality of the fruit upon arrival. It has been reported that 'Elliott' blueberries

harvested at earlier stages of fruit ripening (immediately after achieving blue skin color) stored more satisfactorily than fruit harvested at more advanced stages (Hancock et al., 2008). On the other hand, other cultivars like 'Liberty' and 'Aurora' could be harvested later than usually done in commercial practice, without a loss in post-harvest storage life and an improvement in flavor (Lobos et al., 2014).

Given the above, there is the need to understand the pre- and postharvest behavior of different cultivars, in order to seek strategies to maintain their quality for longer periods. The main goal of this work was to study changes in quality and maturity during fruit development of 'Duke' and 'Brigitta' blueberries, and the postharvest behavior of fruit harvested at three different maturity stages and maintained 45 days under refrigerated storage**.**

2. Materials and methods

2.1. Fruit material and experimental setup

Highbush blueberries (*Vaccinium corymbosum* L., cv. 'Duke' and 'Brigitta') were picked from mature plants (8 and 9 years old, respectively) planted at a commercial field in Río Claro, Maule Region, Chile (35°15'35.16'' South; 71°14'22.53'' West). For fruit development assessments, samples were harvested every two to five days, and classified by visual grading into the following color categories: 100% green (100G); 75% green + 25% pink (25P); 50% green + 50% pink (50P); 25% pink +75% pink-blue (75B), 90-100% blue (100B) and 100% blue allowed to remain on the plant for 5 extra days (100B+5). In order to establish the last two stages, fruit clusters with similar characteristics (number and shape) and canopy position (superior third of the eastern side) were selected and labeled once 75B was reached. As these fruit reached 100% blue, one half thereof were harvested (100B), the other half remaining on the plant for extra 5 days (100B+5), in order to mimic the usual commercial practice. No visual differences in skin color could be perceived between 100B and 100B+5 fruit.

For each sampling date, three replicates of 25 fruit each were collected, in order to characterize maturity and quality at the different developmental stages. Additionally, four replications (125 g clamshells, 50 berries each) were harvested for 75B, 100B and 100B+5 fruit, placed in commercial cardboard boxes, and stored at 2 °C and 85-88% RH for 45 days. Fruit were evaluated for maturity and weight loss, after being kept 1d at 18 °C following removal from cold storage.

2.2. Maturity and quality assessments

For surface color, 25 individual blueberries were measured, at the equatorial zone, using a Minolta Chroma Meter (CR210, Osaka, Japan) calibrated with a white tile; lightness (L), hue angle (h°) and chroma (C*) are reported as proposed by McGuire (1992). Fruit weigh (g) and size (equatorial and polar diameter (mm)) were measured on the same 25 fruit with an electronic balance, and a digital caliper, respectively. Firmness (g mm^{-1}) was measured with a FirmTech 2 (BioWorks, Wamego, Kansas, USA) on four replicates of 25 fruit each. The equipment was set up at maximum and minimum compression forces of 200 g (1.96 N) and 15 g (0.15 N), respectively, and piston speed of 6 mm s^{-1}.

Soluble solids content (SSC) (%) was assessed on five berries per replicate with a digital refractometer (Atago, Pocket PAL-1, Tokyo, Japan). Titratable acidity (TA) (% citric acid) was determined in 10 mL of juice per replicate, after dilution with distilled water and titration with 0.1 N NaOH to pH 8.2.

For the evaluation of respiration rates (RR) (mL kg^{-1} h^{-1} CO_2), three fruit per replicate were placed for 2 h at 18 °C within a 28-mL sealed glass vial, and a Quantek 902P O_2/CO_2 analyzer (Quantek Instruments Inc., MA, USA) was used to measure CO_2 inside the vials. For the quantification of ethylene production (EP) (µL kg^{-1} h^{-1}), a gas sample (1 mL) was withdrawn with a syringe from the headspace volume, and injected onto a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector and an alumina FID 80/100 mesh column. The injector, oven, and detector temperatures were set at 75 °C, 100 °C, and 170 °C, respectively, with helium as the carrier gas. Initial (harvest) and final (45+1) weight of each clamshell was recorded, for the estimation of weight loss (%) after storage.

2.3. Statistical analysis

Data were subjected to ANOVA, using a completely randomized design with maturity stage as the factor, separately for each cultivar. Mean separations ($P \leq 0.05$) were calculated (HSD test) using Statgraphics Centurion XVI (v.16.0.09).

3. Results and Discussion

'Duke' and 'Brigitta' fruit exhibited a raise on both RR and EP (Fig. 1), and as reported previously (Windus et al., 1976; Suzuki et al., 1997), differences were seen in the magnitude of the peaks and the stage of development at which they were reached. 'Brigitta' displayed a maximum RR and EP between 50P and 75B with mean values of 27.6 mL kg^{-1} h^{-1} CO_2 and 0.75 μL kg^{-1} h^{-1} ethylene, respectively. On the other hand, 'Duke' exhibited higher metabolic activity, in terms of both RR and EP; rise for EP of this cultivar (2.63 μL kg^{-1} h^{-1} ethylene) also occurred between 50P and 75B, but the peak in RR (94.2 mL kg^{-1} h^{-1} CO_2) was earlier (25P). According to Gough (1984), cultivars displaying higher respiration rates after harvest are least likely to keep well.

Fruit color (Table 1) as measured by colorimeter (L, chroma and hue angle) resulted in marked differences between 100G, 25P and 50P; but slight or none differences were found between 75B, 100B or 100B+5. Hue values are variable since changes in skin color change from green, to pink and blue; therefore, L and chroma would be better indicators of changes associated to berry development. Additionally no visual differentiation could be perceived between 100B and 100B+5.

The highest increase in fruit weight and diameter was observed during the first three stages of development (100G, 25P and 50P). Maximum fruit weight was reached on 'Duke' at B100 and B100+5 on 'Brigitta'. For both cultivars equator diameter increased up to B100 stage; polar diameter increased until H100+X on 'Duke' and 50P on 'Brigitta' (Table 2).

'Duke' and 'Brigitta' berries were very firm at 100G, but had lost 47% and 45% of initial firmness, respectively, when they reached 75B (considered as the first suitable harvest stage) (Table 3). The highest decline in firmness occurred between 100G and 25P for 'Duke' (30%), and between 50P and 75B for 'Brigitta' (34%), coincident with a peak in RR for 'Duke' and peaks in RR and EP for 'Brigitta'. A second important decrease on firmness was seen between 100B and 100B+5 (18.5% and 15.4% reduction for 'Duke' and 'Brigitta', respectively).

Additionally SSC augmented and TA decreased leading to increased values of SSC/TA ratio between 10.9 and 25.4 from 75B to 100B+5 stages (Table 3). Firmer fruit and SSC/TA ratios <18 have been associated to higher postharvest potential (Hanson et al., 1993; Galletta et al., 1971). In our study,

values for 100B+5 fruit at harvest appear too extreme if long-distance markets are to be reached with acceptable quality.

Although 'Duke' fruit were firmer at harvest, firmness decline for this cultivar was high, since fruit lost 32%, 25% and 18% of initial firmness at 75B, 100B and 100B+5 stages, respectively, after cold storage (Table 4). For 'Brigitta' these decreases were 4%, 0.5% and 19%, respectively. Weight loss ranged from 10 to 21% on 'Duke' and from 6.4 to 9.8% on 'Brigitta'; according to this, final firmness was negatively correlated with weight loss (R^2=0.61), explaining a better postharvest condition for 'Brigitta'. Paniagua et al. (2013) reported similar results for blueberries stored at 4 °C and subjected to different airflow treatments.

4. Conclusions

- Duke' displayed higher RR and EP than 'Brigitta' along fruit development and maturation, and resulted in lower postharvest quality.

- Although no visual or instrumental color differentiations could be perceived between 100B and 100B+5 stages at harvest, 100B+5 fruit consistently resulted in lower firmness and quality after storage.

- In order to avoid fruit heterogeneity that can lead to enhanced differences after longer storage periods, picking intervals should be narrower in cultivars that exhibit higher differences between these two maturity stages.

- Since main differences between cultivars were given by firmness and weight loss after storage, additional physiological, morphological and biochemical changes both on pre- and postharvest, need to be studied. Further emphasis should be given to the cuticle and its components.

Acknowledgements

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Table 1. Fruit color (L. Chroma and Hue) of 'Duke' and 'Brigitta' blueberries picked at six different stages of development[y]

Stage[z]	Duke			Brigitta		
	L[y]	Chroma[y]	Hue[y]	L[y]	Chroma[y]	Hue[y]
100G	62.6 a	33.6 a	121.5 c	62.9 a	21.2 a	104.7 b
25P	55.9 b	16.9 b	90.8 d	55.2 b	15.9 b	76.5 c
50P	43.9 c	12.1 c	19.0 e	39.6 c	12.4 c	12.8 d
75B	36.4 d	5.2 d	288.6 a	33.4 d	4.9 d	275.6 a
100B	35.0 d	4.6 d	266.3 b	34.4 d	5.7 d	267.3 a
100B+5	34.6 d	4.3 d	264.2 b	33.6 d	5.2 d	266.4 a
Significance (p)	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0007*	*0.0000*

[z]:100G (100% green fruit); 25P (75% green + 25% pink fruit); 50P (50% green + 50% pink fruit); 75B (25% green +75% pink-blue fruit); 100B (90-100% blue fruit); 100B+5(100%B fruit plus extra 5 days residing in the plant).
[y]Within a column, represent significant differences (Tukey's test, $p \leq 0.05$).

Table 2. Fruit size (weight and diameter) of 'Duke' and 'Brigitta' blueberries picked at six different stages of development[y]

Stage[z]	Duke			Brigitta		
	Fruit Weight (g)	Fruit diameter Equatorial (mm)	Polar (mm)	Fruit Weight (g)	Fruit diameter Equatorial (mm)	Polar (mm)
100G	0.56 d	7.01 e	4.9 e	0.85 e	12.2 d	8.7 c
25P	0.62 d	10.3 d	7.3 d	1.13 d	12.9 c	9.2 b
50P	1.21 c	14.1 c	9.4 c	1.55 c	14.8 b	10.8 a
75B	1.44 b	14.7 b	9.5 c	1.95 b	14.9 b	10.8 a
100B	1.72 a	15.3 a	10.1 b	2.21 b	15.2 a	11.4 a
100B+5	1.73 a	15.6 a	10.9 a	2.43 a	16.4 a	11.6 a
Significance (p)	*0.0038*	*0.0014*	*0.0000*	*0.0000*	*0.0017*	*0.0003*

[z]:100G (100% green fruit); 25P (75% green + 25% pink fruit); 50P (50% green + 50% pink fruit); 75B (25% green +75% pink-blue fruit); 100B (90-100% blue fruit); 100B+5(100%B fruit plus extra 5 days residing in the plant).
[y]Within a column, different letters represent significant differences (Tukey's test, $p \leq 0.05$).

Table 3. Fruit quality of 'Duke' and 'Brigitta' blueberries picked at six different stages of development[y]

Stage[z]	Duke				Brigitta			
	Firmness (g mm^{-1})	SSC (%)	TA (%)	SSC/TA	Firmness (g mm^{-1})	SSC (%)	TA (%)	SSC/TA
100G	334.1 a	6.0 d	2.90 a	2.8 e	358.4 a	6.8 d	2.80 a	2.4 e
25P	235.3 ab	8.6 cd	1.90 b	5.1 d	300.3 a	7.8 d	1.90 b	4.1d
50P	206.4 ab	9.3 c	1.80 b	8.3 cd	196.7 b	9.8 c	1.50 bc	6.5 c
75B	176.4 b	11.6 bc	1.00 c	11.5 c	162.7 c	12.3 b	1.10 c	10.9 b
100B	169.0 b	13.8 b	0.69 d	20.1 b	154.9 c	14.7 a	0.76 d	19.7 a
100B+5	137.8 c	16.4 a	0.65 d	25.4 a	131.1 d	14.7 a	0.64 d	23.5 a
Significance (p)	*0.0001*	*0.0000*	*0.0000*	*0.0020*	*0.0000*	*0.0000*	*0.0057*	*0.0013*

[z]:100G (100% green fruit); 25P (75% green + 25% pink fruit); 50P (50% green + 50% pink fruit); 75B (25% green +75% pink-blue fruit); 100B (90-100% blue fruit); 100B+5(100%B fruit plus extra 5 days residing in the plant).
[y]Within a column, different letters represent significant differences (Tukey's test, $p \leq 0.05$).

Table 4. Postharvest fruit quality of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages and stored for 45 d at 2 ºC + 1 d at 18 ºC

Stage[z]	Duke					Brigitta				
	Firmness (g mm^{-1})	SSC (%)	TA (%)	SSC/TA	Weight loss (%)	Firmness (g mm^{-1})	SSC (%)	TA (%)	SSC/TA	Weight loss (%)
75B	119.5 ab	13.6 b	1.11 a	12.8 c	20.5 a	156.0 a	12.0 c	0.94 a	13.8 b	7.0 ab
100B	126.5 a	15.4 a	0.73 b	22.0 b	19.1 a	154.1 a	14.6 b	0.60 b	24.9 a	9.8 a
100B+5	112.8 b	16.3 a	0.51 c	34.2 a	10.0 b	105.6 b	15.9 a	0.60 b	27.8 a	6.4 b
Significance (p)	*0.0114*	*0.0001*	*0.0000*	*0.0000*	*0.0071*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.064*

[z]:100G (100% green fruit); 25P (75% green + 25% pink fruit); 50P (50% green + 50% pink fruit); 75B (25% green +75% pink-blue fruit); 100B (90-100% blue fruit); 100B+5(100%B fruit plus extra 5 days residing in the plant).
[y]Within a column, different letters represent significant differences (Tukey's test, $p \leq 0.05$).

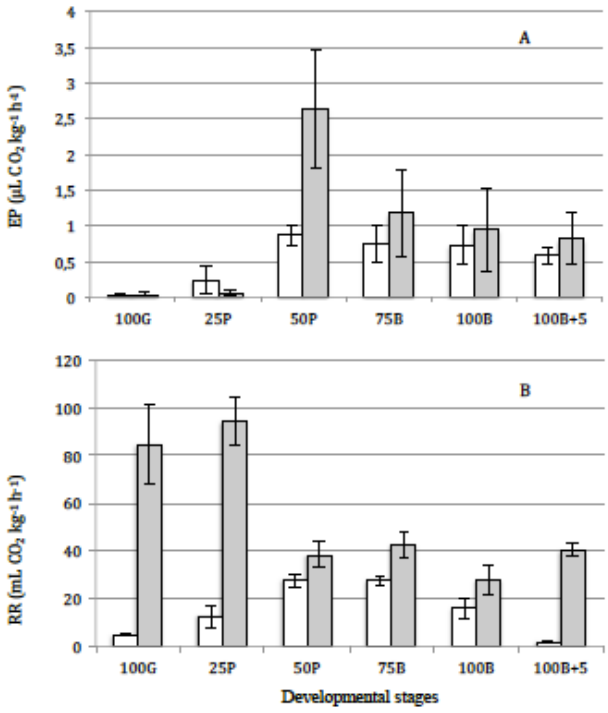

Figure 1. Evolution of (A) Ethylene production (EP) and (B) Respiration Rate (RR) of 'Duke' (grey bars) and 'Brigitta' blueberries (white bars) during different developmental stages: 100G (100% green fruit); 25P (75% green + 25% pink fruit); 50P (50% green + 50% pink fruit); 75B (25% green + 75% pink blue fruit); 100 B (90-100% blue fruit); 100B+5 (100%B fruit plus extra 5 days residing in the plant).

5.2. Fruit Characteristics and Cuticle Triterpenes as Related to Postharvest Quality of Highbush Blueberries.

Abstract

Chilean fresh blueberries take 20-50 days to arrive by boat to the Northern hemisphere, softening and dehydration being the main defects upon arrival. The effect of maturity at harvest (75% blue, 100% blue, and overripe) on cuticular triterpene content, and the possible associated impacts on firmness and weight loss after cold storage were explored for 'Duke' and 'Brigitta' fruit, both non-bagged or bagged in macro-perforated low-density polyethylene bags. Softening and weight loss varied with cultivar and maturity stage: 'Duke' fruit softened faster and were more prone to dehydration than 'Brigitta' samples, whereas overripe fruit were less firm after storage. This is the first report characterizing the triterpenoid fraction in cuticles of fresh blueberries, which may play a role in their postharvest behavior. Weight loss and softening rates were highly correlated to ursolic acid contents at harvest; further research will be required for a better understanding of these relationships.

Key words: blueberry; cuticle; firmness; fruit; triterpenoids; *Vaccinium corymbosum* L.; weight loss

1. Introduction

Chile has a large fresh blueberry-exporting industry (Retamales and Hancock, 2012) and, owing to counter-seasonality, it has the commercial advantage of supplying off-season fresh fruit to the Northern hemisphere. In order to reduce shipping costs, transportation by boat is the preferred means of export (Beaudry et al., 1998). Currently, the proportion of fruit shipped by boat is around 95%, and transport may take 20 to 50 days, from harvest to final consumers. The main market for Chilean fresh blueberries is the USA (82 - 85% of the total volume exported in 2008 - 2011), followed by Europe (12 - 14%) and the Far East (3%), (ODEPA, 2015). Fresh blueberries are relatively perishable, so considering the actual extreme variations in weather patterns due to the climate change (Lobos and Hancock, 2015) and the increasing amount of fruit shipped to long-distance markets, quality upon arrival is likely to become more heterogeneous and this will become a major issue for the blueberry industry (Retamales et al., 2014).

Blueberries are prone to postharvest decay, physiological breakdown, physical damage, shriveling, and water loss. The quality at final markets is dependent on the attributes of fruit at harvest, as well as on handling during and after harvest (Forney, 2009). Fruit softening is one of the major factors limiting the marketing of fresh blueberries (Vicente et al., 2007) and also one of the most critical quality attributes that influence consumer acceptance (NeSmith et al., 2002). According to the industry, the main defects found in Chilean blueberries at final markets are fruit softening and dehydration, accounting for 10 - 45% and 10 - 25% of total defects, respectively (Juillerat, 2014).

In general, fruit softening is estimated by the instrumental measurement of firmness, which declines with maturation. Firmness can vary greatly among cultivars, but also across maturity stages within a singular cultivar (Beaudry, 1992; Lobos et al., 2014). Additionally, blueberries usually soften during the postharvest chain due to deficient temperature management (Ehlenfeldt and Martin, 2002; Tetteh et al., 2004; Ne Smith et al., 2015), although a number of studies have also reported increases in firmness during storage (Miller et al., 1993; Chiabrando et al., 2009; Duarte et al., 2009). Research on blueberry fruit softening has focused on metabolic changes in the cell walls, leading to structural disassembly, which appears to be almost completed by the time of harvest (Vicente et al., 2007; Angeletti et al., 2010), while other possibly involved factors have not been deeply studied. The fruit cuticle, for instance, has a noticeable influence on the postharvest quality of fruits, on three major

aspects: water permeability with the resulting dehydration, susceptibility to infections, and physiological disorders (Lara et al., 2014).

The cuticle is a mostly lipidic external membrane surrounding all non-woody aerial plant organs (Dominguez et al., 2011). Its main component is cutin, a polyester matrix of polyhydroxylated C_{16} and C_{18} fatty acids embedded and covered with amorphous intra- and epi-cuticular waxes, plus a minor fraction of phenolics (Jetter et al., 2000). Cuticular waxes are composed of mixtures of aliphatic (*n*-alkanes, alkanoic acids, alkanols, aldehydes, alkyl esters), and non-aliphatic components (pentacyclic triterpenoids and sterol derivatives) (Kunst and Samuels, 2009). Recent studies on tomato (Lleide et al., 2011), pepper (Parsons et al., 2012), sweet cherry (Belge et al., 2014a), and peach (Belge et al., 2014b) have demonstrated a positive association between water loss rate and the ratio of *n*-alkanes to triterpenoids plus sterol compounds. For the edible berries within the genus *Vaccinium*, most available information refers to cranberry (*Vaccinium macrocarpon*), which is known to be a rich source of the triterpenoids ursolic and oleanolic acids (Crouteau and Fagerson, 1971; Szakielet al., 2012), whereas Kondo et al. (2010) detected the same compounds in lowbush blueberries (*Vaccinium angustifolium*). We are not aware, though, of any reports on the specific composition of *Vaccinium corymbosum* fruit cuticles.

Interestingly, moisture loss has been recently proposed as the major cause of firmness changes during storage of blueberries (Paniagua et al., 2013). There is evidence that cuticle characteristics and composition might play a role on softening of fruits such as pepper and tomato (Bargel and Neinhuis, 2004; Maaleku et al., 2005; Kosma et al., 2010). Noticeable differences have been reported across blueberry cultivars regarding softening rates and water loss during prolonged refrigerated storage (Sargent et al., 2006; Vicente et al., 2007; Alsmairat et al., 2011; Paniagua et al., 2013 and 2014), but to our knowledge, no published study has evaluated the influence of harvest maturity and cuticular wax characteristics on quality parameters during cold storage or transport.

We hypothesize that the triterpenoid content of the highbush blueberry cuticle may impact weight loss and softening of the fruit after storage. The work reported herein is a preliminary study undertaken with the main goal of assessing the relationships, if any, between quality parameters and the cuticular triterpenoids in two highbush blueberry cultivars (‘Duke’ and ‘Brigitta’) harvested at

different maturity stages. Fruit were maintained under refrigerated storage, either unpacked or packed within a low-density macro-perforated polyethylene bag, to mimic shipping to long-distance markets.

2. Material and Methods

2.1. Fruit material and experimental setup

During the season 2014/15, twelve mature highbush blueberry (*Vaccinium corymbosum* L.) plants of 'Duke' and 'Brigitta', 8 and 9 years old, respectively, planted 1.2 m apart in rows spaced at 3 m, were selected and labeled from a commercial field located in Río Claro, Maule Region, Chile (35°15' 35.16" S; 71°14' 22.53" W). Early in the season, when similar percentages of green and pink fruit were reached, clusters with comparable characteristics (fruit number and shape) and canopy position (superior third of the eastern side), were selected and labeled. Fruit ripeness was categorized according to external color as: 75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X). No visual differences in the skin color could be perceived between H100 and H100+X fruit. The latter maturity stage was imposed to mimic the usual commercial harvest practice. The extent of ripening was evaluated every second day, in order to get the different maturity stages. There were three harvest dates for each cultivar: 26 and 29 November, and 5 December (H75, H100 and H100+X, correspondingly) for 'Duke'; 27 and 31 December, and 5 January for 'Brigitta' (H75, H100 and H100+X, respectively).

Fruit from each maturity stage and cultivar were carefully hand-picked and placed directly into plastic clamshells (125 g), containing 50 fruit each. In order to mimic real conditions, fruit were placed in commercial cardboard boxes (containing 12 clamshells), for each cultivar and maturity. Fruit from four clamshells were evaluated at harvest, whereas the remaining fruit were divided into two storage treatments: i) four boxes were placed within a commercial macro-perforated (0.9%), low-density polyethylene (LDPE) unsealed bag, which was used only for weight loss prevention and no gas modification was intended (Pesis et al., 2002; Klaasen et al., 2006; Koutsimanis et al., 2015); and ii) four boxes remained non-bagged as the control. Fruit were stored at 0 °C and evaluated after 45 days at 0 °C plus 1 day at 18 °C (45+1). The general experiment was established under a completely randomized design, with factorial arrangement given by maturity stage (3) and

bagging system (2), thus generating three treatments at harvest and six treatment combinations for the postharvest evaluations. Each treatment had four replicates (one clamshell e.a.).

2.2. Maturity and quality assessments

Fruit weight (g) was measured with an electronic balance, and equatorial and polar diameters (mm) were measured with a digital caliper on four replicates of 25 fruit each. On the same lot, firmness (N) was measured with a compression device (FirmTech 2, BioWorks, KS, USA); the equipment was set up with maximum and minimum compression forces of 1.96 N and 0.15 N, respectively, and piston speed of 6 mm s^{-1} (Ehlenfeldt and Martin, 2002; Saftner et al., 2008). Total soluble solids (TSS, %) were assessed in juice obtained from four replicates of 5 berries each with a digital refractometer (Pocket PAL-1, Atago, Tokyo, Japan). For the determination of titratable acidity (TA, % citric acid), four replicates of 10 mL of juice were diluted to 100 mL with distilled water and titrated with 0.1 mol L^{-1} NaOH to an end-point pH of 8.2. Additionally, the ratio between TSS/TA was calculated. For the evaluation of respiration rate (RR), samples (three fruit × four replicates) were placed within 28-mL sealed glass vials. After 2 h at room temperature (18 °C), CO_2 accumulation inside the vials was measured using a gas analyzer (Quantek 902P, Quantek Instruments Inc., MA, USA) fitted with a thermal conductivity detector; CO_2 production was expressed as μg kg^{-1} s^{-1}. An authenticated standard (2.1 % CO_2 and 2.2 % O_2 in N_2 balance) was used for calibration. Additional samples were also placed in 28-mL vials for the measurement of ethylene production (EP); after 2 h at room temperature (18 °C), a 1 mL gas sample was withdrawn with a syringe from the headspace volume, and ethylene was quantified using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 3 mm i.d. column packed with activated alumina, 80/100 mesh. The injector, oven, and detector temperatures were set at 75 °C, 100 °C, and 170 °C, respectively, with helium as the carrier gas (0.67 mL s^{-1}), in the presence of hydrogen and air (0.67 and 6.67 mL s^{-1}, correspondingly). An ethylene standard (1 μL L^{-1}) was used for calibration, and data were expressed as ng kg^{-1} s^{-1}. For RR and EP, the free headspace of each vial was estimated by subtracting the fruit volume from the total volume of each vial. Fruit volume and surface were calculated using the polar and equatorial diameters of each berry, assuming an oblate spheroid shape. Additionally, surface/volume ratios were estimated for each maturity stage.

After storage removal (45+1), firmness, TSS and TA were measured for both bagged and non-bagged fruit. Firmness was assessed on four replicates of 25 fruit each; TSS and TA were measured on four replicates of 5 berries and four replicates of 10 mL juice, respectively. Weight loss (%) was estimated by the difference between initial and final weight on four replicates of one clamshell, per treatment. Given that fruit size differed between cultivars, weight loss was also expressed as % m^{-2}.

Finally replicates of 50 fruit were visually evaluated to determine the % of sound fruit (edible berries, free of any shriveling and/or rot symptoms) on each clamshell.

2.3. Fruit cuticular wax analysis and triterpenoid identification/quantification

Cuticular wax analyses were undertaken on fruit at harvest. In order to avoid wax removal during picking of fruit used for wax analysis, entire clusters were collected at the field, set into paper bags, and once at the lab, individual fruit were removed from the clusters with tweezers, holding each berry from the pedicel. Fruit wax was extracted (three replicates of 25 fruits e.a.) by dipping the samples in 50 mL distilled dichloromethane, with continuous agitation for 1 min. The solution was filtered and taken to dryness under reduced pressure at 30 ºC in a rotatory evaporator. The solid residue obtained from each replicate sample was dried and weighed to estimate wax yield, per unit surface area (g m^{-2}). The composition of the wax extracts was first assessed by thin layer chromatography (TLC) analysis (silica gel 60 F254, Merck, Darmstadt, Germany) using petroleum ether:ethyl acetate 90:10 (v/v) as the mobile phase. Plates were visualized after spraying with anisaldehyde-sulfuric acid and heating. Several spots were detected with colors suggesting the occurrence of triterpenes and triterpene acids. Selected samples were treated with diazomethane in diethyl ether to obtain the methyl esters of triterpene acids. For triterpenoid identification and quantification by GC-MS, the samples (1 g L^{-1} of wax extract) were treated with 1 mL of diazomethane solution in diethyl ether to obtain the methyl esters of the acids occurring in the mixtures. After evaporation to dryness, the derivatized samples were dissolved in isopropanol and analyzed by GC-MS. The presence of a mixture of triterpene alcohols and triterpene acids was confirmed by ^{1}H NMR analysis (400 MHz, Bruker, Rheinstetten, Germany). The main triterpenes in the samples were identified by analysis of the lipophilic cuticle constituents by TLC, GC-MS and NMR before and after derivatization as the corresponding methyl esters. The identity of the compounds was confirmed by comparison with authentic standards of oleanolic acid, ursolic acid,

lupeol and α-amyrin. Further analysis and quantification were carried out by GC. For quantification, cholesterol (Sigma-Aldrich C 8667, purity ≥ 99%) was used as internal standard.

2.3.1. Chemical Standards and Reagents

Dichloromethane, ethyl acetate, petroleum ether and diethylether were from Merck (Darmstadt, Germany). Isopropanol was from J.T. Baker (Center Valley, PA, USA). Oleanolic acid (O5504, purity ≥97%), ursolic acid (89797, purity ≥ 98.5%), α-amyrin (53017, purity ≥ 98%) and lupeol (L5632, purity ≥ 94%) were from Sigma-Aldrich (St. Louis, MO, USA). Cholesterol (Sigma-Aldrich C8667, purity ≥ 99%) was used as internal standard.

2.3.2. Identification

The identification of the compounds was carried out using a gas chromatograph (GC Trace 1300, Thermo Fisher Scientific, Milan, Italy) coupled to a mass selective detector fitted with an ionization single quadrupole according to Caligiani et al. (2013). A capillary column (0.25 mm i.d., 30 m length × 0.25 μm film thickness) was used (Rtx-5, Restek Corporation, PA, USA). The oven temperature was kept at 240 ºC for 3 min then increased to 280 ºC at 20 ºC min^{-1}, with a total running time of 60 min. The head pressure was 124 kPa. Both the injector and detector temperatures were 290 ºC, with 0.2 min split-less injection mode. One μL was injected, with helium as the carrier gas at 25 μL s^{-1}. For mass spectrometric (MS) analyses, the ion source temperature was 230 ºC (70 eV, m/z 50−700). Under the experiment conditions, the retention time (Rt) of the internal standard and triterpenes were as follows: cholesterol (12 min), α-amyrin (17 min), lupeol (18 min), oleanolic acid methyl ester (23 min) and ursolic acid methyl ester (25 min).

2.3.3. Quantification

Compounds were quantified with a gas chromatograph (GC Trace 1300, Thermo Fisher Scientific, Milan, Italy), coupled to an FID. A capillary column (0.25 mm i.d., 30 m length × 0.25 μm film thickness) (Elite-5MS, PerkinElmer, MA, USA) was used. The oven temperature was held at 240 ºC for 3 min, and then increased to 280 °C at 20 °C min^{-1}, with a total run time of 45 min. Helium was used as the carrier gas (25 μL s^{-1}). The injected volume was 1 μL in all cases, with both injector and detector maintained at 290 ºC, and operated for 0.2 min in a splitless injection mode. Air (5.83 mL s^{-1}) and hydrogen (0.58 mL s^{-1}) were used as the carrier gas. The quantification was done by

integrating the total area of each chromatographic peak with cholesterol as internal standard at a concentration of 1 g L^{-1}. Results were expressed in mg m^{-2} as well as in relative terms (% of each compound over total waxes).

2.4. Statistical analysis

Data were subjected to analyses of variance (ANOVA). Variables measured at harvest were analyzed as one factor experiment whereas those at postharvest were considered as a factorial experiment. When significant differences were found ($p \leq 0.05$), either on the main factors (maturity stage and bagging system) or interactions, Tukey multiple comparison test ($p \leq 0.05$) was applied. In order to aid a preliminary characterization of the influence of the factors considered (cultivar, maturity stage, cuticle triterpenoid composition and bagging) on fruit characteristics, regression analyses were performed to relate weight loss with fruit maturity and characteristics at harvest. Analyses were executed using commercial statistical software (Statgraphics Centurion XVI (v.16.0.09), Statpoint, VA, USA) and R 3.0.0 (R Development Core Team, 2008).

3. Results

3.1. Fruit maturity and quality assessments at harvest

For H75 and H100, fruit firmness was similar, but higher than at H100+X for both cultivars (Table 1). 'Duke' showed significant differences among the three stages for TSS and TSS/TA, whereas for 'Brigitta' there were no differences between H100 and H100+X for TSS, TA, or TSS/TA. Regarding EP, values were below 0.5 ng kg^{-1} s^{-1}, with no differences between maturity stages for either cultivar. For 'Duke', H75 and H100+X fruit had higher RR than H100 fruit, whereas RR values for 'Brigitta' were lower than those for 'Duke', and decreased as maturity increased from H75 to H100+X (Table 1).

Maximum fruit weight was reached at H100 in 'Duke' and H100+X in 'Brigitta' (Table 2). In terms of fruit size, both cultivars grew equatorially until the fruit lost any trace of pink color (H100); polar diameter increased until H100+X in 'Duke', whereas 'Brigitta' did not show differences between stages. Surface/volume ratios were higher for 'Duke' blueberries and decreased from H75 to H100 in both cultivars. No differences in total wax content were found among maturity stages for either cultivar, even though contents were slightly higher in 'Duke' (Table 2).

3.2. Fruit cuticle triterpenoids at harvest

Two triterpenoid alcohols (α-amyrin and lupeol), as well as two triterpenoid acids (oleanolic and ursolic acids), were identified in the triterpenoid fraction of total waxes from 'Duke' and 'Brigitta' blueberries by spectroscopic and spectrometric means. GC traces of the wax constituents are presented as Supplementary Figures S1 and S2. There were no differences in the total % of triterpenoid components between maturity stages for 'Duke' (49% on average), but some dissimilarities were apparent for 'Brigitta', for which the content of triterpenoids was 45% for H75 and H100, and around 35% for H100+X (Table 3).

The main compound identified in both cultivars was lupeol (Fig. 1), which was more abundant in 'Duke', where it increased with maturity stage from 1.16 to 2.03 g m^{-2}. Lower values of this triterpene were found in 'Brigitta' (0.35 to 1.41 g m^{-2}), increasing from H75 to H100, and decreasing towards H100+X.

Large differences between cultivars were also found for oleanolic and ursolic acids. The content of oleanolic acid averaged 0.37 g m^{-2} in 'Brigitta', with no maturity-related differences, the amounts being about two-fold those in 'Duke'. In contrast, 'Duke' waxes were 2- to 7-fold higher in ursolic acid content in comparison with levels in 'Brigitta', although the amounts decreased with maturity. Finally, the triterpene alcohol α-amyrin was detected in 'Brigitta' fruit uniquely, and amounted on average to 0.24 g m^{-2}, regardless of maturity stage.

3.3. Fruit quality and weight loss after storage

Quality assessments and weight loss after storage (45+1), were mainly affected by maturity and bagging as independent factors for both cultivars (Table 4), but additional significant interactions were found for firmness, TA and TSS/TA on 'Brigitta'(Fig. 2). In general, firmness of Duke declined 34% between bagged and non-bagged samples, while reductions of 32, 25 and 18% were observed for H75, H100, and H100+X stages, respectively, in comparison with levels at harvest. For 'Brigitta', a lower impact on firmness preservation was found due to the bagging procedure or the maturity stage; 15% decline occurred between bagged and non-bagged samples, whereas decreases for the three maturity stages were 4, near 0 and 19.8%, when comparing harvest *vs.* storage. The significant interaction between maturity stage and bagging on 'Brigitta' evidenced that

the highest final firmness values were obtained on bagged treatments of H75 and H100 fruit, whilst for H100+X no differences were perceived related to the use of bag. Nonetheless, for both cultivars, H75 and H100 fruit remained firmer than H100+X fruit.

TSS, TA and TSS/TA after storage were significantly affected by harvest maturity, 'Duke' berries showing differences among all three stages for TA and TSS/TA, while H100 and H100+X 'Brigitta' fruit were generally similar. For both cultivars, the TSS/TA increased after storage due to increased TSS and decreased TA. The highest values recorded for TSS/TA ratios in 45+1 fruit were 34.2 for H100+X 'Duke' berries, regardless of bagging. For 'Brigitta', according to the interaction effect (Fig. 2) the highest values occurred for bagged H100, bagged H100+X and unbagged H100+X, with ratios between 26 and 30 (Table 4).

In terms of maturity, for both cultivars the percentage of sound fruit was similar for H75 and H100 samples, which were higher in comparison with H100+X fruit (Table 4); on average, 60 and 90% of the H75 and H100 berries were considered sound for 'Duke' and 'Brigitta', respectively, but only 43 and 80% of the H100+X fruit of 'Duke' and 'Brigitta' were still sound after storage. The effect of bagging on the percentage of visually sound fruit was significant for both cultivars, but differences were larger in 'Duke', where 81.8% of berries were considered healthy under bagged conditions, but only 25.4% resulted free of defects when no bag was used. For 'Brigitta' sound fruit represented 92.2 and 81.4% of bagged and non-bagged treatment, respectively.

The effects of maturity stage and bagging on weight loss showed almost the same statistical significance for values expressed either as % or as % m^{-2}. Large differences were found between cultivars, 'Duke' being more prone to dehydration than 'Brigitta' in all cases (Table 4); 'Duke' had the highest weight loss, particularly for H75 and H100 fruit (14.4 and 12.4%; 5.8 and 5.0% m^{-2}, respectively), whereas values were much lower for 'Brigitta', ranging from 5.0 to 7.2% and 2.1 to 2.6 % m^{-2}. Additionally, the effect of bagging on weight loss was higher for 'Duke', where fruit with no bag lost 3 and 2.3 times more weight (as % and % m^{-2}, respectively) than bagged fruit. For 'Brigitta' differences between bagged and non-bagged fruit were less than 2 times.

Regression analyses for weight loss revealed significant associations ($p \leq 0.05$) between fruit

characteristics and wax compounds at harvest (Table 5). Thus, weight loss values (both as % and % m^{-2}) were highly correlated with surface/volume ratio (r = 0.91 and 0.89), EP (r = 0.94 and 0.92), ursolic acid content (r = 0.96 and 0.95) and initial fruit weight (r = -0.82 for % weight loss uniquely). Additionally, when fruit softening (expressed as % drop between initial and final firmness) was added as the response variable, significant correlations were found against fruit weight (r = -0.96), EP (r = 0.94), RR (r = 0.81), oleanolic and ursolic acid contents (r = -0.83 and 0.95, respectively).

4. Discussion

4.1. Fruit quality vs. weight loss, firmness and softening after storage

The criteria for determining harvest maturity of fresh blueberries rely mainly on surface color, which has to be 100% blue (Gough, 1994; Lobos et al., 2014). Yet, firmness and TSS/TA, which are seldom measured under commercial management, have also been associated to postharvest potential, especially for long-term storage and transport. Firm fruit can more readily withstand harvest handling and subsequent transport (Hanson et al., 1993) and even though some cultivars are only slightly firmer, such small differences can prove very important for postharvest life (Beaudry et al., 1998). Several authors have reported differences in firmness of highbush blueberry cultivars (Ehlenfeldt and Martin, 2002; Saftner et al., 2008), which however seem to be more related to harvest maturity than to genotypic differences (Beaudry et al., 1998; Lobos et al., 2014). In this study, we found that firmness after storage was related to both maturity stage (H75 and H100 fruit remained firmer than H100+X ones) and the use of bag as a moisture barrier. Additionally, between cultivars, ‘Duke’ displayed higher firmness values at harvest, but faster softening after storage, which may explain the highest impact of bagging in this cultivar, regardless of maturity stage. The fact that no visual differences in color could be detected at harvest between H100 and H100+X samples suggests that a relatively wide variation in maturity may exist in any one harvest. In a typical commercial harvest, fruit can be collected every 6 - 10 days, which would practically assure a wide range in fruit maturity. The consistently higher firmness of H100 relative to H100+X samples, both at harvest and after storage, illustrates the problems associated with the presence of fruit with advanced maturity in harvested fruit lots. Similarly, the TSS/TA ratio, which should be balanced in order to achieve optimal flavor, would also be impacted by variation in fruit maturity. Galletta et al. (1971) proposed that good keeping quality could be expected when TSS/TA ratios are < 18, and intermediate keeping quality when values are in the range 18-32. In our study, H75 fruit

had the lowest ratios (around 12); H100 fruit were close to the optimal threshold (roughly 20), but H100+X samples displayed TSS/TA > 24, which appear too high if long-distance markets are to be reached with acceptable quality. In terms of firmness, although no optimum parameters have been defined, mean values for 'Duke' at harvest have been reported between 1.73 and 1.36 N (Ehlenfeldt and Martin, 2002; Saftner et al., 2008) and for 'Brigitta' between 1.88 and 1.46 N (Ehlenfeldt and Martin, 2002). In our study, firmness of 'Duke' fruit was within the mentioned range for all the maturity stages (1.76 N for H75 fruit to 1.38 for H100+X fruit), whereas 'Brigitta' berries were slightly softer (1.63 *vs.* 1.31 N from H75 to H100+X stages).

Since growers often wait for blue fruit to accumulate in the bushes in order to optimize labor costs, it is most likely that, within each harvest, there is a relatively wide range in fruit maturity amidst the uniformly colored fruit harvested. All the fruit may look acceptable when picked, but a fraction of them, the ones picked at more advanced maturity, have a greater likelihood of becoming overripe and unacceptable when reaching the final consumers. This may be an important source of fruit heterogeneity, which will be more deleterious after longer storage and transport periods, and could partially explain quality variations detected at final markets between different seasons (Juillerat, 2014). Results for final firmness and % sound fruit after storage showed that, in terms of maturity stage, H75 and H100 stages of both cultivars, as well as H100+X of 'Brigitta', had a similar behavior but highly differed from those of 'Duke' harvested at H100+X.

Visually, and regardless of cultivar, H75 berries achieved complete blue coverage after storage, but had lower TSS and higher TA than H100 or H100+X fruit. This might have had implications for organoleptic characteristics that were not explored in this study.

'Duke' fruit were firmer at harvest and had slightly higher amount of waxes, but displayed similar TA and TSS/TA values as 'Brigitta'. Yet, these attributes did not result in better condition after storage, since the proportion of sound fruit was substantially lower for 'Duke' (< 60%, depending on maturity stage) than for 'Brigitta' (> 80% at all stages considered herein).

Values for weight loss (expressed both as % and % m^{-2}) were high and varied between both cultivars. The blueberry industry considers acceptable a range of 5 - 7% weight loss in a

commercial 3-week maritime transport where fruit are containerized at 0 °C and held under 90 - 95% RH (Sargent et al., 2006; Paniagua et al., 2014). These values would be consistent with those obtained in this study for 'Brigitta', but not for 'Duke' fruit, for which a higher weight loss was observed, particularly for non-bagged fruit. When Alsmairat et al. (2011) evaluated nine cultivars under different controlled atmosphere storage conditions, weight loss was in the range of 0.6 to 2.3% after eight weeks; among cultivars, 'Duke' showed two-fold higher weight loss compared to 'Brigitta'. Rivera et al. (2013) reported 2.1 and 3.5% weight loss for palletized 'Brigitta' and 'O ´Neal' blueberries, respectively, after 45 d at 0 ºC. In a recent experiment, the use of passive modified atmosphere packaging (MAP) for the storage of 'Brigitta' fruit resulted in decreased percentage of dehydrated fruit and less intense softening when compared to control fruit (Moggia et al., 2014) and interestingly film type had little effect on gas composition within the bag, showing that moisture retention was the main effect of the treatment. In the current study, when comparing values for bagged and non-bagged fruit for each cultivar, 'Brigitta' showed 4.0 *vs*. 7.8% weight loss in bagged and non-bagged samples, respectively. For 'Duke' blueberries, these values were 5.7 and 16.6% for bagged and non-bagged fruit, correspondingly (Table 4). Surprisingly, even though 'Duke' fruit picked at H75 and H100 stages had the highest weight loss after storage, the percentage of visually sound fruit was higher for both stages when compared to H100+X samples. This observation may have arisen from the stronger positive effect of the bagging procedure in this cultivar (81.8% sound fruit and 5.7% weight loss for bagged *vs.* 24.5% and 16.6% for non-bagged fruit, respectively). On the other hand, differences in weight loss between cultivars could be partially associated to fruit size; it is known that surface/volume ratio of fruit affects transpiration (Ben-Yehoshua et al., 1983). In our study 'Duke' fruit had larger surface/volume ratios (Table 2), especially for the H75 stage, which displayed the highest weight loss. Other possible causes might be related to cuticular waxes, as discussed below.

4.2. Wax triterpenoids vs. weight loss, firmness and softening after storage.

The hydrophobic nature of the cuticle has been considered to confer the fruit an effective barrier against water loss (Lara et al., 2014; Martin and Rose, 2014). However, cuticular wax composition and structure, rather than total wax amount, can also impact water permeability (Riederer and Schreiber, 2001). Parsons et al. (2012) found no strong correlation between pepper water loss rate and total wax levels, but an association was seen with specific wax components. Lleide et al. (2011)

reported that the cuticular waxes of the *ps* mutant tomato fruit, which is highly susceptible to water loss, exhibited an almost complete absence of *n*-alkanes and aldehydes, and increased percentage of triterpenoid and sterol derivatives, when compared to the wild type specimens. Belge et al. (2014a) reported ratios of *n*-alkanes to triterpenoids of 0.18 and 0.33 on cuticles of 'Celeste' and 'Somerset' sweet cherries associated with weight loss values of 15.8 and 7.2% after two weeks of refrigerated storage, respectively. Similar results were found on 'October Sun' and 'Jesca' peaches, where ratios of 0.31 and 0.65 were related to 5.6 and 3.9% weight loss 5 days after harvest, correspondingly (Belge et al., 2014b).

The wax barrier in fruit cuticles is viewed as being relatively impermeable to gases including water vapor and existing as a cluster of crystalline waxes (mainly *n*-alkanes), both covering and embedded in a matrix of amorphous material (mostly triterpenoids). Water diffusion is considered to occur mostly in the amorphous fraction, while the crystalline cover would prevent further water transport (Vogg et al., 2004). In this study, four triterpenoids were identified, which represented 35 to 50% of total waxes (Table 3). As reviewed in Lara et al. (2014), published information highlights ursolic and oleanolic acids as the main triterpenoids of many fruit species, while other fruit display mainly triterpenols such as amyrins (tomato, pepper, orange, Asian pear). Lupeol has been reported in pear (Cho et al., 2013), citrus (Lara et al., 2015), tomato, grapes, bell pepper, eggplant and grape fruit (Szakiel et al., 2012). Given the results of our study, further research efforts on a putative relationship between high triterpene amounts, their specific composition, and limited storage potential of blueberry fruit, might help shedding light on this important commercial feature.

The compositional differences in the triterpenoid fraction between 'Duke' and 'Brigitta' was due to the greater content of α-amyrin in the latter cultivar, as well as to the relative ratio of lupeol to oleanolic and ursolic acid (Fig. S1 and S2). Interestingly, α-amyrin and oleanolic acid share a similar carbon skeleton (Neto, 2010). Among triterpenoid compounds, ursolic acid was highly related to weight loss and softening rates: 'Duke', which suffered the highest deterioration rates during postharvest, had 2-4 times higher ursolic acid content than 'Brigitta'. Additionally, oleanolic acid, which was found to be inversely correlated to softening, was more abundant in 'Brigitta'. Remarkable maturity-related differences were found for 'Duke' in the content of the different triterpenoid compounds identified in this work, while changes were very moderate or non-existent

in ‘Brigitta’ fruit (Fig. 1). This might explain, partially, the higher weight loss rates observed after cold storage in ‘Duke’ samples. Non-bagged fruit lost 16.6% weight with respect to harvest. Regarding maturity stage, H75 and H100 fruit lost 14.4 and 12.4%, respectively. In contrast, limited differences in water loss were observed for ‘Brigitta’ samples as related to bagging or maturity stage (Table 3). Actually, chromatographic analyses revealed the presence of a small amount of additional wax compounds eluting at the beginning of the run, which were not identified in this work (Fig. S1 and S2). These unidentified compounds were more abundant in ‘Brigitta’. Future work should elucidate whether they correspond to *n*-alkanes, and hence check if *n*-alkane to triterpenoid ratios are actually higher in this cultivar, which would support a relevant role of this ratio on water loss rates, as suggested for other fruit species (Leide et al., 2011; Parsons et al., 2012; Belge et al., 2014a and b).

Thus, in order to maximize the storage and transport potential of fresh blueberries, a deeper survey of the properties and postharvest behavior of a wider range of cultivars, as well as the effects therein of harvest maturity and cuticle composition, appears advisable for the development of cultivar-specific picking strategies similar to those developed for other fruit (especially apple cultivars).

In conclusion, according to results reported herein, commercial harvest intervals should be narrower for those cultivars showing higher differences between H100 and H100+X fruit. Additionally, the improved firmness retention resulting from the use of a barrier against moisture loss suggests that widespread adoption of some form of vapor barrier be advisable for long-term storage. Beneficial effects of the bagging procedure might be enhanced by the use of MAP for particular cultivars.

The triterpenoid fraction of cuticular waxes of a given cultivar has the potential to play a role in the postharvest behavior of blueberries. This is the first report that characterizes cuticular composition of fresh blueberries, so further research will be required for better understanding the implications of these differences. Additional cuticle and cutin components, as well as the scar morphology may also have important implications on these aspects, and should be considered in future studies.

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Table 1. Fruit maturity and quality assessments at harvest of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

		Firmness[z] (N)	TSS[y] (%)	TA[x] (% citric ac.)	TSS/TA	EP[w] (ng kg^{-1} s^{-1})	RR[v] (µg CO_2 kg^{-1} s^{-1})
Cultivar	Maturity stage						
'Duke'	H75	1.76 a	11.6 c	1.02 a	11.5 c	0.32	17.83 b
	H100	1.69 a	13.8 b	0.69 b	20.1 b	0.25	11.61 a
	H100 +X	1.38 b	16.4 a	0.65 b	25.4 a	0.22	16.97 b
	Significance (p)	***0.0005***	***0.0000***	***0.0000***	***0.0000***	*0.5015*	***0.0003***
'Brigitta'	H75	1.63 a	12.3 b	1.14 a	10.9 b	0.20	11.49 a
	H100	1.55 a	14.7 a	0.76 b	19.7 a	0.19	6.63 b
	H100 +X	1.31 b	14.7 a	0.64 b	23.5 a	0.16	8.85 b
	Significance (p)	***0.0031***	***0.0066***	***0.0010***	***0.0023***	*0.4265*	***0.0000***

For a given cultivar, and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
[z] Firmness: values represent the mean of 4 replicates of 25 fruit each
[y] TSS: Total soluble solids, values represent the mean of 4 replicates of 5 fruit each
[x] TA: Titratable acidity, values represent the mean of 4 replicates of 10 mL juice each
[w] EP: Ethylene production, values represent the mean of 4 replicates of three fruit each
[v] RR: Respiration rate, values represent the mean of 4 replicates of three fruit each

Table 2. Fruit size and cuticular wax content of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

		Fruit weight (g)	Fruit diameter Equatorial (mm)	Polar (mm)	Surface /Volume ratio	Wax content per area (g m^{-2})
Cultivar	Maturity					
'Duke'	H75	1.44 b	13.69 b	9.53 c	5.07 a	3.06
	H100	1.72 a	14.65 a	10.07 b	4.76 a	2.63
	H100 +X	1.72 a	15.59 a	10.91 a	4.44 b	3.32
	Significance (*p*)	***0.0000***	***0.0013***	***0.0000***	***0.0437***	*0.1193*
'Brigitta'	H75	2.11 b	14.92 b	10.81	4.56 a	2.28
	H100	2.21 b	15.12 a	11.38	4.60 a	2.91
	H100 +X	2.43 a	16.36 a	11.36	4.24 b	2.18
	Significance (*p*)	***0.00014***	***0.0029***	*0.1734*	***0.0287***	*0.3081*

For a given cultivar, and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$). Values represent the mean of 4 replicates of 25 fruit each.

Table 3. Triterpene composition (relative %) of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

		Triterpenoid (%)				
		Amyrin	Lupeol	Oleanolic Acid	Ursolic Acid	Total
Cultivar	Maturity					
'Duke'	H75	Nd	32.7 b	3.9 a	13.3 a	49.9
	H100	Nd	34.6 b	3.2 a	9.7 ab	47.5
	H100 +X	Nd	43.2 a	1.4 b	5.7 b	50.3
	Significance (p)		***0.0035***	***0.0413***	***0.0250***	*0.3430*
Brigitta'	H75	6.6	25.1 a	9.8	4.0	45.5 a
	H100	5.8	25.1 a	8.7	4.1	43.8 a
	H100 +X	5.2	15.9 b	9.8	4.1	35.0 b
	Significance (p)	*0.2143*	***0.0031***	*0.7026*	*0.9855*	***0.0070***

For a given cultivar, and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$). Values represent the mean of 3 replicates of 25 fruit each. Nd, non detected.

Table 4. Fruit quality assessments and weight loss of 'Duke' and 'Brigitta' blueberries picked at three different stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)], and stored either bagged or non-bagged for 45 days at 0 ºC + 1 day at 18 ºC.

	Factor	Firmness[z] (N)	TSS[y] (%)	TA[x] (% citric ac.)	TSS/TA	Sound fruit[w] (%)	Weight loss[w] (%)	(% m
'Duke'	***Maturity (M)***							
	H75	1.20 ab	13.6 b	1.11 a	12.8 c	52.9 a	14.4 a	5.8 a
	H100	1.27 a	15.4 a	0.73 b	22.0 b	63.4 a	12.4 a	5.0 a
	H100 +X	1.13 b	16.3 a	0.51 c	34.2 a	42.6 b	6.6 b	2.3 b
	Significance (*p*)							
	Bagging (B)							
	Bag	1.45 a	14.6 b	0.76	23.5	81.8 a	5.7 b	3.4 b
	No Bag	0.95 b	15.6 a	0.80	22.5	25.4 b	16.6 a	7.7 a
	Significance *(p)*							
	M	***0.0114***	***0.000***	***0.0000***	***0.0000***	***0.0000***	***0.0073***	***0.0021***
	B	***0.0000***	***0.0256***	*0.3787*	*0.6204*	***0.0000***	***0.0000***	***0.0000***
	M x B	*0.6225*	*0.1055*	*0.8248*	*0.2001*	*0.0865*	*0.7081*	*0.6061*
'Brigitta'	***Maturity (M)***							
	H75	1.56 a	12.0 c	0.94 a	13.8 b	90.8 a	5.5 ab	2.2
	H100	1.58 a	14.7 b	0.60 b	24.9 a	89.3 a	7.2 a	2.6
	H100 +X	1.06 b	15.9 a	0.60 b	27.8 a	80.3 b	5.0 b	2.1
	Bagging (B)							
	Bag	1.49 a	14.4	0.74	22.4	92.2 a	4.0 b	1.9 b
	No Bag	1.31 b	14.0	0.69	21.9	81.4 b	7.8 a	3.5 a
	Significance *(p)*							
	M	***0.0000***	***0.0000***	***0.0000***	***0.0000***	***0.0003***	***0.00637***	*0.3179*
	B	***0.0026***	*0.2345*	*0.5033*	*0.7405*	***0.0000***	***0.0001***	***0.0000***
	M x B	***0.0047***	*0.0901*	***0.0000***	***0.0010***	*0.7860*	*0.3207*	*0.7081*

For a given cultivar, or factor,and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).

[z] Firmness: values represent 4 replicates of 25 fruit each

[y] TSS: Total soluble solids, values represent 4 replicates of 5 fruit each

[x] TA: Titratable acidity, values represent 4 replicates of 10 mL juice each

[w] Sound fruit and weight loss, values represent 4 replicates of 50 fruit each

Table 5. Linear correlation coefficients (r)[z] between fruit characteristics at harvest and postharvest evaluations (weight loss and softening) of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)], and stored for 45 days at 0 ºC + 1 day at 18 ºC.

	Fruit quality								**Wax compounds (g m^{-2})**				
	Fruit weight (g)	Surface/ Volume Ratio	Firmness (N)	TSS (%)	TA (%)	TSS/ TA	EP (ng kg^{-1} s^{-1})	RR (µg kg^{-1} s^{-1})	Wax content	Alpha amiryn	Lupeol	Olean. acid	Ursolic acid
Weight loss (%)	**-0.82****	**0.91***	0.78^{ns}	-0.51^{ns}	0.17^{ns}	-0.37^{ns}	**0.94****	0.49^{ns}	0.40^{ns}	---	0.34^{ns}	-0.52^{ns}	**0.96****
Weight loss (% m^{-2})	-0.78^{ns}	**0.89***	0.78^{ns}	-0.57^{ns}	0.21^{ns}	-0.41^{ns}	**0.92****	0.47^{ns}	0.30^{ns}	---	0.24^{ns}	-0.52^{ns}	**0.95****
Softening (% firmness loss)	**-0.96****	0.76^{ns}	0.59^{ns}	-0.32^{ns}	0.10^{ns}	-0.21^{ns}	**0.94****	**0.81***	0.55^{ns}	---	0.50^{ns}	**-0.83***	**0.95****

[z] n=6
ns, non significant
* $p \leq 0.05$
** $p \leq 0.01$

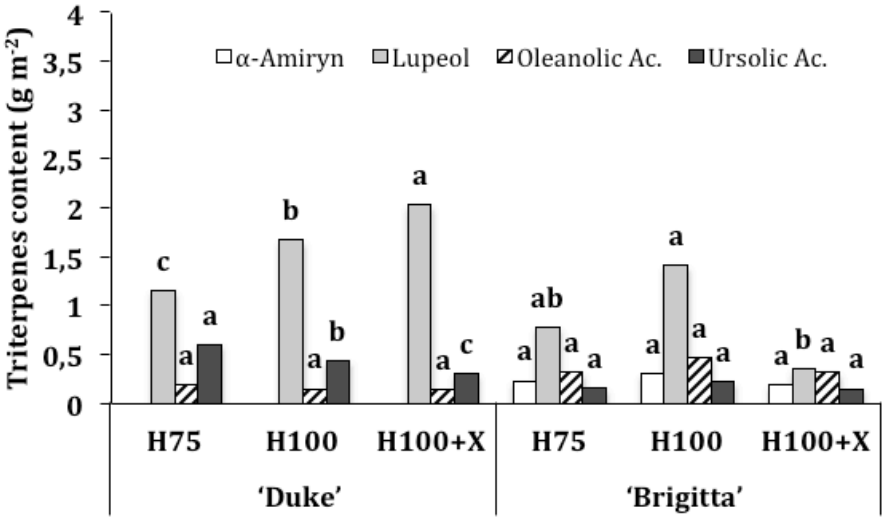

Figure 1. Main triterpene content (g m^{-2}) in cuticular waxes isolated from 'Duke' and 'Brigitta' blueberries harvested at three maturity stages. For each cultivar and component, values bearing different letters are significantly different (Tukey's test, $p \leq 0.05$).

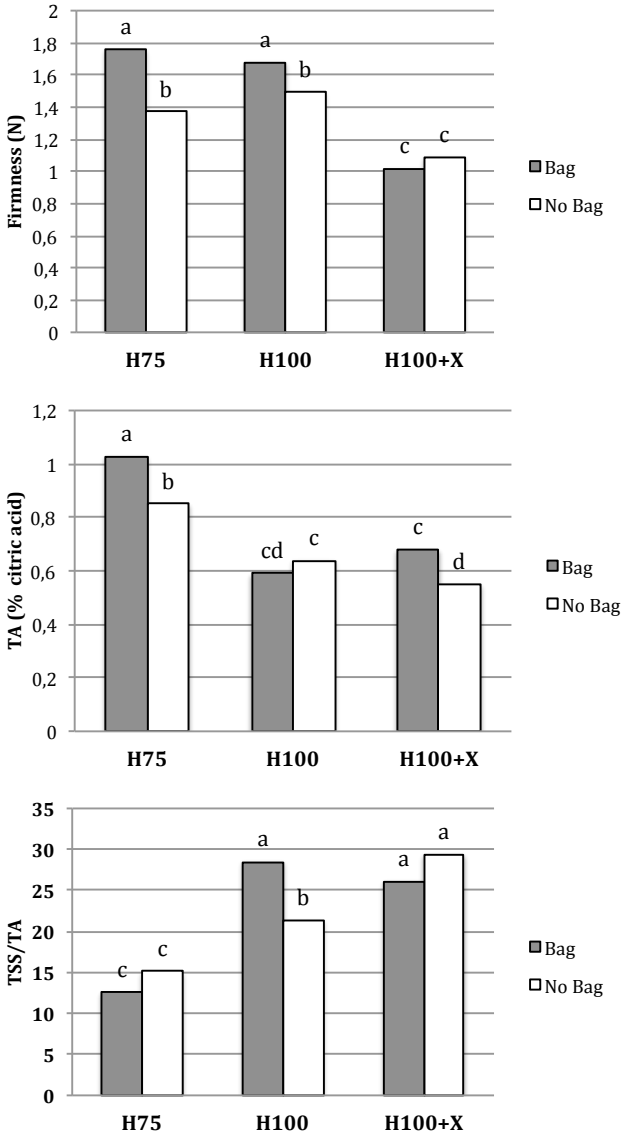

Figure 2. Effect of maturity stage at harvest (H75, H100 and H100+X) × bagging treatment on firmness (top), TA (middle) and TSS/TA (bottom) of 'Brigitta' blueberries after 45 days at 0 °C+1 day at 18 °C. For each variable, bars bearing different letters are significantly different (Tukey's test, $p \leq 0.05$).

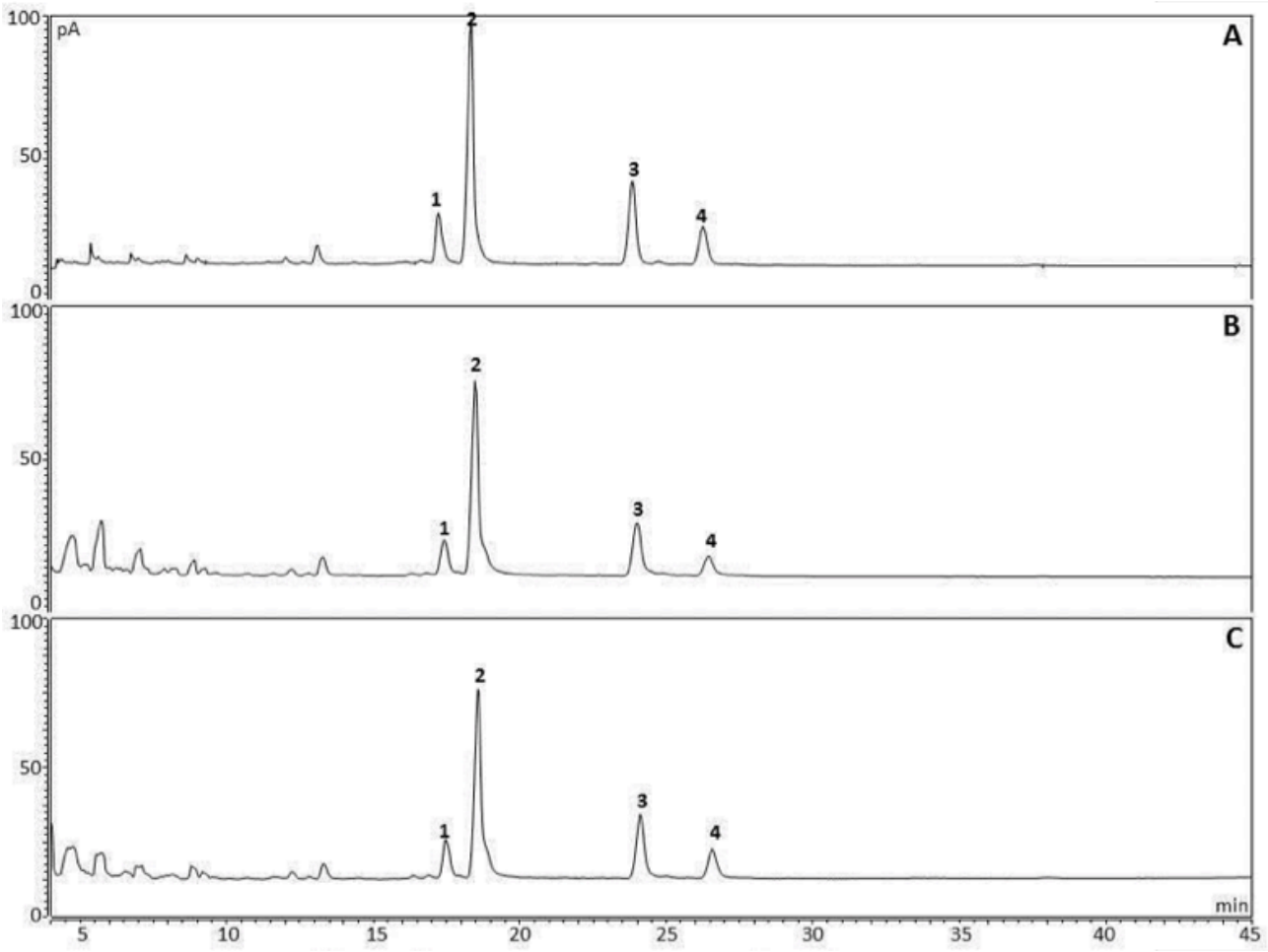

Figure S1. Main triterpenes from the wax of 'Brigitta' blueberries harvested at different maturity stages: A: H75; B: H100; C: H100+X. Peak numbers: (1) α-amyrin; (2) lupeol; (3) oleanolic acid; (4) ursolic acid.

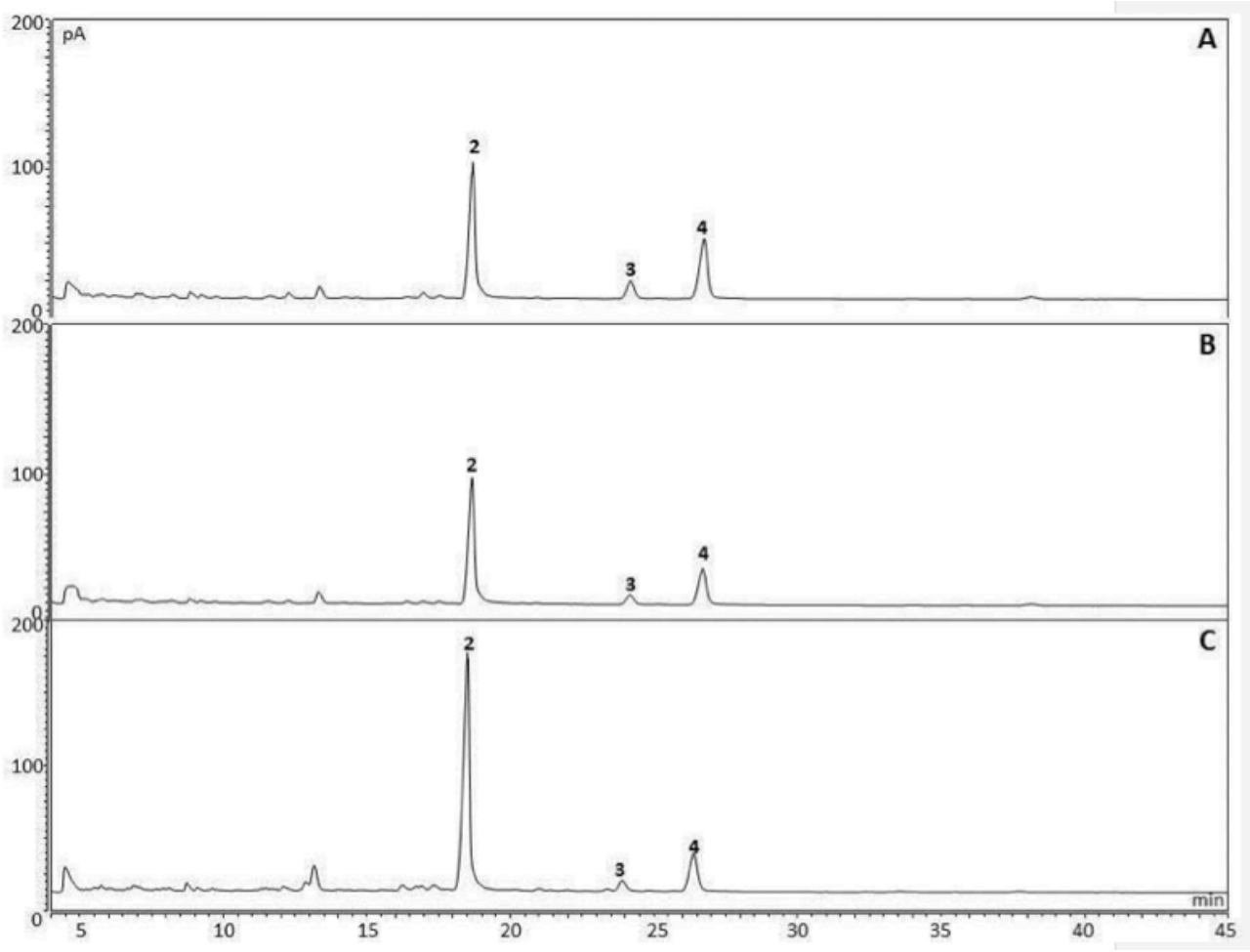

Figure S2. Main triterpenes from the wax of 'Duke' blueberries harvested at different maturity stages: A: H75; B: H100; C: H100+X. Peak numbers: (2) lupeol; (3) oleanolic acid; (4) ursolic acid.

5.3. Firmness at harvest influences development of internal browning of highbush blueberries (*Vaccinium corymbosum* L.)

Abstract

Fresh blueberries are very susceptible to mechanical damage, which limits postharvest life and firmness. Softening and susceptibility of cultivars 'Duke' and 'Brigitta' to developing internal browning (IB) after mechanical impact and subsequent storage was evaluated during a two-year study (2011/12, 2012/13). On each season fruit were carefully hand-picked, segregated into soft (< 1.60 N), medium (1.61–1.80 N) and firm (1.81–2.00 N) categories, and then either were dropped (32 cm) onto a hard plastic surface or remained non-dropped. All fruit were kept under refrigerated storage (0°C and 85 – 88% RH) to assess firmness loss and IB after 7, 14, 21, 28 and 35 d. In general, regardless of cultivar or season, high variability in fruit firmness was observed within each commercial harvest, and significant differences in IB and softening rates were found. 'Duke' exhibited high softening rates, as well as high and significant r^2 between firmness and IB, but little differences for dropped *vs.* non-dropped fruit. 'Brigitta', having lesser firmness rates, exhibited almost no relationships between firmness and IB (especially for non-dropped fruit), but marked differences between dropping treatments. Firmness loss and IB development were related to firmness at harvest, soft and firm fruit being the most and least damaged, respectively. Soft fruit were characterized by greater IB development during storage along with high soluble solids /acid ratio, which could be used together with firmness to estimate harvest date and storage potential of fruit. Results of this work suggest that the differences in fruit quality traits at harvest could be related to the time that fruit stay on the plant after turning blue, soft fruit being more advanced in maturity. Finally, the observed differences between segregated categories reinforce the importance of analyzing fruit condition for each sorted group separately.

Key words: blueberry, bruising, soluble solids, acidity, maturity, firmness segregation, storage.

1. Introduction

Blueberry production has increased rapidly around the world over the last two decades (Lobos and Hancock, 2015). Chile is the second largest global producer, as well as the first exporter of fresh blueberries to the Northern hemisphere (U.S.A, Canada, Europe and Asia). Most of the Chilean fruit is sent by boat, with transit periods of 20 to 50 d depending on destination. Blueberries are highly perishable, so fruit quality upon arrival to the final markets has major relevance to ensure economic returns (Beaudry et al., 1998; Retamales et al., 2014).

Several quality (dust, contaminants, size, bloom, russet/scars, attached stems, flower remains, and color) and condition (decay, mold, wounds, dehydration, firmness, and shriveling) traits are evaluated by inspection companies at destination markets. Among them, and regardless of season, dehydration and softening are the most common defects causing shipment rejections (Moggia et al., 2016b).

At present, due to low availability and high costs of labor for hand picking, farmers are being forced to invest in the mechanization of this critical production phase (Takeda et al., 2008; Xu et al., 2015). Mechanical harvesting of blueberries has the advantages of increasing capacity and efficiency as well as of reducing labor costs, but there are discrepancies as to their real contribution for the fresh fruit market. In general, machine harvest leads to the reduction of the acceptable amount of fruit that can be exported as a result of softening and excessive bruising; nevertheless, promising results have been reported on the use of a particular shaker, this being a viable alternative during critical periods (Lobos et al., 2014b). Fruit can also develop bruising during transport from the field to the packing-house, or when being processed on the packing-lines (Xu et al., 2015).

Blueberries are especially susceptible to mechanical damage, with injured berries resulting in loss of firmness that leads to reduced fruit quality and shelf-life (Xu et al., 2015). Bruises develop in the flesh of the damaged fruit as internal browning (IB) areas, resulting from tissue breakage and oxidation of phenolic compounds (Studman, 1997; Opara and Pathare, 2014). In order to relate the effect of mechanical damage with bruise damage, as done on large fruits and vegetables with instrumented spheres, a blueberry impact-recording device (BIRD) has been developed (Yu et al., 2011 and 2014). Recently, Xu et al. (2015) measured the mechanical impacts on packing lines with

the BIRD, showing that most of them occurred at the transfer points and that the highest impacts were recorded in one of the final handling steps, when the sensor dropped into the hopper above the clamshell filler.

Unfortunately, blueberry bruising can be expected to continue occurring, not only because of the use of mechanical/semi-mechanical harvest, or of differences between packing-line designs (e.g. number and height of transfer points, presence/absence of cushion materials), but also because of the lack of enough processing facilities during harvest peaks. Because of this, operators are forced to increase the speed at the sorting/packing lines, increasing the risk that fruit develop softening and IB during postharvest.

By simulating mechanical impact damage (as for other fruit species such as apples), the resistance of blueberries to IB has been evaluated by dropping fruit from different heights onto diverse surfaces; damage is rated on an internal bruise severity scale (affected area) after a period of cold storage (Brown et al., 1996; Yu et al., 2014). When berries were dropped from 15 to 30 cm onto hard surfaces, Brown et al. (1996) concluded that fruit developed IB on up to 50% of fruit area, and firmness declined significantly in samples having 25% or more damaged area. Yu et al. (2014) also reported a genotype effect, soft-textured cultivars being more susceptible than firm-textured ones when dropped on a hard plastic surface. However, all reported studies omit the high variability in firmness that occurs within an commercial clamshell, and hence the question arises whether results obtained for a given cultivar may be reproducible when variations in maturity stage, environmental conditions, and management procedures affect the proportions of soft, medium and firm fruit on a particular picking.

To the best of our knowledge, there are no previous reports on the implications of firmness segregation at harvest for the development of IB and softening of blueberries maintained under refrigerated conditions. Thus, the objective of this study was to understand how initial firmness and a single mechanical impact could affect the evolution of these traits during postharvest. For this, during two seasons, ‘Duke’ and ‘Brigitta’ fruit were segregated into soft, medium and firm categories at harvest, evaluating firmness loss and IB development of dropped (32 cm) and non-dropped fruit during 35 d under cold storage.

2. Material and Methods

2.1. Plant material

During two consecutive seasons (2011/12: Y1 and 2012/13: Y2), highbush blueberry (*Vaccinium corymbosum* L.) fruit of cultivars 'Duke' and 'Brigitta' (6- and 4-year old, correspondingly) were collected at the peak of the commercial harvest from Chilean orchards located in Longaví (36°00' S; 71°35' W) and Santa Bárbara (37°29' S; 72°19' W), respectively. Both cultivars were planted on raised beds, at 3 × 1 m in a loam soil. Each bed had two drip irrigation lines (2.4 L h^{-1} each 50 cm); irrigation frequency and timing were determined according to tensiometers established on each block at 30 and 50 cm depth. Pruning (May to July) was oriented to contribute for light entrance and air circulation, assuring a balance between canes of different ages and a stable production over time; pruning consisted in removing canes either unproductive or causing excessive shade on the plant. Fertigation was applied according to soil/foliar analysis and yield estimations; main nutrients were N (90 – 120 and 10 – 25 kg ha^{-1} for 'Duke' and 'Brigitta, correspondingly), K_2O (25 – 30 kg ha^{-1}), and P_2O_5 (150 –180 kg ha^{-1}). Environmental conditions are summarized in Supplementary Table 1.

In order to mimic the marketable characteristics of exported fresh fruit, all fruit were harvested upon commercial criterion, which is based on 100% blue color ('Duke' 5/12/2011 and 3/12/2012; 'Brigitta' 29/12/11 and 3/01/2013). Berries were hand-picked by qualified workers belonging to each orchard. To avoid potential differences in sorting and packaging facilities, and to reduce IB damage, fruit were harvested directly into plastic clamshells (125 g). Fruit were immediately transported to the laboratory facilities at Universidad de Talca (35º24' S; 71º38' W), for further analysis and treatment establishment.

2.2. Experimental set-up and measurements

Upon arrival to the research facilities, fruit were initially characterized in terms of firmness and IB, and then subjected to firmness segregation, impact damage simulation, and finally stored under refrigerated conditions as described below.

2.2.1. Firmness and IB at harvest

In order to assess firmness and IB variability on commercial fruit coming from the field, a sample of 200 fruit were evaluated on each cultivar and season prior to firmness segregation. Firmness (N) was assessed using a compression device (FirmTech 2, BioWorks, KS, USA) with the force thresholds set between 200 g (max) and 15 g (min) (Ehlenfeldt and Martin, 2002; Saftner et al., 2008). IB was assessed by slicing fruit equatorially and then rating flesh browning on each individual fruit, according to the extent of the bruised area, as 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), or 4 (> 75%) (Fig. 1).

2.2.2. Firmness segregation and initial condition on each category

Using the same equipment as for firmness assessments, fruit were assigned to one of three firmness categories: soft (<1.60 N), medium (1.60 – 1.80 N), and firm (1.81 – 2.00 N). For each season, this segregation represented 50 clamshells (125 g) per cultivar and category, from which each replicate was withdrawn. Then, for each firmness group, the following traits were assessed as initial condition: i) firmness on five replicates of 20 fruit each; ii) total soluble solids (TSS, %) using a digital refractometer (Pocket PAL-1, Atago, Tokyo, Japan), from juice obtained from five replicates of five berries each; iii) titratable acidity (TA, % citric acid equivalents) from five replicates; each one consisted of 10 mL of blueberry juice diluted to 100 mL with distilled water and titrated with 0.1 mol L^{-1} NaOH to an end-point pH of 8.2; iv) TSS/TA ratio; and v) IB on slices of five replicates of 20 fruit each.

2.2.3. Impact damage simulation

In order to study the evolution of IB and softening originated by impact damage, half of the fruit within each firmness category group were dropped from 32 cm onto a 30 × 30 cm of a hard plastic surface (6.4 mm-thick plexiglass), while the other half remained non-dropped. Dropping height was selected based on previous findings (data not published), as well as reports on extensive bruising resulting from 15 – 30 cm drop heights onto hard surfaces (Brown et al., 1996; Xu et al., 2015). For each cultivar, both dropped (32 cm) and non-dropped (0 cm) fruit were placed within clamshells into cardboard boxes, and then stored during 35 days at 0°C and 85 – 88% RH.

2.2.4. Firmness and IB evolution during postharvest

For each cultivar, firmness category group, and dropping treatment, firmness and IB evaluations were undertaken in samples (five replicates of 20 fruit each) from clamshells removed from cold storage after 7, 14, 21, 28 and 35 d. After each storage removal, fruit were acclimated to room temperature (18ºC) for 3 h prior to perform measurements. Individual fruit were first assessed for firmness and then cut transversally for IB rating.

2.3. Statistical analysis

Firmness and IB condition of commercial fruit at harvest (before firmness segregation) was described for each cultivar and season, through box and whisker plots. Quality traits of fruit segregated at harvest were analyzed considering a completely randomized design with factorial arrangement, considering three firmness categories (soft, medium, and firm) × two seasons (Y1 and Y2). Data of parametric variables were subjected to analysis of variance (ANOVA), and significance of the differences was determined by Tukey´s test ($p \leq 0.05$). IB data was subjected to non-parametric ANOVA with aligned rank for non-parametric analysis of multifactor designs (Oliver-Rodríguez and Wang, 2013) and mean separation by Tukey´s test ($p \leq 0.05$) for ranked data.

For the postharvest study, in order to determine the relationships between firmness and IB during storage, data were subjected to regression analysis (r^2) and models were fitted for each cultivar, season, firmness category, and drop heights. Additionally, statistical comparisons of slopes and intercepts between models for dropped *vs.* non-dropped fruit and, between firmness categories of each dropping treatment (soft *vs.* medium; medium *vs.* firm and soft *vs.* firm) were performed. Data were transformed to obtain linearized models between firmness (x) and IB (y). The best-fitted model was 1/x for both cultivars. Analyses were executed using commercial statistical software Statgraphics Centurion XVI (v.16.0.09, Statpoint, VA, USA) and R 3.0.0 (R Development Core Team, 2008).

3. Results

3.1. Fruit condition at harvest

3.1.1. Firmness and IB before fruit segregation

When commercial fruit sample was assessed for firmness at harvest, both cultivars displayed a wide range of values (Fig. 2A). 'Duke' firmness showed similar mean values during seasons 2011/12 (Y1) and 2012/13 (Y2) (1.55 and 1.60 N, respectively), whereas higher disparity was found on 'Brigitta' (1.52 and 1.92 N, correspondingly). Yet, comparison by Kolmogorov-Smirnov test ($p \leq 0.05$) evidenced significant differences in frequency distribution between years for both varieties (data not shown). Additionally, on both cultivars, fruit harvested on Y1 had greater variability (largest and smallest data values, wider quartile distributions, greater number of outliers) than berries picked on Y2. For 'Duke', 55% and 50% of fruit were below 1.6 N (upper threshold of the soft firmness category) for Y1 and Y2, respectively. For 'Brigitta' these values reached 60% and 15% for Y1 and Y2, correspondingly. If a threshold of 1.4 N for very soft fruit is considered, 25% (Y1) and 10% (Y2) of 'Duke' fruit were below that level, whereas values for 'Brigitta' were 42% and 5% for Y1 and Y2, in that order (Fig. 2).

Although not subjected to the dropping procedure, fruit displayed some internal browning at harvest (Fig. 2B), with mean IB scores of 0.15 to 0.19 for 'Duke', and 0.10 to 1.01 for 'Brigitta', on Y1 and Y2, correspondingly. The most heterogeneous IB values were found for 'Brigitta' fruit harvested on Y1. Yet, overall percentages of non-bruised fruit (Cat. 0) at harvest were higher for 'Brigitta' (90.0 to 92.5%) than for 'Duke' (83.1 to 89.8 %) (data not shown).

3.1.2 Fruit quality after firmness segregation

Once samples were segregated by firmness, the analysis of variance proved that fruit quality at harvest was influenced by initial firmness (Table 1). On both cultivars, firmer fruit was related to higher TA but lower TSS/TA and IB; TSS were significant only on 'Brigitta', and higher on the softer group (< 1.60 N). Differences between years occurred for TSS, TA and TTS/TA for 'Duke' and for TSS, TA and IB on 'Brigitta', reinforcing the higher variability found on this last trait during Y1. Significant interactions occurred for TA on 'Duke' (with differences between categories on Y1, but no differences on Y2) and for IB on 'Brigitta' (with differences only on soft fruit between years, having Y1 higher IB than Y2) (Supplementary Fig. 1).

3.2. Firmness and IB evolution of dropped and non-dropped fruit during postharvest

In comparison to 'Brigitta', 'Duke' berries showed lower firmness retention along time, irrespective of firmness category, dropping treatment or season (Fig. 3). Between harvest and the end of storage, and for both seasons, firmness of 'Duke' blueberries was reduced on average by 39.8, 33.6 and 38.6% (Fig. 3A, C and E) for soft, medium and firm fruit, respectively (data not shown), whereas firmness loss in 'Brigitta' averaged 17.3, 24.4 and 23.8%, correspondingly (Fig. 3B, D and F). When dropped and non-dropped fruit were compared, 'Brigitta' fruit appeared to be more sensitive to initial firmness, since significant differences between damaged and non-damaged fruit were found for most of storage evaluations (medium on Y1; soft, medium and firm on Y2). In contrast, for 'Duke' samples consistent differences between dropped and non-dropped fruit along the whole storage period were observed on soft fruit harvested on Y1 uniquely. Additionally, the magnitude of the differences between dropped and non-dropped fruit, as well as between seasons, were higher for 'Brigitta'.

In general, IB was higher after storage than at harvest, particularly for soft fruit (Fig. 4), regardless of cultivar, year or dropping treatment. 'Duke' fruit exhibited relatively low IB values up to 21 d of storage, with the highest IB at 35 d for soft (Y1 and Y2) and medium firmness fruit (Y2) (Fig. 4A and C). Similarly to the evolution of firmness in postharvest (Fig. 3), 'Duke' fruit also developed less IB in response to dropping, given that no significant differences between treatments were found at most of the evaluation dates. 'Brigitta', on the other hand, showed marked differences in IB development between dropped and non-dropped fruit for all firmness categories (Fig 4B, D and F). Compared to 'Duke' and regardless of dropping treatment, 'Brigitta' fruit developed lower IB within medium and firm categories (Fig 4B and D).

3.3 Relationship between IB and firmness

For 'Duke' samples, the regression analyses (r^2) between IB and firmness (Table 2, Fig. 5) revealed significant effects on dropped and non-dropped fruit for all three firmness categories and for both seasons. Although r^2 varied among comparisons, soft and firm fruit showed in general the highest values. In contrast, nine out of the twelve models fitted for 'Brigitta', which included all non-dropped fruit of both years and dropped fruit of Y2, showed no significant associations. During Y1, the highest r^2 values for dropped fruit were found on soft and medium fruit of this cultivar (72.7 and

80.6, respectively). The comparisons of slopes and intercepts between dropping treatments (Table 2) showed that significant differences for 'Duke' were found only between intercepts of firm fruit harvested in Y2. In contrast, equations developed for 'Brigitta' differed in slopes (soft and medium fruit of Y1) and intercepts (medium fruit of Y1, all three categories on Y2) on five out of the six instances.

When firmness categories were contrasted within the same dropping treatment (Table 3), outcomes varied among seasons. On non-dropped fruit of Y1, three comparisons resulted on different intercepts (medium *vs*. firm on 'Duke'; soft *vs*. medium, and soft *vs*. firm on 'Brigitta'), but no differences were found between slopes. For the same treatment, differences on Y2 occurred amid slopes of 'Duke' (medium *vs*. firm, and soft *vs*. firm) and intercepts of 'Brigitta' (soft *vs*. medium). Within dropped fruit of Y1 no significant differences were found for any comparison on 'Duke', whereas two cases were statistically significant for 'Brigitta' (soft *vs*. medium differed on intercept and slope; medium *vs*. firm differed on slopes). On Y2, differences between intercepts of medium *vs*. firm, and soft *vs*. firm occurred for 'Duke', meanwhile for 'Brigitta' the only significant difference happened between slopes of soft *vs*. firm fruit.

4. Discussion

The analysis of fruit characteristics at harvest revealed two important aspects that have not been reported previously. The first one is that, regardless of cultivar or season, high variability in fruit firmness occurred within each commercial harvest. In comparison with other fruit species such as apple, for which very soft fruit (58 – 62 N) represent less than 0.5 – 0.8% (Herregods and Goffings 1993; De Silva et al., 2000), a high percentage of 'Duke' and 'Brigitta' blueberries showed this characteristic (< 1.4 N) in Y1 (25% and 42%, respectively) and Y2 (10% and 5%, respectively). The second one refers to the noticeable differences in quality traits found between firmness categories, which highlights the relevance of analyzing the development of softening and IB for each sorted group separately. These two aspects will be covered during the discussion.

4.1 Susceptibility of blueberries to develop IB

IB was detected at harvest in this study, even though fruit were carefully hand-picked and not subjected to sorting or packing. Gołacki et al. (2009) indicated that vibration forces, usually occurring during transportation from the field, are difficult to avoid and may also cause damage. In

addition to possible damage sources before harvest (e.g. due to wind or machinery), fruit samples used herein underwent a ~3-h trip from the field to the laboratory, and hence transportation may have impacted the basal IB found. Indeed, unless a packinghouse facility is available at the producing orchard, it is common that fruit travel 2 – 3 h until being processed. This observation highlights the importance of careful handling of the fruit throughout the whole production and distribution chain, and evidences high differences within a particular cultivar among seasons. In fact, variability in firmness and IB at harvest showed dissimilarities between cultivars, with 'Duke' fruit being more homogeneous for both seasons, whereas 'Brigitta' berries showed higher differences within and between years. The high IB values in Y1 at harvest for 'Brigitta' were associated to softer fruit (Fig. 2). Variations in ambient temperature between both seasons (Supplementary Table 1) may partially account for the differences in fruit condition between seasons and cultivars, especially for higher heterogeneity of 'Brigitta' samples on Y1. Although there is not much information, it has been suggested that an ideal range of temperatures for northern highbush blueberries might range 20 to 25ºC (Davies and Flore, 1986); values above 30ºC (also associated with high light intensity as in Chile) cause plant damage (Trehane 2004; Lobos and Hancock, 2015), as well as lowered wax coverage of fruit, which tend to be smaller and softer (Mainland 1989). With the exception of precipitation (Y1: 32.9 mm and Y2: 102 mm), Longaví does not usually register substantial differences in environmental conditions from early October (full bloom) to early December (harvest) (Supplementary Table 1). This might in part explain the lower variability between seasons observed for 'Duke'. On the other hand, different temperature patterns for each season were registered in Santa Bárbara in December. Even though more favorable temperatures occurred in Y1 (20 – 25ºC), more temperature extremes took place (greater number of hours or days hotter than 27, 29 and 32ºC), probably leading to early softening of fruit.

It is also highly likely that blueberries can be damaged on packing-lines. Xu et al. (2015) studied 11 commercial packing lines using the blueberry impact recording device (BIRD) and found that the tested lines differed in their combinations and alignments, thus creating different points for potential impact damage. Yet, all the impacts occurred at transfer points, the highest drop heights being 35 – 36 cm. Additionally, the latter part of the packing line, where fruit drop into the hopper for loading clamshells, is another point for potential damage due to the combination of hard contact surface (usually stainless steel) and high drop height (Xu et al., 2015), and especially when the first berries

drop into the hopper, since they will impact directly onto the hard surface. As more fruit get into the line, ever more fruit-to-fruit impacts will take place, this being a source of impact that has not been fully incorporated in studies dealing with mechanical damage. Results obtained in the present study show that significant differences in IB development between 'Duke' and 'Brigitta' occurred with drop heights of 32 cm, evidencing a differential effect of season, cultivar and firmness category.

In order to standardize sorting/packing-lines and to establish some basic recommendations to improve condition, it is critical to identify which fruit would be more prone to softening and IB during postharvest. Unfortunately, given that the main criterion for establishing harvest date of blueberries is skin color, and that high labor costs are associated to this operation (Brown et al., 1996; Takeda et al., 2008; Lobos et al., 2014b), growers wait for blue fruit to accumulate in the bush before starting commercial pickings. This practice results in fruit with similar external appearance but, as found in the present study, with important heterogeneity in maturity status, that will lead to a wide range of firmness levels at harvest, as well as in softening rates during postharvest. Previous works have proved that delaying harvest increases TSS and TSS/TA but reduces TA and firmness (Woodruff et al., 1960; Ballinger et al., 1963; Kushman and Ballinger, 1963; Lobos et al., 2014a), since TSS increase and acids decrease due to fruit respiration in the course of maturation (Dai et al., 2009; Famiani et al., 2005). In fact, when fruit showing no differences in skin color at harvest (determined either visually or instrumentally) were picked 2 or 6 d after turning 100% blue on the bush, important differences in fruit condition were demonstrated associated to these two maturity stages (Moggia et al., 2016a and b). In those previous studies, when similar percentages of green and pink fruit were reached early in the season, clusters with similar characteristics and canopy position were selected and labeled. Fruit development was followed until both maturity stages were reached: 100% blue and residing on the plant for a maximum of 2 d (ripe), and 100% blue and residing on the plant for 6 d (overripe). That methodology allowed the authors to conclude that, when these two maturity stages were selectively picked, important differences were found, 'Duke' being more sensitive than 'Brigitta' to this factor. The elapsed time between harvests was enough to increase TSS and TSS/TA of 'Duke' samples, and to reduce fruit firmness in both cultivars. These findings reinforce the importance of the time that fruit stay on the plant after turning 100% blue for fruit heterogeneity. In the present study, segregation by firmness at harvest revealed similar trends for these traits, suggesting that fruit

within the soft category had actually stayed longer in the plant after turning completely blue. Accordingly, when fruit were segregated based on firmness, berries assigned to the soft category displayed the highest IB, TSS and TSS/TA values (Table 1). Given the variability found at harvest (box and whisker plots), these dissimilarities would be higher for Y1 'Brigitta' fruit, thus accounting for the greater differences found according to the dropping treatment between fruit within the soft and the medium categories. In fact, according to the Chilean blueberry industry, overall commercial defects (including softening, dehydration and mechanical damage) differ between seasons, and the affected produce may account for 10 – 45% of the fresh fruit reaching final markets (Moggia et al., 2016b).

4.2 Bruising as related to firmness

Firmness is one of the characteristics most frequently measured to evaluate quality of fresh fruit (Timm et al., 1996). As for many other fruit species, firmer blueberries can more readily withstand harvest handling, and will therefore have longer storage potential (Hanson et al., 1993; Yu et al., 2014). Differences in firmness among highbush blueberry cultivars seem to be more dependent on physiological maturity at harvest than on genotypic differences (Beaudry et al., 1998; Lobos et al., 2014a); yet there is limited information on the relevance of firmness at harvest for postharvest quality of fruit within a particular cultivar. Wolfe et al. (1983) demonstrated that firmness separation of blueberries at harvest allows better control of postharvest decay, since soft, medium and firm fruit show different susceptibility to rot, and fruit segregation enhanced disease control when combined with a hot water dip. Similarly, the present study demonstrates that softening and IB development are related to firmness at harvest of individual fruit, and that high IB can be expected in soft fruit of both cultivars after prolonged storage.

Since in this study the highest IB rates were always found for soft berries (< 1.60 N), our findings strengthen the idea that mid-to-firm berries can better withstand a long trip to distant markets. Therefore, any strategy oriented to increase the percentage of these firmness classes into the clamshells will assure higher and more homogeneous quality upon arrival to final destination.

Dropping the fruit did not always lead to higher IB values, and this observation was more evident for 'Duke' samples, in which high softening rates but small differences in IB between dropped and

non-dropped fruit occurred (Fig. 4). This finding agrees with the lack of differences between slopes and intercepts of the models fitted for fruit of this cultivar (0 *vs.* 32 cm drop heights) (Table 2); the only difference was found between intercepts of firm fruit, but not between slopes, which indicates similar rates of change in IB per firmness unit both for dropped and non-dropped fruit. (Table 2, Fig. 5). Yet, significant associations between firmness and IB, and generally higher r^2 coefficients, both for dropped and non-dropped fruit were obtained for 'Duke' as compared to 'Brigitta' samples (Table 2). On the other hand, the fact that 'Brigitta' fruit did not show significant associations for most of the equations indicates a weak relationship between firmness and IB development for this cultivar, especially for samples harvested in Y2. However, higher IB levels in dropped than in non-dropped fruit, regardless of fruit firmness at harvest should be expected for this cultivar (Table 2, Fig. 5). The analyses undertaken for 'Brigitta' samples corresponding to Y1 (more heterogeneous in initial condition, and significant r^2 values for dropped fruit uniquely) reveal that differences in slopes and intercepts occurred for all three firmness categories, with different rates of change between dropping treatments. When equations were compared between firmness categories within each dropping treatment (Table 3), variability between seasons became more evident, since significances were not the same in both years considered. Moreover, different slopes (meaning dissimilar rate of change in IB per firmness unit) were found on 'Duke'- 0 cm and 'Brigitta'- 32 cm, whereas different intercepts (indicating similar rates, but different damage threshold) occurred on 'Duke'- 32 cm and 'Brigitta' - 0 cm. Additionally, most of these differences were observed between soft and firm fruit, which emphasizes the negative effects on quality resulting from a high proportion of soft fruit on a particular picking.

According to these results, each cultivar would display a different pattern of IB development when subjected to mechanical damage. Therefore, and depending upon fruit condition at harvest (initial firmness), fruit might not necessarily exhibit severe IB symptoms but would probably show different softening patterns. Another important aspect to consider is that sectioning berries through the equator detects bruising caused by impacts occurring onto that area, but this procedure does not take into account damage at or near the calyx or stem ends, and it would hence lead to an underestimation of the actual mechanical damage (Yu et al., 2014).

The present study demonstrated the different susceptibility to IB development and softening rates in blueberry fruit among different cultivars and firmness categories at harvest, and suggests that fruit displaying firmness lower than 1.6 N at harvest should be avoided if long-term storage is intended. Galletta et al. (1971) proposed that good keeping quality could be expected when TSS/TA ratios are < 18, whereas intermediate keeping quality would result from higher TSS/TA values. Given that TSS/TA ratios at harvest of medium and firm fruit ranged from 15 to 21, and that soft fruit values ranged 19 – 29, it is suggested that this ratio could be used as an additional index to define harvest time and destination of the fruit (long- *vs*. short-term storage).

Overall, 'Duke' fruit were characterized by high rates of firmness loss, as well as by a strong association between firmness and IB, but little differences were found between dropped and non-dropped fruit. 'Brigitta' berries had slower softening rates, and displayed very weak relationships between firmness and IB (especially for non-dropped fruit), but marked differences between dropping treatments were found.

5. Conclusions

Results of this work suggest that the mean firmness value may be not adequate as an indicator of blueberry fruit condition at harvest, and that the differences in fruit quality traits associated to the initial firmness level might be related to the time that fruit stay on the plant after turning blue, softer fruit displaying more advanced maturity. This finding suggests that, during seasons in which adverse environmental events occur (probably associated to high temperatures close to harvest), the proportion and evolution of soft fruit during shipments would enhance rejections at destination markets. Future research should include a more detailed study on potential sources of fruit heterogeneity. Furthermore, more systematic measurements of changes throughout fruit development from early stages, as done for other species, could help in modeling softening and IB during postharvest. Finally, long-time studies are needed to quantify the real genotypic and environmental effects on softening and IB development in blueberries.

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Table 1. Analysis of variance for fruit quality traits[x] at harvest of 'Duke' and 'Brigitta' blueberries according to three-firmness category groups, during seasons 2011/12 (Y1) and 2012/13 (Y2).

Cultivar	Factor	TSS (%)	TA (% citric ac.)	TSS/TA	IB (scale 0 – 4)
'Duke'	*Firmness Category (F)*				
	Soft (< 1.60 N)	11.2	0.59 c	19.4 a	0.38 a
	Medium (1.60 – 1.80 N)	11.1	0.71 b	17.5 ab	0.44 a
	Firm (1.81 – 2.00 N)	11.1	0.83 a	15.1 b	0.19 b
	Year (Y)				
	Y1	12.4 a	0.93 a	13.7 b	0.34
	Y2	9.9 b	0.49 b	20.9 a	0.31
	Significance (p-value)				
	F	*0.974*[z]	*0.000*	*0.049*	*0.000*
	Y	*0.000*	*0.000*	*0.000*	*0.069*
	F x Y	*0.527*	*0.002*	*0.118*	*0.352*
'Brigitta'	*Firmness Category (F)*				
	Soft (< 1.60 N)	15.2 a	0.57 b	28.4 a	1.28 a
	Medium (1.60 – 1.80 N)	13.5 b	0.64 ab	21.4 b	0.26 b
	Firm (1.81 – 2.00 N)	13.2 b	0.75 a	18.0 b	0.19 c
	Year (Y)				
	Y1	15.1 a	0.74 a	22.3	0.69 a
	Y2	12.8 b	0.57 b	22.9	0.45 b
	Significance (p-value)				
	F	*0.008*	*0.030*	*0.002*	*0.000*
	Y	*0.000*	*0.007*	*0.692*	*0.000*
	F x Y	*0.243*	*0.329*	*0.844*	*0.000*

For a given cultivar, or factor, and significance $p \leq 0.05$, different letters within a column represent significant differences (Tukey´s test, $p \leq 0.05$)

[y] Traits: total soluble solids (TSS), titratable acidity (TA), and internal browning (IB) damage categories: 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), or 4 (> 75%).

[z] In red, color p-values lower than 0.05.

Table 2. Internal browning (IB*) vs.* firmness (F) regression analysis for non-dropped (0 cm) and dropped (32 cm) fruit. Intercept and slope comparison between IB_0 and IB_{32}, of 'Duke' and 'Brigitta' blueberries according to three firmness category groups, during seasons 2011/12 (Y1) and 2012/13 (Y2).

Cultivar	Year	Firmness category		Model					Model comparisons (*p-values*)	
						Equation	n^x	r^{2y}	Intercept	Slope
'Duke'	Y1	Soft	(< 1.60 N)	IB_0	=	-1.669 + 3.188 • (1/F)	30	69.2 ***	*0.772*[z]	*0.740*
				IB_{32}	=	-1.429 + 2.966 • (1/F)	30	53.7 ***		
		Medium	(1.60 – 1.80 N)	IB_0	=	-1.213 + 2.745 • (1/F)	16	25.7 *	*0.643*	*0.600*
				IB_{32}	=	-0.796 + 1.994 • (1/F)	16	34.3 *		
		Firm	(1.81 – 2.00 N)	IB_0	=	-1.936 + 0.825 • (1/F)	18	72.1 ***	*0.585*	*0.369*
				IB_{32}	=	-1.365 + 2.711 • (1/F)	20	44.5 *		
	Y2	Soft	(< 1.60 N)	IB_0	=	-2.233 + 4.311 • (1/F)	50	50.3 ***	*0.528*	*0.864*
				IB_{32}	=	-2.185 + 4.173 • (1/F)	50	58.0 ***		
		Medium	(1.60 – 1.80 N)	IB_0	=	-2.256 + 4.203 • (1/F)	50	55.0 ***	*0.747*	*0.834*
				IB_{32}	=	-2.013 + 3.953 • (1/F)	50	20.3 *		
		Firm	(1.81 – 2.00 N)	IB_0	=	-1.039 + 2.656 • (1/F)	50	35.3 ***	*0.002*	*0.118*
				IB_{32}	=	-2.265 + 3.899 • (1/F)	50	59.7 ***		
'Brigitta'	Y1	Soft	(< 1.60 N)	IB_0	=	0.439 + 1.584 • (1/F)	48	8.57 n.s.	*0.814*	*0.000*
				IB_{32}	=	-3.333 +5.576 • (1/F)	48	72.7 ***		
		Medium	(1.60 – 1.80 N)	IB_0	=	0.963 – 0.340 • (1/F)	20	0.57 n.s.	*0.016*	*0.000*
				IB_{32}	=	-8.393 + 12.921 • (1/F)	20	80.6 ***		
		Firm	(1.81 – 2.00 N)	IB_0	=	-0.359 + 1.354 • (1/F)	26	4.48 n.s.	*0.507*	*0.219*
				IB_{32}	=	-2.060 + 3.798 • (1/F)	26	43.9 **		
	Y2	Soft	(< 1.60 N)	IB_0	=	-0.780 + 2.153 • (1/F)	40	12.4 n.s.	*0.028*	*0.904*
				IB_{32}	=	-0.597 + 2.338 • (1/F)	40	11.7 n.s.		
		Medium	(1.60 – 1.80 N)	IB_0	=	-1.580 + 2.910 • (1/F)	40	7.06 n.s.	*0.012*	*0.947*
				IB_{32}	=	-1.081 + 2.763 • (1/F)	40	10.6 n.s.		
		Firm	(1.81 – 2.00 N)	IB_0	=	-0.444 + 1.280 • (1/F)	28	0.97 n.s.	*0.000*	*0.609*
				IB_{32}	=	0.757 + 0.244 • (1/F)	28	0.36 n.s.		

[x] Sample size.

[y] Significance: n.s. (non-significant), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

[z] In red, color p-values lower than 0.05.

Table 3. Intercept and slope comparisons of internal browning *vs.* firmness regression analysis, between firmness category groups for non-dropped (0 cm) and dropped (32 cm) 'Duke' and 'Brigitta' blueberries, during seasons 2011/12 (Y1) and 2011/12 (Y2).

Cultivar	Drop height (cm)	Firmness category	Model comparisons (*p*-values)			
			Y1		Y2	
			Intercept	Slope	Intercept	Slope
'Duke'	0	Soft *vs*. Medium	*0.273*[z]	*0.746*	*0.304*	*0.902*
		Medium *vs*. Firm	*0.035*	*0.572*	*0.779*	*0.045*
		Soft *vs*. Firm	*0.840*	*0.783*	*0.152*	*0.066*
	32	Soft *vs*. Medium	*0.911*	*0.517*	*0.908*	*0.851*
		Medium *vs*. Firm	*0.164*	*0.492*	*0.007*	*0.702*
		Soft *vs*. Firm	*0.576*	*0.877*	*0.001*	*0.870*
'Brigitta'	0	Soft *vs*. Medium	*0.000*	*0.443*	*0.019*	*0.691*
		Medium *vs*. Firm	*0.148*	*0.399*	*0.678*	*0.558*
		Soft *vs*. Firm	*0.007*	*0.954*	*0.126*	*0.689*
	32	Soft *vs*. Medium	*0.029*	*0.002*	*0.397*	*0.810*
		Medium *vs*. Firm	*0.346*	*0.000*	*0.148*	*0.081*
		Soft *vs*. Firm	*0.783*	*0.370*	*0.146*	*0.034*

[z] In red, color p-values lower than 0.05.

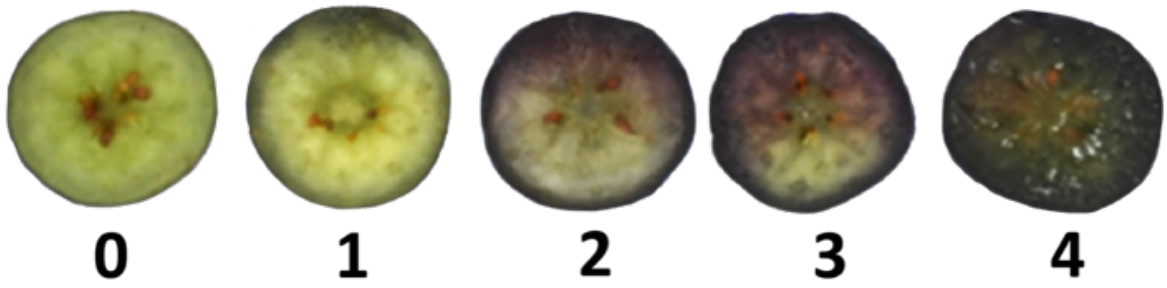

Figure 1. Scale used for assessing internal browning (IB) severity in blueberry fruit. Categories were assigned based on the extent of bruised equatorial area: 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), and 4 (> 75%).

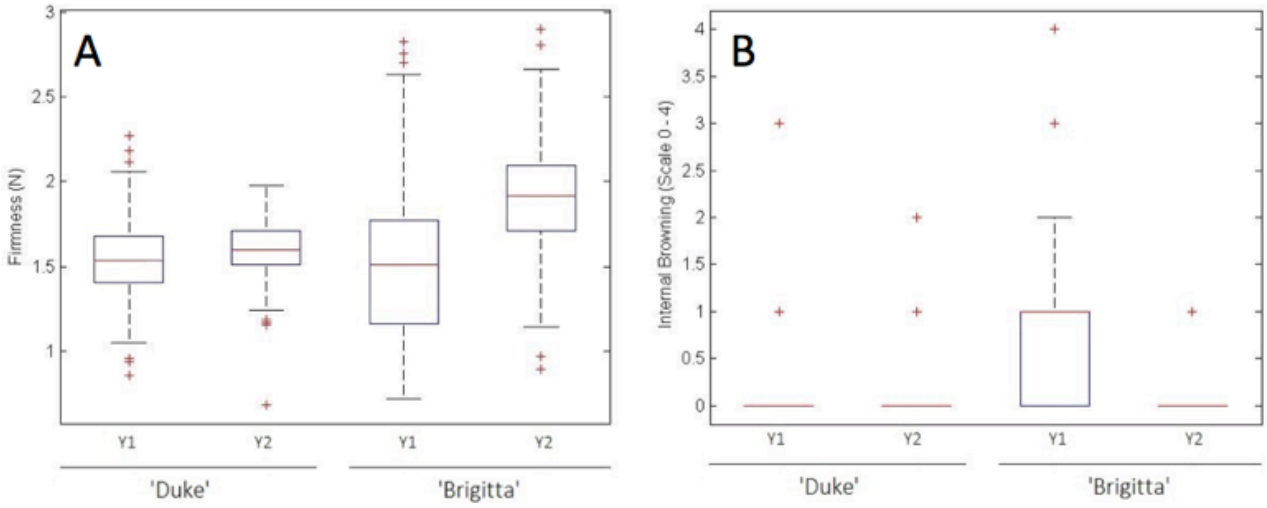

Figure 2. Fruit firmness (A) and internal browning (B) variability at commercial harvest of 'Duke' and 'Brigitta' blueberries, during seasons 2011/12 (Y1) and 2012/13 (Y2). IB categories: 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), and 4 (> 75%). n=200 per year and cultivar.

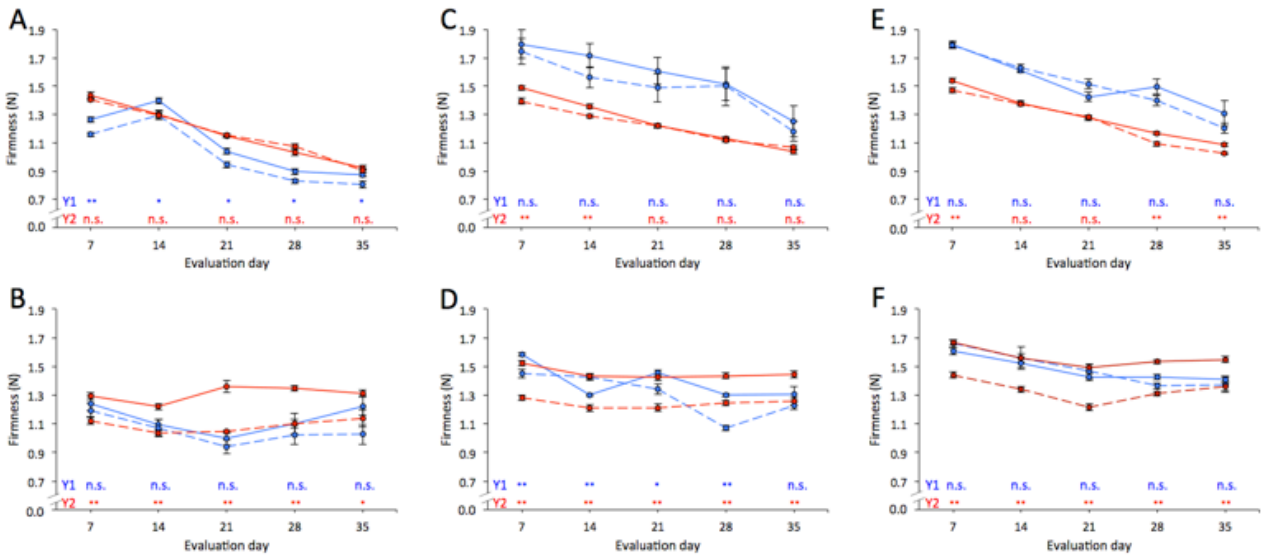

Figure 3. Firmness (N) changes during refrigerated storage (0°C and 85-88% RH) of 'Duke' (A, C, and E) and 'Brigitta' (B, D, and F) blueberries, according to firmness segregation at harvest: soft (< 1.60 N; A and B), medium (1.61 – 1.80 N; C and D), and firm (1.81 – 2.00 N; E and F). Assesments were taken during 2011/12 and 2012/13 (Y1, Y2; blue and red lines, respectively) on dropped (32 cm, dashed lines) and non-droped (0 cm, solid lines) fruit. Each value represents the mean of 5 replicates of 20 fruit. Significance: n.s. (non-significant), * ($p < 0.05$), and ** ($p < 0.01$).

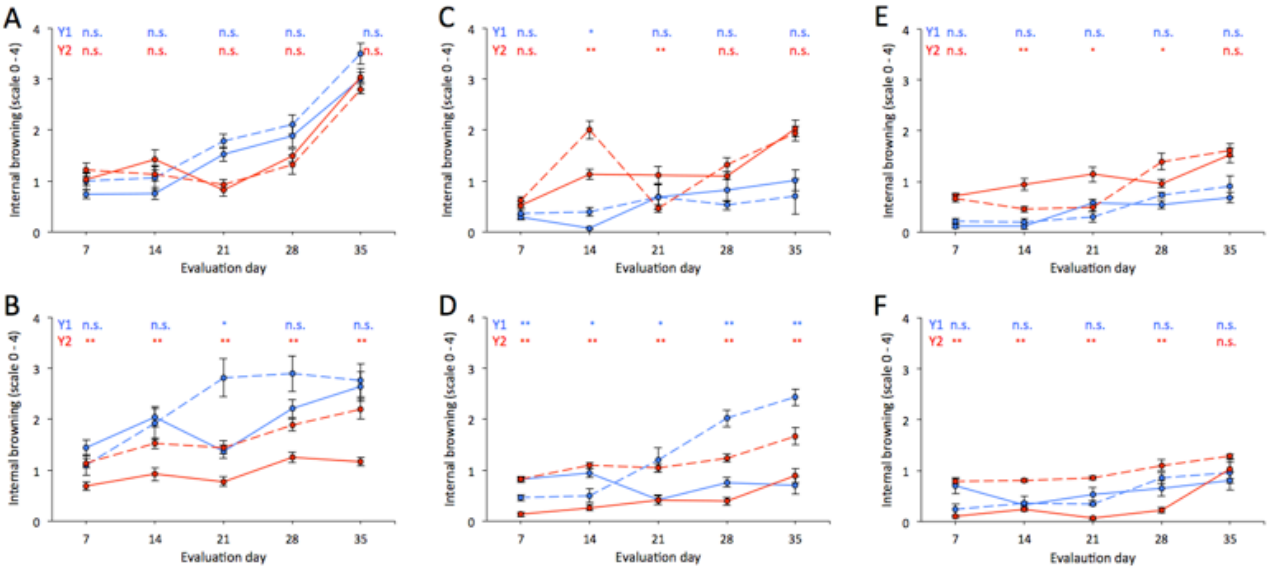

Figure 4. Internal browning changes during refrigerated storage (0°C and 85-88% RH) of 'Duke' (A, C, and E) and 'Brigitta' (B, D, and F) blueberries, according to firmness segregation at harvest: soft (< 1.60 N; A and B), medium (1.61 – 1.80 N; C and D), and firm (1.81 – 2.00 N; E and F). Assesments were taken during 2011/12 and 2012/13 (Y1, Y2; blue and red lines, respectively) on dropped (32 cm, dashed lines) and non-droped (0 cm, solid lines) fruit. Each value represents the mean of 5 replicates of 20 fruit. IB scale: 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), and 4 (> 75%). Significance: n.s. (non-significant), * ($p < 0.05$), and ** ($p < 0.01$).

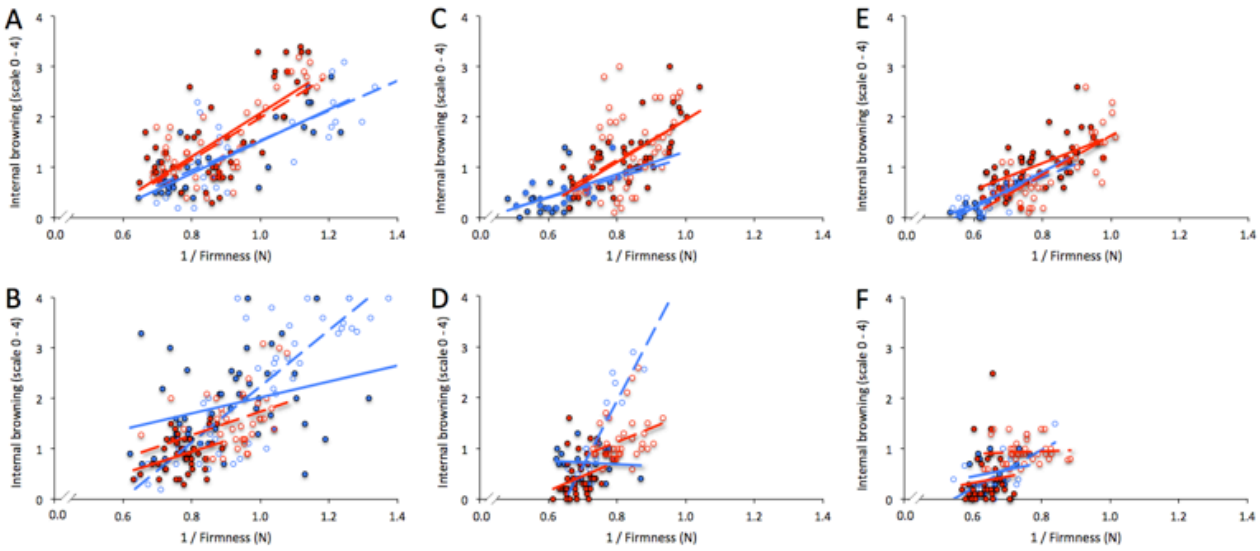

Figure 5. Regression analysis between firmness (N) and internal browning for 'Duke' (A, C, and E) and 'Brigitta' (B, D, and F) blueberries, according to firmness segregation at harvest: soft (< 1.60 N; A and B), medium (1.61 – 1.80 N; C and D), and firm (1.81 – 2.00 N; E and F). Assesments were taken during 2011/12 and 2012/13 (Y1, Y2; blue and red lines, respectively) on dropped (32 cm, dashed lines) and non-droped (0 cm solid lines) fruit. Each value represents individual fruit. IB scale: 0 (0 – 5%), 1 (6 – 25%), 2 (26 – 50%), 3 (51 – 75%), and 4 (> 75%).

5.4. Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding

Abstract

The role of fruit scar on water loss from fresh harvested, fully blue highbush blueberry (*Vaccinium corymbosum* L.) fruit was studied on three germplasm lines from each of three half-sib families at University of Talca, Chile. The stem scar of half of the harvested fruit was sealed using nail polish and weight loss of sealed and non-sealed fruit determined daily at 20 °C (5 d storage) and bi-weekly at 0 °C (15 d storage). Fruit firmness was determined at the end of the storage period. The stem scar accounted for approximately 40 % of the moisture lost at 20 °C, but percentages varied considerably between lines. While the stem scar covered 0.19 % to 0.74 % of the fruit surface area, its rate of transpiration was 170-times higher than for the cuticle at 20 °C. The larger the fruit scar area, the greater was the absolute rate of water loss, but scar size scar did not affect the rate of weight loss expressed on a per gram fruit basis. Higher levels of water loss were associated with a greater loss in firmness; fruit having a large scar had a greater rate of water loss and were less firm than those having medium or small scars. The water permeance of the fruit cuticle varied two-fold and the apparent permeance of the scar varied three-fold among the 9 lines evaluated when held at 20 °C. Interestingly, one line exhibited a 75 % lower rate of water loss from its stem scar than the other lines than would be predicted based on its scar diameter. Storage at 0 °C reduced the rate of water loss by 90 % but the cuticle permeance was not affected by temperature. Sealing the stem scar increased fruit firmness retention at 0 °C and 20 ºC, but provided less benefit at 0 °C *vs.* 20 °C. The highly variable nature of water loss through the stem scar and the cuticle in this study suggests that large gains in reductions in water loss are possible for the highbush blueberry once the mechanisms for transpiration are better understood.

Key words: transpiration; softening; water loss; permeance; maturity; cold storage

1. Introduction

Blueberries are highly perishable, with softening and dehydration as major factors that can limit their marketability (Ehlenfeldt and Martin, 2002; Vicente et al., 2007) or increase rejections at final markets (Prussia et al., 2006). Firmness is considered one of the most important attributes influencing acceptance of fresh blueberries with firmer fruit being preferred (Ne Smith et al., 2002; Lobos et al., 2014). The rate of water loss varies substantially for blueberry cultivars and is a major contributor to softening during long-term refrigerated storage (Paniagua et al., 2013). Cultivar, cuticle characteristics, maturity stage, and the use of a moisture barrier are also important factors affecting moisture loss (Moggia et al., 2016).

Transpiration accounts for most of the weight loss in the majority of horticultural species (Burton, 1982). Gaseous exchange may take place from harvested produce to the atmosphere by four major routes: the stem scar region, stomata/lenticels, the calyx, and the cuticle (Ben-Yehoshua and Rodov, 2003; Díaz-Perez, 1998). Tomato (*Solanum lycopersicum*) fruit have a moderately thick waxy cuticle with no pores (Wilson and Sterling, 1976; Das and Barringer, 1999; Thompson, 2001) and sealing the stem scar significantly reduces gas exchange, reducing the ripening rate and prolonging storage life (Yang and Shewfelt, 1999). In eggplant (*Solanum melonena*) the fruit calyx is the main route for fruit water loss, accounting for at least 60 % of fruit transpiration (Díaz-Perez, 1998).

Blueberries have a cuticle and wax-covered epidermis that, like tomato and eggplant, have no stomata (Gough, 1994). The cuticle, composed of a cutin polyester polymer with waxes and embedded with epicuticular waxes, is considered a major barrier against water loss (Lara et al., 2014; Lownds et al., 1993; Martin and Rose, 2014). In this context, the question arises as to the relative contributions of the stem scar (where the pedicel detaches) and the cuticle to fruit dehydration.

To our knowledge, selection for water loss rates has not been a priority in any blueberry breeding program. Nevertheless, moisture loss and shrivel are major quality concerns for blueberry industries (Paniagua et al., 2014; USDA, 1995). The blueberry industry in Chile permits no more than 5-7 % weight loss in a commercial 3-week period at 0 ºC (Paniagua et al., 2014). However, less than optimal temperatures can occur in real supply chains (Sargent et al., 2006). Given the

potential value of blueberry germplasm with the quality characteristic of shrivel resistance, a good argument can be made for evaluating water loss physiology and assessing its potential for improvement through breeding.

The objective of this study was to evaluate morphometric fruit variables of stem scar size, fruit surface area, and the ratio between the two on fruit dehydration and softening using breeding lines from an active blueberry breeding program. Fruit exhibiting a wide range in stem scar size were selected from three half-sib families grown in Talca, Chile. Three lines were selected per family; one line had small-sized stem scars, a second had medium-sized stem scars and the third had large-sized stem scars. To determine the contribution of the stem scar to water loss, shrivel and firmness, half of the fruit had their stem scar sealed during storage at 20 °C and 0 °C.

2. Material and Methods

2.1. Plant material

During 2015/2016 season, ripe fruit (100 % blue) were collected from adult highbush blueberry plants grown at Panguilemo Experimental Station, University of Talca, Maule Region (35°22'15"S; 71°35'50"W). Plants were from a germplasm collection representing crosses made in a University of Talca blueberry-breeding program; the planting was established in 2009. For this study three families were selected, having the following female and male parents, respectively: Family 6 (F6; Legacy x Brigitta); Family 16 (F16; Chandler x Legacy) and Family 40 (F40; Orus 344 x Legacy). Three plants, each representing a different line, were selected per family based on visual assessments of stem scar size; one line had small-sized stem scars, a second had medium-sized stem scars, and the third had large-sized stem scars (Fig. 1A).

Fully ripe fruit with 100 % blue color coverage were hand-picked into plastic clamshells and transported within 30 min of harvest to the laboratory facilities at University of Talca, for treatment establishment.

2.2. Experimental set-up

2.2.1. Experiment 1: Effect of family, scar size and stem scar sealing at room temperature

From each germplasm line, a minimum of 30 fruit was harvested, on December 28th, 2015. Upon arrival at the laboratory, twenty uniform, undamaged fruit were selected per line and each individual berry was measured for scar width, fruit weight, fruit length and width, and fruit firmness. To evaluate contribution of stem scar to fruit transpiration, the scar on half (10) of the berries of each family was sealed with nail polish (Fig. 1B) to permit calculation of water loss via the cuticle and stem scar independently. Fruit were placed into depressions on plastic trays to prevent fruit-to-fruit contact and stored at room temperature in the laboratory (20 ºC, 65 % RH). Fruit weight was determined daily for each fruit over a period of 5 d to determine the rate of weight loss as percent per day and water loss as μg s^{-1}. Average room temperature and relative humidity were determined using a calibrated portable temperature humidity sensor (HOBO U23 Pro v2, Onset Computer Corp., Bourne, MA, USA) placed adjacent to the trays holding the fruit. On day 5, firmness and the degree of shrivel were determined for each fruit (see 2.3).

2.2.2. Experiment 2: Effect of family and scar sealing under refrigerated storage

From each family, forty fruit from lines designated as having a small stem scar were harvested on January 4th , 2016 and handled as described in 2.2.1. These lines differed from those in Experiment 1. For this experiment, half of the fruit were placed in the laboratory (20 ºC, 65 % RH) and fruit weight was determined daily for each fruit for 7 d. The remaining half were placed in refrigerated storage (0 ºC, 88 % RH) and fruit weight determined every 2-3 d for a total of 15 d to estimate the rate of weight loss. Half of the fruit at each temperature had their stem scar sealed as previously described to permit calculation of water loss via the cuticle and stem scar. Room temperature and humidity were determined as previously described. During the final evaluation (day 15) each individual berry was evaluated for firmness and shrivel severity.

2.3. Measurements and estimations

Firmness and morphometric variables (fruit weight, fruit diameter, fruit length, and scar diameter) were measured on each fruit. A digital caliper (Truper, Model CALDI-6MP, Mexico) was used to measure fruit and stem scar dimensions to the nearest tenth of a millimeter. Fruit surface area (cm^2) was calculated for an oblate spheroid using length (*LEN*) and diameter (*DIA*) as follows: Area = $(2\pi(DIA/2)^2)(1+((1-(1-((LEN/2)^2/(DIA/2)^2)))/((1-((LEN/2)^2/(DIA/2)^2))^{0.5}))\text{Arctanh}((1-((LEN/2)^2/$

$(DIA/2)^2))^{0.5}))/100$. Scar area (mm^2) was estimated assuming the scar was circular. From these measures, the scar area to fruit surface area ratio (%) was calculated.

Firmness (N mm^{-1}) was measured as N per mm deformation using an automated compression tester (FirmTech 2, BioWorks, Inc., Wamego, KS, USA), which measured compressive load as a function of compression distance between loads of 0.15 and 2 N. The compression rate was 6 mm s^{-1}. Fruit firmness loss was calculated as the percent difference between pre- and post-storage firmness within each treatment.

Fruit weight (g) was measured with an electronic balance (LSV-6200g, Veto y Cía. Ltda., Santiago, Chile). The decline in weight with time was assumed to be primarily due to water loss. The water loss rate was expressed as μg s^{-1}. Weight loss as a result of transpiration was expressed on a percentage basis as the daily weight loss relative to initial weight. To account for differences in surface area to mass ratio among fruit and the gradient in water vapor pressure, permeance to water vapor (P_{H2O}, μmol m^{-2} s^{-1} Pa^{-1}) was calculated for the fruit cuticle and the stem scar as proposed by Díaz-Perez et al. (2007). The P_{H2O} of the stem scar was termed 'apparent P_{H2O}' because the mechanism of diffusion is from a free water source and is technically not permeance. However, calculation of this value permitted direct comparison of the rate of water loss from both surfaces on a per area basis. Additionally, for the stem scar, pore diffusivity (PD) was expressed as nmol s^{-1} m^{-1} Pa^{-1} to normalize the rate of water loss for stem scar diameter (mm) and for partial pressure differential of water vapor between the interior and the exterior of the fruit (Brown and Escombe, 1900).

Shrivel severity was based upon comparison to images numerically scaled as 1 (no apparent shrivel), 2 (shrivel only at stem scar) a 3 (shrivel at stem scar and on lateral portions of the fruit) (Fig. 1C).

2.4. Experimental design and statistical analysis

At harvest, fruit characteristics from each family were analyzed as a completely randomized design, with scar size as treatments. Experiment 1 (storage at room temperature) was analyzed for each family as a completely randomized 3×2 factorial design considering scar size and scar sealing as

main factors. Experiment 2 (cold storage at 0 °C) was analyzed for each family as a completely randomized design with scar sealing as the treatment. No direct comparison of fruit held at 0 and 20 ºC was possible because the rate of air movement was much higher at 0ºC. All data were subjected to analysis of variance and means separation done by Tukey´s multiple comparison test ($p \leq 0.05$). Shrivel index was analyzed through a non-parametric mixed ANOVA with aligned rank test (Oliver-Rodriguez and Wang, 2013). Pearson correlation coefficients were calculated to establish associations between apparent stem scar P_{H2O}, cuticle P_{H2O}, PD, percent weight and water loss rate *vs.* fruit characteristics at harvest. All analyses were performed using commercial statistical software (Statgraphics Centurion XVI v.16.0.09).

3. Results

3.1. Experiment 1

3.1.1. Initial condition

Scar area (mm^2) for the three stem scar area categories (S, M, L) differed for each family, confirming the visual classification made at harvest (Table 1). Fruit from the germplasm lines with a large scar were bigger (greater in weight, length, and diameter) than those having medium or small scar for F6 and F40, but not for F16. The highest firmness values were found for the medium stem scar line from F6 (1.87 N mm^{-1}) and the softest fruit were from the large stem scar line from F40 (1.50 N mm^{-1}). The large stem scar line from F40 had the largest stem scar area (6.29 mm^2) and the small stem scar line from F16 had the smallest stem scar area (1.30 mm^2). When data were pooled together for the three families (Supplementary Table S1), scar area was highly and positively correlated with fruit weight (r=0.86), fruit length (r=0.79), fruit diameter (r=0.83), fruit area (r=0.78) and scar area/fruit area ratio (r=0.95); the association of scar area with firmness at harvest was not significant.

3.1.2. Effect of stem scar and cuticle on water loss at 20 °CÇ

The decline in weight for individual fruit was very linear over the five days of storage. The r^2 for regressions of weight versus time averaged 0.999 (data not shown). The three families differed in the rate of transpiration from the stem scar, with F40 (0.286 μg s^{-1}) having roughly twice as much water loss from the stem scar as F16 (0.140 μg s^{-1}) (Table 2). Small-scar lines had less water loss through the stem scar than the medium- or large-scar lines. There was an interaction for water loss

between family and the scar size of the lines within the families. The range in stem scar water loss varied markedly between lines from a low of 0.033 μg s^{-1} for the small-scar line of F16 to 0.330 μg s^{-1} for the large-scar line of F40.

Cuticular water loss was also affected by family and stem scar size and there was an interaction between these two factors (Table 2). The range in cuticular water loss varied only 1.5-fold between lines from a low of 0.261 μg s^{-1} to 0.325 μg s^{-1}. Pore diffusivity (PD) differed between all families and small-scar lines had lower values than medium- or large-scar lines. The significant interaction between factors revealed that the lowest PD occurred on fruit with small scars of F16 (2.02 nmol s^{-1} m^{-1} Pa^{-1}), which was about $1/4^{th}$ that of the rest of the lines.

The apparent P_{H2O} of the stem scar did not differ between families, but was lower for the large-scar lines than medium- or small-scar lines (Table 2). There was an interaction for P_{H2O} between family and the scar size. The range in the P_{H2O} of the stem scar varied about 2.5-fold between lines, with the small scar line of F16 being about half that of the other lines. Cuticular P_{H2O} was affected by family and stem scar size and there was an interaction between these two factors (Table 2). Cuticular P_{H2O} varied about 2-fold between lines from a low of 0.0205 μmol m^{-2} s^{-1} Pa^{-1} to 0.0419 μmol m^{-2} s^{-1} Pa^{-1}. The ratio of the apparent P_{H2O} for the stem scar to that of the cuticle varied from 57 for the small-scar line of F16 to 271 for the medium scar line of F40 (Table 2).

Sealing the stem scar consistently resulted in higher final firmness, less softening, lower rates of percent weight loss and water loss, and a lower shrivel index across all three families (Table 3). Statistical analysis demonstrated that for F6 and F40 stem scar size and stem scar sealing affected percent weight loss rate and water loss rate, but there was no interaction between these two main factors. The percent weight loss of non-sealed fruit was 1.7, 1.5 and 2.2 times higher than sealed fruit for F6, F16 and F40, respectively. The greatest difference in shrivel was found between sealed and non-sealed treatments of F40 (1.3 *vs.* 2.9, respectively). Scar size and scar sealing affected final firmness of all families with large-scar fruit having the lowest values and sealed fruit exhibiting 1.2 times higher firmness than non-sealed berries. Fruit softening was affected by scar size on F6 and F16 only, whereas sealing resulted in 34 %, 22 % and 46 % less softening for F6, F16 and F40, respectively. Non-sealed fruit with large scars were least firm after 5 d at 20 ºC for the three

families and the large stem scar line was softer than the small-scar line only for F6 and F16.

Significant correlations were found between characteristics of berries at harvest *vs.* PD, cuticle P_{H2O}, percent weight loss rate and water loss rate after storage at room temperature (Supplementary Table S2). However, the nature of the correlation for pooled data (data for all three families combined) often differed from those of the individual families.

The PD of the stem scar increased as the diameter and area of the stem scar increased for pooled date, but the behavior of the families differed (Supplementary Table S2, Fig. 3). The F16 correlations differed in sign from F6 and F40 due to the unique behavior of the small-scar line in F16. When regressions were performed for PD *vs*. stem scar diameter for each line, negative slopes were obtained for every line (Supplementary Table S3).

Cuticle P_{H2O} was highly and inversely correlated with fruit diameter, fruit area, and fruit weight for pooled data (r values ranging from -0.67 to -0.73), generally reflecting the relationships found for each family. Cuticle P_{H2O} was not related to scar diameter or scar area for any of the families.

The rate of weight loss (percent per day) was negatively correlated with fruit diameter, fruit area, and fruit weight for pooled data (r values ranging from -0.54 to -0.57) and for each of the families with the exception of fruit diameter of F16. F16 was the only family for which percent weight loss correlated with scar diameter or scar area. The rate of water loss ($\mu g\ s^{-1}$) was positively correlated with fruit diameter, fruit area, and fruit weight for pooled data (r values ranging from 0.45 to 0.52) and the relationship was dependent upon the stem scar being open (Fig. 3A). However, the relationships for water loss rate *vs.* fruit diameter and fruit area were inconsistent for individual families. The rate of water loss was also positively correlated with stem scar diameter (Fig. 4) and stem scar area for pooled data (r values of 0.71 and 0.69, respectively), but not when the stem scar was sealed (Fig. 3B). Similar relationships were found for all families. In general, all the lines tended to have a similar relationship between the rate of water loss and stem scar diameter except for the small-scar fruit from F16, which had much lower water loss rate than the other lines for the size of stem scar they possessed (Fig. 4, Supplementary Table S3).

3.2. Experiment 2

3.2.1. Initial condition

Scar area for fruit of the three families were 1.35, 1.22 and 1.55 mm^2 for F6, F16 and F40, respectively. Similar characteristics were found between F6 and F16 for fruit weight, fruit diameter, fruit area, and scar area. No differences were found between families for scar area/fruit area ratio (Table 1).

3.2.2. Effect of scar sealing during refrigerated storage

Similar to fruit of Experiment 1, the weight loss for individual fruit was very linear over the 15 days of storage, with an average r^2 for regressions of weight vs. time of 0.990 (data not shown).

Transpiration via stem scar and via cuticle at 0 ºC was considerably less than that for fruit stored at room temperature (Table 4). The average ratio for cuticle/stem scar transpiration was approximately 1.2 at 0°C; at 20 °C, the average ratio for cuticle/stem scar transpiration was approximately 9. PD was higher at 0 ºC compared to 20 ºC, with the greatest PD in fruit from F16 and F40 at both 0 and 20 °C. Stem scar P_{H2O} was several times higher at 0 ºC than at 20 ºC; the greatest values occurred on F16 and F40 fruit. Cuticle P_{H2O} was 1.2 times higher at 0 ºC *vs.* 20 ºC, with the highest value on berries from F16 (0.0518 μmol m^{-2} s^{-1} Pa^{-1}) at 0 °C. The P_{H20ss}/P_{H2Ocut} ratio varied from 295 to 570 for F40 at 0 °C and 27 to 335 at 20 °C.

Sealing of the stem scar did not affect the firmness of fruit held at 0 °C for 15 d at 88 % RH (Table 5). On the contrary, sealing of the stem scar affected water loss rate for all three families, although percentage of weight loss relative to 20 °C was markedly reduced by the use of low temperature. Sealing the stem scar had its greatest impact on water loss rate for fruit belonging to F40. Sealing also impacted shrivel; the shrivel index did not exceed 1.3 on sealed fruit, whereas for non-sealed berries, values varied between 2.0 and 2.2.

4. Discussion

Water loss through transpiration is an important cause of deterioration of horticultural crops, resulting not only in direct quantitative losses (less salable weight), but also in losses in appearance, texture, and nutritional quality (Kader, 2002). Blueberries have an outer epidermis with no stomata

or lenticels (Gough, 1994), so moisture loss is strictly through the stem scar area and the cuticle. P_{H2O} of whole tomato fruit (Shirazi and Cameron, 1993) was similar to that found for whole blueberry in the current study (data not shown). However, whole fruit permeability is actually a combination of the apparent permeability for stem scars and cuticle. Given that the mechanism for diffusion from a pore differs from that from a non-perforated surface (Brown and Escombe, 1900), the data in the current study was segregated for stem scar and cuticle.

Although large fruit and a small stem scar are considered important traits in the selection of commercial blueberry cultivars, larger stem scars were associated with larger fruit in all families in this study. This agrees with reports by Galleta and Ballington (1996), but not with Parra et al. (2007), who found commercial cultivars 'Bonita', 'Reveille' and 'Premier' having medium- and large-sized fruit had a small or medium scar while small-fruited cultivars 'Georgia Gem', 'Snowflake', and 'Marimba' had relatively large scars. Further, Parra et al. (2007) reported that the scar width/fruit width ratio for a given germplasm line varied from year to year. Thus, perhaps another important quality trait to minimize water loss would be a consistent stem scar size.

While a small stem scar is desirable, the observation that PD and apparent P_{H2O} of the stem scar increased as stem scar area decreased (Fig. 2, Supplementary Fig. S1, Supplementary Table S3) suggests that the benefit from selecting small stem scars is less than might be anticipated. This finding is in accordance with those of Brown and Escombe (1900), who first described the mechanism for the phenomenon of increasing permeance with decreasing pore diameter. They proved that water loss through a pore increased linearly with the pore diameter rather than pore area. In the current study, the rate of moisture loss as a function of stem scar diameter (Fig. 4) is within 10 % of the measurements of Brown and Escombe (1900) for pores in a membrane. Importantly, the small stem scar line from F16 had a distinctly different relationship between stem scar diameter and the rate of water loss. The rate of water loss for this line as a function of stem scar diameter was about $1/4^{th}$ that of the other 8 lines. This suggests physical features not related to stem scar area could affect water loss rates. What this feature may be in the present study is not clear, but possibilities include stem scar occlusion through tissue collapse or lignification. Identification of the factor limiting stem scar moisture loss may be a valuable feature for the selection of shrivel-resistant blueberry lines.

The data of Experiment 2 suggests the cuticle is a much more important route of moisture loss at elevated temperatures and that, conversely, the influence of the stem scar on water loss increases as temperature declines. Given that essentially all blueberry fruit are refrigerated when stored or shipped, this finding suggests there would be some benefit to further understanding mechanisms that might limit water loss through the stem scar.

The data on water loss through the stem scar is not unlike that found for other fruits in which the calyx or stem scar contributes to moisture loss. For tomato, at least half of fruit water loss occurs through the stem scar/calyx (Cameron, 1982; Ehret and Ho, 1986). For large-size eggplant fruit, 65 % of whole fruit water loss was attributed to the calyx, which covered about 10 % of fruit surface area, (Díaz-Perez, 1998). In our study, depending on family and scar size, scar area accounted for 0.19 to 0.74 % of berry total surface and yet the scar released 39-67 % of the water loss of the whole fruit. The high rate of water loss through the blueberry stem scar permitted scar size to negatively influence the firmness of stored fruit.

Transpiration expressed as percent loss per day can be affected by fruit size or shape (Burton, 1982). For nearly spherical fruit, like blueberries, there is a reduction in the area/mass ratio as fruit increase in size (Ben-Yehoshua et al., 2002). This partially explains the negative correlations between water loss rate *vs.* fruit diameter, fruit area, and fruit weight (Supplementary Table S2). Interestingly, however, the P_{H2O} (μmol m^{-2} s^{-1} Pa^{-1}) of the cuticle, which is expressed on a per area basis, decreased as fruit size increased, suggesting that there is a mechanism that reduces cuticular transpiration for larger fruit. The nature of this mechanism is not clear but may be related to cuticle development.

The cuticle is one of the most important plant barriers (Heredia-Guerrero et al., 2014) and one of its main functions is the protection against uncontrolled water loss (Burghartd and Riederer, 2006). A large portion of the total water loss for blueberry fruit was via the cuticle. Thus, cuticular water loss properties should also bear scrutiny in the selection of new cultivars for long-term storage. In this study, calculations of cuticular P_{H2O} revealed a two-fold difference between lines, which is similar to that found for pepper (*Capsicum annuum*) (Lownds et al., 1993).

A number of studies on peppers, tomatoes, cherries (*Prunus avium*) and peaches (*Prunus persica*), have demonstrated a correlation between wax characteristics (in terms of composition and structure, rather than total wax amount) and transpiration properties of the cuticle, which differ between cultivars, resulting in different water loss rates (Banaras et al., 1994;Vogg et al., 2004; Lleide et al., 2011; Parsons et al., 2012; Belge et al., 2014; Lara et al., 2015). A high content of ursolic acid in the cuticle of blueberries was highly correlated with water loss and softening (Moggia et al., 2016). The level of ursolic acid in cuticles of the fruit of the families in the present study is unknown.

The absolute water loss rates at 20 °C were roughly 10-fold higher than the water loss rates at 0 °C. This is likely due to the difference in the vapor pressure deficit in the two storage environments. At 20 °C, the RH was 65 %, which would lead to a VPD of approximately 820 Pa if one assumes the internal atmosphere of the blueberry fruit is saturated with water vapor. At 0 °C, the 88 % RH would have generated a VPD of 73 Pa, which is a little less than $1/10^{th}$ that at 20 °C. The reduced rate of water loss at low temperature was likely a factor in the superior firmness retention and low level of shrivel of fruit held at 0 °C compared to those held at 20 °C and contributed to the marginal impact of stem scar sealing at 0 °C. The rapid rate of water loss at 20 °C highlights the importance of rapidly cooling the fruit after harvest and maintaining the cool chain throughout the entire handling and marketing process.

The water loss values for this study correspond to singulated fruit, but given that fruit are usually packed into clamshells for commercial storage and shipping, water loss rates could be expected to differ from those published here. In fact, fruit held 15 d at 0 ºC and 88 % RH and stored in clamshells had 1.2 % and 1.8 % weight loss for sealed and non-sealed fruit, respectively (data not shown). Thus, sealing the stem scar still reduced weight loss of clamshell-stored fruit by over 30 %.

Water loss, unlike many visual characteristics, is a not a simple phenotype to assess, but given the very highly linear nature of weight loss found here, needed data can be reduced to two measurements per fruit over a relatively short time period. Individual measurements take seconds, permitting the analysis of hundreds of lines per day and should readily permit the selection of shrivel-resistant blueberry lines. Additionally, further studies to better understand the impact of cuticle and stem scar morphology, structure, and chemical composition on water loss in blueberry

are needed to identify the underlying the underlying physical and biological mechanisms controlling water loss to further improve selection for this important trait.

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Table 1. Fruit firmness and morphometric fruit and stem scar characteristics for selected germplasm lines of three different families of highbush blueberry based on visual classification as having a small (S), medium (M) or large (L) stem scar. Fruit from Experiment 1 was harvested on December 28th, 2015; fruit from Experiment 2 was harvested on January 4th, 2016.

		Fruit					Scar	
Experiment 1								
Family	Scar size	Weight (g)	Length (mm)	Diameter (mm)	Area (cm^2)	Firmness (N mm^{-1})	Area (mm^2)	Scar area/frui area ratio
F6	S	1.95 b	12.0 b	15.7 a	6.61 ab	1.78 ab	2.25 c	0.35 b
	M	1.78 b	12.2 b	15.0 b	5.21 b	1.91 a	3.19 b	0.51 a
	L	2.20 a	13.2 a	15.9 a	7.04 a	1.71 b	4.02 a	0.58 a
	Significance	**	**	*	**	*	**	**
F16	S	1.46 b	11.4 a	14.2 b	5.27 b	1.77	1.30 c	0.23 c
	M	1.79 a	11.6 a	15.3 a	6.19 a	1.65	2.05 b	0.34 b
	L	1.44 b	10.9 b	14.0 b	5.30 b	1.59	2.78 a	0.53 a
	Significance	**	**	**	**	*n.s*	**	**
F40	S	1.89 c	12.6 b	15.4 c	6.56 c	1.84 a	2.45 c	0.38 b
	M	2.26 b	12.8 b	16.8 b	7.50 b	1.71 b	3.64 b	0.49 b
	L	2.68 a	13.6 a	18.0 a	8.55 a	1.53 c	6.29 a	0.74 a
	Significance	**	**	**	**	**	**	**
Experiment 2								
F6	S	1.54 b	11.9 a	14.4 b	5.80 b	No data	1.35 ab	0.23
F16	S	1.60 b	11.5 b	14.9 b	5.94 b	No data	1.22 b	0.21
F40	S	1.84 a	11.8 ab	15.5 a	6.43 a	No data	1.55 a	0.24
	Significance	**	*	**	**		*	*ns*

For a given family, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant)

Table 2. Impact of size of the stem scar of harvested highbush blueberry fruit held for 5 d at room temperature (20 ºC) on water loss via stem scar and fruit cuticle, pore diffusivity of the stem scar, apparent permeance (P_{H20}) of the stem scar, cuticle P_{H2O}, and the ratio of the apparent P_{H2O} of the stem scar versus the P_{H2O} of the cuticle, for three families of blueberry used in Experiment 1

		Water loss ($\mu g\ s^{-1}$)		Pore diffusivity ($nmol\ s^{-1}\ m^{-1}\ Pa^{-1}$)	P_{H20} ($\mu mol\ m^{-2}\ s^{-1}\ Pa^{-1}$)		
Factor		Via stem scar	Via cuticle	stem scar	stem scar (ss)	cuticle (cut)	$P_{H20}ss/\ P_{H20}cut$
Family (F)							
F6[x]		0.220 b	0.275 b	7.54 b	5.04 a	0.0284 b	182.7 a
F16		0.140 c	0.325 a	5.77 c	4.64 a	0.0390 a	126.5 b
F40		0.286 a	0.261 b	8.64 a	5.16 a	0.0252 c	217.1 a
Scar Size (SS)							
S[y]		0.146 b	0.273 b	6.14 b	5.00 a	0.0326 a	173.6 b
M		0.236 a	0.264 b	8.14 a	5.50 a	0.0272 b	212.8 a
L		0.264 a	0.324 a	7.67 a	4.34 b	0.0329 a	139.8 b
F x SS							
F6	S	0.178 c	0.240 cd	7.64 ab	6.25 ab	0.0256 c	249.6 a
	M	0.118 c	0.235 cd	4.36 b	4.20 c	0.0274 bc	153.4 bc
	L	0.278 ab	0.329 a	8.28 a	4.67 c	0.0321 ab	145.0 bc
F16	S	0.033 d	0.310 a	2.02 c	2.37 d	0.0416 a	57.5 d
	M	0.205 c	0.323 a	8.75 a	7.10 a	0.0336 b	213.5 ab
	L	0.183 c	0.342 a	6.56 b	4.44 c	0.0419 a	108.5 c
F40	S	0.228 bc	0.269 bc	8.76 a	6.36 ab	0.0305 ab	213.8 ab
	M	0.301 ab	0.215 d	8.98 a	5.20 bc	0.0205 d	271.6 a
	L	0.330 a	0.301 ab	8.18 a	3.91 c	0.0246 cd	166.0 bc
Significance							
F		**	**	**	*ns*	**	**
SS		**	**	**	**	**	**
F x SS		**	**	**	**	**	**

For a given family, scar size, or interaction effect, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant)
[x] Data correspond to non-sealed fruit
[y] Small, medium and large stem scars are indicated by S, M and L

Table 3. Impact of size and sealing of the stem scar of harvested highbush blueberry fruit held for 5 d at 20 ºC on firmness after storage (final firmness), percent firmness loss relative to initial firmness (softening), rate of percent weight loss, rate of water loss, and shrivel index (1-3) for three families of blueberry used in Experiment 1.

Family	Factor Scar Size (SS)	Final firmness (N mm^{-1})	Softening (%)	Weight loss (percent per day)	Water loss (μg s^{-1})	Shrivel index
F6	S[x]	1.21 a	31.2 b	1.49 b	0.324 b	2.4
	M	1.18 a	37.2 a	1.76 a	0.359 b	2.7
	L	1.00 b	40.7 a	1.86 a	0.463 a	2.7
	Sealing (B)					
	Yes[z]	1.23 a	28.9 b	1.25 b	0.278 b	2.2 b
	No	1.03 b	43.8 a	2.16 a	0.498 a	2.9 a
	Significance					
	SS	**	**	**	**	*n.s*
	B	**	**	**	**	**
	SS x B	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
F16	Scar Size (SS)					
	S	1.07 a	38.3 b	1.99 b	0.324 b	2.7
	M	1.04 a	35.8 b	2.09 b	0.428 a	2.8
	L	0.78 b	51.2 a	2.62 a	0.440 a	3.0
	Sealing (B)					
	Yes	1.03 a	36.5 b	1.90 b	0.324 b	2.6 b
	No	0.89 b	47.0 a	2.57 a	0.521 a	3.0 a
	Significance					
	SS	**	**	**	**	*n.s*
	B	**	**	**	**	**
	SS x B	*n.s*	*n.s*	*n.s*	**	*n.s*
F40	Scar Size (SS)					
	S	1.29 a	30.1	1.83 a	0.382 b	2.3
	M	1.16 b	31.7	1.43 b	0.370 b	2.01
	L	1.01 c	33.6	1.52 b	0.463 a	2.2
	Sealing (B)					
	Yes	1.32 a	22.2 b	0.99 a	0.255 b	1.3 a
	No	0.99 b	41.4 a	2.19 b	0.544 a	2.9 b
	Significance					
	SS	**	*n.s*	**	**	*n.s*
	B	**	**	**	**	**
	SS x B	**	*n.s*	*n.s*	*n.s*	*n.s*

On each family, for a given scar size, sealing or interaction effect, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$). Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant).
[x]Small, medium and large stem scars are indicated by S, M and L, respectively.
[z]Sealed an non-sealed stem scars are indicated by Yes and No, respectively.

Table 4. Effect of family on transpiration via stem scar and fruit cuticle, pore diffusivity of the stem scar, apparent permeance (P_{H2O}) of the stem scar, cuticle P_{H2O}, and the ratio of the later two measures for highbush blueberry fruit held for 7 d at 20 °C or 15 d at 0 ºC. Data for the two temperatures evaluated cannot be directly compared since air flow was not controlled (n=10 for each family at each temperature).

Factor		Water loss ($\mu g\ s^{-1}$)		Pore diffusivity ($nmol\ s^{-1}\ m^{-1}\ Pa^{-1}$)	P_{H20} ($\mu mol\ m^{-2}\ s^{-1}\ Pa^{-1}$)		
Family (F)	Temp. (°C)	Stem scar	Cuticle	Stem scar	Stem scar (ss)	Cuticle (cut)	P_{H20}ss/ P_{H20}cut
F6[x]	0	0.019 b	0.026 b	10.81 b	10.35 b	0.0348 b	295.4 b
F16	0	0.024 b	0.041 a	15.07 a	15.99 a	0.0518 a	312.4 b
F40	0	0.032 a	0.024 b	17.07 a	15.74 a	0.0291 b	569.8 a
Significance		**	**	**	**	**	**
F6[x]	20	0.015 c	0.316 a	0.882 c	1.01 c	0.0386 a	26.9 c
F16	20	0.055 a	0.315 a	2.899 b	2.99 b	0.0363 a	88.1 b
F40	20	0.185 b	0.211 b	8.434 a	7.43 a	0.0225 b	334.7 a
Significance		**	**	**	**	**	**

For each temperature, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant).
[x]Data correspond to non-sealed fruit

Table 5. Impact of sealing the stem scar of harvested highbush blueberry fruit held for 15 d at 0 ºC on final firmness, rate of percent weight loss, rate of water loss, and shrivel index (1-3) for small stem scar lines from three families of blueberry used in Experiment 2.

		Final Firmness	Weight loss	Water loss	Shrivel index
Family	Sealing	(N mm^{-1})	(percent per day)	(μg s^{-1})	
F6	Yes[x]	1.92	0.10 b	0.0254 b	1.2 b
	No	1.70	0.24 a	0.0446 a	2.0 a
	Significance	*n.s*	**	**	*
F16	Yes	1.58	0.19 b	0.0409 b	1.3 b
	No	1.47	0.31 a	0.0650 a	2.2 a
	Significance	*n.s*	**	**	**
F40	Yes	1.22	0.09 b	0.0241 b	1.2 b
	No	1.24	0.27 a	0.0557 a	2.2 a
	Significance	*n.s*	**	**	**

For each family, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant).
[x]Sealed and non-sealed stem scars are indicated by Yes and No, respectively.

Supplementary Table S1. Pearson correlation coefficients (r) between morphometric fruit characteristics at harvest for highbush blueberry families F6, F16, and F40 for Experiment 1.

	Fruit length	Fruit diameter	Fruit area	Fruit firmness	Scar area	Scar area/fruit area ratio
Fruit weight	0.93 **[x]	0.99 **	0.96 **	-0.34 *ns*	0.86 **	0.70 **
Fruit length		0.88 **	0.85 **	-0.09 *ns*	0.79 *	0.64 *ns*
Fruit diameter			0.96 **	-0.37 *ns*	0.83 **	0.63 *ns*
Fruit area				-0.48 *ns*	0.78 *	0.59 *ns*
Fruit firmness					-0.51 *ns*	-0.50 *ns*
Scar area						0.95 **

[z] Correlation coefficient (r); n=9
[x] Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant)

Supplementary Table S2. Correlation coefficients (r) for fruit characteristics at harvest for three families of highbush blueberry fruit (F6, F16, and F40) and for all families *vs*. pore diffusivity, cuticle permeance (P_{H2O}), percent weight loss rate, and water loss rate for non-sealed fruit measured over a 5 d holding period at 20 °C in Experiment 1.

Fruit characteristic	Pore diffusivity (nmol s^{-1} m^{-1} Pa^{-1})				Cuticle P_{H2O} (µmol m^{-2} s^{-1} Pa^{-1})				Weight loss (percent per day)				Water loss (µg s^{-1})			
	F6	F16	F40	All	F6	F16	F40	All	F6	F16	F40	All	F6	F16	F40	All
Scar diam.	-0.32[x]	0.61	-0.39	0.36	0.29	0.04	0.01	-0.35	0.21	0.52	0.11	0.004	0.52	0.79	0.74	0.71
	30[y]	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	ns[z]	**	*	**	*ns*	*ns*	*ns*	**	*ns*	**	*ns*	*ns*	**	**	**	**
Scar area	-0.31	0.56	-0.37	0.28	0.26	0.09	0.08	-0.30	0.18	0.53	0.16	0.005	0.52	0.76	0.76	0.69
	30	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	ns	**	*	*	*ns*	*ns*	*ns*	**	*ns*	**	*ns*	*ns*	**	**	**	**
Fruit diam.	0.15	0.71	-0.29	0.48	-0.43	-0.66	-0.65	-0.71	-0.53	-0.28	-0.65	-0.54	0.18	0.70	0.19	0.45
	30	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	ns	**	*ns*	**	*	**	**	**	**	*ns*	**	**	*ns*	**	*ns*	**
Fruit Area	0.16	0.68	-0.30	0.47	-0.32	-0.73	-0.62	-0.73	-0.45	-0.38	-0.67	-0.58	0.32	0.63	0.22	0.46
	30	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	ns	**	*ns*	**	*ns*	**	**	**	*	*	**	**	*ns*	**	*ns*	**
Fruit weight	0.15	0.66	-0.15	0.50	-0.20	-0.65	-0.48	-0.67	-0.44	-0.39	-0.63	-0.57	0.38	0.65	0.35	0.52
	30	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	ns	**	*ns*	**	*ns*	**	**	**	*	*	**	**	*	**	*	**
Scar area/ fruit area	-0.36	0.37	-0.30	0.23	0.38	0.37	0.26	-0.11	0.34	0.72	0.40	0.24	0.45	0.59	0.76	0.66
	30	30	30	90	30	30	30	90	30	30	30	90	30	30	30	90
	*	*	*ns*	*ns*	*	*	*ns*	*ns*	*ns*	**	*	*	*	**	**	**

[x] Correlation coefficient
[y] Sample size (n)
[z] Significance: ** ($p < 0.01$); * ($p < 0.05$); *ns* (non-significant)

Supplementary Table S3. Values for slope and intercept for fitted regression equations for Fig. 3 and Fig. 5 for each highbush blueberry germplasm line in Experiment 1. The intercept for Fig. 4 was set to zero since it is assumed that moisture loss through a pore would be zero for a pore with a diameter of zero, hence when the fit was poor, no r^2 could be calculated.

		Fig. 3 - pore diffusivity *vs.* stem scar area			Fig. 5- water loss *vs*. stem scar diameter
Family	Line	slope (nmol s^{-1} m^{-1} Pa^{-1}) per mm^2	intercept (nmol s^{-1} m^{-1} Pa^{-1})	r^2	slope (µg s^{-1}) per mm
6	S	-2.19	12.02	0.504	0.110
	M	-1.48	11.90	0.850	0.096
	L	-1.03	12.60	0.424	0.120
16	S	-0.64	2.64	0.313	0.029
	M	-0.64	10.03	0.086	0.128
	L	-0.96	9.39	0.349	0.096
40	S	-1.67	12.99	0.339	0.127
	M	-0.19	9.71	0.073	0.131
	L	-0.26	9.65	0.162	0.118

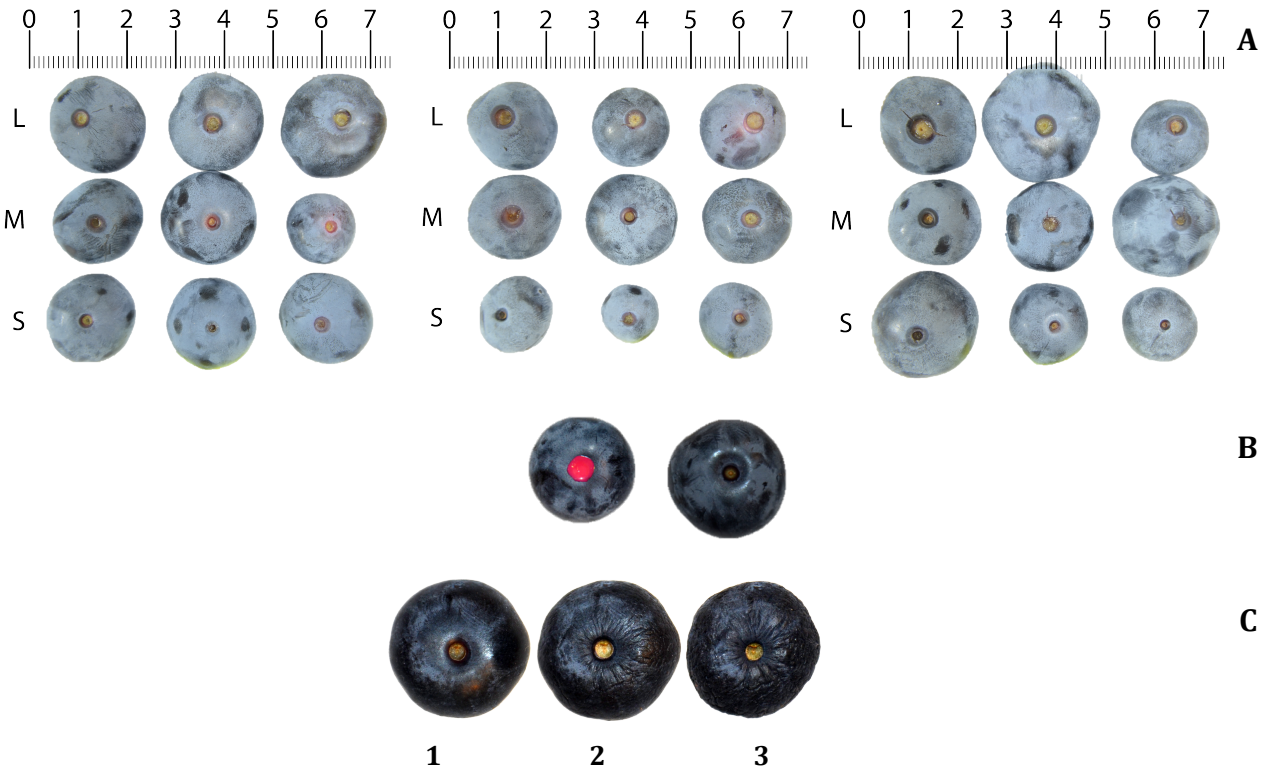


Figure 1. Fruit characteristics of: three representative fruits from F6 (left), F16 (middle) and F40 (right) families, with large (L), medium (M) and small (S) scars. The scales provided are in cm with mm divisions (**A**); sealed (left) vs. non-sealed (right) blueberry (**B**); the scale used for shrivel index: 1(none); 2 (moderate); 3 (severe) (**C**). Female and male parents of the three families correspond to: F6 (Legacy x Brigitta); F16 (Chandler x Legacy); F40 (Orus 344 x Legacy).

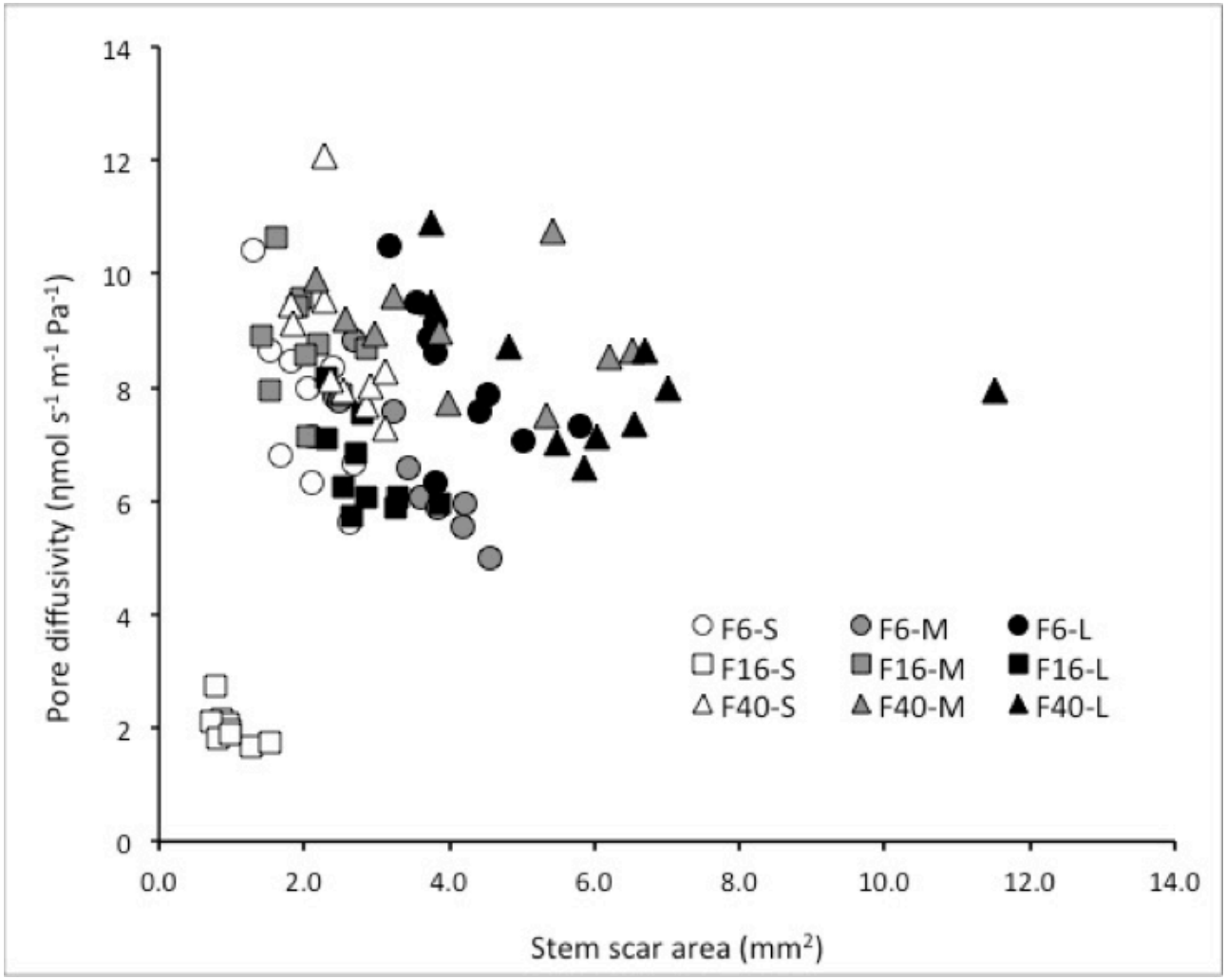
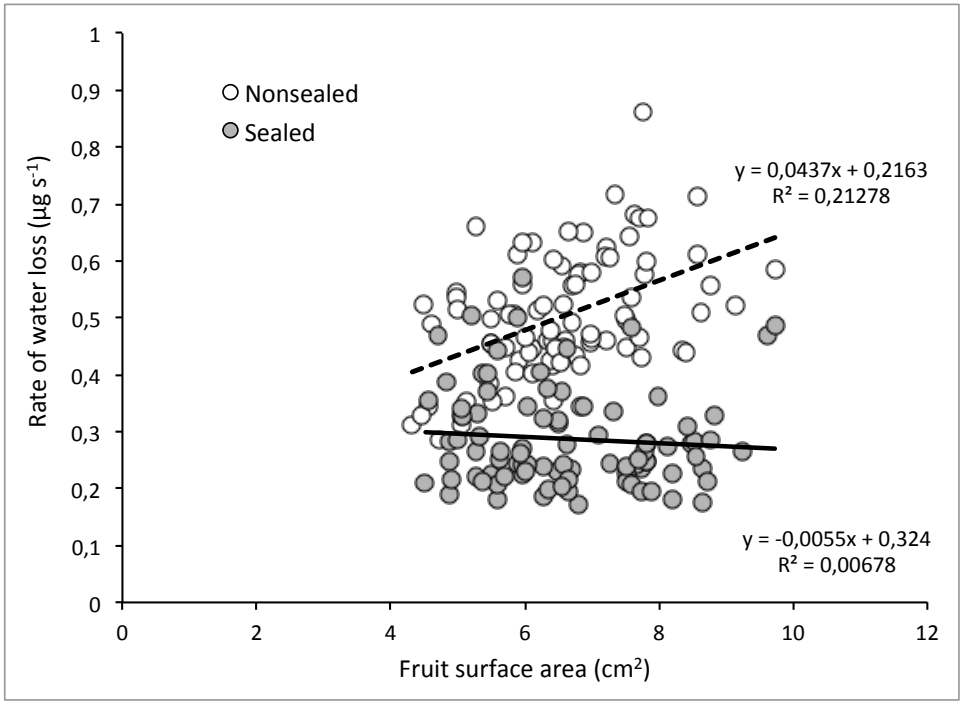


Figure 2. Relationship between stem scar area and the pore diffusivity of the stem scar for highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines having small, medium and large stem scars (S, M, and L, respectively) from each of three families (F6, F16, F40) in the blueberry germplasm repository at the University of Talca, Chile.



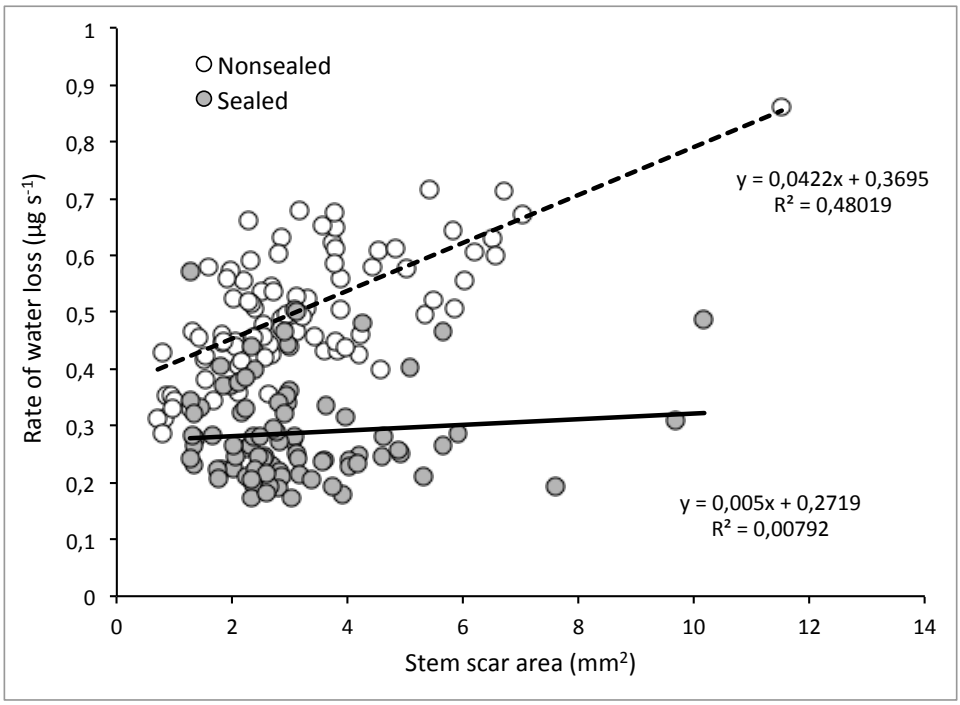


Figure 3. Relationship between fruit surface area (**A**) or stem scar area (**B**) and the absolute rate of moisture loss for highbush blueberry fruit with sealed or non-sealed stem scars when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines (10 fruit per line) from each of three families in the blueberry germplasm repository at the University of Talca, Chile.

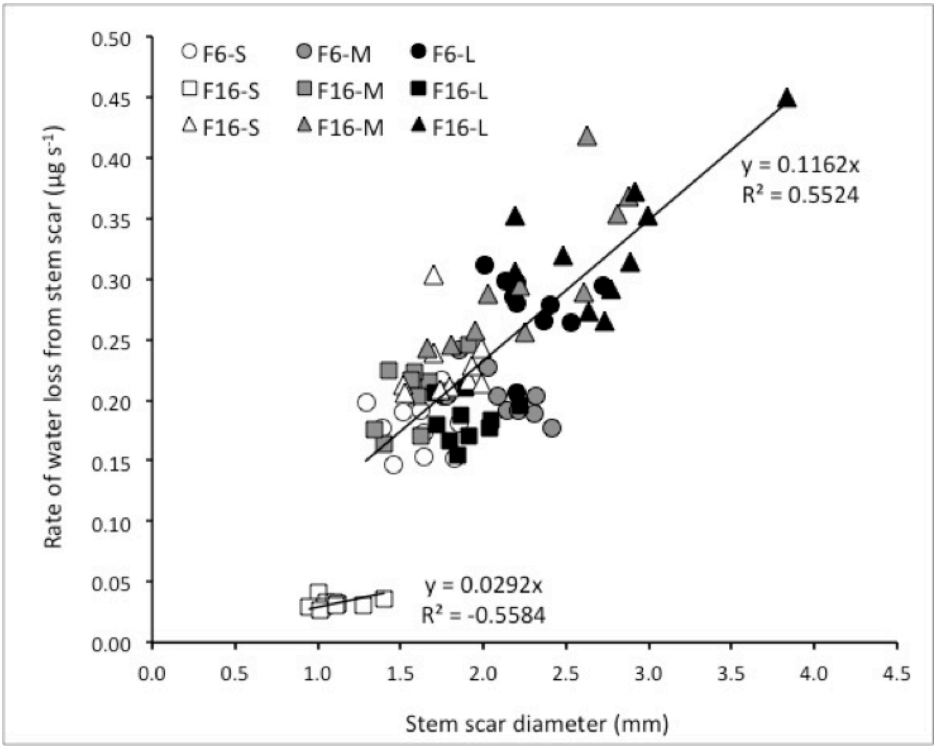


Figure 4. Relationship between stem scar diameter and the absolute water loss rate from the stem scar for highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines having small, medium and large stem scars (S, M, and L, respectively) from each of three families (F6, F16, F40) in the blueberry germplasm repository at the University of Talca, Chile.

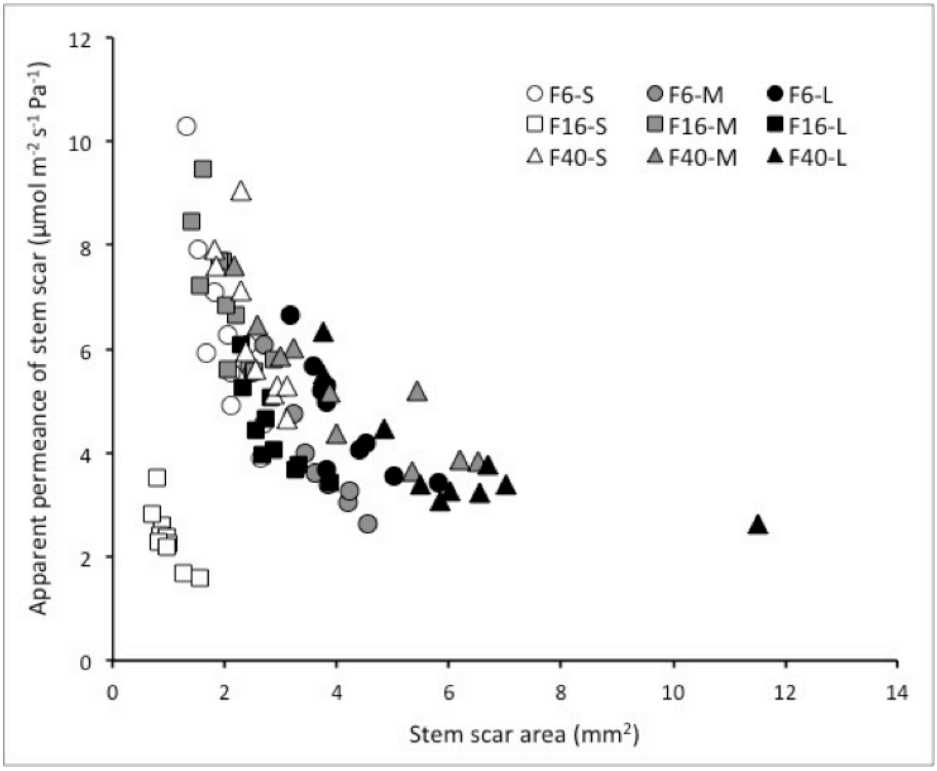


Figure S1. Relationship between stem scar area and the apparent permeance of the stem scar for highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines having small, medium and large stem scars (S, M, and L, respectively) from each of three families (F6, F16, F40) in the blueberry germplasm repository at the University of Talca, Chile.

5.5. Maturity at harvest and position within canopy affect highbush blueberry (*Vaccinium corymbosum* L.) fruit firmness during postharvest

Abstract

For blueberry, harvest readiness is based on skin color, with fruit being considered ready to pick when the berry skin reaches 100% blue coverage. The extended bloom period for the blueberry inflorescence and uneven developmental rates yield 100% blue fruit that often vary widely in physiological maturity on any given harvest date. The objective of this study was to determine the inherent variability in the firmness of a synchronized cohort of blueberry fruit and determine effect of harvest delay and position within the canopy on fruit characteristics at harvest and after refrigerated storage. During two seasons, regions of the canopy of 12 'Duke' and 'Brigitta' plants were designated as east (E) and west (W) sides. Fruit of a specific developmental stage from each side were either harvested when reaching 100% blue coverage (ripe: B100) or allowed to stay on the plant for 6 additional days (over-ripe: B100+6). Despite the very narrow maturity range as defined by blue color development, variation in firmness was extensive, with firmness drops, ranging from 0 to 24% between B100 and B100+6 fruit, respectively, depending on year and cultivar. Interestingly, the firmness distribution for B100 fruit did not broaden when the fruit were allowed to remain on the bush for an additional 6 days. Even so, the six days of additional development was enough to increase the amount of soft and very soft fruit at harvest and after storage, demonstrating the importance of frequent harvests to improve firmness at final destinations. In general, the E side of the plant produced softer fruit. Both the percentage of blue fruit at each harvest date and total fruit produced were higher on the E side of the plant. Year-to-year variation in firmness exceeded that from the imposed treatment, highlighting the need to understand which environmental factors contribute to fruit softening. This is the first report on in-plant fruit variability for blueberry and its effect on postharvest performance.

Keywords: within plant, within canopy, variability, heterogeneity, softening, TSS, TA, ethylene, respiration

1. Introduction

Postharvest performance of fresh blueberries is critical for long-term storage of produce, especially for Chilean fruit, which are exported mainly by boat, and typically take 20 – 50 days to reach final markets (Beaudry et al., 1998; Lobos et al., 2014b; Moggia et al., 2016). Fruit homogeneity is essential to get high quality produce, firmness being one of the most critical attributes influencing consumer acceptance (NeSmith et al., 2002). Firmer fruit will better stand harvest and postharvest management (Hanson et al., 1993). Shippers have reported that the rate of rejections of Chilean fruit at destination has increased in the past few years. Rejections provide evidence of high variability between seasons and between shipments within a particular season. The root causes of this variability are uncertain.

The blooming and fruit development periods in northern highbush blueberries usually span 3-4 weeks (Retamales and Hancock, 2012) and 42-90 days (Darnell, 2006), respectively. Thus fruit will develop under different environmental conditions along the season (Gough, 1994; Lobos et al., 2014a). For instance, earlier flowers set fruit that are subjected to lower initial temperatures compared to fruit set at the end of the blooming time.

Environmental factors such as temperature and light have important effects on fruit texture (Sams, 1999). Although a cluster fully exposed to sunlight is considered to be growing under enhanced conditions for fruit quality (Smart, 1985), high temperatures could also have negative effects on their metabolism (Bergqvist et al., 2001), indirectly affecting cell structure and other texture-determining components (Vicente et al., 2007). Temperatures higher than 32 ºC during blueberry maturation have been associated with smaller and softer fruit (Mainland, 1989), as well as with reduced anthocyanin production (Prange and DeEll, 1997). Lobos et al. (2013) proved that the lower light incidence and temperature at lower canopy positions contributed to an increase in fruit weight, fruit water content, titratable acidity, and firmness, but lead to a decrease in soluble solids content at harvest.

Fruit position within the canopy could be an additional source of variability. In the main Chilean northern blueberry production area (latitude 35 – 38° S) (Lobos and Hancock, 2015), where orchards are primarily planted in the N-S direction, differences in daily integration of radiation and

temperature are expected for leaves and fruit according to their situation either at the east or the west side of the plant. For instance, the side of the plant receiving lower radiative flux during the warm afternoon might experience reduced photoinhibition and by increasing stomatal conductance, experience a lower gradient in vapor pressure deficit, thus reducing leaf temperature and increasing net CO_2 accumulation rates in that side of the canopy (Dale, 1992; Syvertsen et al., 2003), and favoring the accumulation of carbohydrates in surrounding fruit.

Accordingly, since most of the physical-chemical fruit traits are influenced by environmental factors (Sams, 1999), it would be expected that more perishable fruits, such as blueberries, have a higher variability in fruit condition at harvest. In addition, harvest index for blueberries is based almost uniquely on skin color, fruit being considered ready to pick when reaching 100% blue coverage. It is then most likely that within each harvest (typically separated by 4 – 10 d intervals), fruit with similar external appearance, but different physiological maturity, are picked and packed together in the same clamshell.

Concerns regarding over-ripe fruit in packed commercial units started very early in the history of blueberry postharvest research (Bailey, 1947; Woodruff et al., 1960). However, except for some initiatives (Vicente et al., 2007; Moggia et al., 2016), this problem has not been fully addressed. Therefore, the objective of this study was to determine the effect of fruit maturity stage at harvest (ripe *vs*. over-ripe) and the possible role of fruit positioning within the canopy (east *vs*. west) on fruit characteristics at harvest and after medium and long-term refrigerated storage. Although macro and microclimatic factors were not a part of this study, some basic assessments supporting their importance are included.

2. Material and Methods

2.1. Plant material and treatments

Trials were conducted on mature highbush blueberry plants (*Vaccinium corymbosum* L.), cvs. ‘Duke’ and ‘Brigitta’ (9- and 8-year old, respectively), established on a commercial field located in Río Claro, Maule Region - Chile (35° 15’ 33.80’’ S; 71° 14’ 17.70’’ W; 339 m.a.s.l.; N-S orientation: 331.75º), during seasons 2013/14 (Y1) and 2014/15 (Y2). Honeybees (*Apis mellifera*) were used as pollinators in a ratio of 8 beehives per ha.

Twelve plants of similar characteristics (height, canopy volume, and number and age of canes) were selected for each cultivar, to study the effects of maturity stage and canopy position at the peak of each commercial picking (~40 – 50% of annual production).

Plants were divided into east (E), top, and west (W) sectors and fruit were collected only from the E and W sides (Fig. 1). For each canopy side and harvest date, fruit were picked at two different maturity stages: (1) 100% blue coloration (B100) and (2) over-ripe 100% blue coloration (B100+6). To identify and isolate fruit of these specific maturities, clusters were initially stripped of all fruit that had greater than 75 % blue coloration. The timing of this step was such that only a small portion (~10 % to 15 %) of the total fruit was removed. Fruit color development was monitored until the first fruits in the selected clusters developed 100 % blue coloration. Two days after this point in time, all 100% blue fruit were harvested on half of the clusters. For the remaining clusters, all fruit with greater than 50 % blue coloration and less than 100 % blue coloration were removed, effectively retaining the 100 % blue fruit and primarily green fruit. After another six days on the bush, the 100 % blue fruit were harvested (B100+6). In order to evaluate differences between years, the same elapsed time (6 d) was considered between 100 % blue and over-ripe fruit during Y1 and Y2. According to this procedure harvest dates for 'Duke' were 12/03/13 (Y1, B100); 12/09/13 (Y1, B100+6); 11/28/14 (Y2, B100) and 12/03/14 (Y2, B100+6). For 'Brigitta' pickings were done on 01/03/14 (Y1, B100); 01/08/14 (Y1, B100+6); 12/29/14 (Y2, B100) and 01/02/15 (Y2, B100+6).

2.2 Fruit evaluations

At each harvest, fruit was characterized in terms of weight (g), firmness (N), total soluble solids (TSS, %), titratable acidity (TA, % citric acid), respiration rate (RR, µg CO_2 kg^{-1} s^{-1}), and ethylene production (EP, ng k^{-1} s^{-1}). After 30 or 45 d of refrigerated storage (0 ºC and 88 – 90% RH) plus one day at 15 ºC, fruit were analyzed for firmness, TSS, TA and TSS/TA.

Measurements were done on six replicates (each replicate taken from two plants) for each treatment combination as follows: (1) fruit weight was measured with an electronic balance (LSV-6200g, Veto y Cía. Ltda., Santiago, Chile) for 25 fruit per replicate; (2) firmness was estimated using a compression device (FirmTech 2, BioWorks Inc., KS, USA), using the maximum slope of the curve as compressive force increased from 15 g to 200 g under a loading rate of 16 mm s^{-1} for 25 fruit per

replicate; (3) TSS were assessed with a digital thermo-compensated refractometer (Master–T, Atago, Tokyo, Japan) for five fruit per replicate; (4) TA was determined once per replicate, each one consisting of 10 mL juice diluted (distilled water) to 100 mL and titrated with 0.1 mol L^{-1} NaOH to an end-point pH of 8.2; (5) TSS and TA data were used to calculate the TSS/TA ratio; (6) RR was recorded from six replicates of 20 fruit each that were placed in 200 ml sealed glass jars and left in darkness for 2 h at room temperature (18 °C). CO_2 accumulation inside the jars was measured using a gas analyzer (Mocon, Inc., PacCheck 325, Minneapolis, USA). An authenticated standard (2.1 % CO_2 and 2.2 % O_2 in N_2 balance) was used for calibration; and (7) for EP, from the same jars used for RR, a 1 mL gas sample was withdrawn with a syringe from the headspace volume, and injected on a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 3 mm i.d. column packed with activated alumina, 80/100 mesh. The injector, oven, and detector temperatures were set at 75, 100, and 170 °C, respectively, with helium as the carrier gas (0.67 mL s^{-1}), in the presence of hydrogen and air (0.67 and 6.67 mL s^{-1}, correspondingly). An ethylene standard (1.0 μL L^{-1}) was used for calibration.

In order to assess the proportion (%) of ripe fruit on E and W sides of the canopy during the season, and the total amount of fruit produced (kg per plant) in each case, six additional plants were selected on both cultivars during the Y2 season. Using the hoop-count technique (Hancock et al., 2000), the percentage of ripe fruit (100% blue by visual assessments) was recorded weekly, and all ripe fruit present on the bush were harvested and weighed.

2.3 Environmental characteristics.

Ambient temperature (ºC) and relative humidity (%) in the field were recorded every 15 minutes with automatic sensors (HOBO S-THB, Onset Computer, Bourne, MA, USA), and this information plotted every hour. Additionally, daily precipitation was recorded using a rain gauge.

2.4 Statistical analyses

For each season (Y1 and Y2) and day of measurement (0, 30 and 45 d of refrigerated storage) data was assessed using an analysis of variance (ANOVA) for a split-plot design (orientation as the main plot, with maturity stage as sub-plot). When significant differences were found, Tukey's multiple comparison test ($p \leq 0.05$) was applied. Additionally, the Kolmogorov-Smirnov test (D K-S) was

used to estimate maximum distances (in absolute values) between cumulative frequency distributions of individual fruit firmness (n = 150); comparisons were done between orientations (E *vs.* W) for each maturity stage and between maturity stages (B100 *vs.* B100+6) within each orientation. Statistical analyses were performed using Statgraphics Centurion XVI (v.16.0.09, Statpoint, VA, USA) and R 3.0.0 (R Development Core Team, 2008).

3. Results

3.1 Fruit characteristics at harvest

The firmness frequency distributions of pooled data (maturity stages and orientations) (Fig. 2) indicate that a wide range in firmness existed within each harvest for both cultivars, and also between seasons. For 'Duke', fruit firmness ranged between 1.89 and 2.07 N in Y1 and between 1.37 and 1.80 N in Y2. 'Brigitta' berries displayed lower firmness values in both seasons ranging between 1.56 to 1.89 in Y1, and between 1.32 and 1.55 N in Y2. In general, during the first season, 'Duke' berries had a lower proportion of soft fruit than 'Brigitta' samples while differences were less evident during the second year.

For the factorial analyses of all fruit traits, 'Duke' berry orientation on the bush only affected firmness in Y1, with firmer fruit coming from the W side of the plant (Table 1). In contrast fruit maturity affected almost all fruit characteristics in both years, with consistent differences as maturity advanced: B100+6 fruit was lower in firmness and TA, as well as higher in weight and TTS/TA compared to B100 fruit. Two significant interactions (TA and TSS/TA) were found between factors and only in Y1.

For 'Brigitta' (Table 2), orientation on the plant affected firmness in Y1 (higher at the W side), and EP in Y2 (higher at the E side). Increased fruit maturity (B100+6) reduced weight (Y2), firmness (Y1 and Y2), TA (Y2) and EP (Y1 and Y2), but increased TSS/TA ratios and EP in Y2. Significant interactions occurred only in Y2 for TA and TSS/TA.

3.2 Fruit characteristics after storage

After 30 and 45 d of cold storage, orientation of 'Duke' fruit affected several variables but there was relatively little consistency across years (Table 3). In contrast, the effect of maturity stage was more

evident, with greater maturity associated with lower firmness, higher TSS, lower TA and higher TSS/TA ratio on almost all the evaluations. Significant interactions were detected only for firmness (Y1, 30 d).

Similar to 'Duke', the E-W orientation of the fruit on ‘Brigitta' bushes (Table 4) had little impact on the characteristics of stored fruit, with the exception of firmness (higher on E side for Y1, 45d). Again, the effect of maturity stage at harvest was more pronounced, with the less mature berries (B100) being higher in firmness and TA, but lower in TSS and TSS/TA ratios than the B100+6 fruit. No interactions were detected in this cultivar.

3.3 Firmness cumulative frequency distributions at harvest and after storage

The analysis of cumulative frequencies curves for fruit firmness (Figs. 3 and 4; Table 5) allowed a more comprehensive characterization of the differences originating from fruit orientation and maturity stage at harvest. Under this approach, it is possible to compare treatments according to different firmness classes for a given cumulative frequency (e.g., 50% represented by an horizontal dashed line) or to look at the proportion of fruit being equal to or lower than a given firmness threshold (e.g., 2 N represented by a vertical dashed line). As previously described, Y2 fruit were softer regardless of cultivar, orientation or maturity stage, since these samples displayed in all cases larger percentages of fruit with firmness values < 2 N in comparison with Y1 produce. Additionally at harvest very soft fruit (< 1.4 N) varied between 0 and 31 % for Y1 and between 11 and 67 % depending on cultivar, maturity stage and orientation (Fig. 3 and 4).

We used the Kolmorogov-Smirnov test for the maximum distance between two cumulative frequency distributions. For ‘Duke’ fruit, the effect of orientation (E *vs*. W) was significant for Y1 at 0 d for both maturity levels with those fruit from the E side being less firm (Fig. 3A and Table 5). The only other effect of canopy orientation for 'Duke' was for fruit stored for 30 d, in which B100+6 samples from the E side were softer (Fig. 3B and Table 5). When comparisons were performed for maturity level under the same orientation (either E or W), differences between maturity stages were evident (Fig. 3), with a greater proportion of firmer fruit at the B100 stage. During Y1, significant distances between maturity stages were detected only for fruit harvested from the E side, both at harvest and after storage for 30 d (Figs. 3A and B, respectively), whereas

after 45 days, maturity impacted the firmness frequency curves for both canopy positions (Fig. 3C). During Y2, maturity impacted firmness frequency curves at time 0 and after 30 and 45 d storage for fruit from both E and W sides of the bush (Fig. 3D–F, Table 5).

The frequency curve differences observed for 'Brigitta' (Fig. 4 and Table 5) were less marked than those for 'Duke': there was an effect of canopy orientation only for Y1, with B100 fruit from the E side being softer than those from the W side at day 0 (Fig. 4A and Table 5), while B100+6 samples from the W side were softer at 45 d (Fig. 4C and Table 5). When comparisons were performed for a given orientation (either E or W), the more mature B100+6 fruit were typically softer than B100 fruit. Significant shifts in the firmness frequency curves were found in Y1 at harvest for fruit from E and W sides (Fig. 4A) and after 45 d for fruit from the E side only (Fig. 4C). In Y2, maturity lead to significant differences in the frequency curves for all storage periods for both canopy positions (Fig. 4D–F).

3.4 Maturity and productivity on each side of the canopy.

For each cultivar and evaluation date, more blue fruit were produced from the E than from the W sides of the canopy (Fig. 5A). 'Duke' and 'Brigitta' plants displayed similar percentages of blue fruit, both at the beginning (~80% E and ~20% W) and at the end of the season (~50% on each side). Productivity followed the same trend, the E side producing more kilograms of fruit per plant than the W side (Fig. 5B). Additionally, 'Duke' plants produced more fruit per plant than 'Brigitta' (4.2 and 3.1 kg *vs*. 3.6 and 2.2 for the E and the W sides of the canopy, respectively).

3.5 Environmental characteristics.

The Y1 season registered higher temperatures (Fig. 6 A) and lower relative humidity (Fig. 6 B) along the whole period of fruit development from the early green tip until harvest. TheY2 season had higher daytime temperatures and lower relative humidity later in the day, especially for dates close to harvest. Total rainfall was also higher for Y2 than for Y1, with significant rain events (>5 mm) after early bloom and before the harvest of 'Brigitta' berries (Fig. 6 C).

4. Discussion

Several studies, primarily on apples, have reported that multiple sources of variation, both between-plants and especially within-plant can be found, resulting in heterogeneous quality of harvested fruit (Heinicke, 1966; Jackson, 1967; Robinson et al., 1983; Perring, 1989; Broom et al., 1998; De Silva et al., 2000). Despite the importance of these aspects, no formal studies have been published for blueberries. The high variability in firmness of harvested blueberries of similar maturities in the current study (6-d interval between B100 and B100+6 fruit), regardless of cultivar or season (Y), is concerning. The broad range in blueberry fruit firmness is consistent with the report of Moggia et al. (2017), who found that percentages of very soft fruit (firmness < 1.4 N) varied between 25% and 42% for 'Duke' and between 5 and 10% for 'Brigitta' in two consecutive years. This is very different in comparison to other fruit species such as apple, where very soft fruit (58 – 62 N) represent less than 0.5 – 0.8% of the total batch harvested (Herregods and Goffings, 1993; De Silva et al., 2000).

In accordance with Moggia et al. (2017), firmness values found in the present study varied between seasons and dates of evaluation, and cumulative frequency analysis further revealed that there was an important percentage of fruit displaying very low firmness values, along with consistently higher in TSS/TA ratios, due mostly to fruit that had remained on the plant for some days after achieving full color (B100+6). This highlights the importance of maturity stage at harvest, especially for long distance shipments. Additionally the differences on fruit firmness found between Y1 and Y2 also emphasizes the importance of climatic conditions during fruit growth and maturation, reinforcing the idea that elapsed time between harvests should be based on physiological changes that derivate from environmental conditions rather than a fixed interval. Unfortunately this is a common practice since growers wait for blue fruit to accumulate in order to optimize harvesting labor costs.

The dearth of interactions between maturity and fruit position within the canopy would suggest that fruit ripen evenly on both E and W sides of the bush. For perspective, however, it is worth noting that in experiments with a factorial design, the average of each level within one factor is calculated considering the combination of the levels of the other factor (Lawal, 2014). Therefore, the much bigger effects due to maturity stages (B100 *vs*. B100+6) compared to canopy orientation (E *vs*. W) could be masking the possible orientation effect. These results, which are probably influenced also

by the high variability found within the samples, suggest that ANOVA procedures, which are based on mean values, might be not necessarily the best approach for finding differences in fruit firmness associated to a particular canopy side. Hence, the analysis of the cumulative frequency distribution of all data was used as an alternative approximation. This approach not only allowed the comparison between treatments, but also the visualization of important information, such as different firmness categories and the percentage in which each was present. Despite the advantages and disadvantages of both methodologies (Bailey, 1947; Woodruff et al., 1960), results illustrate that for blueberries, depending on growing conditions and intervals between pickings, a wide range of firmness can be found at a particular harvest, with the consequent risks associated to the presence of over-ripe fruit within commercial units. Our study suggests that the proportion of over-ripe fruit therein might play an important role on the chances of shipment rejections at final destinations.

Although variation in firmness associated with the position of the fruit within the canopy at a given maturity stage was less marked, fruit coming from the E side was often softer at harvest. These orientation-related differences could be explained, in part, by the daily microclimatic fluctuations integrated during the whole season. Even though the influence of preharvest environmental conditions on postharvest behavior of fruit is still not well understood for this species, it is known that optimal temperatures for gas exchange are between 20 and 25 ºC (Davis and Flore, 1986). It has been speculated that gas exchange could be associated to fruit condition because of the cooling due to evaporation from the canopy during the generation of carbohydrates for fruit growth and development. Due to the relatively inefficient water-conducting system of blueberry plants (Gough, 1994), when transpirational demand exceeds capacity, blueberry fruit would be under stress during part of the day (Chen et al., 2012; Estrada et al., 2015). According to the present results, this might have been the case during the last portion of the maturation period, especially during the afternoons when direct sunlight illuminates the W side of the canopy. Because of this, there is not a clear explanation for the E side of the canopy having softer fruit. Other morphological and anatomical changes during fruit development may be responsible for firmness and shelf life behavior: a progressive increase in thickness of the epicuticular wax layer and cuticle as well as in the cell walls of the epidermis and hypodermis has been reported in blueberries (Konarska, 2015). While many of these qualitative traits have a genetic background, they also depend to great extent on environmental conditions and maturity stage at harvest (Connor et al., 2002).

Interestingly, significant differences in firmness between plant sides were only detected for Y1, values for B100 and B100+6 fruit at harvest being higher than those observed in Y2. Orientation-related differences in fruit firmness remained during postharvest evaluations of B100+6 fruit, both after 30 and 45 d of storage, suggesting that depending on firmness distribution at harvest and environmental conditions during fruit growth and development, fruit position might be a source of variability. These thresholds, have not been determined and should be further studied.

Variation in fruit firmness between seasons has been previously reported (Ehlenfeldt and Martin, 2002), and it was suggested that softer fruit might be linked to average to above-average rainfall patterns. Pritts and Hancock (1992) stated that rain during harvest can adversely affect fruit quality of highbush blueberries by delaying harvest, washing off fungicides, softening berries, moistening stem scars, and splitting berries. High temperature combined with rain exacerbates these problems. The second season considered in this study (Y2) had more rain, not only at the beginning of the season (Aug. – Sep.) but also during the maturation and harvest periods. Environmental information for both seasons suggests that even the length of each raining event would influence berry softening. In comparison to Y1, Y2 displayed higher temperatures and lower relative humidity later in the day near-harvest dates, which would represent another possible source of variability between seasons.

Environmental conditions could also be responsible for the difference in fruit productivity we found between the E and W sides of the canopy. Although productivity was measured only in one year, results are consistent for both cultivars. No measurements were taken at the beginning of the season in order to determine whether the E side started with more or with better quality (higher number of flowers per cluster) flower buds, but pollination dynamics could be a possible explanation for these canopy asymmetry. Honeybees modify their foraging visits according to the nectar production rhythms (Moore et al., 1989), and may use volatile floral emissions (attractant and repellent) as information to regulate their activity by assessing the quality of flowers prior to the contact (Dobson, 2006; Raguso, 2008). According to Rodríguez-Saona (2011), after bees visit and pollinate highbush blueberry flowers, the production of nectar is reduced, concomitant with relatively predictable changes in the emission of particular volatiles. The same authors also found that the amount of volatiles released were two-fold greater between 09:00 and 12:00 h than earlier or later

periods of the day. Additionally, nectar production is also higher between 09:00 and 11:00 h. Honey bee foraging has a lower ambient temperature threshold of 12 to 14 ºC (Winston, 1987). Mornings are when the E side of the plant is more fully illuminated and so is the warmer side of the plant during the mornings when volatiles and nectar rewards are at their maximum. This suggests that within orchards oriented in the N-S direction, conditions may favor pollination and fruit set on the E side of the plant.

5. Conclusions

Frequency analysis of firmness within a well-defined maturity stage (B100 and B100+6) demonstrates a high degree of variability exists within populations of blueberry fruit related to cultivar, season and orientation on the bush. The reasons for the high degree of firmness variation are not known. In addition, to these factors fruit maturity stage contributes meaningfully to the overall variation expected in typical commercial harvests. Our data show that even the short time elapsed between B100 and B100+6 stages was enough to increase the amount of soft and very soft fruit, and make clear the importance of more frequent harvests to improve firmness at final destinations, especially when preharvest environmental conditions could accelerate fruit softening as they did in Y2 of this study. Even though the effect of fruit position in the canopy was less consistent than that of maturity stage, cumulative frequency distributions suggest that the E side of the plant would produce softer fruit. Data also support the idea that, within the same picking date, export-destined clamshells would contain a greater proportion of fruit coming from the E side of the canopy.

Increasing temperatures and weather variability expected with climate change, suggests that blueberry growers will face an increase in the already high variability in fruit firmness. It will be therefore critical to establish the main environmental factors contributing and predisposing fruit to softening.

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Table 1. Analysis of variance of fruit traits[z] for 'Duke' berries coming from different orientations (East and West) and picked at different maturity stages (B100 and B100+6). Fruit were assessed at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2).

		Quality traits						
		W (g)	**F (N)**	**TSS (%)**	**TA (%)**	**TSS/TA**	**EP (ng kg^{-1} s^{-1})**	**RR (µg CO_2 kg^{-1} s^{-1})**
Y1	**Orientation (O)**							
	East	1.81	1.92 b	16.8	0.79	24.6	0.49	33.58
	West	1.79	2.10 a	16.5	0.79	21.9	0.46	32.48
	Significance (p)	*0.3539*	***0.0000***	*0.7119*	*0.9704*	*0.3291*	*0.5772*	*0.3156*
	Maturity (M)							
	B100	1.69 b	2.07 a	15.6 b	0.96 a	16.5 b	0.48	37.53 a
	B100+6	1.90 a	1.95 b	17.6 a	0.62 b	30.1 a	0.48	28.52 b
	Significance (p)	***0.0000***	***0.0012***	***0.0002***	***0.0004***	***0.0001***	*0.9558*	***0.0000***
	O x M							
	Significance (p)	*0.9355*	*0.8124*	*0.2212*	***0.0322***	***0.0213***	*0.3117*	*0.2821*
Y2	**Orientation (O)**							
	East	1.60	1.57	15.0	0.66	23.8	0.26	14.28
	West	1.61	1.59	14.8	0.66	23.6	0.23	14.33
	Significance (p)	*0.8089*	*0.5568*	*0.7387*	*0.9483*	*0.9572*	*0.8318*	*0.9227*
	Maturity (M)							
	B100	1.52 b	1.80 a	14.8	0.76 a	19.7 b	0.12 b	12.43 b
	B1006	1.70 a	1.37 b	15.1	0.56 b	27.7 a	0.38 a	16.18 a
	Significance (p)	***0.0000***	***0.0000***	*0.5237*	***0.0040***	***0.0080***	***0.0000***	***0.0016***
	O x M							
	Significance (p)	*0.9891*	*0.2368*	*0.0598*	*0.9344*	*0.4540*	*0.1957*	*0.1627*

[z] Traits: fruit weight (W), firmness (F), TSS (total soluble solids), TA (titratable acidity), ethylene production (EP), and respiration rate (RR). Mean separation by Tukey test ($p \leq 0.05$).

Table 2. Analysis of variance of fruit traits[z] for 'Brigitta' berries coming from different orientations (East and West) and picked at different maturity stages (B100 and B100+6). Fruit were assessed at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2).

		Quality traits						
		W (g)	**F (N)**	**TSS (%)**	**TA (%)**	**TSS/TA**	**EP (ng kg^{-1} s^{-1})**	**RR (µg CO_2 kg^{-1} s^{-1})**
Y1	**Orientation (O)**							
	East	1.73	1.67 b	16.2	0.84	20.5	0.05	20.58
	West	1.72	1.78 a	16.2	0.79	21.1	0.05	19.22
	Significance (p)	*0.6294*	***0.0000***	*0.9633*	*0.5054*	*0.7231*	*0.8914*	*0.4197*
	Maturity (M)							
	B100	1.70	1.89 a	16.2	0.82	20.9	0.07 a	20.67
	B100+6	1.74	1.56 b	16.1	0.81	20.7	0.03 b	19.13
	Significance (p)	*0.2510*	***0.0000***	*0.8996*	*0.8298*	*0.9243*	***0.0129***	*0.1283*
	O x M							
	Significance (p)	*0.8390*	*0.0669*	*0.4215*	*0.7913*	*0.8294*	*0.2242*	*0.7730*
Y2	**Orientation (O)**							
	East	1.77	1.44	15.5	0.65	26.5	0.07 a	7.28
	West	1.79	1.43	14.9	0.59	30.4	0.06 b	7.26
	Significance (p)	*0.7548*	*0.6608*	*0.2583*	*0.7519*	*0.9587*	***0.0091***	*0.6502*
	Maturity (M)							
	B100	1.92 a	1.55 a	14.9	0.82 a	18.5 b	0.08 a	5.20 b
	B100+6	1.64 b	1.32 b	15.5	0.43 b	38.4 a	0.06 b	9.35 a
	Significance (p)	***0.0000***	***0.0000***	*0.2097*	***0.0000***	***0.0000***	***0.0280***	***0.0001***
	O x M							
	Significance (p)	*0.3514*	*0.3169*	*0.4470*	***0.0018***	***0.0024***	*0.8397*	*0.2933*

[z] Traits: fruit weight (W), firmness (F), TSS (total soluble solids), TA (titratable acidity), ethylene production (EP), and respiration rate (RR). Mean separation by Tukey test ($p \leq 0.05$).

Table 3. Analysis of variance of fruit traits[z] for '**Duke**' berries coming from different orientations (East and West) and picked at different maturity stages (B100 and B100+6). Fruit were harvested at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2), and assessed after 30 or 45 days under cold storage.

		Quality traits							
		F (N)		**TSS (%)**		**TA (%)**		**TSS/TA**	
		30	**45**	**30**	**45**	**30**	**45**	**30**	**45**
Y1	**Orientation (O)**								
	East	2.07 b	1.87	17.6	15.4	0.69	0.69 b	26.3	25.5 a
	West	2.23 a	1.87	17.4	15.0	0.73	0.76 a	24.7	21.2 b
	Significance (p)	***0.0039***	*0.9139*	*0.6632*	*0.1703*	*0.5544*	***0.0447***	*0.5273*	***0.0039***
	Maturity (M)								
	B100	2.18	2.18 a	16.1 b	14.6 b	0.78 a	0.92 a	21.1 b	16.0 b
	B100+6	2.12	1.56 b	18.9 a	15.7 a	0.65 b	0.52 b	29.8 a	30.7 a
	Significance (p)	*0.1010*	***0.0000***	***0.0000***	***0.0006***	***0.0494***	***0.0010***	***0.0023***	***0.0002***
	O x M								
	Significance (p)	***0.0333***	*0.4834*	*0.1337*	*0.2604*	*0.9445*	*0.4391*	*0.6292*	*0.0587*
Y2	**Orientation (O)**								
	East	1.64	1.86	13.9	15.1 b	0.66 a	0.60	21.9 b	26.1
	West	1.62	1.94	14.1	16.0 a	0.50 b	0.64	32.6 a	26.1
	Significance (p)	*0.4026*	*0.0876*	*0.5925*	***0.0091***	***0.0214***	*0.7101*	***0.0013***	*0.9973*
	Maturity (M)								
	B100	1.72 a	2.00 a	12.3 b	15.1 b	0.66 a	0.63	18.7 b	24.5
	B100+6	1.54 b	1.79 b	15.8 a	16.0 a	0.49 b	0.60	35.8 a	27.8
	Significance (p)	***0.0000***	***0.0003***	***0.0000***	***0.0489***	***0.0105***	*0.5627*	***0.0013***	*0.1628*
	O x M								
	Significance (p)	*0.2957*	*0.2352*	*0.3378*	*0.6187*	*0.1647*	*0.1034*	*0.0844*	*0.0970*

[z] Traits: firmness (F), TSS (total soluble solids), and TA (titratable acidity). Mean separation by Tukey test ($p \leq 0.05$).

Table 4. Analysis of variance of fruit traits[z] for '**Brigitta**' berries coming from different orientations (East and West) and picked at different maturity stages (B100 and B100+6). Fruit were harvested at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2), and assessed after 30 or 45 days under cold storage.

		Quality traits							
		F (N)		TSS (%)		TA (%)		TSS/TA	
		30	45	30	45	30	45	30	45
Y1	**Orientation (O)**								
	East	1.52	1.58 a	15.2	14.7	0.69	0.71	23.7	21.7
	West	1.61	1.44 b	15.3	14.9	0.71	0.67	21.4	24.4
	Significance (p)	*0.0691*	***0.0088***	*0.8656*	*0.3100*	*0.2072*	*0.1608*	*0.5000*	*0.4593*
	Maturity (M)								
	B100	1.53	1.53	15.3	14.4 b	0.72	0.76	21.2	20.0 b
	B100+6	1.60	1.49	15.2	15.2 a	0.67	0.62	23.9	26.1 a
	Significance (p)	*0.0941*	*0.9203*	*0.8232*	***0.0020***	*0.5178*	*0.0620*	*0.4226*	***0.0254***
	O x M								
	Significance (p)	*0.1843*	*0.4293*	*0.5038*	*0.4388*	*0.6609*	*0.4576*	*0.6667*	*0.7028*
Y2	**Orientation (O)**								
	East	1.72	1.62	15.9	14.7	0.49	0.55	33.6	28.2
	West	1.68	1.66	16.2	14.7	0.51	0.56	32.7	28.2
	Significance (p)	*0.2393*	*0.3101*	*0.4535*	*0.8836*	*0.7212*	*0.8290*	*0.8286*	*0.9784*
	Maturity (M)								
	B100	1.87 a	1.79 a	16.3	14.9	0.57 a	0.68 a	29.7 b	22.8 b
	B100+6	1.53 b	1.49 b	15.8	14.6	0.43 b	0.44 b	36.5 a	33.6 a
	Significance (p)	***0.0000***	***0.0000***	*0.2147*	*0.3493*	***0.0247***	***0.0006***	***0.0493***	***0.0029***
	O x M								
	Significance (p)	*0.6103*	*0.4328*	*0.0586*	*0.5213*	*0.6803*	*0.9635*	*0.5382*	*0.7436*

[z] Traits: firmness (F), TSS (total soluble solids), and TA (titratable acidity). Mean separation by Tukey test ($p \leq 0.05$).

Table 5. Firmness cumulative frequency distance (absolute values) and significance using the Kolmogorov-Smirnov analysis for 'Duke' and 'Brigitta' berries coming from different orientations (East-E and West-W) and maturity stages (B100 and B100+X). Fruit were harvested at the peak of the seasons 2013/14 (Y1) and 2014/15 (Y2), and assessed at harvest (0 d), and after 30 (30 d) or 45 (45 d) days under cold storage

			Y1			Y2		
'Duke'	**Level**	**Comparison**	0 d	30 d	45 d	0 d	30 d	45 d
Maturity	B100	East *vs*. West	0.33***[z]	0.15[n.s.]	0.09[n.s.]	0.14[n.s.]	0.17[n.s.]	0.13[n.s.]
	B100+6	East *vs*. West	0.35***	0.38***	0.11[n.s.]	0.16[n.s.]	0.09[n.s.]	0.16[n.s.]
Orientation	East	B100 *vs*. B 100+X	0.24**	0.22**	0.56***	0.44***	0.40***	0.34***
	West	B100 *vs*. B 100+X	0.20[n.s.]	0.16[n.s.]	0.45***	0.63***	0.26**	0.29***
			Y1			**Y2**		
'Brigitta'			0 d	30 d	45 d	0 d	30 d	45 d
Maturity	B100	East *vs*. West	0.40***	0.22[n.s.]	0.23[n.s.]	0.12[n.s.]	0.08[n.s.]	0.06[n.s.]
	B100+6	East *vs*. West	0.16[n.s.]	0.11[n.s.]	0.19*	0.06[n.s.]	0.09[n.s.]	0.07[n.s.]
Orientation	East	B100 *vs*. B 100+X	0.27***	0.13[n.s.]	0.23**	0.48***	0.44***	0.37***
	West	B100 *vs*. B 100+X	0.49***	0.08[n.s.]	0.15[n.s.]	0.42***	0.45***	0.31***

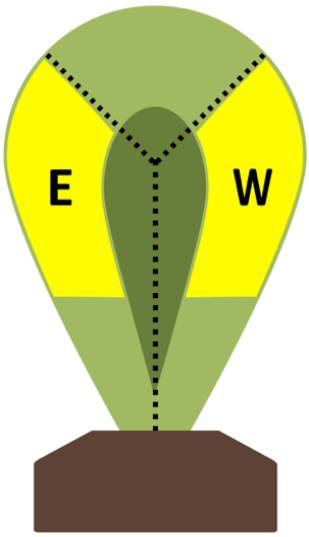

Figure 1. Scheme of bush segmentation into East (E) and West (W) orientation. On each side, delimited by dashed lines, fruit was harvested from the yellow area

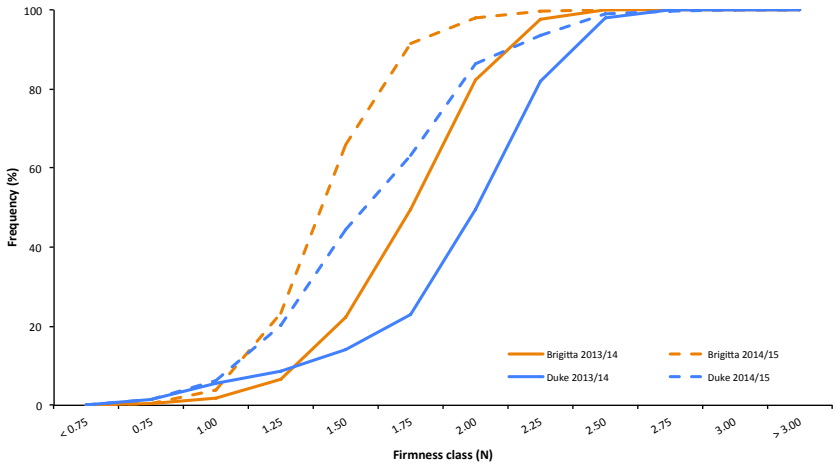

Figure 2. Firmness (N) cumulative frequency distribution of pooled data for 'Duke' (blue lines) and 'Brigitta' (orange lines) fruit at the peak of harvest. Season 2013/14 (Y1) is represented by solid lines and 2014/15 (Y2) is represented by dashed lines. Each curve represents B100 and B100+6 fruit, from the East and West side of the plat; n = 600 fruit.

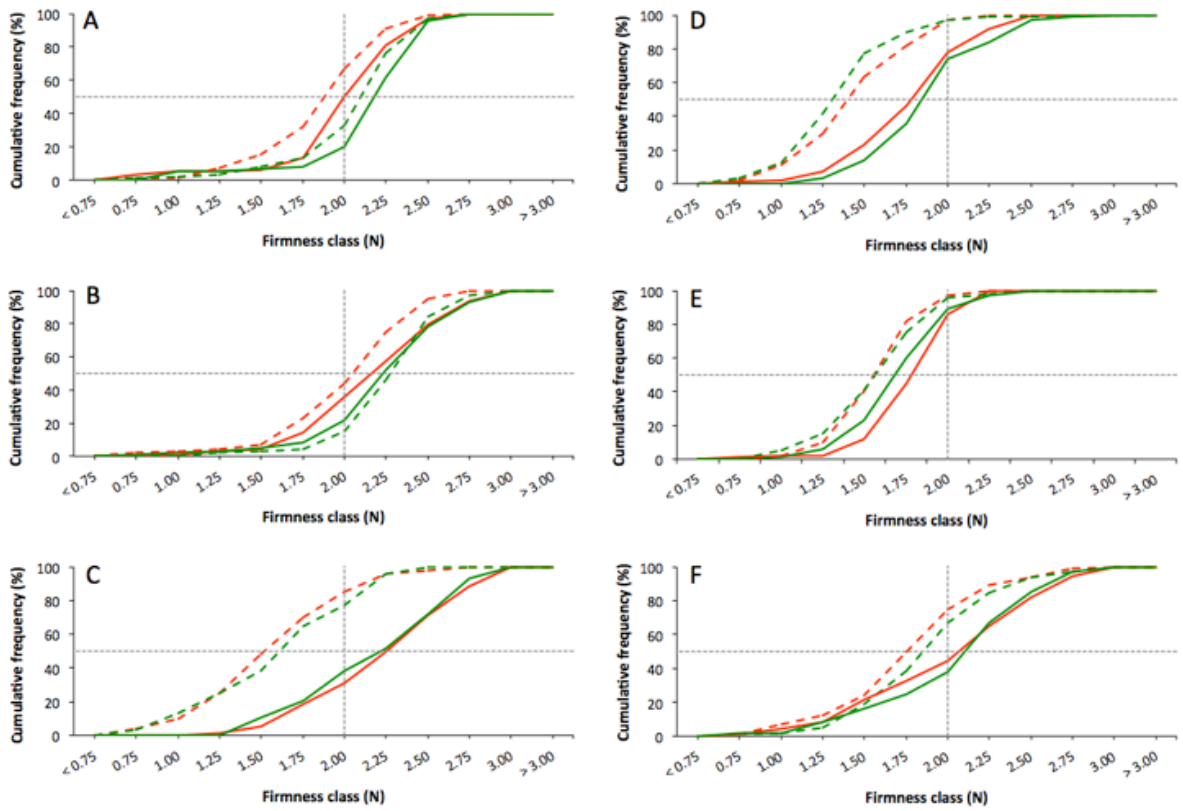

Figure 3. Firmness (N) cumulative frequency distribution of 'Duke' harvested at the peak of seasons 2013/14 (A, B and C) and 2014/15 (D, E and F). Measurements taken at harvest (A and D), and after 30 (B and E) and 45 (C and F) days of cold storage. Berries were picked from East (red lines) and West (green lines) side of the plant, when fruit reached 100% blue color within a maximum of 2 days (B100: solid lines) or left remaining on the plant for additional 6 days (B100+6: dashed lines). For a better understanding, each graph includes a horizontal (50% of the cumulative frequency) and vertical (firmness class at 2 N) dashed lines; n = 150 fruit

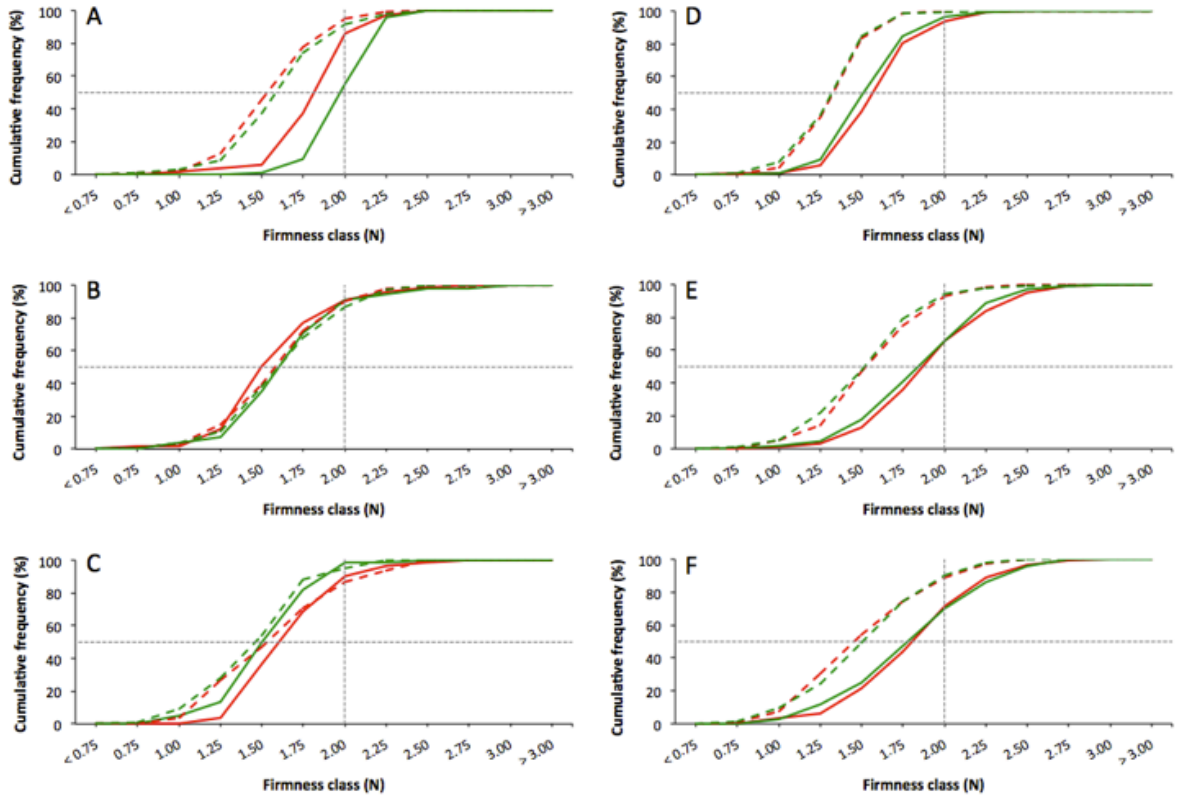

Figure 4. Firmness (N) cumulative frequency distribution of 'Brigitta' harvested at the peak of seasons 2013/14 (A, B and C) and 2014/15 (D, E and F). Measurements taken at harvest (A and D), and after 30 (B and E) and 45 (C and F) days of cold storage. Berries were picked from East (red lines) and West (green lines) side of the plant, when fruit reached 100% blue color within a maximum of 2 days (B100: solid lines) or left remaining on the plant for additional 6 days (B100+6: dashed lines). For a better understanding, each graph includes a horizontal (50% of the cumulative frequency) and vertical (firmness class at 2 N) dashed lines; n = 150 fruit.

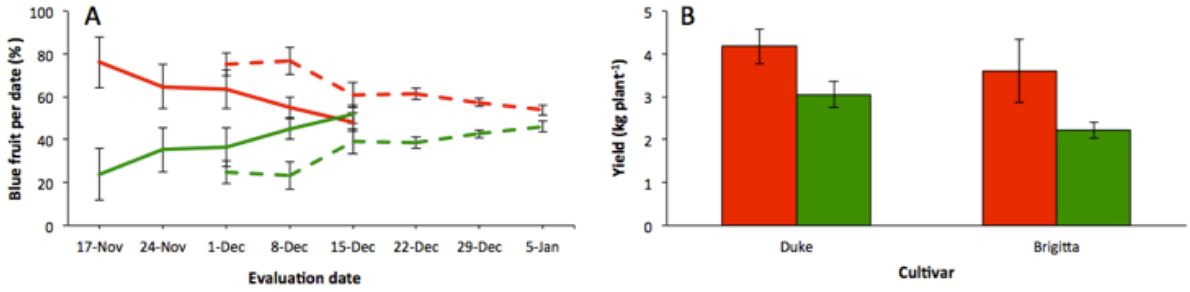

Figure 5. Weekly proportion of 100% blue fruit (A; 'Duke' with solid lines and 'Brigitta' with dashed lines) and total amount of fruit (B) produced by the East (red lines and bars, respectively) and West (green lines and bars, respectively) sides of the canopy during season 2014/15. Vertical black bars indicate standard error.

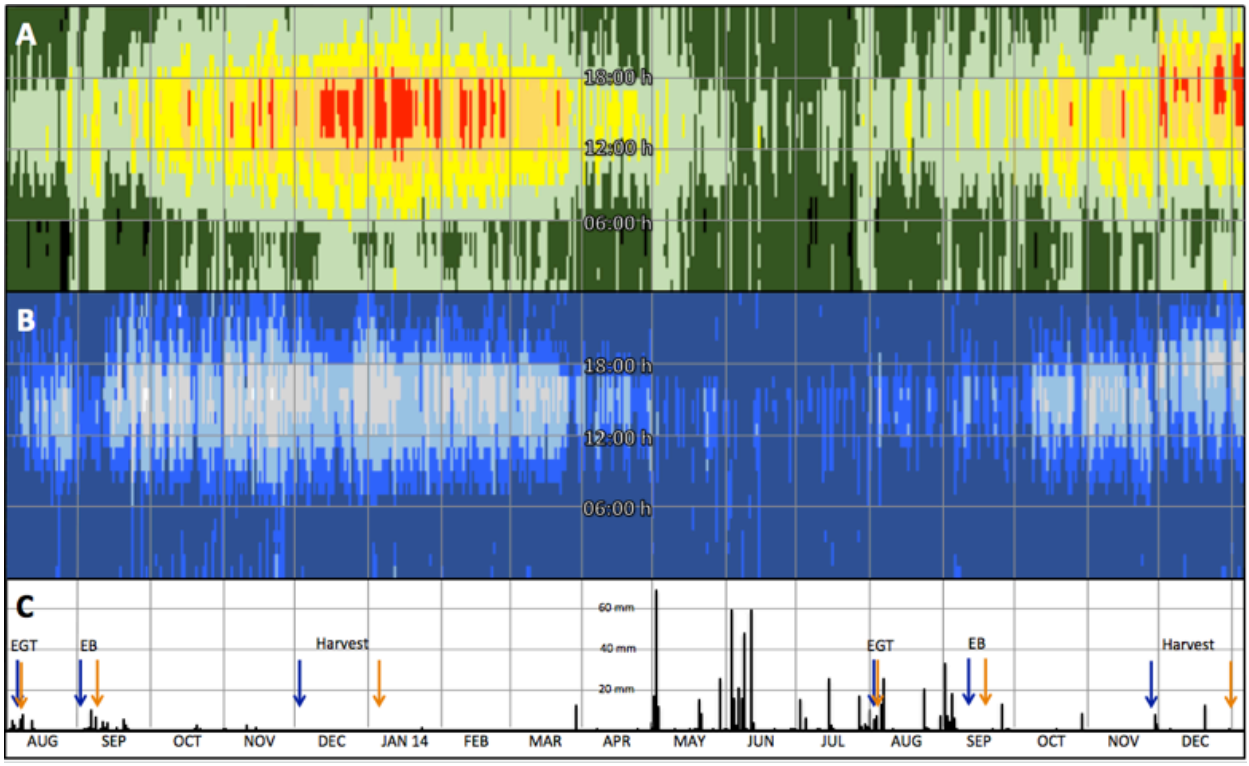

Figure 6. Hourly ambient temperature (A) and relative humidity (B) bands, and daily precipitation (C), from August 01, 2013 until January 05, 2015. Data is color coded by temperature range (ºC) (black: < 0; dark green: 0–10; light green: 10–18; yellow: 18–24; orange: 24–29; and red: 29–38) and relative humidity (%) (white: < 20; grey: 20–40; light blue: 40–60; blue: 60–80; and dark blue: > 80) bands. Phenological stages (early green tip – EGT, early bloom – EB, and Harvest, respectively) of 'Duke' and 'Brigitta' are denoted with blue and orange arrows, respectively.

6. DISCUSIÓN

Introducción

Los cultivares utilizados en la presente Tesis fueron seleccionados por su importancia económica, al ser los más plantados en la zona productiva de Chile y por presentar fechas diferenciales de cosecha; 'Duke' es un cultivar de recolección temprana, en tanto 'Brigitta' es considerado un cultivar de media estación.

Para una mayor claridad en esta sección se hace mención a los estados de madurez de los frutos con las siguientes siglas: G (frutos verdes); 25P (frutos con 25% de coloración rosa); 50P (frutos con 50% de coloración rosa), 75B (frutos con 75% de coloración azul), 100B (frutos con 100% de coloración azul) y 100B+X (frutos 100% coloración azul, sobremaduros).

Es de relevancia indicar que para conseguir los estados 100B y 100B+X se realizaron seguimientos diarios en campo desde temprano en la temporada; cuando se alcanzaron porcentajes similares de fruta en los estados G y 25P, se marcaron racimos con similares características en plantas homogéneas, previamente seleccionadas. Desde ese momento se monitoreó el desarrollo del fruto cada 2 días, hasta que se alcanzaron los dos estados: 100B (fruta que alcanza coloración azul y es cosechada máximo 2 días después del evento) y 100B+X (fruta que después de alcanzar el estado azul se mantuvo por 5 a 7 días adicionales en la planta).

6.1. Variabilidad de la fruta en función de estado de desarrollo, cultivar, madurez y años.

- *Diferencias entre cultivares y estados de madurez del fruto (Capítulo 5.1)*

Tres temporadas de ensayos permitieron corroborar las diferencias entre los cultivares estudiados, en cuanto a potencialidad de almacenaje: los frutos del cv. 'Duke' tienden a ser más firmes en cosecha, pero presentan una mayor tasa de ablandamiento durante el almacenaje posterior; los frutos del cv. 'Brigitta' por su parte, con valores más bajos de firmeza inicial, presentan una caída de firmeza menos acentuada en el tiempo de almacenaje. Adicionalmente se logró determinar, para ambos cultivares, cambios importantes en la calidad del fruto en función del estado de madurez en cosecha; con especial énfasis se discuten los resultados que demuestran las marcadas diferencias entre frutos cosechados en los estados de madurez 75B, 100B y 100B+X. Es importante destacar que esta diferenciación se logró realizar a nivel metodológico experimental; sin embargo, por el

momento no existen formas prácticas de distinguir los estados 100B y 100B+X a nivel de huerto comercial.

El potencial de almacenaje diferencial de ambos cultivares de arándano podría explicarse por la evolución que tienen los frutos desde el estado verde inmaduro hasta el estado azul maduro. Los frutos correspondientes a ambos cvs. exhibieron un alza en la respiración y la producción de etileno (Windus et al., 1976; Suzuki et al., 1997); sin embargo, hubo diferencias en la magnitud de estos parámetros fisiológicos y en el momento en que se alcanzaron sus valores máximos. Para el cv. 'Brigitta' los máximos valores de respiración y producción de etileno ocurrieron entre las coloraciones 50P y 75B; para el cv. 'Duke', el alza de la producción de etileno también se produjo entre los mismos estados de madurez, sin embargo, el máximo de respiración ocurrió previamente, en fruta 25P. Adicionalmente, 'Duke' presentó una mayor actividad metabólica, observándose en este cv. máximos valores de respiración y producción de etileno de 3 a 3.5 veces superiores que en el cv. 'Brigitta'. De acuerdo a Gough (1994) cultivares con mayor tasa de respiración en cosecha (en este caso 'Duke') tendrían un menor potencial de almacenamiento después de la recolección.

Durante su desarrollo en campo los frutos presentaron dos disminuciones importantes de su firmeza; la primera de ellas, responsable de más del 30 % del ablandamiento respecto de la firmeza inicial, coincidió con el máximo de respiración, similar a lo reportado por Windus et al. (1976) y El-Agamy et al. (1982). Si bien los arándanos son considerados frutos climatéricos, el momento en que ocurre el alza respiratoria diferencia a esta especie de otros frutos climatéricos, ya que su cosecha se realiza en un estado postclimatérico, donde los frutos no mejoran en calidad posterior a la recolección y almacenaje (NeSmith et al., 2005; Ne Smith, 2011). La segunda disminución en firmeza, que fue de menor magnitud (15.4 a 18.5 %) ocurrió entre los estados de madurez 100B y 100B+X. Los cambios más importantes asociados con la evolución de la maduración, que incluyen el aumento del contenido de sólidos solubles (SS) y la reducción de la acidez titulable (AT), resultaron en relaciones SS/AT que incrementaron de 10.9 a 25.4 entre los estados 75B y fruta sobremadura (100B+X). Fruta firme y con ratios de SS/AT < 18 se asocian a un mayor potencial de almacenaje de arándanos (Hanson et al., 1993). Galletta et al. (1971) establecieron tres categorías basadas en el ratio SS/AT: buena calidad de almacenaje (con valores de ratio < 18); calidad intermedia (para ratios de 18-32); y muy mala calidad (para ratios > 32). De acuerdo con ello, los

valores de SS/AT para fruta sobremadura en nuestros estudios serían muy extremos y limitarían su posibilidad de envíos a mercados lejanos. Dado que habitualmente la cosecha comercial de arándanos se basa en la coloración de la epidermis del fruto, que debe ser 100 % azul (Gough,1994; Lobos et al., 2014), frutos maduros y sobremaduros son generalmente recolectados en forma conjunta sin que sea posible distinguirlos visualmente. En el presente estudio, mediciones objetivas con colorímetro (L*, "Chroma", "hue angle"), tampoco fueron efectivas para diferenciar estos dos estados.

Si bien los frutos del cv. 'Duke' eran más firmes en el momento de la cosecha, su ablandamiento en almacenaje fue mayor, ya que los frutos cosechados en los estados 75B, 100B y 100B+X, perdieron un 32, 25 y 18 % de su firmeza inicial, respectivamente, después del almacenaje refrigerado. Para el cv. 'Brigitta' estos descensos de firmeza fueron de 4, 0.5 y 19 % para los mismos estados de madurez, respectivamente. Adicionalmente, la pérdida de peso de los frutos varió entre un 10 y 21 % en el cv. 'Duke' y de 6.4 a 9.8 % en el cv. 'Brigitta'; esto se tradujo en una relación negativa entre firmeza y pérdida de peso (r^2 =0.61), confirmando una mayor potencialidad de almacenaje para el cv. 'Brigitta'. Paniagua et al. (2013) reportaron resultados similares para arándanos almacenados a 4 °C y sometidos a diferentes tratamientos de flujos de aire.

- *Efecto del estado de madurez del fruto y uso de bolsa plástica macro-perforada en almacenaje refrigerado (Capítulo 5.2)*

La fruta firme es más resistente a las labores de cosecha y posterior transporte y procesamiento (Hanson et al., 1993). Diversos autores han descrito importantes diferencias entre cultivares de arándano (Ehlenfeldt y Martin, 2002; Saftner et al., 2008); sin embargo, éstas diferencias parecen estar más relacionadas con el estado de madurez en cosecha que con diferencias genotípicas (Beaudry et al., 1998; Lobos et al., 2014). Dado los elevados niveles de pérdida de peso encontrados en los ensayos del Capítulo 5.1, se incorporó, en un estudio paralelo, un tratamiento de envasado con bolsa macro-perforada. Los resultados obtenidos indican que la firmeza final después del almacenamiento estuvo relacionada tanto con el estado de madurez en cosecha (ya que la fruta cosechada en 75B y 100B permaneció más firme que aquella sobremadura) como con el uso de una bolsa plástica como barrera a la pérdida de humedad. Nuevamente se observó una mayor tasa de

ablandamiento en el cv. 'Duke', destacándose en éste un mayor beneficio en la retención de la firmeza, producto de la utilización de la bolsa, independiente del estado de madurez.

Por el momento no existen parámetros de firmeza óptima para la cosecha de la fruta de esta especie; se han reportado valores promedio en cosecha para el cv. 'Duke' entre 1.73 y 1.36 N (Ehlenfeldt y Martin, 2002; Saftner et al., 2008) y entre 1.88 y 1.46 N para el cv.'Brigitta' (Ehlenfeldt y Martin, 2002). Los valores obtenidos en el presente estudio concuerdan para el cv. 'Duke' (entre 1.76 N para 75B y 1.38 N para 100B+X), pero no para el cv. 'Brigitta', en el que los valores fueron ligeramente más bajos (1.63 vs. 1.31 N entre los estados 75B y 100B+X).

Como se ha mencionado previamente, a nivel visual no es posible distinguir frutos de los estados 100B y 100B+X. En una típica recolección comercial la fruta es cosechada cada 6-10 días, en espera de que se acumule fruta azul, con el fin de optimizar costos de mano de obra, lo que claramente incide en una amplia variación de los frutos en cuanto a la madurez. En el presente estudio, la fruta 100B mostró, de forma consistente, una mayor firmeza respecto de la sobremadura (100B+X), tanto en cosecha como después de almacenamiento, confirmándose la problemática que puede acarrear la presencia de fruta con avanzada madurez en lotes comerciales. Adicionalmente, la relación SS/AT de fruta con 75B (12 aproximadamente) y 100B (cercano a 20) confirman estar en valores cercanos al óptimo propuesto por Galleta et al. (1971); no así la fruta sobremadura que alcanzó ratios > 24.

Dado lo comentado anteriormente, toda la fruta puede aparecer aceptable en cosecha, pero aquellas que se encuentran en estado sobremaduro poseen una alta probabilidad de volverse inaceptables cuando lleguen al consumidor final. Esta potencial heterogeneidad en unidades comerciales será más detrimental en la medida que transcurra mayor tiempo entre cosecha y consumo. Esto revela que la frecuencia de cosecha es de vital importancia para aquellas situaciones donde se planifican envíos a larga distancia y destaca, como lo mencionan Bailey (1974) y Woodruff et al. (1960), el riesgo de tener fruta sobremadura en las unidades de comercialización. Adicionalmente explicaría, en parte, las variadas situaciones de rechazo que se han dado en los mercados de destino en diferentes temporadas de comercialización de arándanos desde Chile (Chilean Blueberry Committee, 2015; Juillerat, 2014).

Considerando los valores de firmeza final y el porcentaje (%) de fruta sana después de almacenamiento, el cv. 'Brigitta' tuvo un comportamiento relativamente homogéneo para los tres estados de madurez; en tanto que para el cv. 'Duke' la fruta sobremadura se diferenció negativamente de aquella cosechada en los estados 75B y 100B. Para ambos cultivares, la fruta del estado 75B logró el 100 % de coloración azul al finalizar el almacenaje, dado que las antocianinas continúan produciéndose después de cosecha (Mitcham, 2007). Esto a su vez implicaría que para cultivares de arándano con ablandamiento rápido, que requieran ser enviados a mercados lejanos, se podría intentar la recolección antes de alcanzar el 100% de coloración azul (El-Agamy, 1982); sin embargo, falta por determinar cómo se verían afectadas las características organolépticas del fruto, ya que en nuestro estudio los frutos cosechados en el estado 75B finalizaron el almacenaje con bajo contenido de SS (13.6% y 12.0%) y alta acidez (1.1% y 0.94%), para 'Duke y 'Brigitta', respectivamente.

Los valores de pérdida de peso de los frutos fueron elevados y variaron en función del cultivar, estado de madurez y uso de bolsa. En la industria de comercialización de arándanos frescos, rangos de 5 a 7 % de pérdida de peso son aceptables para fruta que permanece almacenada durante 3 semanas a 0 °C y 90-95 % HR (Sargent et al., 2006; Paniagua et al., 2014). En nuestro estudio la pérdida de peso estuvo dentro de este rango en el cv. 'Brigitta', no así en el cv. 'Duke', en especial cuando no se utilizó bolsa para su envasado. Diferencias entre cultivares han sido previamente reportadas; así, Alsmairat et al. (2011) encontraron valores de pérdida de peso entre 0.6 y 2.3 % después de 8 semanas en Atmósfera Controlada (AC), y en particular 'Duke' perdió el doble de peso que 'Brigitta'. Rivera et al. (2013) indicaron valores del 2.1 y 3.5 % de pérdida de peso para los cvs. 'Brigitta' y 'O`Neal' después de 45 días a 0 °C. Al utilizar envasado en AM pasiva (MAP) durante el almacenamiento de 'Brigitta', se obtuvo una disminución del % de frutos que mostraban síntomas de deshidratación y presentaban un menor ablandamiento, respecto de los frutos control, a pesar del escaso efecto que tuvo el envasado con film sobre el cambio en la composición de gases en el interior del envase, demostrando que la retención de humedad fue el principal efecto del tratamiento (Moggia et al., 2014). En nuestro estudio los valores de deshidratación para fruta con y sin bolsa fueron de 4.0 vs. 7.8 % para el cv. Brigitta' y de 5.7 vs. 16.6 % para el cv.'Duke'.

Las diferencias en pérdida de peso entre los cultivares podrían estar, en parte, asociadas al tamaño del fruto, ya que la relación superficie/volumen del mismo afecta a su tasa de transpiración (Ben-Yehoshua et al., 1983); efectivamente, frutos de 'Duke' poseían mayor relación superficie/volumen, en especial para frutos de 75B, siendo éstos los que presentaron la mayor deshidratación. Otras posibles causas estarían relacionadas con las características de la cutícula y la cicatriz del fruto como se discutirá más adelante.

- *Diferencias entre campañas: efecto sobre Pardeamiento Interno (PI) y ablandamiento del fruto (Cap.5.3 y 5.5)*

Los ensayos realizados durante dos campañas para el estudio de pardeamiento interno (PI) y variabilidad dentro de la planta revelaron que, independiente del cultivar, ocurrió una alta variabilidad en la firmeza, entre temporadas, para cada una de las fechas de cosecha comercial (Fig. 2 Cap. 5.3; Fig 2. Cap. 5.5). El aspecto más relevante de esta observación proviene del alto porcentaje de fruta blanda que es posible encontrar en una partida de fruta cosechada en una determinada fecha. Ello difiere de otras especies, tales como el manzano, donde fruta muy blanda (58–62 N) no representa más del 0.5 a 0.8 % en una cosecha comercial (Herregods and Goffings, 1993; De Silva et al., 2000).

En oposición, nuestros resultados arrojaron porcentajes muy variables de fruta considerada muy blanda (<1.4 N); así para el ensayo de PI, la proporción de esta categoría fue mayor en el año 1, alcanzando en cada campaña, valores del 25 y 10 % para el cv. "Duke" y del 42 y 5 % para el cv. 'Brigitta', respectivamente.

Los ensayos de variabilidad se realizaron en localidades y campañas diferentes, y la firmeza de los frutos se analizó por distribución de frecuencia acumulada, para fruta recolectada diferencialmente en los estados 100B y 100B+X. Esto permitió confirmar la variabilidad entre años y corroborar que las proporciones de fruta con valores inferiores a 1.4 N, se presentaron principalmente en los frutos de100B+X, representando valores de 8 y 55 % para el cv. 'Duke' y de 19 y 50 % para el cv. 'Brigitta', en los años 1 y 2, respectivamente. Como ha sido discutido previamente, la fruta de menor firmeza fue aquella que permaneció más tiempo en la planta después de alcanzar su coloración azul. Esto vuelve a destacar la importancia del momento y los intervalos de la cosecha

para fruta destinada a almacenaje prolongado. Como se explicará más adelante las campañas también influyeron en la calidad de la fruta según su posición en la planta.

Variaciones climáticas entre campañas podrían explicar parte de las diferencias descritas. Si bien la información es escasa, se ha sugerido un rango ideal de temperatura para el crecimiento de arándanos de arbusto alto entre 20 y 25 °C (Davies y Flore, 1986); en tanto valores sobre 30 °C (que en Chile además se asocian a elevada intensidad lumínica), podrían causar daño a la planta (Trehane, 2004; Lobos y Hancock, 2015), junto a una disminución de la capa cerosa en los frutos, que además tienden a ser de menor tamaño y más blandos (Mainland, 1989). Adicionalmente, fruta blanda también se asocia a patrones de precipitación superiores a los normales en momentos cercanos al momento de la cosecha (Ehlenfeldt y Martin, 2002).

Para las localidades de Longaví (donde se cosechó 'Duke' para los ensayos de PI) y de Santa Bárbara (lugar de procedencia de 'Brigitta' para los mismos ensayos), la mayor proporción de frutos blandos ocurrió en el año 1 (25 y 45 % para 'Duke' y 'Brigitta', respectivamente). El análisis de las condiciones climáticas indica que si bien, en general se dieron temperaturas medias máximas cercanas al rango considerado favorable (20 a 25 °C, Davies y Flore, 1986) desde plena flor (Octubre) hasta cosecha (Diciembre), hubo más presencia de temperaturas extremas dada por mayor número de horas o días en que se superaron los 27, 29 y 32 °C, en especial en el mes de Diciembre, que explicarían un ablandamiento temprano de la fruta (Trehane, 2004; Lobos y Hancock, 2015).

Respecto de los ensayos de variabilidad, realizados en la localidad de Río Claro, en ambas temporadas hubo elevadas temperaturas en las fechas cercanas a cosecha, pero durante el año 2 (mayor proporción de fruta blanda) hubo además, mayor precipitación tanto al inicio de la temporada (Agosto-Septiembre) como durante el período de maduración y cercano a cosecha, lo que podría acrecentar las condiciones de ablandamiento en la fruta (Trehane, 2004; Lobos y Hancock, 2015).

- *Variabilidad dentro de la planta (Cap. 5.5)*

Numerosos estudios demuestran la existencia de fuentes de variación entre plantas y principalmente dentro de una misma planta, que resultan en una calidad heterogénea de la fruta en cosecha (Heinicke, 1966; Jackson, 1967; Robinson et al., 1983; Perring, 1989; Broom et al., 1998; De Silva et al., 2000); sin embargo, no existen trabajos al respecto en el caso de los arándanos.

En la presente Tesis, los resultados para los ensayos de variabilidad dentro de la planta indicaron que, para algunas campañas, existen diferencias en la firmeza de la fruta en función de su ubicación en la planta (Este-Oeste); sin embargo, éstas se harían más evidentes al separar la fruta 100B de aquella 100B+X (situación que no ocurre en una recolección comercial), y analizar su distribución acumulada de frecuencia. Por otra parte estas diferencias serían dependientes de las condiciones climáticas bajo las cuales se desarrolla la fruta en una campaña particular.

Así, en general, las diferencias en firmeza debido a la ubicación de la fruta en la planta fueron menos marcadas que las diferencias entre los estados de madurez, siendo el efecto variable según el año de cosecha. No obstante, cuando ocurrieron diferencias, se observó de forma consistente que fruta proveniente del lado Oriente era más blanda, para ambos estados de madurez. Es interesante señalar que este efecto se observó en el año 1, donde la fruta fue más firme. Es posible pensar que en temporadas con condiciones climáticas extremas (elevadas temperaturas o exceso de precipitaciones en precosecha), el nivel de ablandamiento de la fruta ocurra a nivel global en toda la planta.

Las diferencias detectadas en cuanto a la orientación de la fruta en la planta se pueden explicar, en parte, por las diferencias microclimáticas que ocurren durante el día, las cuales integradas a lo largo de la temporada pueden resultar en fruta con diferente condición y potencial de postcosecha. Para nuestros ensayos la máxima temperatura e iluminación así como la mínima HR ocurrieron entre las 12:00 y las 18:00 h en el lado Poniente (plantación orientada N-S). Si se considera el umbral de 20 a 25 ºC, como óptimo para un normal intercambio gaseoso que permita la síntesis de carbohidratos basado en una respiración normal, una demanda atmosférica por sobre estos valores haría que la planta experimente un estrés fisiológico, ya que al poseer un sistema radical carente de pelos radicales, no es capaz de responder lo suficientemente rápido para reponer agua en la parte área

(Chen et al., 2012; Estrada et al., 2015). Esta situación estaría ocurriendo durante la última parte del período de desarrollo y maduración, y en especial pasado el mediodía, cuando el sol ilumina directamente la cara Poniente de la planta, lo que no permitiría explicar la mayor proporción de fruta blanda ocurriendo en el lado Oriente. Otros aspectos no considerados en este estudio pero que podrían ser interesantes, son los cambios a nivel de pared celular, que llevan al desmontaje estructural, y que difieren de lo que ocurre en frutos de otros "berries" (Vicente et al., 2007), o los cambios morfológicos y anatómicos durante el desarrollo del fruto (tal como aumento en el grosor de la capa de ceras y la cutícula) (Konarska, 2015). Muchas de estas características tienen un fuerte componente genético; sin embargo, también dependen de las condiciones medioambientales y la madurez del fruto en cosecha (Connor et al., 2002).

Por otra parte, las condiciones climáticas podrían ser responsables de la diferencias observadas en la evolución de la madurez de los frutos en cada lado de la planta. A lo largo de la temporada de crecimiento de los frutos, siempre hubo mayor proporción de fruta azul en lado Oriente de la planta, coincidente con mayores rendimientos. En futuras investigaciones sería aconsejable realizar mediciones al inicio de la temporada para determinar si el lado Oriente comienza con más yemas florales o flores de mejor calidad que justifiquen una mayor cantidad de fruta en dicho lado. Adicionalmente, la dinámica del proceso de polinización podría justificar la asimetría en productividad. Se ha documentado que las abejas de miel modifican sus visitas en función de los ritmos de producción de néctar (Moore et al., 1989) y que la emisión de volátiles a nivel de flor (atrayentes o repelentes) serían utilizados como información para regular su actividad (Dobson, 2006; Raguso, 2008). Adicionalmente, la cantidad de volátiles emitidos y la producción de néctar varían durante el día y deben coordinarse con los requerimientos de temperatura para el vuelo de las abejas, cuyo umbral está por sobre los 12 a 14 ºC (Rodríguez-Saona, 2011). Basado en esto, se puede pensar que en plantaciones orientadas N-S, la cara Oriente que recibe luz directa en la mañana presentaría condiciones más favorables para la polinización, respecto de la cara Poniente.

6.2. Efecto de la firmeza de los frutos en cosecha sobre el desarrollo de Pardeamietno Interno (PI) (Cap. 5.3)

La información existente en relación al desarrollo de PI, derivado de daños por impacto en frutos (Brown et al., 1996; Yu et al., 2014; Xu et al., 2015), no considera la gran variabilidad en firmeza

que se da en forma natural en las unidades de comercialización ("clamshells"), por lo que se consideró relevante realizar un estudio donde la fruta fuera segregada por firmeza en cosecha.

A pesar de los cuidados en la recolección, igualmente se detectó PI en la medición inicial de la fruta. Según Gołacki et al. (2009) las fuerzas de vibración que ocurren durante el transporte desde la finca a la central de almacenaje son difíciles de evitar y pueden causar ya daños. Los frutos de estos ensayos fueron sometidos a un viaje de 3 h hasta llegar a las dependencias del laboratorio; ello no difiere de un proceso comercial, ya que a menos que un productor posea una línea de selección y embalaje dentro de su finca, es común que la fruta viaje entre 2 y 3 h antes de ser procesada. Esto refuerza la importancia de mantener un extremo cuidado a lo largo de toda la cadena de producción y evidencia que, dependiendo de la temporada, la incidencia basal de PI será variable. En este caso la fruta del cv. 'Duke' fue más homogénea que la del cv. 'Brigitta', tanto en valores iniciales de PI como en valores promedio de firmeza.

La altura de caída seleccionada para los ensayos realizados en esta Tesis proviene de experiencias previas que mostraban un mayor desarrollo de PI con golpes a partir de 32 cm de altura (datos no publicados) y de experiencias previas con el sensor BIRD en numerosas líneas de selección y embalaje; estas líneas constituyen una de las instancias de mayor riesgo de daño para la fruta, donde los puntos de transferencia causantes de potenciales impactos tenían 35-36 cm de altura (Xu et al., 2015). Con el fin de estandarizar las líneas de "packing" y establecer algunas recomendaciones básicas es indispensable identificar cuál es la fruta más propensa al ablandamiento y la predisposición al desarrollo de PI durante la postcosecha. Los resultados indican que el nivel de PI encontrado en frutos de los cvs. 'Duke' y 'Brigitta' varió entre temporadas, cultivar y categoría de firmeza, sugiriendo que fruta de la categoría más blanda correspondería mayoritariamente a un estado 100B+X. De esta forma, cuando los frutos fueron segregados en base a firmeza, aquellos de la categoría blanda presentaron el mayor nivel de PI, en paralelo con mayores valores de SS y SS/AT.

La relevancia de la firmeza en cosecha en el comportamiento postcosecha de arándanos no está bien documentada. Wolfe et al. (1983) concluyeron que la separación por firmeza en cosecha de arándanos cvs. 'Weymouth' y 'Bluecrop', en combinación con un tratamiento de inmersión en agua

caliente, proporcionaba un mejor control de pudriciones en postcosecha, debido a la susceptibilidad diferencial de frutos blandos, medios y firmes. De forma similar, nuestros estudios demuestran que el ablandamiento y el desarrollo de PI están relacionados con la firmeza individual de los frutos en cosecha, y por lo tanto, hay mayor probabilidad de un elevado PI en fruta blanda, de ambos cultivares, después de un almacenaje prolongado. Dado que los mayores valores de PI se encontraron siempre en fruta blanda (< 1.6 N), nuestros resultados refuerzan la idea que son aquellos frutos de firmeza media a firme los que mejor podrán resistir un viaje a mercados distantes. Por lo tanto, es importante generar estrategias orientadas a incrementar el porcentaje de esta fruta en los "clamshells" con el fin de asegurar una mayor y más homogénea calidad en destino.

Una observación apreciable es que golpear los frutos no siempre se tradujo en altos valores de PI, situación que fue más evidente en el cv. 'Duke', con elevadas tasas de ablandamiento, pero escasa diferencia para PI entre frutos golpeados y no golpeados. En efecto, si bien los modelos ajustados presentaron valores significativos de r^2, los valores de pendientes y ordenada en el origen prácticamente no mostraron diferencias entre fruta con y sin golpe. La excepción se presentó en el caso de fruta firme, con tasas similares de cambio en PI por unidad de firmeza, para ambos tratamientos.

En el caso del cv. 'Brigitta' las asociaciones entre firmeza y PI fueron menos consistentes (menor número de instancias con r^2 significativo) revelando una débil relación entre las variables para este cultivar, en especial para frutos provenientes del año 2, donde la fruta resultó ser más homogénea en cosecha. Sin embargo, en este cultivar se esperaría mayor desarrollo de PI en frutos golpeados, independiente de la firmeza en cosecha (dado por las diferencias tanto en ordenada en el origen como en pendiente entre los tratamientos). La mayor parte de las diferencias ocurrió entre fruta firme y fruta blanda, lo que enfatiza los efectos negativos sobre la calidad que se derivan al tener una elevada proporción de fruta blanda en una cosecha particular.

Basado en estos resultados, es esperable que cada cv. de arándano presente un patrón diferente de desarrollo de PI y ablandamiento al ser sometido a daño mecánico por impacto, por cual sería aconsejable repetir estos ensayos en más variedades, con el fin de establecer un rango de

susceptibilidad, de acuerdo a la condición de la fruta en cosecha (en particular según su firmeza inicial).

La evaluación de PI se realiza principalmente a través de la observación de síntomas en el corte ecuatorial de los frutos, para inspeccionar así la superficie de la pulpa dañada. Este procedimiento no considera daños en zonas cercanas al cáliz o al extremo pedicelar y podría de alguna forma estar subestimando la severidad del daño mecánico (Yu et al., 2014).

Dada la susceptibilidad diferencial al ablandamiento y desarrollo de PI, en función de los cultivares y las categorías de firmeza, debiera evitarse fruta con menos de 1.6 N en el momento de la cosecha, para aquellas partidas destinadas a almacenaje prolongado. Por otra parte, los frutos de las categorías firme y media mostraron valores de SS/AT en un rango de 15 a 21, respecto a valores entre 19 y 29 para frutos de la categoría blanda; esto sugiere que el ratio SS/AT podría ser utilizado como un índice adicional para definir el momento óptimo de cosecha y el destino final del producto (breve, medio o prolongado período de almacenaje).

6.3. Rol de la cutícula en el ablandamiento y la deshidratación del fruto (Cap. 5.2)

Se considera que la naturaleza hidrofóbica de la cutícula le confiere a la fruta una barrera efectiva contra la pérdida de agua por transpiración (Lara et al., 2014; Martin and Rose, 2014). En el presente estudio, los frutos del cv. ‘Duke’ eran más firmes y presentaban un contenido levemente mayor de ceras en el momento de la cosecha en comparación con los frutos del cv. ‘Brigitta’. Sin embargo, estas características no resultaron en una mejor condición del fruto después de almacenaje, dado que la proporción de frutos sanos fue considerablemente menor para el cv. ‘Duke’ (< 60% dependiendo del estado de madurez) en comparación con ‘Brigitta’, donde más del 80% de los frutos fue clasificado como sano, para todos los estados de madurez estudiados.

Diversos estudios indican que la composición y estructura de las ceras de la cutícula, más que su contenido total, influenciarían en su permeabilidad al agua (Riederer y Schreiber, 2001; Parsons et al., 2012). Leide et al. (2011) encontraron escasa presencia de *n*-alcanos y aldehídos en las ceras cuticulares, junto a un alto porcentaje de triterpenoides y derivados de esterol, al comparar un cultivar de tomate altamente susceptible a la pérdida de agua, con una especie silvestre más

resistente. Ratios de *n*-alcanos/triterpenoides de 0.18 y 0.33 se asociaron en cutículas de cerezas cvs. 'Celeste' y 'Somerset' a valores de 15.8 y 7.2 % de pérdida de peso, después de 2 semanas de almacenaje refrigerado (Belge et al., 2014a); para melocotones 'October Sun' y 'Jesca', ratios de 0.31 y 0.65 resultaron en valores de 5.6 y 3.9 % de deshidratación (Belge et al., 2014b).

La cutícula de los frutos es considerada relativamente impermeable a los gases, incluyendo el vapor de agua, y está conformada por un grupo de ceras cristalinas (principalmente constituidas por *n*-alcanos) que la cubren y a la vez se embeben en una matriz de material amorfo (esencialmente compuesta de triterpenoides). La difusión de agua ocurriría principalmente a través de la fracción amorfa, en tanto la fracción cristalina prevendría la translocación de agua (Vogg et al., 2004).

En nuestro estudio, se identificaron cuatro triterpenoides que representaban del 35 al 50 % del total de las ceras extraídas de los frutos de arándano: alfa-amirina, lupeol, ácidos ursólico y ácido oleanólico. Las diferencias en composición entre los cvs. 'Duke' y 'Brigitta' se debieron a un mayor contenido de alfa-amirina, así como una mayor relación de lupeol *vs*. ácido oleanólico y ursólico en el cv.'Brigitta'. El ácido ursólico estuvo altamente relacionado con la pérdida de peso y las tasas de ablandamiento de los frutos; así los frutos de 'Duke', que se caracterizaron por un mayor nivel de deterioro en postcosecha, presentaron 2 a 4 veces más contenido de ácido ursólico que los frutos de 'Brigitta'. Por su parte, el ácido oleanólico, que fue más abundante en el cv. 'Brigitta', presentó una correlación inversa con el ablandamiento de la fruta durante el almacenaje.

Otra observación interesante entre cultivares ocurrió respecto del nivel de triterpenoides según el estado de madurez: en el cv. 'Duke' hubo una gran diferencia entre los estados 75B, 100B y 100B +X, en tanto que en el cv. 'Brigitta' los cambios de estos compuestos fueron escasos o inexistentes. Esto explicaría en parte, la mayor tasa de pérdida de agua y las diferencias entre estados de madurez observadas en 'el cv. Duke' después del almacenaje, respecto de los menores valores de deshidratación y limitadas diferencias entre estados encontradas en frutos del cv. 'Brigitta'.

Los estudios de cromatografía revelaron la presencia de una pequeña cantidad de ceras que eluyen al inicio del análisis, que no fueron identificados en este trabajo, pero que eran más abundantes en el cv. 'Brigitta'. Futuros trabajos debieran corroborar si estos compuestos corresponden a *n*-alcanos,

lo que podría ratificar en arándanos el efecto de las relaciones reportadas para otras especies. Otros componentes de la cutícula y la cutina, así como la morfología de la cicatriz del fruto, pueden tener importantes influencias en el comportamiento postcosecha de un cultivar particular, por lo que también debieran ser abordados en el futuro.

6.4. Rol de la cicatriz del arándano en su ablandamiento y deshidratación. (Cap. 5.4)

La pérdida de agua vía transpiración es una causa importante de deterioro de los productos hortofrutícolas frescos (Kader, 2002). Los arándanos se caracterizan por tener una epidermis sin estomas o lenticelas (Gough, 1994), por lo que la pérdida de humedad está restringida a la cutícula y al área de la cicatriz pedicelar. En este ensayo se utilizaron 3 líneas híbridas provenientes de un programa de mejoramiento genético en arándano, eligiendo en cada una de ellas plantas que tenían frutos con tamaños diferenciales de cicatriz (pequeña, mediana y grande), con lo cual se obtuvo 9 líneas o familias para ser usadas en el presente estudio.

Nuestras primeras aproximaciones arrojaron valores de permeabilidad al agua (P_{H2O}) para los frutos enteros de 0.035 a 0.085 μmol m^{-2} s^{-1} Pa^{-1} a 20 °C, similar a lo encontrado para tomates, cuyos valores fluctuaron entre 0.050 y 0.085 μmol m^{-2} s^{-1} Pa^{-1} a 20 °C (Shirazi and Cameron, 1993). Sin embargo, la permeabilidad de un fruto es en realidad la combinación de la permeabilidad aparente de la cutícula y de la cicatriz, y dado que el mecanismo de difusión desde un poro difiere de aquel de una superficie no-perforada (Brown and Escombe, 1900), los datos del presente estudio fueron diferenciados en permeabilidad para la cutícula (P_{H2O}) por un lado,y en difusión a través de un poro (PD) para la cicatriz por otro lado.

Si bien en la selección de cultivares comerciales para consumo fresco, se buscan frutos de mayor tamaño y pequeña cicatriz, nuestros resultados muestran que los frutos más grandes estuvieron asociados a cicatrices más grandes, en todas las familias estudiadas, acorde con lo reportado por Galleta y Ballington (1996). Sin embargo, Parra et al. (2007) encontraron que diversos cultivares comerciales con frutos de tamaño mediano a grande poseían cicatrices pequeñas o medianas, en tanto otros cultivares de fruto pequeño presentaban cicatrices relativamente grandes. Además, la relación entre diámetro de fruto y diámetro de la cicatriz para un híbrido determinado, variaba entre temporadas.

Existe consenso en que una cicatriz pequeña es deseable, sin embargo, es interesante observar que nuestros análisis muestran que la PD de la cicatriz aumenta cuando el área de la cicatriz decrece, sugiriendo ello que el beneficio de seleccionar una cicatriz pequeña puede ser menor al que se le confiere. Este resultado concuerda con los hallazgos de Brown y Escombe (1900), quienes fueron los primeros en describir el mecanismo de aumento en la permeabilidad por disminución del diámetro de un poro. Ellos determinaron que la pérdida de agua a través de un poro se incrementa de forma lineal con el diámetro del poro, y no con el área, derivando las ecuaciones para describir esta relación.

De forma excepcional, se comprobó que fruta de una de las líneas, con cicatriz pequeña, presentó una relación diferente entre el diámetro de la cicatriz y la tasa de pérdida de agua, resultando 4 veces menor que aquella de las otras 8 líneas en estudio. Este caso particular sugiere que hay otros factores, no relacionados con el área de la cicatriz, que podrían estar afectando la pérdida de agua, como podría ser una oclusión a nivel de cicatriz por colapso o lignificación de los tejidos en la zona de abscisión.

En fruta mantenida bajo almacenaje refrigerado (0 °C), las pérdidas de agua a través de la cutícula fluctuaron entre 0.024 y 0.041 μg s^{-1}; en tanto para la cicatriz los valores estuvieron en un rango de 0.019 y 0.032 μg s^{-1}, arrojando un promedio para la relación cutícula/cicatriz de 1.2. Las pérdidas atribuidas a la cutícula a 20 °C variaron entre 0.211 y 0.316 μg s^{-1} y las de la cicatriz entre 0.015 y 0.185 μg s^{-1}, obteniéndose una relación cutícula/cicatriz de aproximadamente 9. Esto sugiere que la cutícula es una ruta más importante de pérdida de humedad a temperaturas elevadas y que, por el contrario, la influencia de la cicatriz aumenta cuando la temperatura desciende. Considerando que en la práctica de postcosecha los arándanos se manejan bajo almacenaje refrigerado, sería beneficioso alcanzar un mejor entendimiento sobre los mecanismos que pueden limitar la pérdida de agua a través de la cicatriz. Los valores de pérdida de agua por la cicatriz no difieren de aquellos encontrados para otras especies, en donde el cáliz o la cicatriz pedicelar/peduncular contribuyen a la pérdida de humedad. Así en tomates al menos la mitad del agua se pierde por la cicatriz peduncular (Cameron y Yang, 1982; Ehret and Ho, 1986). En frutos grandes de berenjena, un 65 % de la pérdida total de agua ocurrió por el cáliz, que cubre cerca del 10 % de la superficie del fruto (Díaz-Pérez, 1998). En nuestro estudio, dependiendo de la familia y el tamaño de la cicatriz, el área de

ésta última representaba 0.19 a 0.74 % del área total del fruto; sin embargo, fue responsable del 39 a 67 % de su pérdida total de agua.

El tamaño de la cicatriz influenció la firmeza final y la pérdida de agua en todas las familias estudiadas; frutos con el mayor tamaño de cicatriz resultaron tener los valores más bajos de firmeza, en tanto frutos con cicatriz mediana y grande fueron los de mayor pérdida de peso.

La transpiración, expresada como porcentaje (%) de pérdida de agua por día, puede verse afectada por el tamaño o forma del fruto (Burton, 1982). Para aquellos con una forma cercana a una esfera, como los arándanos, hay una reducción en la relación superficie/volumen en la medida que el fruto aumenta de tamaño (Ben-Yehoshua y Rodov, 2002), lo que explicaría las correlaciones negativas entre la tasa de pérdida de agua y el diámetro, superficie y peso del fruto. Por su parte, la P_{H2O} (µmol m^{-2} s^{-1} Pa^{-1}) de la cutícula, que se expresa en base a la superficie, decreció a medida que el fruto incrementó su tamaño, sugiriendo que hay otros mecanismos, aparte de la relación superficie/ volumen, que reducen la transpiración cuticular en frutos de mayor tamaño. La naturaleza de este mecanismo no está bien estudiada, pero debiera estar vinculada al proceso de desarrollo y características composicionales y estructurales de la cutícula.

Dado que una gran proporción de la pérdida de agua ocurrió a través de la cutícula del fruto, la caracterización de sus propiedades debiera ser incluida en los programas de selección varietal para nuevos cultivares con mayor potencial de almacenaje refrigerado.

Como se discutió en la sección 6.3 numerosos estudios han demostrado la asociación entre las composición y estructura de las ceras y las propiedades de transpiración para una amplia gama de especies (Banaras et al., 1994;Vogg et al., 2004; Leide et al., 2011; Parsons et al., 2012; Belge et al., 2014a y 2014b; Lara et al., 2015), incluyendo el arándano (Moggia et al., 2016).

La tasa absoluta de pérdida de agua fue aproximadamente 10 veces mayor a 20 °C que a 0 °C. Esto se explica en parte por las diferencias en déficit de presión de vapor de agua (DPVA) resultantes en ambos ambientes. A 20 °C, la HR fue de 65 %, originando un DPVA de 0.820 kPa, asumiendo que

la atmósfera interna del fruto está saturada con vapor de agua. A 0 °C y 88 % HR se genera un DPVA de 0.073 kPa, que es aproximadamente 1/10 de aquel déficit a 20 °C.

La reducción en la tasa de pérdida de agua fue un factor importante en la retención de firmeza y bajo nivel de deshidratación en los frutos a 0 °C, en comparación con aquellos otros mantenidos a 20 °C. Producto de esto, la reducción en transpiración a través del sellado de la cicatriz fue menos efectiva a 0 °C que a 20 °C. Los frutos cuya cicatriz fue sellada tuvieron < 3 % de pérdida de peso y los no sellados < 5 %, después de 15 días a 0 ºC. Esta pérdida de agua está dentro de los límites aceptables para la industria de los arándanos, que considera permisible un 5 a 7 % de pérdida de peso en un lapso de 3 semanas a 0 ºC (Paniagua et al., 2014). Por otra parte, la alta tasa de pérdida de agua experimentada a 20 °C resalta la importancia y necesidad de un enfriamiento rápido de la fruta después de cosecha y el mantenimiento de la cadena de frío durante todo el proceso postcosecha hasta la llegada al consumidor.

Es importante considerar que los valores de pérdida de agua reportados en este estudio corresponden a frutos individuales, que se mantuvieron separados unos de otros, a diferencia de lo que ocurre en un proceso comercial donde los frutos se envasan conjuntamente en "clamshells". Esto podría generar alguna diferencia en las cifras calculadas para cutícula y cicatriz, con respecto a otros estudios. En efecto, ensayos realizados con frutos de otros híbridos del mismo programa de mejoramiento, que fueron almacenados en "clamshells", presentaron valores del 1.2 y 1.8 % de pérdida de peso (frutos con cicatriz sellada y frutos no sellados, respectivamente), después de 15 días a 0 ºC y 88 % HR (datos no mostrados). Por lo tanto, sellar la cicatriz redujo la pérdida de peso en más de un 30 % para fruta almacenada en "clamshells", mientras que la reducción en frutos individuales fue de un 46 % en promedio. Los valores similares encontrados en ambas condiciones, validan la recomendación de seguir realizando esfuerzos en estudios futuros que permitan minimizar la pérdida de agua a través de la cicatriz.

Finalmente, es importante ahondar en los estudios que permitan una mayor comprensión del impacto de la cutícula y la cicatriz, tanto en composición como estructura, sobre la pérdida de agua del fruto de arándano.

6.5. Estrategias y recomendaciones para el manejo postcosecha de arándanos

La presente Tesis, aparte de obtener nuevos conocimientos científicos acerca de la fisiología y calidad postcosecha de los arándanos, pretendía desde el inicio aportar una serie de recomendaciones prácticas que fueran útiles para el manejo por parte del sector productor y comercializador de dichos frutos.

En primer lugar, cabría señalar que, dada la alta variabilidad detectada en la firmeza de los frutos en cada cosecha comercial, y con el fin de mejorar la homogeneidad en las unidades de comercialización, sería recomendable realizar seguimientos periódicos de la fruta durante el período precosecha, para definir diferencias entre cultivares y determinar con mayor precisión la necesidad de acortar el intervalo entre cosechas. Esto parece ser más relevante para aquellos cultivares que desarrollan una mayor diferencia de calidad entre los estados de madurez 100 y 100+X.

Por otra parte, una estrategia que parece razonable es corroborar la calidad de la fruta, en función de su orientación en la planta. De comprobarse diferencias, que podrían ser tanto de rendimiento como de calidad, se podrían cosechar diferencialmente los frutos de cada lado de la planta, asignándoles un determinado potencial de almacenaje acorde a sus características, principalmente de firmeza (comercialización rápida *vs.* mercados distantes). Por ello, la industria de arándanos para consumo fresco debería incorporar la medición instrumental de la firmeza en los frutos, como un parámetro objetivo de la calidad y madurez de los mismos, que permita estandarizar la condición o el estado de las distintas partidas de fruta destinadas a ser almacenadas en frío y/o a ser transportadas a mercados distantes, si se desea que su potencial postcosecha sea adecuado y homogéneo; ello ha de permitir hacer llegar fruta con excelente calidad al consumidor final.

Además, para minimizar los daños de pardeamiento que habitualmente pueden producirse en estos frutos a causa de daños mecánicos, se puede deducir de la presente Tesis la recomendación de aplicar un manejo muy cuidadoso tanto en el momento de la cosecha en campo como en el posterior procesado en las líneas de selección y clasificación en las centrales. A este nivel también se hace relevante conocer las diferencias varietales y evitar fruta de baja firmeza que es la de mayor susceptibilidad a los daños.

Con respecto a evitar la deshidratación de los arándanos, se comprueba en la presente Tesis la importancia del manejo de baja temperatura después de la cosecha y la mantención de la cadena de frío durante todo el período postcosecha, así como la conveniencia del uso de coberturas plásticas, que aporten una cierta barrera a la difusión del vapor de agua. Ambas estrategias de manejo permitirían además disminuir la tasa de ablandamiento durante el almacenaje refrigerado de los frutos.

6.6. Futuras investigaciones

Los estudios realizados en esta Tesis constituyen el primer reporte que caracteriza la composición de las ceras en arándanos; en ese sentido nuestros resultados sugieren que la fracción de triterpenoides en esas ceras tiene un rol potencial sobre el comportamiento postcosecha. Futuras investigaciones debieran ahondar en definir estas diferencias y sus implicaciones para la calidad de la fruta en postcosecha, tanto para distintos cultivares como para diversos estados de madurez del fruto en el momento de la cosecha. Al respecto, se dispone de resultados preliminares derivados de una segunda temporada de ensayos, donde se analizaron además de los triterpenoides, los componentes de cutina de ambos cultivares estudiados, así como las características de grosor de la cutícula y morfología de las células epidermales del fruto en el momento de cosecha.

Dada la alta variabilidad encontrada en función de las condiciones climáticas propias de cada año, sería interesante profundizar en los cambios que pueda sufrir la fruta a causa de la influencia de dichas condiciones, generando eventos controlados de temperaturas extremas, precipitaciones o daños mecánicos.

Por otra parte, se pretende continuar con los estudios a nivel de cicatriz pedicelar en el fruto de arándano, para mejorar el conocimiento de su morfología y su rol en la deshidratación y ablandamiento del fruto en postcosecha. Al respecto, estamos desarrollando actualmente estudios para definir el efecto de diferentes niveles de humedad relativa en las cámaras frigoríficas sobre éstos parámetros.

La alta variabilidad encontrada en los frutos de arándano, en especial, en relación a su firmeza en el momento de la cosecha, indica la necesidad de ampliar los estudios a un mayor número de

cultivares y sugiere, para ello, mantener la segregación por firmeza de los frutos en cosecha. Sería interesante abordar la obtención de modelos aptos para predecir el ritmo y extensión del ablandamiento del fruto a lo largo de toda la cadena desde el campo hasta el consumo.

Finalmente, se hace necesario profundizar en el conocimiento de los otros parámetros y cambios que suceden a lo largo de toda la etapa del desarrollo y maduración del fruto del arándano; especial énfasis se debiera dar a entender los cambios de color de la epidermis y el rol del etileno en la maduración de esta especie, aspecto que sigue en cierta controversia.

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7. CONCLUSIONES

VARIABILIDAD DE LOS FRUTOS EN COSECHA Y POSTCOSECHA EN FUNCIÓN DEL ESTADO DE DESARROLLO, CULTIVAR, MADUREZ Y AÑOS.

1. Durante su desarrollo en campo los frutos del cv. 'Duke' exhibieron una mayor tasa respiratoria y una mayor producción de etileno que los frutos del cv. 'Brigitta', resultando ello en una menor calidad postcosecha de dichos frutos.

2. Los frutos de ambos cultivares presentaron dos disminuciones importantes de la firmeza, a lo largo del desarrollo del fruto, siendo la primera de mayor magnitud (más del 30 % respecto del valor inicial) y coincidente con el máximo de la tasa de respiración.

3. Para ambos cultivares se corroboró una alta variabilidad de los frutos en cuanto a su firmeza en cosecha, con proporciones de fruta muy blanda (< 1.4 N), que variaron entre 5 y 50 %; esto ocurrió entre campañas, entre cosechas de una misma campaña y entre estados de madurez dentro de una misma cosecha.

4. Para ambos cultivares, a pesar de no existir diferencias visuales o instrumentales del color de la epidermis entre los estados de madurez 100B y 100B+X en cosecha, los frutos del estado 100B+X presentaron, de forma consistente, un mayor ablandamiento y una menor calidad después del posterior almacenaje refrigerado.

5. Los frutos del cv. 'Duke' fueron más firmes en el momento de la cosecha; sin embargo, presentaron un mayor ablandamiento durante el posterior almacenaje, con valores de 32, 25 y 18 % de pérdida de firmeza después de 45 días a 0 °C, para los estados 75B, 100B y 100B+X, respectivamente. Para frutos del cv. 'Brigitta' las disminuciones de firmeza fueron de 4, 0.5 y 19 % para los mismos estados y condiciones de almacenaje.

6. Para ambos cultivares, la menor tasa de ablandamiento en postcosecha se observó en frutos que presentaron una relación SS/AT menor a 21 en el momento de la cosecha.

7. El uso de una bolsa plástica macroperforada en el almacenaje de los frutos benefició la calidad final de los mismos, permitiendo ello una menor pérdida de peso y una mayor retención de la firmeza inicial, siendo estos efectos más marcados en 'Duke'.

8. La mayor proporción de frutos blandos en cosecha ocurrió, para ambos cultivares, en huertos y campañas con temperaturas máximas extremas en el ambiente (> 27-32 °C) durante la última fase de maduración de los frutos (Diciembre) ó en huertos y campañas en los que, junto con temperaturas elevadas, se presentaron eventos de precipitaciones al inicio del periodo de crecimiento (Agosto-Septiembre) y durante el posterior período de maduración de los frutos (Diciembre).

9. El efecto de la ubicación de los frutos en la planta (Oriente vs. Poniente) sobre la firmeza en cosecha fue menos marcado que las diferencias entre estados de madurez (100B *vs.* 100B+X), siendo variable para cada campaña. Cuando se produjeron diferencias, las cosechas provenientes del lado Oriente presentaron mayor proporción de fruta blanda que aquellas del lado Poniente. Sin embargo, esta diferencia no siempre se mantuvo durante el posterior almacenaje refrigerado.

10. Para ambos cultivares, el lado Oriente de la planta produjo mayor proporción de frutos con epidermis de color azul al inicio del periodo de crecimiento (80 % en el lado Oriente vs. 20 % en el lado Poniente). También se obtuvo mayor productividad total (4.2 vs 3.1 kg por planta en 'Duke' y 3.6 vs 2.2 kg por planta en 'Brigitta') para los lados Oriente y Poniente, respectivamente.

EFECTO DE LA FIRMEZA EN COSECHA SOBRE EL DESARROLLO DE DAÑOS POR PARDEAMIENTO INTERNO (PI)

11. El nivel de PI basal de los frutos así como su firmeza en cosecha variaron entre cultivares y campañas; los frutos del cv. 'Duke' exhibieron valores promedios de 0.15 y 0.19 (en escala de 0 a 4) de PI, así como porcentajes del 10 y 25 % de fruta con firmeza < 1.4 N, en las campañas 1 y 2, respectivamente. Para los frutos del cv. 'Brigitta' estas cifras fueron de 0.10 a 1.01 para PI y de 42 y 5 % para fruta < 1.4 N, respectivamente.

12. El ablandamiento y el desarrollo de PI dependieron fuertemente de la firmeza de los frutos en cosecha, resultando frutos blandos (< 1.6 N) y firmes (1.8 -2.0 N) aquellos con mayor y menor daño PI, respectivamente.

13. Al final del almacenaje, los frutos del cv. 'Duke' presentaron mayores tasas de ablandamiento (39.8, 33.6 y 36.8 %, para fruta de las categorías blanda, media y firme) y mayor asociación entre firmeza y desarrollo de PI, a la vez que escasas diferencias entre frutos golpeados y no golpeados.

14. Los frutos del cv. 'Brigitta' tuvieron una menor tasa de ablandamiento (17.3, 24.4 y 23.8 % para las categorías blanda, media y firme) y escasa relación entre firmeza y desarrollo de PI en almacenaje (en especial para fruta no golpeada); sin embargo, se exhibieron marcadas diferencias entre fruta golpeada y no golpeada.

15. Los resultados de estos ensayos sugieren que fruta con menos de 1.6 N en el momento de la cosecha debieran evitarse para almacenaje prolongado.

ROL DE LA CUTÍCULA EN EL ABLANDAMIENTO Y LA DESHIDRATACIÓN DE LOS FRUTOS

16. En la cutícula de los frutos de arándano se identificaron cuatro triterpenoides, alfa-amirina, lupeol, ácido ursólico y ácido oleanólico que, dependiendo del cultivar y del estado de madurez en cosecha, representaban entre 35 y 50% del total de ceras extraídas.

17. El principal triterpenoide identificado fue lupeol, cuya concentración en frutos de 'Duke' se incrementó de 1.16 a 2.03 g m^{-2} entre los estados 75B y 100B+X, mientras que en frutos de 'Brigitta' fluctuó entre 0.35 y 1.41 g m^{-2}.

18. El contenido de ácido oleanólico promedió 0.37 g m^{-2} en frutos del cv. 'Brigitta', sin diferencias entre estados de madurez, y duplicando los niveles encontrados en frutos del cv. 'Duke'.

19. Los frutos del cv. 'Duke' presentaban 2 a 7 veces más contenido de ácido ursólico que los del cv. 'Brigitta'; sin embargo, dichos contenidos decrecieron con el avance de la madurez del fruto.

20. La pérdida de peso de los frutos después de almacenaje (expresada como % o % m^{-2}) mostró asociaciones significativas con el contenido de ácido ursólico en cosecha (r=0.96 y 0.95, respectivamente).

21. El ablandamiento final de los frutos se correlacionó negativamente con el contenido de ácido oleanólico en cosecha (r= -0.83) y positivamente con el contenido de ácido ursólico en cosecha (r = 0.95).

ROL DE LA CICATRIZ EN EL ABLANDAMIENTO Y LA DESHIDRATACIÓN DEL FRUTO

22. La cicatriz pedicelar del fruto, que cubrió entre 0.19 y 0.74 % de la superficie total del mismo, fue responsable de aproximadamente un 40 % de la pérdida de peso de los frutos mantenidos a 20 °C; sin embargo, los porcentajes variaron en forma considerable entre los diferentes híbridos estudiados.

23. En el caso de los frutos mantenidos a 20 °C la permeabilidad al agua de la cutícula varió el doble, mientras que la permeabilidad aparente de la cicatriz varió el triple entre los 9 híbridos estudiados.

24. La influencia de la cicatriz del fruto en la pérdida de agua de los frutos fue mayor a baja temperatura; la relación entre pérdida de agua a través de la cutícula y pérdida de agua a través de la cicatriz fue en promedio 1.2 para fruta almacenada a 0 °C y de aproximadamente 9 para fruta almacenada a 20 °C.

25. Elevados niveles de deshidratación en los frutos (> 5%) se asociaron con mayor ablandamiento de los mismos, especialmente en aquellos con mayor tamaño de cicatriz.

26. El almacenaje de los frutos a 0 °C redujo la tasa de pérdida de agua de los mismos en un 90 %; sin embargo, la permeabilidad de la cutícula no se vio afectada por la temperatura.

27. El sellado artificial de la cicatriz de los frutos incrementó la retención de firmeza de los mismos mantenidos a 0 y 20 ºC; sin embargo, hubo mayor diferencias de firmeza entre frutos sellados y no sellados a 20 °C. (18 % mayor firmeza) con respecto de aquellos almacenados a 0 °C (6 % mayor firmeza) .