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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

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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 °C, 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.

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 (NeSmith 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, 2002; 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

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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 °C (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 °C, 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 °C, 65% RH) and fruit weight was determined daily for each fruit for 7 d. The remaining half were placed in refrigerated storage (0 °C, 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–6200 g, 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 × 2factorial design considering