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 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

88 in a University of Talca blueberry-breeding program; the planting was established in 2009. For
89 this study three families were selected, having the following female and male parents,
90 respectively: Family 6 (F6; Legacy x Brigitta); Family 16 (F16; Chandler x Legacy) and Family
91 40 (F40; Orus 344 x Legacy). Three plants, each representing a different line, were selected per
92 family based on visual assessments of stem scar size; one line had small-sized stem scars, a
93 second had medium-sized stem scars, and the third had large-sized stem scars (Fig. 1A).
94 Fully ripe fruit with 100 % blue color coverage were hand-picked into plastic clamshells and
95 transported within 30 min of harvest to the laboratory facilities at University of Talca, for
96 treatment establishment.
97
98 *2.2.Experimental set-up*
99 *2.2.1. Experiment 1: Effect of family, scar size and stem scar sealing at room temperature*
100 From each germplasm line, a minimum of 30 fruit was harvested, on December 28th, 2015.
101 Upon arrival at the laboratory, twenty uniform, undamaged fruit were selected per line and each
102 individual berry was measured for scar width, fruit weight, fruit length and width, and fruit
103 firmness. To evaluate contribution of stem scar to fruit transpiration, the scar on half (10) of the
104 berries of each family was sealed with nail polish (Fig. 1B) to permit calculation of water loss
105 via the cuticle and stem scar independently. Fruit were placed into depressions on plastic trays to
106 prevent fruit-to-fruit contact and stored at room temperature in the laboratory (20 ºC, 65 % RH).
107 Fruit weight was determined daily for each fruit over a period of 5 d to determine the rate of
108 weight loss as percent per day and water loss as μg s^{-1}. Average room temperature and relative
109 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: $\text{Area} = (2\pi(DIA/2)^2)(1+((1-(1-((LEN/2)^2/(DIA/2)^2)))/((1-((LEN/2)^2/(DIA/2)^2))^{0.5}))\text{Arctanh}((1-((LEN/2)^2/(DIA/2)^2))^{0.5}))/100$. 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

156 sealing as main factors. Experiment 2 (cold storage at 0 °C) was analyzed for each family as a
157 completely randomized design with scar sealing as the treatment. No direct comparison of fruit
158 held at 0 and 20 ºC was possible because the rate of air movement was much higher at 0ºC. All
159 data were subjected to analysis of variance and means separation done by Tukey´s multiple
160 comparison test ($p \leq 0.05$). Shrivel index was analyzed through a non-parametric mixed ANOVA
161 with aligned rank test (Oliver-Rodriguez and Wang, 2013). Pearson correlation coefficients were
162 calculated to establish associations between apparent stem scar P_{H2O}, cuticle P_{H2O}, PD, percent
163 weight and water loss rate *vs.* fruit characteristics at harvest. All analyses were performed using
164 commercial statistical software (Statgraphics Centurion XVI v.16.0.09).
165
166 **3. Results**
167 *3.1 Experiment 1*
168 *3.1.1 Initial condition*
169 Scar area (mm^2) for the three stem scar area categories (S, M, L) differed for each family,
170 confirming the visual classification made at harvest (Table 1). Fruit from the germplasm lines
171 with a large scar were bigger (greater in weight, length, and diameter) than those having medium
172 or small scar for F6 and F40, but not for F16. The highest firmness values were found for the
173 medium stem scar line from F6 (1.87 N mm^{-1}) and the softest fruit were from the large stem scar
174 line from F40 (1.50 N mm^{-1}). The large stem scar line from F40 had the largest stem scar area
175 (6.29 mm^2) and the small stem scar line from F16 had the smallest stem scar area (1.30 mm^2).
176 When data were pooled together for the three families (Supplementary Table S1), scar area was
177 highly and positively correlated with fruit weight (r=0.86), fruit length (r=0.79), fruit diameter

178 (r=0.83), fruit area (r=0.78) and scar area/fruit area ratio (r=0.95); the association of scar area
179 with firmness at harvest was not significant.

180 *3.1.2 Effect of stem scar and cuticle on water loss at 20 °C*

181 The decline in weight for individual fruit was very linear over the five days of storage. The
182 r^2 for regressions of weight versus time averaged 0.999 (data not shown). The three families
183 differed in the rate of transpiration from the stem scar, with F40 (0.286 μg s^{-1}) having roughly
184 twice as much water loss from the stem scar as F16 (0.140 μg s^{-1}) (Table 2). Small-scar lines had
185 less water loss through the stem scar than the medium- or large-scar lines. There was an
186 interaction for water loss between family and the scar size of the lines within the families. The
187 range in stem scar water loss varied markedly between lines from a low of 0.033 μg s^{-1} for the
188 small-scar line of F16 to 0.330 μg s^{-1} for the large-scar line of F40.

189 Cuticular water loss was also affected by family and stem scar size and there was an
190 interaction between these two factors (Table 2). The range in cuticular water loss varied only
191 1.5-fold between lines from a low of 0.261 μg s^{-1} to 0.325 μg s^{-1}.

192 Pore diffusivity (PD) differed between all families and small-scar lines had lower values than
193 medium- or large-scar lines. The significant interaction between factors revealed that the lowest
194 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
195 of the rest of the lines.

196 The apparent P_{H2O} of the stem scar did not differ between families, but was lower for the
197 large-scar lines than medium- or small-scar lines (Table 2). There was an interaction for P_{H2O}
198 between family and the scar size. The range in the P_{H2O} of the stem scar varied about 2.5-fold
199 between lines, with the small scar line of F16 being about half that of the other lines. Cuticular
200 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

224 correlations differed in sign from F6 and F40 due to the unique behavior of the small-scar line in
225 F16. When regressions were performed for PD *vs*. stem scar diameter for each line, negative
226 slopes were obtained for every line (Supplementary Table S3).

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

231 The rate of weight loss (percent per day) was negatively correlated with fruit diameter, fruit
232 area, and fruit weight for pooled data (r values ranging from -0.54 to -0.57) and for each of the
233 families with the exception of fruit diameter of F16. F16 was the only family for which percent
234 weight loss correlated with scar diameter or scar area.

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

245 *3.2 Experiment 2*

246 *3.2.1 Initial condition*

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

251 *3.2.2 Effect of scar sealing during refrigerated storage*

252 Similar to fruit of Experiment 1, the weight loss for individual fruit was very linear over the
253 15 days of storage, with an average r^2 for regressions of weight vs. time of 0.990 (data not
254 shown). Transpiration via stem scar and via cuticle at 0 ºC was considerably less than that for
255 fruit stored at room temperature (Table 4). The average ratio for cuticle/stem scar transpiration
256 was approximately 1.2 at 0°C; at 20 °C, the average ratio for cuticle/stem scar transpiration was
257 approximately 9. PD was higher at 0 ºC compared to 20 ºC, with the greatest PD in fruit from
258 F16 and F40 at both 0 and 20 °C. Stem scar P_{H2O} was several times higher at 0 ºC than at 20 ºC;
259 the greatest values occurred on F16 and F40 fruit. Cuticle P_{H2O} was 1.2 times higher at 0 ºC *vs.*
260 20 ºC, with the highest value on berries from F16 (0.0518 μmol m^{-2} s^{-1} Pa^{-1}) at 0 °C. The
261 P_{H20ss}/P_{H2Ocut} ratio varied from 295 to 570 for F40 at 0 °C and 27 to 335 at 20 °C.

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

268

269 **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

293 mechanism for the phenomenon of increasing permeance with decreasing pore diameter. They
294 proved that water loss through a pore increased linearly with the pore diameter rather than pore
295 area. In the current study, the rate of moisture loss as a function of stem scar diameter (Fig. 4) is
296 within 10 % of the measurements of Brown and Escombe (1900) for pores in a membrane.
297 Importantly, the small stem scar line from F16 had a distinctly different relationship between
298 stem scar diameter and the rate of water loss. The rate of water loss for this line as a function of
299 stem scar diameter was about $1/4^{th}$ that of the other 8 lines. This suggests physical features not
300 related to stem scar area could affect water loss rates. What this feature may be in the present
301 study is not clear, but possibilities include stem scar occlusion through tissue collapse or
302 lignification. Identification of the factor limiting stem scar moisture loss may be a valuable
303 feature for the selection of shrivel-resistant blueberry lines.
304 The data of Experiment 2 suggests the cuticle is a much more important route of moisture
305 loss at elevated temperatures and that, conversely, the influence of the stem scar on water loss
306 increases as temperature declines. Given that essentially all blueberry fruit are refrigerated when
307 stored or shipped, this finding suggests there would be some benefit to further understanding
308 mechanisms that might limit water loss through the stem scar.
309 The data on water loss through the stem scar is not unlike that found for other fruits in which
310 the calyx or stem scar contributes to moisture loss. For tomato, at least half of fruit water loss
311 occurs through the stem scar/calyx (Cameron, 1982; Ehret and Ho, 1986). For large-size
312 eggplant fruit, 65 % of whole fruit water loss was attributed to the calyx, which covered about 10
313 % of fruit surface area, (Díaz-Perez, 1998). In our study, depending on family and scar size, scar
314 area accounted for 0.19 to 0.74 % of berry total surface and yet the scar released 39-67 % of the

315 water loss of the whole fruit. The high rate of water loss through the blueberry stem scar
316 permitted scar size to negatively influence the firmness of stored fruit.

317 Transpiration expressed as percent loss per day can be affected by fruit size or shape (Burton,
318 1982). For nearly spherical fruit, like blueberries, there is a reduction in the area/mass ratio as
319 fruit increase in size (Ben-Yehoshua et al., 2002). This partially explains the negative
320 correlations between water loss rate *vs.* fruit diameter, fruit area, and fruit weight
321 (Supplementary Table S2). Interestingly, however, the P_{H2O} (μmol m^{-2} s^{-1} Pa^{-1}) of the cuticle,
322 which is expressed on a per area basis, decreased as fruit size increased, suggesting that there is a
323 mechanism that reduces cuticular transpiration for larger fruit. The nature of this mechanism is
324 not clear but may be related to cuticle development.

325 The cuticle is one of the most important plant barriers (Heredia-Guerrero et al., 2014) and
326 one of its main functions is the protection against uncontrolled water loss (Burghartd and
327 Riederer, 2006). A large portion of the total water loss for blueberry fruit was via the cuticle.
328 Thus, cuticular water loss properties should also bear scrutiny in the selection of new cultivars
329 for long-term storage. In this study, calculations of cuticular P_{H2O} revealed a two-fold difference
330 between lines, which is similar to that found for pepper (*Capsicum annuum*) (Lownds et al.,
331 1993).

332 A number of studies on peppers, tomatoes, cherries (*Prunus avium*) and peaches (*Prunus*
333 *persica*), have demonstrated a correlation between wax characteristics (in terms of composition
334 and structure, rather than total wax amount) and transpiration properties of the cuticle, which
335 differ between cultivars, resulting in different water loss rates (Banaras et al., 1994;Vogg et al.,
336 2004; Lleide et al., 2011; Parsons et al., 2012; Belge et al., 2014; Lara et al., 2015). A high
337 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

361 the impact of cuticle and stem scar morphology, structure, and chemical composition on water
362 loss in blueberry are needed to identify the underlying the underlying physical and biological
363 mechanisms controlling water loss to further improve selection for this important trait.
364
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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/fruit 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 a	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)

514 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
515 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
516 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.
517

		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		**	**	**	**	**	**

518 For a given family, scar size, or interaction effect, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$).
519 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

523 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
524 storage (final firmness), percent firmness loss relative to initial firmness (softening), rate of percent weight loss, rate of water loss, and
525 shrivel index (1-3) for three families of blueberry used in Experiment 1.
526

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.

Family	Sealing	Final Firmness ($N\ mm^{-1}$)	Weight loss (percent per day)	Water loss ($\mu g\ s^{-1}$)	Shrivel index
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.

546 Supplementary Table S1. Pearson correlation coefficients (r) between morphometric fruit characteristics at harvest for highbush
547 blueberry families F6, F16, and F40 for Experiment 1.
548

	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 **

549 [z] Correlation coefficient (r); n=9
550 [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

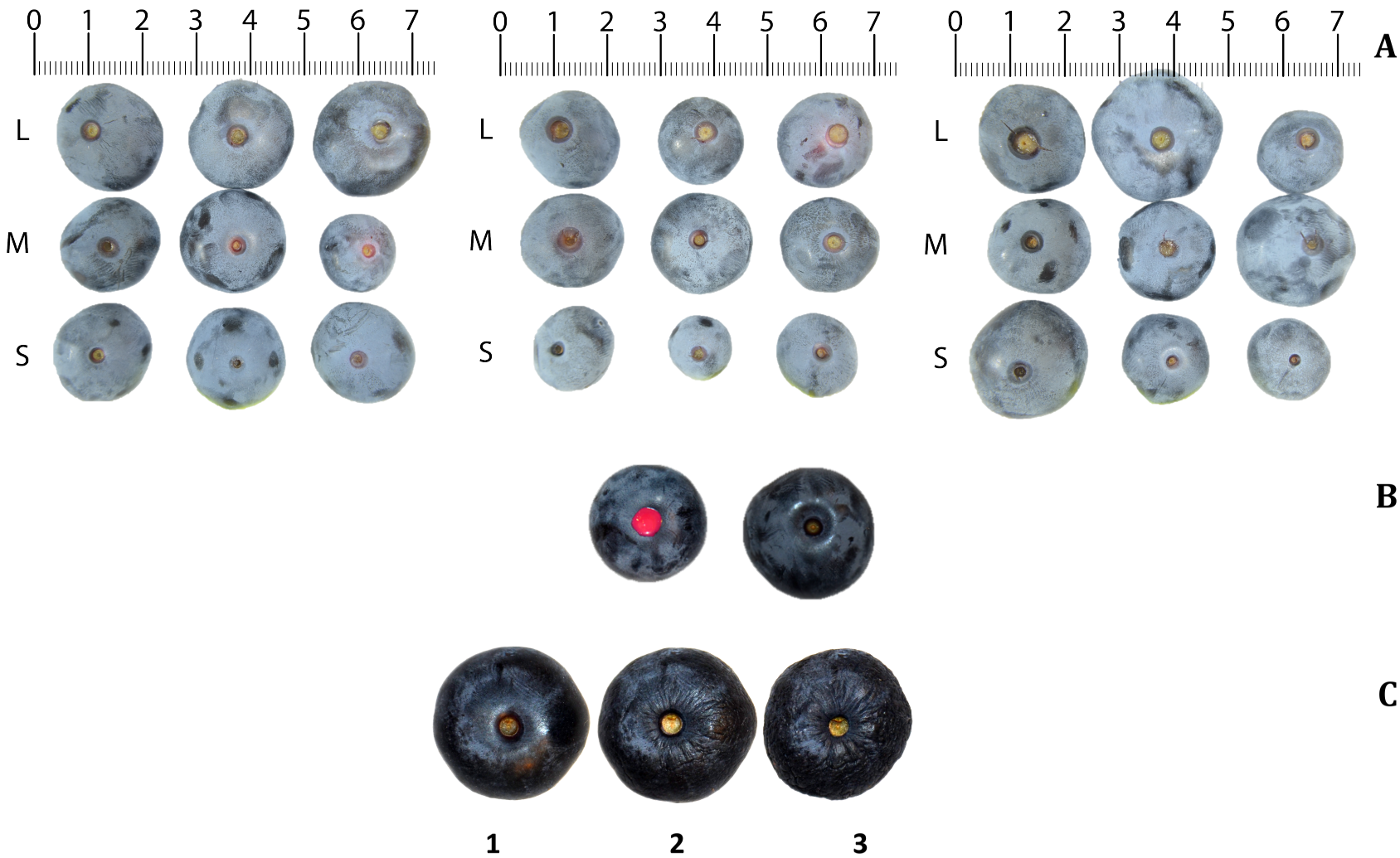

Figure 1: Fruit characteristics of: three representative fruits from F6 (left), F16 (middle) and F40 (right) families, with large (L), medium (M) and small (S) scars. The scales provided are in cm with mm divisions (**A**); sealed (left) vs. non-sealed (right) blueberry (**B**); the scale used for shrivel 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).

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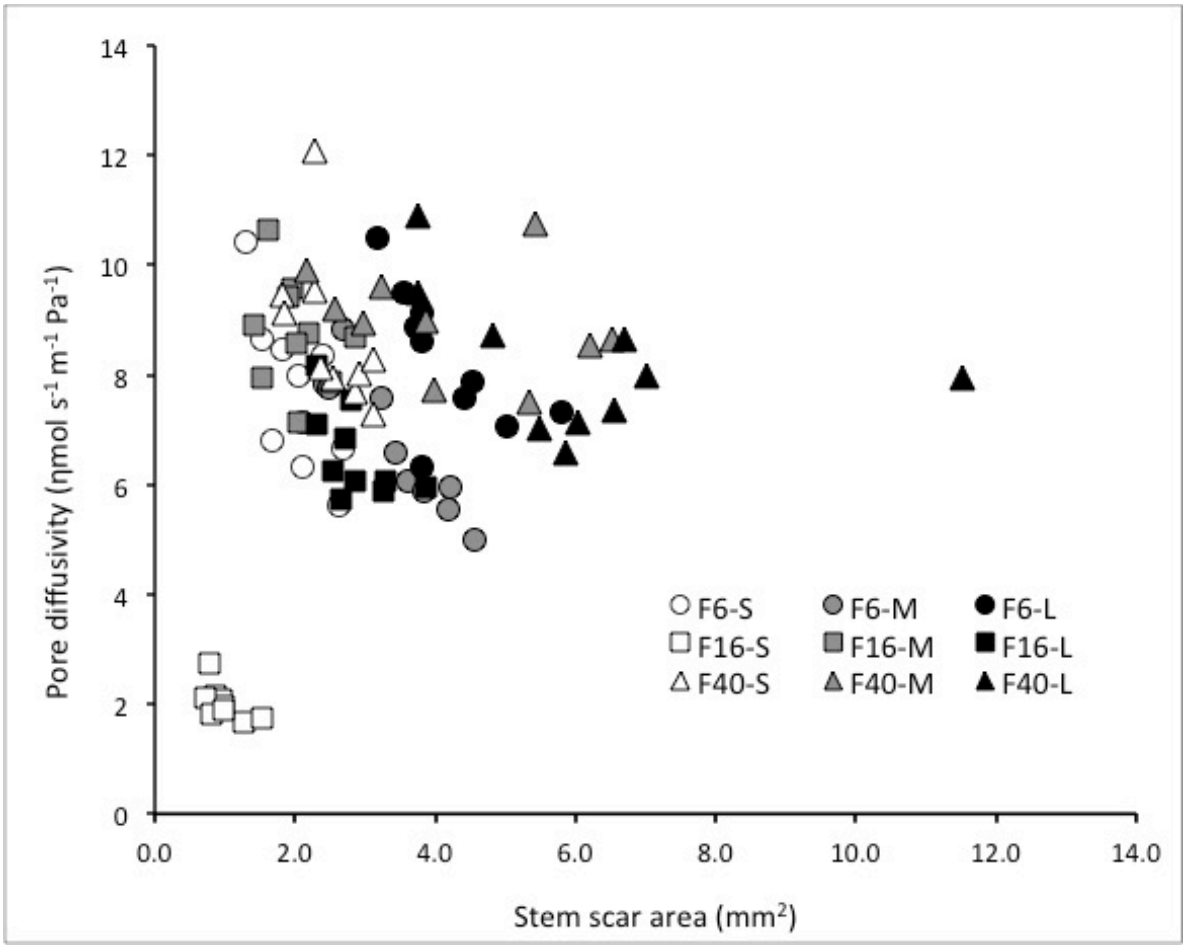

568
569 Figure 2. Relationship between stem scar area and the pore diffusivity of the stem scar for
570 highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a
571 single berry from one of three lines having small, medium and large stem scars (S, M, and
572 L, respectively) from each of three families (F6, F16, F40) in the blueberry germplasm
573 repository at the University of Talca, Chile.
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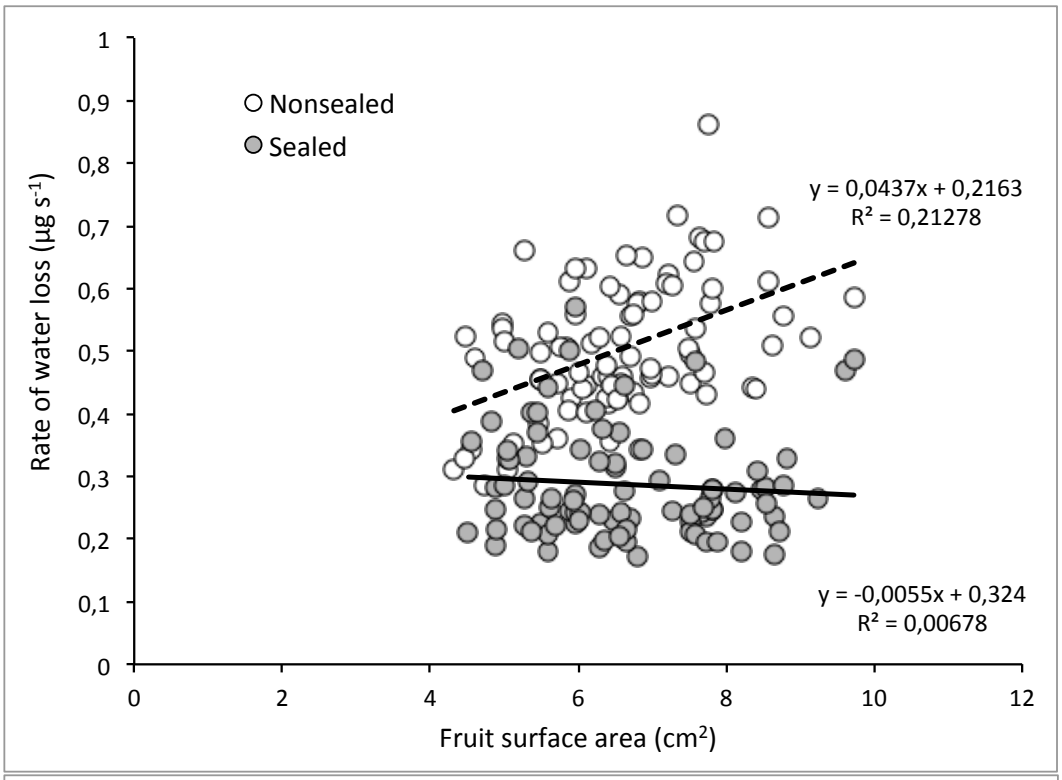

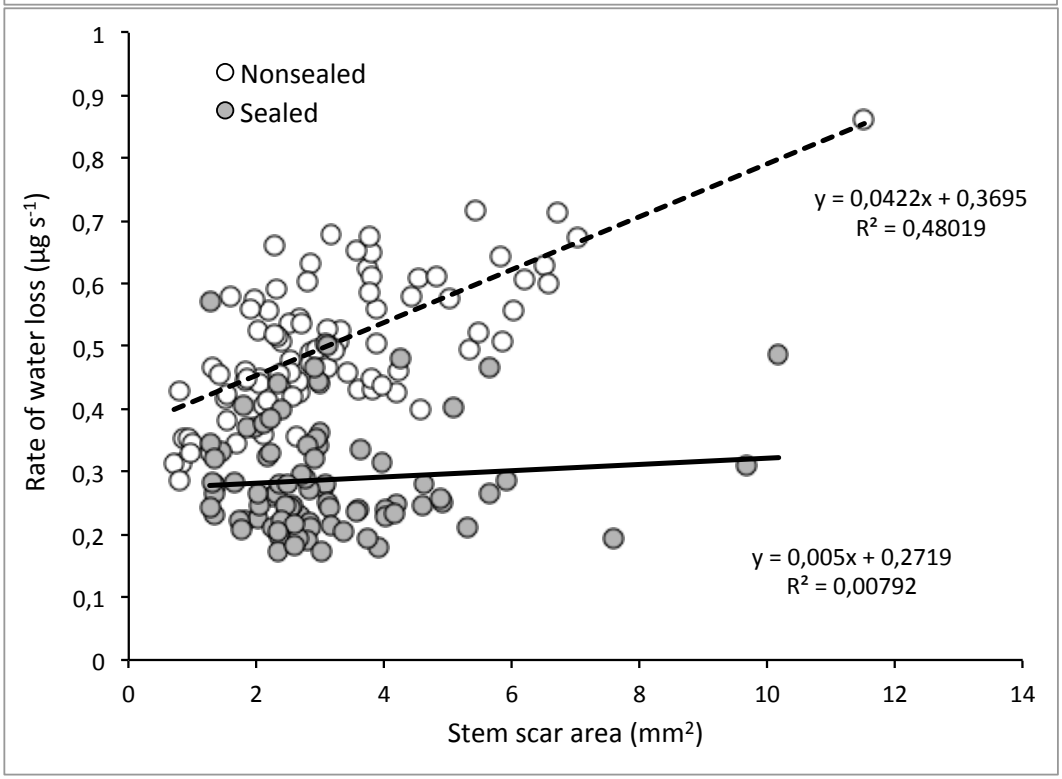

Figure 3. Relationship between fruit surface area (**A**) or stem scar area (**B**) and the absolute rate of moisture loss for highbush blueberry fruit with sealed or non-sealed stem scars when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines (10 fruit per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

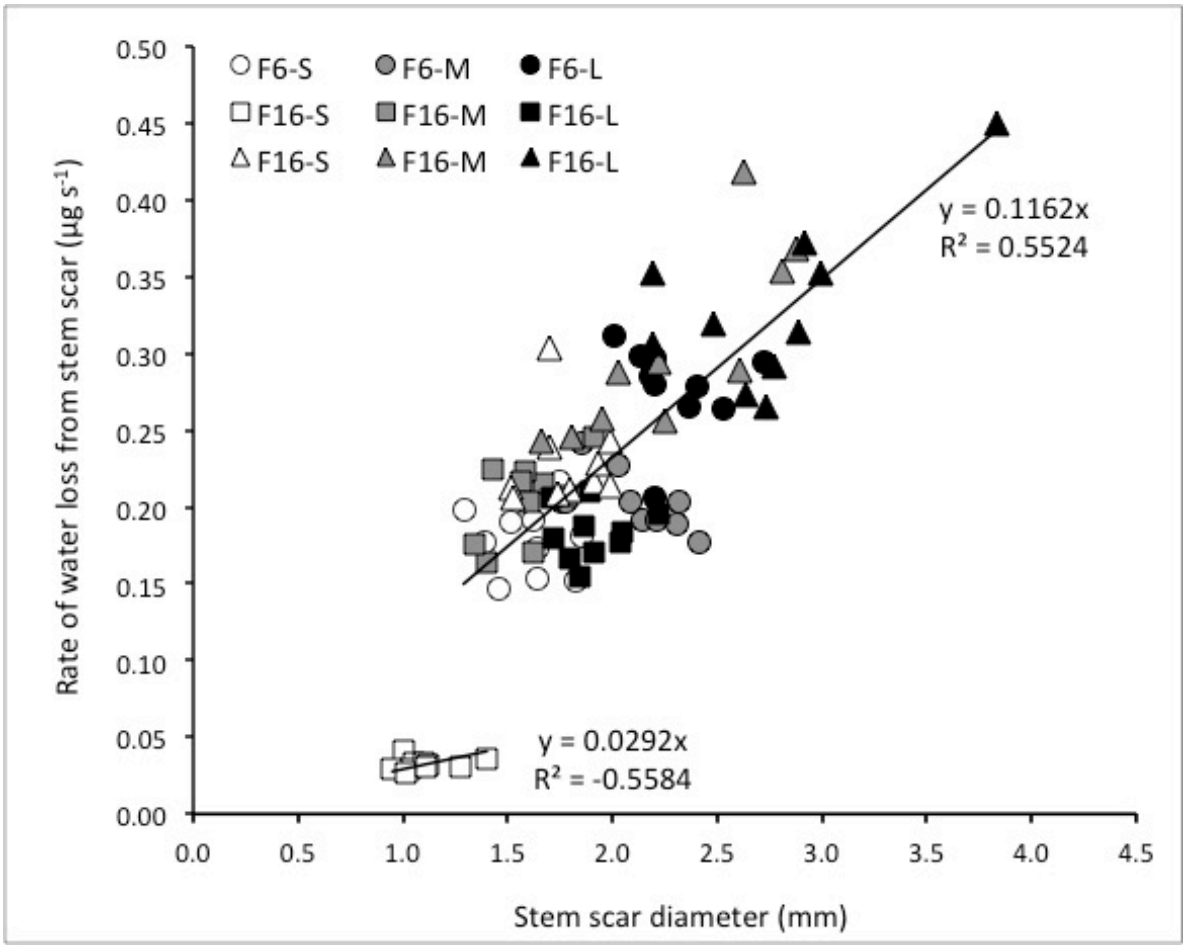

Figure 4. Relationship between stem scar diameter and the absolute water loss rate from the stem scar for highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines having small, medium and large stem scars (S, M, and L, respectively)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

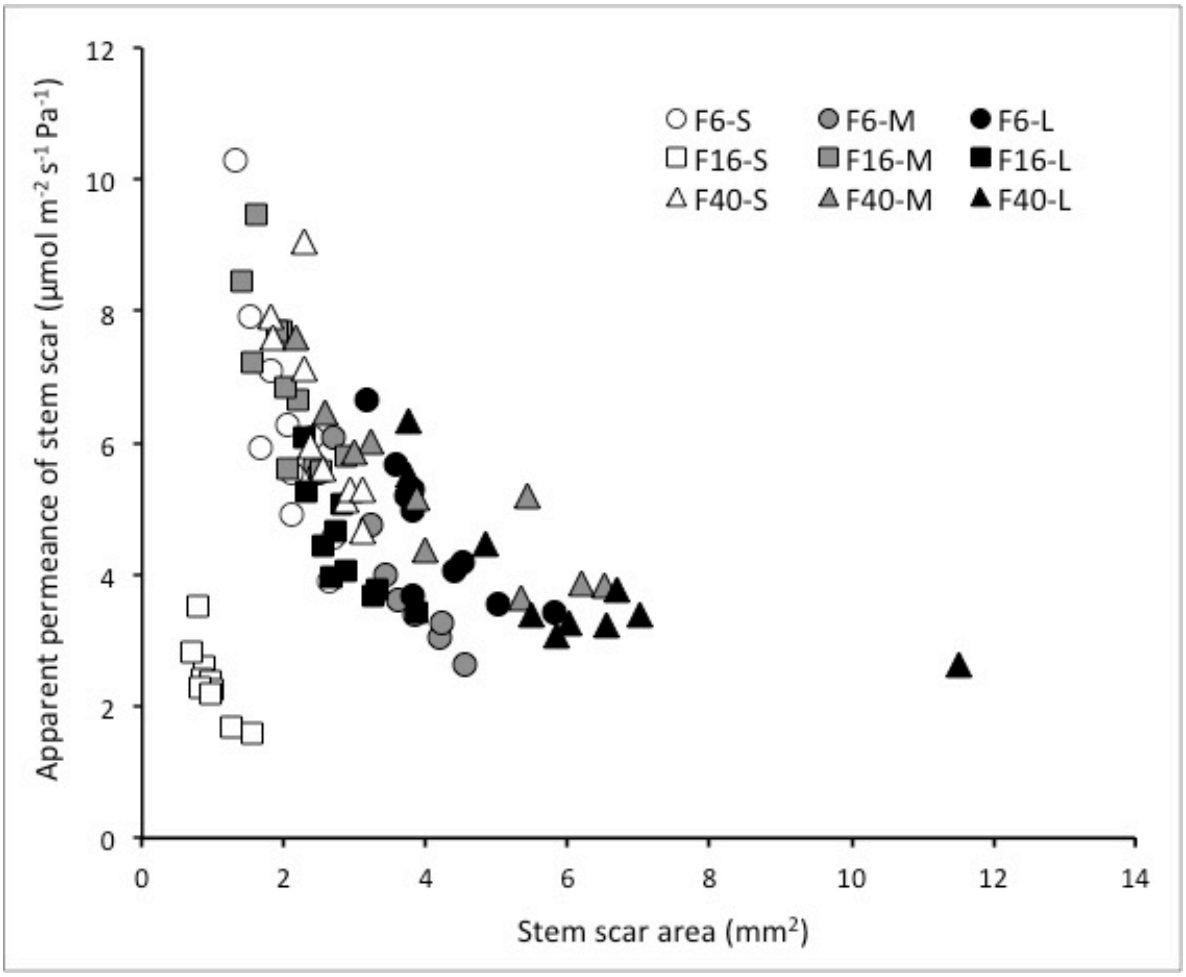

Figure S1. Relationship between stem scar area and the apparent permeance of the stem scar for highbush blueberry fruit when held at 20 °C for 5 d at 65 % RH. Each point represents a single berry from one of three lines having small, medium and large stem scars (S, M, and L, respectively) from each of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