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3	A simple allometric model for estimating blueberry fruit weight from diameter measurements
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27 Abstract. Monitoring of fruit growth is a measurement widely used in physiology and agronomy 28 studies. This is normally done from detached fruits, which can lead to erroneous results when fruits grow asynchronously in clusters as occurs in blueberry plants (Vaccinium spp.). The aim of this 29 communication is to develop a simple allometric model for estimating blueberry weight from 30 diameter measurements. In three growth seasons, weight and diameter of a total of 416 fruits were 31 measured from four field-grown blueberry cultivars. The obtained data set was then randomly 32 divided resulting in 200 data for calibrating model and 216 for validating. Prior to calibration 33 procedure, the use of cultivar-specific models or a common model for four cultivars was evaluated 34 35 by Analysis of Covariance between logarithm transformed diameter and cultivar/season variables. 36 From analysis, no interactive effect was observed, so a common allometric model was constructed, 37 giving a power functional relationship between berry diameter and weight. The allometric model 38 was validated with the independent data, giving errors ranging from 4.7% to 6.9%. Residuals showed a random dispersion pattern. Our results showed that a common model was adequate for 39 40 providing a reliable means of estimating blueberry fruit weight of different cultivars. 41

42 Key words: Regression, validation, non-destructive methods

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53 1. Introduction

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Monitoring of fruit growth is a measurement widely used in studies of physiology and evaluation of 55 management practices (e.g. Godoy et al., 2008; Minchin et al., 2010). Thus, its knowledge has been 56 an important parameter in understanding source-sink relationships, water and nutrient use 57 efficiency, crop quality and yield potential (Génard and Lescourret, 2004, Grossman and DeJong, 58 59 1995, Spreer et al., 2009). Habitually, fruit growth curves are developed from detached samples; however, this method can increase variance and lead to erroneous results for species in which fruits 60 grow in clusters (Coombe, 1976). This is because several development stages of the fruit can be 61 62 observed on a plant at a given time, as occur typically in several fruits species including blueberry (e.g. Tuccio et al., 2011; Godoy et al., 2008). A common method for non-destructively estimating 63 biomass of plant organs is to fit simple equations to data relating biomass to some dimensional 64 characteristic of the plant such as diameter, height, length and width (e.g. Antunes et al., 2008; 65 66 Roxburgh et al., 2015). In this line, estimating fruit weight from equations using simple measurements of fruit diameter can represent an inexpensive and rapid alternative for accurately 67 68 assessing fruit weight. In blueberry industry, fruit segregation on the basis of weight and size, is 69 crucial to obtain higher export market value. The aim of this communication is to develop a simple 70 allometric model for estimating blueberry weight from diameter measurements.

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72 2. Materials and methods

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74 **2.1. Fruit data**

In three growth seasons, a total of 416 healthy berries from three northern highbush blueberry
cultivars (*V. corymbosum* cultivars 'Bluegold', 'Brigitta' and 'Elliot') and one southern blueberry
cultivar (*V. corymbosum* x *Vaccinium darrowi* Camp. cultivar 'Legacy') were collected from three
commercial orchards located in the Región de La Araucanía, Chile (Table 1).

79 The cultivars used differing in fruiting season; Bluegold and Elliot are early- and late-season 80 cultivars, respectively, whereas Legacy and Brigitta are mid-season cultivars. Plants of the orchards ranged from three to five-year-old and were established at a spacing of 3×0.9 m, in north-south 81 oriented rows on an Andisol. In each orchard, the average fertilization was 70 kg N ha⁻¹, 40 kg P₂O₅ 82 ha⁻¹ and 75 kg K₂O ha⁻¹, the irrigation was applied as needed, and pests and diseases were controlled 83 according to the locally recommended practices. Winter pruning was annually done in each orchard, 84 85 removing old wood, damaged canes, unproductive branches, and short shoots that develop from the base of the plant. 86

Fruits were weekly picked from fruit coloring phase (stage in which berries are changing from green to pink to blue in the cluster) up to beginning of commercial harvest for each season. After picking, samples were placed in a cooler and then rapidly transported to the laboratory for evaluating equatorial diameter and fresh weight by using digital caliper (Mitutoyo, Japan, accuracy ± 0.01 mm) and technical balance (Gibertini Elettronica S.r.l., Italy), respectively.

92 The data set was randomly divided to obtain data for both calibrating (n=200) and validating 93 (n=216) model (external validation).

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95 2.2. Allometric model calibration

96 Preliminary regression analysis showed that the relationships between fruit weight and diameter
97 followed a power fit typical of allometric models, for all situations in which fruit was obtained (Eq.
98 1):

$$99 \quad FW = aFD^b \tag{Eq. 1}$$

100 where FW is berry weight, FD is berry diameter, a and b are allometric coefficients.

In order to discriminate the use of cultivar-specific models (including the season effect) or a
 common model for four cultivars, an Analysis of Covariance was performed on data linearized by
 natural logarithm transformation (Eq. 2)

 $104 \quad \ln FW = \ln a + b \times \ln FD$

(Eq. 2)

105 Thus, when interaction between logarithm transformed diameter (continuous variable) and 106 cultivar/season (categorical variable) is not significant, the slopes among groups are similar, so a 107 common model for four cultivars can be constructed (Warton and Weber, 2002; Chen et al., 2010).

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109 2.3. Model goodness-of-fit and validation

Model results were compared with independent data through graphical procedures. The Relative
Root Mean Squared Error (RRMSE, Eq. 3), a common criterion to quantify the mean difference
between estimated data and observed data (Kobayashi and Us Salam, 2000) was calculated:

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$$RRMSE = \left(\sqrt{\frac{\sum n_i (x_i - y_i)^2}{N}}\right) / \bar{y}$$
 (Eq. 3)

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where n_i , x_i , and y_i are the number of observed data, the estimated data, and the observed data at the measurement date *i*, respectively, *N* is the total number of observed data and \bar{y} the mean of observed values. The smaller the value of RRMSE, the closer to the measurement the estimation is (Grechi et al., 2008).

Model residuals were also calculated and plotted against data estimated by the model to checktheir normal distribution (i.e. homoscedastic behaviour).

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123 **2.4. Data analysis**

124 In order to characterize the data set for calibrating model, results obtained from cultivars/season 125 were subjected to Multiple Comparison of Mean (MCM) by using Scheffé's test ($\alpha = 0.05$). This 126 test was selected because the sample size among groups was different (Ruxton and Beauchamp, 127 2008). The MSM, analysis of covariance and model fitting were performed with R software version 128 2.15.0 (R Development Core Team, 2012).

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132 **3.1. Berry diameter and weight**

The mean fruit diameter and weight from different cultivars/seasons ranged from 13.35 to 134 17.75 mm and from 1.19 to 2.58 g, respectively (Table 2). As observed, the highest values were 135 measured for cv. Legacy in the season 2015-2016, while the lowest values for cv. Brigitta. When 136 comparing cultivars, the mean diameter and weight of the fruit was significantly higher in Legacy 137 and Bluegold than in Elliot and Brigitta (Table 2).

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139 **3.2.** Evaluation of regression slopes and allometric model

140 Diameter and weight of the fruits were regressed on logarithm transformed data in order to evaluate whether the interactive effect of berry diameter (continuous variable) and cultivar/season 141 (categorical variable) is significant for estimating berry weight (Table 3). Regression slopes of 142 143 logarithm transformed data were no statistically different among cultivars/season, thereafter whole 144 data set for calibration procedure was used for constructing a common model for four cultivars. 145 Then, fruit weight data were plotted against fruit diameter data, giving a power functional relationship as shown in the Fig. 1. The relation between these variables was highly significant with 146 *p*-values of the coefficients lower than 2×10^{-16} . 147

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149 3.3. Model goodness-of-fit and validation

For model validation, estimated versus actual berry weights were plotted (Fig. 2). As observed, the values were closely distributed alongside the 1:1 line, although there was some tendency to overestimate the weight, which can be noticed on regression slope, being slightly higher than one (Fig. 2A). The external validation of the model showed that the model goodness-of-fit (RRMSE) decreased from cv. Bluegold (4.7%) to cv. Elliot (6.9%), with cv. Legacy and Brigitta showing intermediate values (Fig. 2A). Residual were randomly distributed along estimated data, whichindicates absence of heteroscedasticity (Fig. 2B).

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158 **4. Discussion**

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Empirical models are required for the non-destructive estimation of biomass of a plant organ of interest from easily obtainable measurements. These models, which are based on allometric theory, use the statistic relations between a variable of interest and one or several variables used as predictor (Niklas, 1994).

Many abiotic and biotic factors may potentially affect allometric relationships, including 164 genetics, ontogeny, size, age, structure, site, climate, and their interactions (Misle et al., 2013; 165 Niklas, 1994), however our results indicated that the relationship between fresh weight and 166 diameter was sufficiently stable irrespectively to the genotype and growth seasons (Table 3). From 167 168 this, a common allometric model was calibrated, giving a power functional relationship as showed 169 in the Fig. 1. On the other hand, according to the principle of parsimony and the objective of 170 proposing easy-to-use models, this finding was convenient. In the model, as a fruit grows, its 171 dimensions change in ways that maintain their functional balance, but not in the ways that maintain 172 constant ratios between dimensions (Castellan-Estrada et al., 2002). Similar allometric relationships 173 have been reported in studies of fruit physiology. In most of these, fruit diameter was included as 174 predictor of fresh and dry weight (e.g. Gautier et al., 2001; Gilbert et al., 2005; Díaz-Pérez et al., 175 2007; Grechi et al., 2008). Our allometric model was validated with independent data, giving higher 176 goodness-of-fit for evaluated cultivars (Fig. 2A). This showed that a common model was adequate 177 for providing a reliable means of estimating blueberry fruit weight of different cultivars. On the other hand, residuals follow a normal distribution, with data randomly distributed, which indicates 178 absence of heteroscedasticity (Fig. 2B). When the dispersion pattern of residuals follows a 179

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model for estimating blueberry fruit weight from diameter measurements. Scientia Horticulturae, 219, 131 - 134. , DOI : 10.1016/j.scienta.2017.03.009 heteroscedastic behavior, the estimated standard errors of the regression coefficients are often
underestimated, giving an incorrect perception of accuracy (Chatterjee and Hadi, 2006).

In conclusion, blueberry fruit weight was satisfactorily estimated on the basis of diameter measurements via allometric approach. Despite that model showed to be reliable for estimating berry weight of four blueberry cultivars, it must be validated when applied to other genetic materials. Further studies must be done in blueberry fruits to establish allometric equations for relating other fruit quality traits to easily obtainable non-destructive measurements

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193 6. References

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	Cultivar	Season	Orchard location
	Bluegold	2015-2016	38°84' S 72°69' W
	Brigitta	2010-2011	38°29' S 72° 23'
	Elliot	2008-2009	39°19' S 72°25' W
	Elliot	2015-2016	38°84' S 72°69' W
	Legacy	2010-2011	38°29' S 72° 23'
	Legacy	2015-2016	38°84' S 72°69' W
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257 Table 1. Cultivars, season and orchard locations

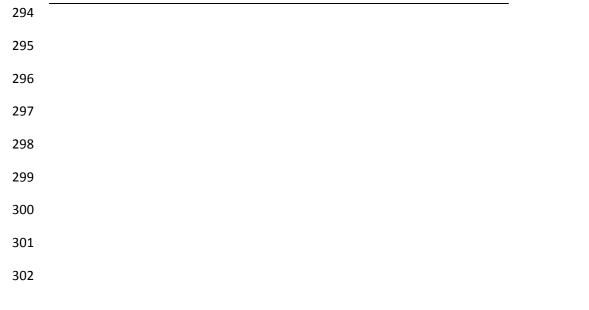
Table 2. Mean fruit diameter and weight for four field-grown blueberry cultivars on data set used
for calibrating model. Standard deviation (in brackets) and letters indicating statistic differences by

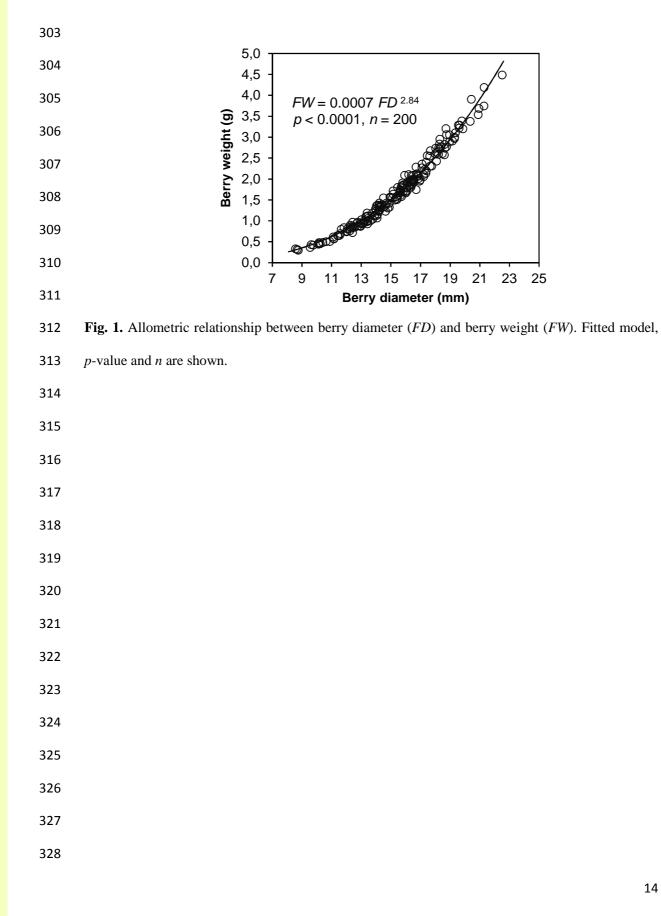
Cultivar	Season	Berry diameter (mm)	Berry weight (g)	Ν
Bluegold	2015-2016	16.00 (±2.25) b	1.95 (±0.74) b	50
Brigitta	2010-2011	13.35 (±3.24) d	1.19 (±0.85) c	50
Elliot	2008-2009	15.52 (±1.30) bc	1.65 (±0.36) bc	21
Elliot	2015-2016	15.83 (±1.31) cd	1.14 (±0.31) c	27
Legacy	2010-2011	14.97 (±1.81) bcd	1.55 (±0.57) bc	16
Legacy	2015-2016	17.75 (±2.09) a	2.58 (±0.79) a	33
Comparison d	among cultivars			
Bluegold		16.00 (±2.25) A	1.95 (±0.74) A	53
Brigitta		13.35 (±3.24) B	1.19 (±0.85) B	50
Elliot		14.57 (±1.54) B	1.36 (±0.41) B	48
Legacy		16.84 (±2.39) A	2.24 (±0.86) A	49

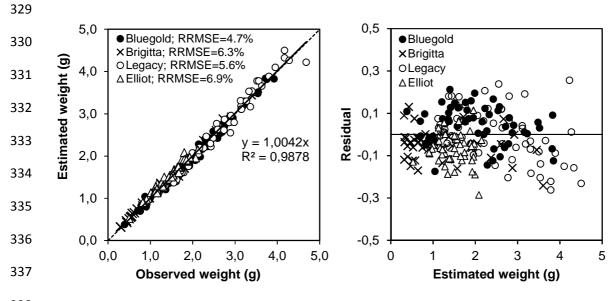
278	Scheffe's test ($\alpha = 0.05$) are shown.
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- **Table 3.** Linear regression between log-transformed weight and log-transformed diameter data, and ANCOVA for log-transformed diameter and cultivar/season variables. Determination coefficient (R^2) and significance values are shown. Abbreviations: ln = natural logarithm, *FW* = fresh weight,
- 293 FD = fruit diameter.

C-14:	Linear regressions on	
Cultivar / season	logarithmic transformed data	
Legacy / 2015-16	$\ln(FW) = 2.68 \ln(FD) - 6.79$	0.98
Legacy / 2008-09	$\ln(FW) = 2.77 \ln(FD) - 7.10$	0.97
Bluegold / 2015-16	$\ln(FW) = 2.74 \ln(FD) - 6.97$	0.98
Elliot / 2015-16	$\ln(FW) = 2.77 \ln(FD) - 7.17$	0.97
Elliot / 2008-09	$\ln(FW) = 2.97 \ln(FD) - 7.68$	0.98
Brigitta / 2010-11	$\ln(FW) = 2.87 \ln(FD) - 7.43$	0.99
ANCOVA		
Variable	p-value	
ln (FD)	2.20 x10 ⁻¹⁶	
Cultivar / season	2.67 x10 ⁻¹⁵	
ln (FD) x cultivar / season	0.07	







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Fig. 2. Estimated versus observed berry weight (A), and residuals versus estimated fruit weight (B)
for four field-grown blueberry cultivars. In A, Relative Root Mean Squared Error (RRMSE), line
1:1 (dotted line) and regression between plotted data are showed.

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