[1§]Tiago Camponogara Tomazetti, [1§]Márcia Denise Rossarolla, [1]Alberto Fontanela Brighenti, [2]Lirio Luiz dal Vesco, [3]André Kulkamp de Souza, [4]Emílio dela Bruna, [5]Marco Stefanini, [6]Oliver Trapp, [1]José Afonso Voltolini, [1]Aparecido Lima da Silva, [2*]Leocir José Welter
§: These authors contributed equally to this work

Exploring phenotypic plasticity leaf trait relationships in fungal-resistant grapevines using linear regression: Implications of the genotype environment interaction

Affiliations

[1]Federal University of Santa Catarina – UFSC, Department of Plant Science, campus Florianópolis-SC, Brazil

[2]UFSC, Department of Natural and Social Sciences, campus de Curitibanos, Curitibanos–SC, Brazil

[3]EPAGRI, Experimental Station of Videira-SC, Brazil

[4]EPAGRI, Experimental Station of Urussanga-SC, Brazil

[5]Research and Innovation Centre, Fondazione Edmund Mach (FEM), E. San Michele all'Adige, TN, Italy

[6]JKI – Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

Correspondence

Tiago Camponogara Tomazetti: tctomazetti@gmail.com, Márcia Denise Rossarolla: mdrossarolla@gmail.com, Alberto Fontanela Brighenti: alberto.brighenti@ufsc.br, Lirio Luiz Dal Vesco: lirio.luiz@ufsc.br, André Kulkamp de Souza: andresouza@epagri.sc.gov.br, Emílio dela Bruna: emilio@epagri.sc.gov.br, Marco Stefanini: marco.stefanini@fmach.it, Oliver Trapp: oliver.trapp@julius-kuehn.de, José Afonso Voltolini: afonso.voltolini@ufsc.br, Aparecido Lima da Silva: aparecido.silva@ufsc.br,
*Corresponding author: Leocir José Welter: leocir.welter@ufsc.br

Abstract

Accurate and non-destructive models for predicting leaf area (LA) are essential for monitoring vineyard growth and developing automated algorithms. In this study, we developed and compared the performance of eight linear regression models for predicting LA in eleven fungal-resistant grapevine genotypes. We also explored the phenotypic plasticity of leaf traits and their relationship with LA using kernel density estimation analysis. We found that genotype played a major role in defining leaf shape, and genotype-environment interaction was observed. The best models for LA estimation were identified for each genotype, and a leaf deformation index was proposed. Our results provide accurate and robust models for estimating LA in fungal-resistant grapevine genotypes and demonstrate the relationship between leaf traits and the environment. Additionally, we present a method for defining leaf asymmetry. Overall, this study contributes to the development of non-destructive and automated techniques for monitoring vineyard growth.

Keywords

Disease resistance; modeling; pilzwiderstandsfähig; sustainable viticulture; Vitis vinifera.

Abbreviations

aLV: average of lateral veins; eLA: estimated leaf area; L: length; LA: Leaf Area; LDI: Leaf Deformation Index; oLA: observed leaf area; PIWI: pilzwiderstandsfähig (German for fungus disease resistant); VIVC: Vitis International Variety Catalogue; W: Width

Introduction

Viticulture is a crucial agricultural activity with global significance, providing a source of income and employment for millions of people. In 2021, the total worldwide area covered by vineyards was 6,7 million hectares, with a total grape production of approximately 75,5 million tons (FAO 2023). Grapevine is grown on almost all continents in the world, and *Vitis vinifera* cultivars are the most widely cultivated due to their exceptional fruit quality, either for fresh consumption or winemaking. Over the centuries, viticulturists/breeders have selected a wide range of *V. vinifera* cultivars with distinct characteristics and adaptability to different environments.

Despite their exceptional fruit quality, *V. vinifera* cultivars are highly susceptible to a range of fungal diseases, which pose a significant challenge for viticulturists. Outbreaks of fungal diseases can lead to severe losses in grape production, requiring large amounts of fungicides to produce grapes in commercially required amount and quality (Pirrello *et al.* 2019). The unintentional introduction of North American endemic pathogens *Plasmopa viticola* and *Erysiphe necator*, causal agents of downy and powdery mildew, in the second middle of the 19th century, caused significant losses to European viticulture (Gobbin *et al.* 2006). Since then, grapevine breeding focused on the development of fungal-diseases resistant scion cultivars, both for table and wine production (Brown *et al.* 1999, Eibach *et al.* 2007, Vezzulli *et al.* 2018).

2 | Original Article

Regarding wine production, the first generation of cultivars developed by crossing American and/or Asian species with *V. vinifera* cultivars revealed low oenological quality, due to the wild nature of the resistance donors. However, persistent, and visionary breeders have made successive modified backcrossings between selected disease-resistant hybrids and *V. vinifera* cultivars, donor of quality attributes, which increased the proportion of *V. vinifera* genome and, consequently, the wine quality. This breeding strategy led to the release of modern fungus-resistant cultivars, which combine high oenological quality with disease resistance (Töpfer *et al.* 2011).

Currently, dozens of fungal-resistant cultivars, particularly resistant to mildew diseases, are available for cultivation, mainly in Europe (https://www.piwi-international.de/en/varieties.html). The European continent is the most traditional and largest wine producer, and their Common Agriculture Policy (PAC) has clear environmental goals, aiming at the promotion of organic farming and the responsible management of inputs, such as pesticides and fertilizers. The current regulations (2017/625 and 1107/2009) dealing with the topic promote the adoption of low environmental impact cultivation methods (EUR-LEX 2009, 2017). Thus, the member countries are implementing policies to promote breeding and cultivation of fungus-resistant grapevines (Montaigne *et al.* 2016). This trend of migrating from traditional *V. vinifera* to fungus-resistant grapevine cultivars is also driven by the increased demand for healthy products with lower environmental and economic impacts, as well as intended reduction of exposure of producers and consumers to pesticide residues (Gadoury *et al.* 2012, Kapusta *et al.* 2017, Montaigne *et al.* 2016, Narduzzi *et al.* 2015, Pertot *et al.* 2017).

However, despite the positive economic, social, and environmental impacts of disease-resistant cultivars, studies regarding their adaptation to different environments worldwide are limited. In Santa Catarina, Southern Brazil, disease-resistant cultivars are being tested under contrasting edaphoclimatic conditions in traditional viticulture regions, both in the highlands of the Santa Catarina plateau (Brighenti *et al.* 2019, Souza *et al.* 2019), and in low-lying areas, such as the Goethe Grape Valley (Stefanini *et al.* 2019). Resistance scoring to downy mildew has shown that these cultivars have a great potential to reduce chemical input in viticulture (Zanghelini *et al.* 2019).

Accurate estimation of leaf area (LA) is critical for advancing agronomical and ecophysiological research. LA estimation is essential for studies related to light interception, leaf transpiration, photosynthesis, plant nutrition, ecological competition, plant-water relations, leaf cover, cultivation ecosystem, and plant productivity (Beslic *et al.* 2010, Costanza *et al.* 2004, Rouphael *et al.* 2010, Silvestroni *et al.* 2018). LA can be estimated using either destructive or non-destructive methods. Destructive methods are precise and accurate, but are laborious and do not allow monitoring of LA evolution during the phenological cycle (Beslic *et al.* 2010, Jiang *et al.* 2015). On the other hand, non-destructive methods do not require leaf removal or damage and allow for continuous monitoring of LA evolution (Buttaro *et al.* 2015, Tondjo *et al.* 2015). Various combinations of measurements and simple linear mathematical models have been utilized for non-destructive LA estimation in numerous plant species (Carvalho *et al.* 2017, Cirillo *et al.* 2017, Fascella *et al.* 2018, Keramatlou *et al.* 2015, Liu *et al.* 2017). As a non-destructive and rapid method, LA estimation using linear regression models can provide an accurate and efficient means for monitoring vineyard growth and developing automated algorithms for grape production.

Grapevine LA is commonly estimated using a simple linear regression model with leaf length and width as predictors (Buttaro *et al.* 2015, Williams and Martinson 2003). However, multiple linear regression models that incorporate additional leaf traits (X_0, X_1, ..., X_n) may provide better fit to the observed LA with the same equation $\widehat{LA} = b_0X_0 + b_1X_1 \dots b_nX_n$ (Cankaya *et al.* 2006). These models can be employed through automatized LA estimation by computational algorithms that use acquired trait data (Easlon and Bloom 2014, Hu *et al.* 2018, Tech *et al.* 2018).

LA estimation models can have a generalist or specific scope. Specific models are more precise and accurate, incorporating genotype-environment interaction (GxE) effects, and should be developed using data collected from different environments to enable model application for specific genotypes (Teobaldelli *et al.* 2019). The leaf shape is a trait strongly influenced by GxE interaction in several species, such as *Capsicum annum* (Cemek *et al.* 2011), *Ipomoea batatas* (Gupta *et al.* 2020), *Campanula thyrsoides* (Scheepens *et al.* 2010), *Malus* spp. (Migicovsky *et al.* 2018) and grapevine (Baumgartner *et al.* 2020, Demmings *et al.* 2019). Current models available for *V. vinifera* cultivars are cultivar-adjusted (Beslic *et al.* 2010, Borghezan *et al.* 2010, Cirillo *et al.* 2017), and to our knowledge, no LA models have been developed for fungus-resistant grapevine cultivars.

In this study, we developed and validated precise and accurate non-destructive specific linear models for LA estimation of modern fungus-resistant grapevine cultivars. In addition, we applied the kernel density estimation (KDE) to describe the phenotypic plasticity of grapevine leaf traits. KDE is a non-parametric method that allows for the estimation of probability density functions from a given sample (Plesovskaya and Ivanov 2021).

Leaf plasticity is crucial in identifying GxE interaction effects on genotype growth and development. Using KDE, we determined how leaf traits distribution changed across different environments and quantified trait variability among genotypes. This information aids in predicting the performance of disease-resistant cultivars under varying conditions and screening new cultivars. Additionally, developing mathematical models for LA estimation provides valuable insights for automating processes using machine learning and deep learning, as reported in predicting grapevine yield (Mohimont *et al.* 2022). The authors emphasize the need to include new features in models to reduce the impact of leaf coverage.

Material and Methods

Plant material

LA estimation models were constructed for eleven disease-resistant genotypes as follow: 1) the white cultivars 'Aromera'

(VIVC: 25400), developed by Innovitis (Bolzano, Italy); 'Bronner' (VIVC: 17129) and 'Helios' (VIVC: 17133), developed by Staatliches Weinbauinstitut Freiburg (WBI); 'Calardis Blanc' (VIVC: 22828) and 'Felicia' (VIVC: 20348), developed by Julius Kühn-Institut (JKI) – Institut für Rebenzüchtung Geilweilerhof, and 2) the red cultivars 'Baron' (VIVC: 20010) and 'Prior' (VIVC: 19993), developed by WBI, 'Calandro' (VIVC: 21797) and 'Regent' (VIVC: 4572), developed by JKI. In addition, the models were generated for two advanced breeding selections (ABS15 and ABS24), containing resistance alleles pyramided, developed by JKI, for breeding purposes.

Experimental vineyards

The disease-resistant genotypes were grown in five experimental vineyards located at different edaphoclimatic regions of Santa Catarina State, in Southern Brazil. Two vineyards were located at the Experimental Station of Santa Catarina Agricultural Research and Rural Extension Company (EPAGRI) in the municipalities of Urussanga (28° 32' S and 49° 18' W) and Videira (27° 01' S and 51° 08" W); one at the Experimental Station of the Federal University of Santa Catarina (UFSC) in Curitibanos (27° 16' S and 50° 35' W); and two in private wineries, Suzin Winery in São Joaquim (28° 3' S and 50° 04' W) and Villaggio Grando Winery in Água Doce (26° 43' S and 51° 30' W). The vines were trained in vertical shoot positioning trellis at the spacing of 3.0 m × 1.2 m, grafted on 'Paulsen 1103', and pruned in a double spur cordon. Climatic data were obtained from weather stations located near the experimental vineyards and kindly provided by the Center for Environmental Resources and Hydrometeorology Information of Santa Catarina (EPAGRI/CIRAM). The geographical location, altitude, annual average temperature, and annual total rainfall are shown in Fig. 1. The climatic data during the grapevine growing season are shown in Fig. 2.

Leaf sample collection

The leaves were collected from adult plants in the pre-veraison stage. One hundred leaves per genotype from each experimental vineyard were randomly collected in the growing season of 2016/17. The leaves were collected from the upper, middle, and lower thirds of the shoots, with at least 30 mm in length. All collected leaves were healthy and without anomalies. The leaf traits were measured immediately after sampling in all locations.

For each leaf, the leaf length (L) and width (W), and the length of lateral veins (left and right) were measured using a millimeter ruler (Fig. 3). The average length of lateral veins (aLV) was calculated by the average between left and right veins (LVL_r and LVL_l). The real LA (rLA) was determined by the portable leaf area meter ADC, model AM 30 (BioScientific, England).

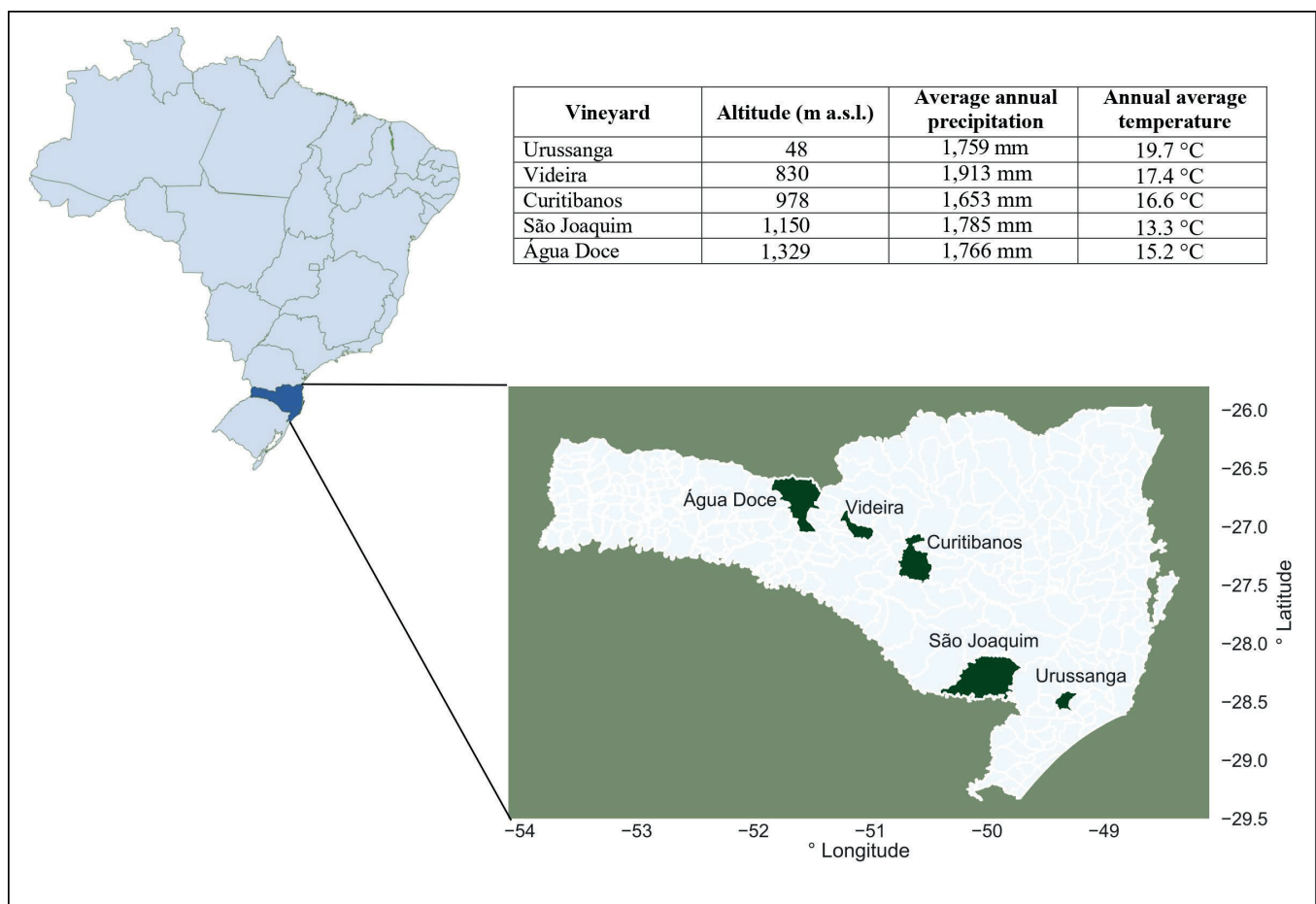| Vineyard	Altitude (m a.s.l.)	Average annual precipitation	Annual average temperature
Urussanga	48	1,759 mm	19.7 °C
Videira	830	1,913 mm	17.4 °C
Curitibanos	978	1,653 mm	16.6 °C
São Joaquim	1,150	1,785 mm	13.3 °C
Água Doce	1,329	1,766 mm	15.2 °C

Fig. 1: Geographic location and climatological conditions (mean of the last 30 years) of the five vineyards containing the eleven grapevine disease-resistant genotypes evaluated.
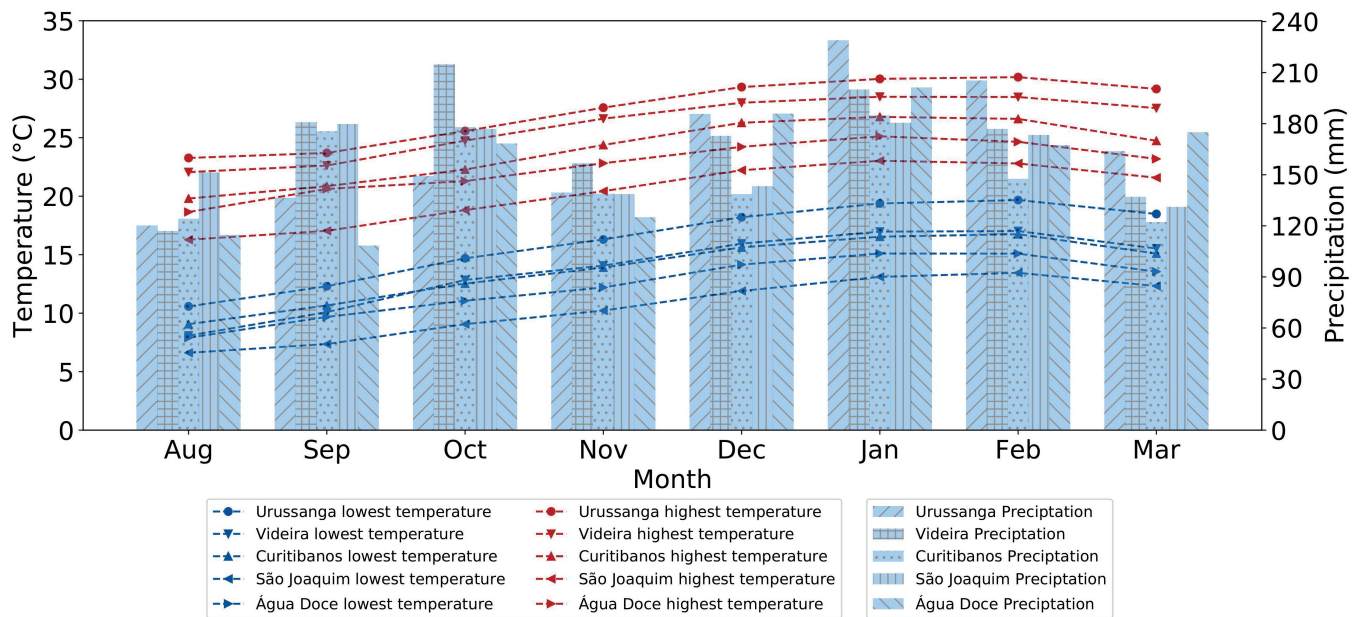
Fig. 2: Climatological conditions, that represents the mean of the last 30 years, in the five experimental vineyards during the period from august to march, covering the grapevine full productive cycle.
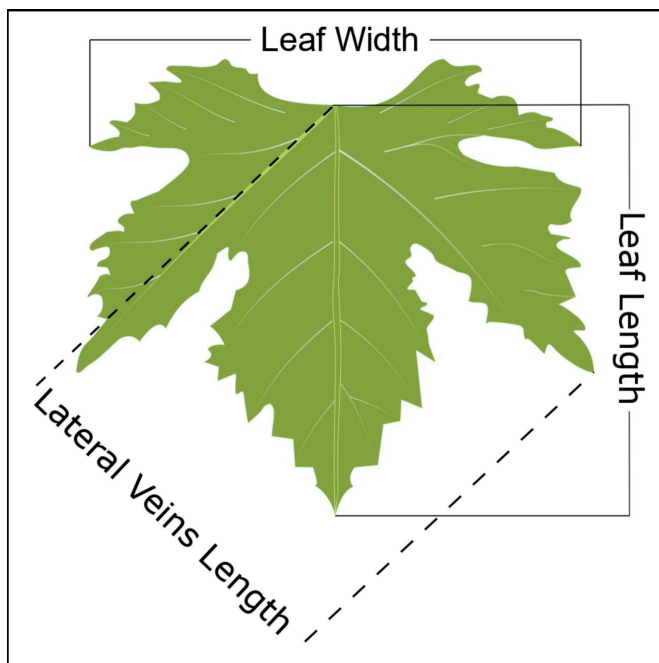

Fig. 3: Graphical representation from the measured leaf traits used to develop mathematical linear models for the estimation of leaf area in eleven grapevine disease-resistant genotypes.

LA models construction

The linear regressions used for the LA estimation were performed with 60% of the random data. Simple linear regression (sLR) was fitted through the relationships between rLA and L, L^2 W, W^2, aLV, aLV^2, or L·W using the Ordinary Least Squares (OLS) optimizer. Moreover, a multiple linear regression (mLR) was fitted with L^2, W^2, and aLV^2. In total, seven simple linear regressions and one multiple linear regression models were tested. The significance of the angular and intercept coefficients, the coefficient of determination (R^2),

and the square standard error (SSE) were obtained for each model. The generated models were considered for validation when their residuals presented normal distribution, tested with the Shapiro-Wilk test ($\alpha=0.05$).

LA models validation

The generated models were tested with the remaining 40% of the random data, ensuring data independence. The estimated LA (eLA), obtained by applying the generated models for each genotype, was compared with rLA, using the t-test ($\alpha=0.05$). Additionally, the generalist model proposed by Buttaro *et al.* (2015) was tested. The models were discarded when the eLA was significantly different from rLA. The remaining models were ranked based on the values of significance of the angular coefficient and intercept of the linear regression by eLA and rLA, the Pearson correlation coefficient (r) between eLA and rLA, the Root Mean Square Error (RMSE), the model bias (%) and efficiency. When two or more models had the same goodness of fit, the model that required the measurement of a lower number of leaf traits was selected.

Kernel Density Estimation (KDE) Analysis

The scikit-learn library in Python (Pedregosa *et al.* 2011) was used to perform a Kernel Density Estimation (KDE) analysis (Plesovskaya and Ivanov 2021). The KDE method is a non-parametric technique that was applied to estimate the probability density function for the two Principal Components obtained from the Principal Component Analysis (PCA) of leaf traits from each grapevine genotype across the five different environments. Furthermore, we used the seaborn library in Python (Waskom 2021) to generate plots of the estimated probability density functions. By analyzing the distribution of these traits, we were able to quantify the degree of phenotypic plasticity and its relationship with the environment,

providing insights into how grapevine genotypes respond to different environmental conditions.

Genotype × Environment Interaction

To avoid collinearity, the variable LA, LDI, and the ratio L W^{-1}, L aLV$^{-1,}$ and W aLV^{-1} were selected for statistical analysis. They were contrasted using ANOVA (α=0.05) and when the variation was significant the Scott Knott (α=0.05) test was performed to understand GxE effects. The Scott Knott analysis was performed using the statistical program R v. 3.5.1 (R Core Team 2013), employing the packages SkottKnot (Jelihovschi *et al.* 2014).

The same data were normalized and employed to perform a Principal Component Analysis (PCA). The two principal components (PC1 and PC2) were used to plot the KDE to demonstrate the behavior and the phenotypic plasticity from all genotypes in each environment and the influence of all environments on each genotype.

Leaf Deformation Index

To complement the allometric study, we proposed a methodology to calculate the leaf deformation index (LDI), which expresses the average difference of the lateral veins, right (LV$_r$) and left (LV$_l$) in mm cm^{-1}. Thus, the LDI was calculated by the equation $LDI = \dfrac{|LV_r - LV_l|}{aLV}\, x\, 10.$

Results

Genotype × environment interaction (GxE)

The variation of the measured leaf traits from all genotypes is represented in Fig. 4. The shortest L measured was 3.0 cm from leaves of 'Baron', 'Calardis Blanc', and ABS15, whereas the longest L (18.2 cm) was from ABS24. The W ranged from 3.6 cm (ABS15) to 24.1 cm (ABS24), while the ratio L W^{-1} ranged from 0.49 cm ('Prior') to 1.46 cm ('Felicia'). The aLV ranged from 2.50 cm ('Baron', 'Calardis Blanc', and 'Regent') to 17.90 cm (ABS24). The rLA ranged from 9.0 cm^2 ('Baron') to 430.4 cm^2 (ABS15), while the LDI ranged from 0.00 mm cm^{-1} (obtained in few leaves of any cultivars) to 5.03 mm cm^{-1} reported from one leaf of 'Aromera'.

The relationship between the traits L W^{-1}, L aLV^{-1}, and W aLV^{-1}, as well as the LDI and the LA are good parameters to access the leaf shape. Quantifying the variation of these traits in contrasting environmental conditions is important to better understand the magnitude of the genetic and environmental influences and their interaction in the leaf shape. For all leaf traits measured, significant differences were observed among the genotypes and among the environments. The GxE interaction was also significant (Table 1).

The scott-knott test revealed that 'Baron' had the smallest LA in Urussanga, Curitibanos, and Água Doce. In São Joaquim, 'Aromera', 'Bronner', and 'Calandro' presented the smallest leaves (Table S1). ABS15, ABS24, 'Calandro', and 'Regent' showed the largest LA at the lowest (Urussanga) and the

highest altitude (Água Doce). However, they didn't differ from 'Bronner' in Urussanga and from 'Calardis blanc', 'Felicia', and 'Prior' in Água Doce. In Videira, Curitibanos, and São Joaquim, the largest LA was reported for ABS15, 'Calandro', and 'Prior', respectively. All genotypes had the smallest LA in Urussanga and/or Água Doce (Table S1), although for a few of them, no significant difference was observed when compared to São Joaquim ('Aromera' and 'Calandro'), and Videira ('Regent').

In Fig. 5, PCA captures 69.2% of the variation in the first two dimensions. PC1 (46.2% variance) positively loads traits L W^{-1}, L aLV^{-1}, and negatively loads W aLV^{-1}. PC2 (23.0% variance) correlates positively with L aLV^{-1} and leaf area ratio, and negatively with LDI. When analyzing all genotypes within each environment, larger clusters indicate greater data variability, emphasizing the stronger influence of genetic factors on allometric traits (Fig. 6). However, environmental factors also contribute to the variation, with separate clusters forming when analyzing all environments within each genotype (Fig. 5).

Fitted LA models

The generated models to estimate the LA for all genotypes presented an angular coefficient significantly different from zero and a few of them had non-significant intercepts (Table S2_A). A high coefficient of determination was observed for all models generated, except those generated from the L for 'Calandro' and 'Regent'. The mLR models (fitted by L^2, W^2, and aLV^2) revealed that the effect of the L was only not significant at 1% in 'Regent' and had a negative significant coefficient in 'Calandro', while W and aLV were significant at 1% for all genotypes evaluated (Table S2_B). These results corroborate with the lower coefficient observed for L in the sLR models for 'Regent' and 'Calandro', which suggests that it is not adequate to estimate the LA in these two cultivars.

LA models validation

From all models generated in this work, only the models obtained from L, L^2, W, aLV for 'Baron', and L and aLV for 'Prior' were excluded from the validation because they returned eLA significantly different from the rLA (t-test < 0,05; Table S3). The Buttaro general model returned non-significant differences between eLA and rLA only for 'Baron', 'Felicia', and ABS24 (Table S3) and these were also validated.

The linear regression between eLA and rLA demonstrates the high correlation between these areas in models generated for any cultivar (Fig. S1). The ranking of the validated models for each genotype is shown in Table S4. The models to estimate the LA that best ranked for each genotype are listed in Table 2. In addition, Table 1 shows the best-ranked model using a single measure. The best-ranked model for 'Calandro', 'Calardis Blanc', and 'Felicia' requires the measurement of a single trait (W^2), while for 'Aromera', 'Helios', 'Prior', ABS15, and ABS24 models based on L W ranked best. Finally, for 'Baron', 'Bronner', and 'Regent' the multiple linear regression fitted best for LA estimation. When considering the use of a single measurement (L or W) for LA estimation, the use of W (W^2)
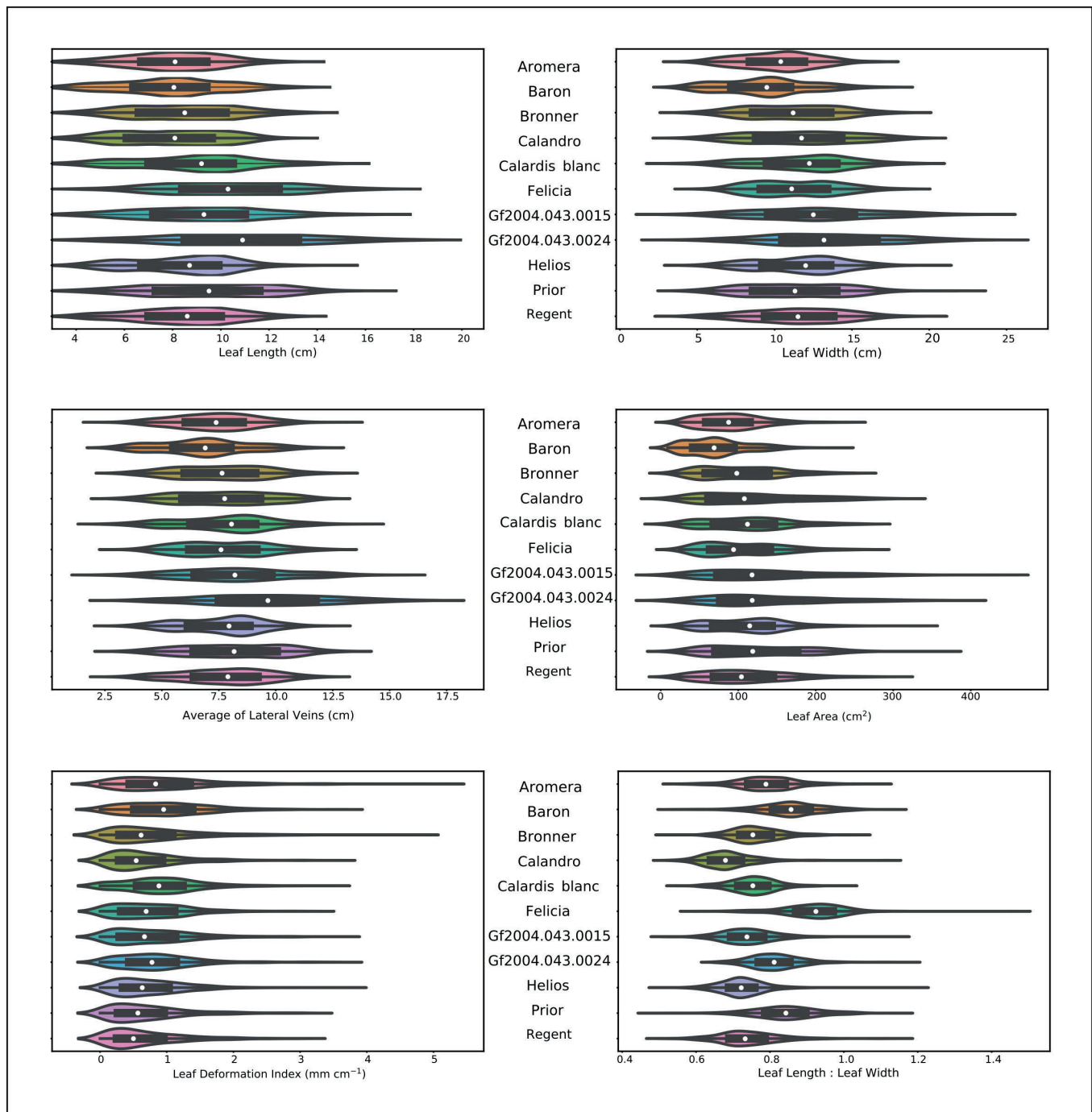
Fig. 4: Values variation of the measured leaf traits from eleven grapevine disease-resistant genotypes, cultivated in five locations of Santa Catarina State, Southern Brazil.

returned the most accurate models for all genotypes, except for 'Helios', which was L (L^2).

Discussion

Allometric plasticity is the differential behavior presented by the same population or the same genotype in different environments and has an important role in genotype adaptation (Lane *et al.* 2019, Vitasse *et al.* 2013, Weiner 2004). Thus, to determine the magnitude of GxE interactions, it is important to predict the behavior of these genotypes in contrasting climatic scenarios (Lande and Shannon 1996). Our results demonstrate a clear GxE interaction of leaf morphology-related traits and the LA, as previously reported for other plant species, such as *Eugenia calycina* (Cardoso and Lomônaco 2003) and *Berberis lycium* (Rahman *et al.* 2019).

Grapevine leaf traits are polygenic (Chitwood *et al.* 2014, Welter *et al.* 2007). This pattern of inheritance is also described in other species, such as maize (Cui *et al.* 2017) and wheat (Hussain *et al.* 2017). The quantitative nature of leaf traits explains both the significant phenotypic variation promoted by the environment and the GxE interaction. But for most traits evaluated, such as L W^{-1}, L aLV^{-1} and W aLV^{-1}, the genetic effect was superior to the effect of the environment.

Tab. 1.: ANOVA performed with allometric traits from leaves of fungal resistant genotypes (*V. vinifera*) grown in five locations of Santa Catarina State, Brazil. The measured traits used were leaf area, leaf deformation index (LDI), relationship between leaf length and width (L W^{-1}), length and average of lateral veins (L aLV^{-1}) and width and average of lateral veins (W aLV^{-1}).

	Sum square	df	F	p value
----------------------------------- Leaf Area -----------------------------------				
Genotype	1,635,937	10	58.15	< 0.01
Environment	1,667,849	4	148.21	< 0.01
Interaction GxE	1,201,854	40	10.68	< 0.01
Residual	15,318,250	5,445	–	–
---------------------------------- LDI ----------------------------------				
Genotype	65.75	10	18.94	< 0.01
Environment	38.15	4	27.47	< 0.01
Interaction GxE	51.27	40	3.69	< 0.01
Residual	1,890.56	5,445	–	–
------------------------------------- L W^{-1} -------------------------------------				
Genotype	22.65	10	456.25	< 0.01
Environment	2.26	4	113.60	< 0.01
Interaction GxE	1.15	40	5.80	< 0.01
Residual	27.03	5,445	–	–
-------------------------------------L aLV^{-1} -------------------------------------				
Genotype	32.08	10	468.31	< 0.01
Environment	1.42	4	51.76	< 0.01
Interaction GxE	1.44	40	5.27	< 0.01
Residual	37.29	5,445	–	–
--------------------------------- W aLV^{-1} ---------------------------------				
Genotype	19.91	10	194.77	< 0.01
Environment	1.83	4	44.66	< 0.01
Interaction GxE	2.95	40	7.21	< 0.01
Residual	55.65	5,445	–	–

The availability of nondestructive methods of LA estimation is fundamental for studies related to crop growth, such as ecology, physiology, pathology, and agronomic performance (Colaizzi *et al.* 2017, Forrester *et al.* 2017, Hang *et al.* 2019, Kraus *et al.* 2018, Pudumalar *et al.* 2020, Zeist *et al.* 2017). We developed simple, rapid, inexpensive, robust, precise, accurate, and nondestructive LA models for nine disease-resistant cultivars. To our knowledge, these are the first models available for these cultivars. These models can now be implemented to provide concrete applications, particularly for studies related to the adaptation of these cultivars in new growing regions and management practices. These models may also be utilized in automating phenotyping processes, potentially integrated into an AI-powered tool.

As demonstrated in Table 1, the leaf shape, in addition to the genotype effect, is influenced by the environment and GxE interaction. That highlights the importance of the development of LA estimation models with specific scope (Teobaldelli *et al.* 2019). For the development of the models, we measured the leaf traits in five contrasting environments, which allowed us to incorporate GxE interaction effects, and in turn, to increase

the precision and accuracy of the models developed. In addition, we used multiple statistics to rank the validated models. This methodology allowed us to mitigate the limitations that each statistic has when applied individually.

Multiple linear regression is often assumed to return the best models for LA estimation. However, in the present work, simple linear regression returned the best-ranked models for LA estimation for eight out of the eleven genotypes investigated. Five of these models are dependent on the measurement of two traits (L W) and best fit for LA estimation of 'Aromera', 'Helios', 'Prior', ABS15, and ABS24, and three are dependent on one single measurement (W^2) and best fit for LA estimation of 'Calandro', 'Calardis Blanc', and 'Felicia'. These findings agree with previous investigations. For instance, sLR returned also accurate models for LA estimation of nine *V. vinifera* varieties using the product of L and W (Buttaro *et al.* 2015). Multiple linear regression returned only the best-ranked model of LA estimation for 'Baron', 'Bronner', and 'Regent'. The use of lateral veins and L is reported in the literature for the estimation of LA from 'BRS-Violeta' and 'Cabernet Sauvignon' (Amarante *et al.* 2009, Malagi *et al.* 2010). However, these previous

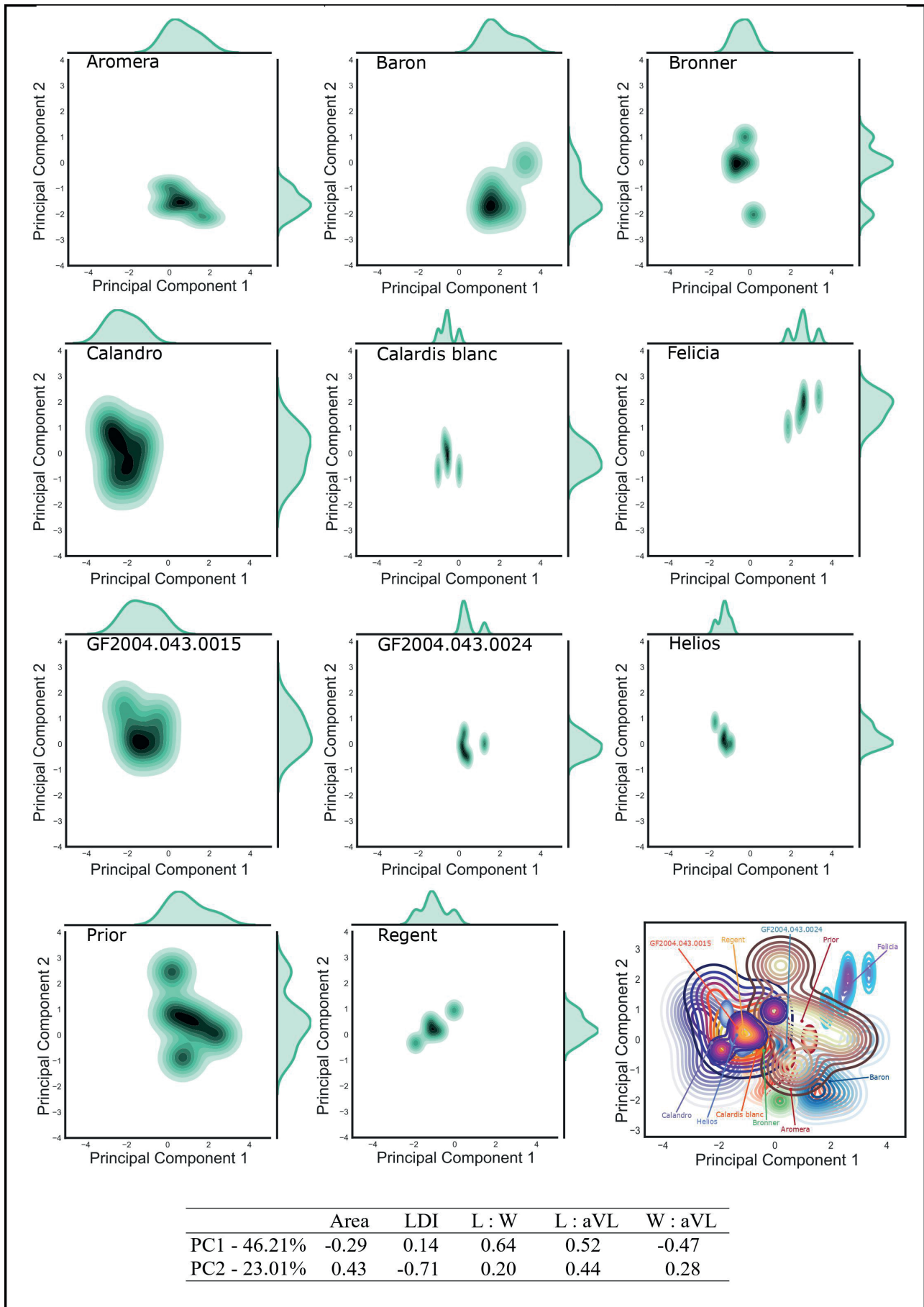|	Area	LDI	L : W	L : aVL	W : aVL
PC1 - 46.21%	-0.29	0.14	0.64	0.52	-0.47
PC2 - 23.01%	0.43	-0.71	0.20	0.44	0.28

Fig. 5: Principal component analysis from eleven grapevine disease-resistant genotypes cultivated in five locations of Santa Catarina State, Brazil, using the leaf traits leaf area, leaf deformation index (LDI), ratio between leaf length and width (L W^{-1}), length and average of lateral veins (L aLV^{-1}), and width and average of lateral veins (W aLV^{-1}).
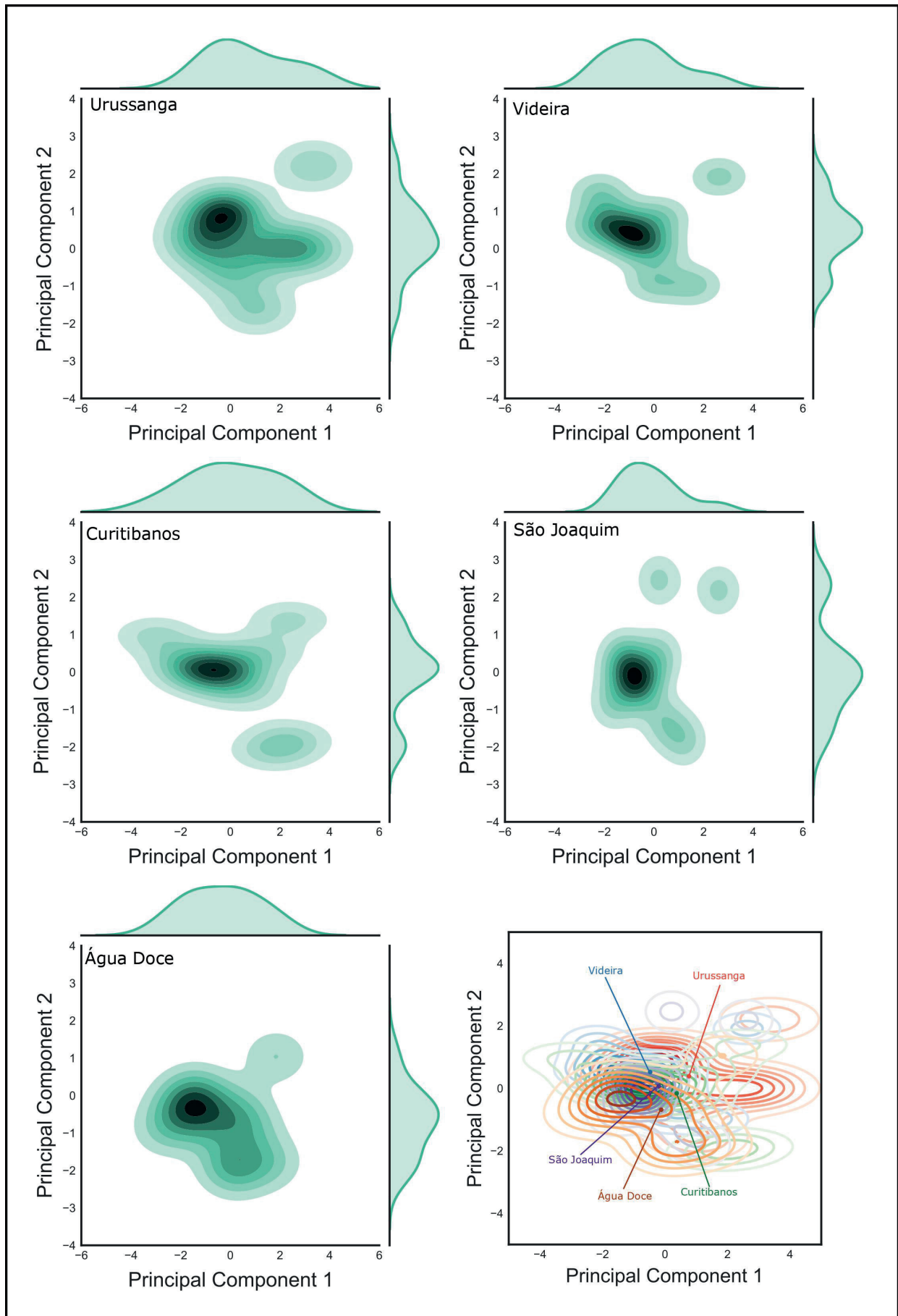
Figure 6. Principal component analysis from five locations of Santa Catarina State, Brazil cultivated with eleven grapevine disease-resistant genotypes using leaf traits, leaf area, leaf deformation index (LDI), ratio between leaf length and width (L W^{-1}), length and average of lateral veins (L aLV^{-1}), and width and average of lateral veins (W aLV^{-1}).

Table 2. Models to estimate the leaf area (LA) from eleven grapevine disease-resistant genotypes (*Vitis vinifera*) obtained from simple and multiple linear regression employing the measured leaf traits length (L), width (W) and average lateral veins (aLV). For each genotype two models are presented: 1) the best ranked model, and 2) the best ranked model considering the use of a single measurement. The accuracy was determined by the bias (BIAS), the precision was determined by the correlation coefficient (r) and Root Mean Square Error (RMSE), while robustness was determined by angular coefficient and model efficiency (EF). The statistics were obtained in the model's validation, comparing estimated and observed LA.

	Model	r	RMSE	BIAS	EF
	--- Aromera ---				
1	$LA = 0.99LW + 2.76$	0.98	9.88 cm^2	-3.68%	0.96
2	$LA = 0.77W^2 + 3.95$	0.96	13.05 cm^2	-1.77%	0.94
	--- Baron ---				
1	$LA = 0.24L^2+0.32W^2+0.47aLV^2+1.85$	0.98	3.98 cm^2	-0.34%	0.95
2	$LA = 0.73W^2+2.98$	0.97	11.34 cm^2	-0.21%	0.94
	--- Bronner ---				
1	$LA = 0.44L^2+0.30W^2+0.46aLV^2+0.12$	0.98	5.82 cm^2	0.20%	0.96
2	$LA = 0.75W^2+4.21$	0.98	14.15 cm^2	4.54%	0.95
	--- Calandro ---				
1	$LA = 0.82W^2 - 1.05$	0.99	16.35 cm^2	-1.32%	0.98
	--- Calardis Blanc ---				
1	$LA = 0.75W^2 - 0.25$	0.97	11.99 cm^2	-0.02%	0.93
	--- Felicia ---				
1	$LA = 0.78W^2 - 1.91$	0.98	9.90 cm^2	0.75%	0.97
	--- ABS15 ---				
1	$LA = 1.08LW - 3.33$	0.98	23.56 cm^2	-1.90%	0.95
2	$LA = 0.73W^2+3.88$	0.98	24.59 cm^2	-2.82%	0.96
	--- ABS24 ---				
1	$LA = 0.85LW - 0.42$	0.98	16.83 cm^2	0.09%	0.96
2	$LA = 0.66W^2+2.96$	0.98	18.66 cm^2	-0.68%	0.95
	--- Helios ---				
1	$LA = 1.06LW + 3.07$	0.97	14.57 cm^2	1.50%	0.95
2	$LA = 1.41L^2+5.68$	0.96	16.27 cm^2	0.05%	0.92
	--- Prior ---				
1	$LA = 1.05LW + 3.60$	0.97	18.51 cm^2	-2.21%	0.95
2	$LA = 0.84W^2+6.71$	0.96	22.27 cm^2	-2.19%	0.92
	--- Regent ---				
1	$LA = 0.11L^2+0.40W^2+0.77aLV^2-4.15$	0.98	2.83 cm^2	-1.70%	0.97
2	$LA = 0.73W^2+3.38$	0.98	13.07 cm^2	-2.49%	0.95

studies relied solely on the coefficient of determination (R^2) for model selection, lacking proper validation procedures. In the present study also aLV generated models with reliability, precision, and accuracy, with high efficiency and low bias (Table S3). The high correlation between aLV dimensions and LA was also reported for *V. vinifera* varieties 'Sauvignon Blanc' and 'Cabernet Sauvignon' (Borghezan *et al.* 2010).

The most laborious activity in implementing LA estimation models is the trait measurement. The fewer measurements, the easier the application of the model. Taking into consider-

ation one single measure for LA estimation, W returned the best-ranked models for ten out of the 11 genotypes investigated. Only for 'Helios' the best-ranked model was based on L. These results contrast with those reported for interspecific hybrids from *V. vinifera* and American species, such as 'De Chaunac' and 'Niagara' where LA is best simulated by L (Williams and Martinson 2003). This probably is related to the different genetic background that modulates the leaf shape. The modern disease-resistant cultivars were obtained by successive modified backcrosses with *V. vinifera* varieties, restor-

ing most of the *V. vinifera* genome. Therefore, the leaves of disease-resistant cultivars best match *V. vinifera* leaf shape.

In addition to the fact that LVs measures can improve the robustness of LA estimation by the implementation of the mLR models, these measures can also be used to estimate the LDI. The proposed methodology to describe the leaf asymmetry is new and can be helpful in research aiming to know the effect of some external factors, such as biotic or abiotic stresses, on the leaf shape. Although the environmental influence on the leaf shape is already known (Chitwood *et al.* 2014), this research marks a pioneering effort in examining the behavior of the LDI. The LDI opens promising avenues for future research in the field of grapevine ecophysiology, allowing for a deeper exploration of GxE interactions and facilitating studies that can provide valuable insights into grapevine growth, health, and adaptation. Its versatility makes it a valuable tool for characterizing leaf responses to various environmental conditions and cultural practices, offering opportunities to enhance our understanding of vineyard management and grapevine cultivation.

Conclusion

1. The trait(s) that returned the most accurate LA estimation model varied according to the genotypes evaluated.
2. For the majority of genotypes, the best-fitted model for the LA estimation involved at least two measurements (traits), however for all genotypes the use of a single measurement maintained a high precision and accuracy.
3. In the case of using a single measure for LA estimation, the leaf width is the most adequate trait for all genotypes evaluated, except for 'Helios', for which leaf length was more accurate.
4. There was a significant genotype-environment interaction for all leaf traits evaluated, reinforcing the importance of developing genotype-specific models.
5. The generic model could only be used to estimate the LA for 'Baron', 'Felicia', and ABS24, however, even for these genotypes the model returned values with lower precision and accuracy.

Supplementary Material

The Supplementary Material can be found online:

https://doi.org/10.5073/vitis.2024.63.08

Acknowledgement

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Amarante, C. V. T. do, Zanardi, O. Z., Miqueloto, A., Steffens, C. A., Erhart, J., Almeida, J. A. de, 2009: Quantificação da área e do teor de clorofilas em folhas de plantas jovens de videira 'Cabernet Sauvignon' mediante métodos não destrutivos. Revista Brasileira de Fruticultura, 33(3), 680–686.

Baumgartner, A., Donahoo, M., Chitwood, D. H., Peppe, D. J., 2020: The influences of environmental change and development on leaf shape in *Vitis*. American Journal of Botany, 107(4), 676–688, DOI:10.1002/ajb2.1460.

Beslic, Z., Todic, S., Tesic, D., 2010: Validation of non-destructive methodology of grapevine leaf area estimation on cv. Blaufränkisch (*Vitis vinifera* L.). South African Journal of Enology and Viticulture, 31(1), 22-25.

Borghezan, M., Gavioli, O., Pit, F. A., Silva, A. L., 2010: Modelos matemáticos para a estimativa da área foliar de variedades de videira à campo (*Vitis vinifera* L.). Ciência e Técnica Vitivinícola, 25, 1–7.

Brighenti, E., Souza, A. L. K., Brighenti, A. F., Stefanini, M., Trapp, O., Gardin, J. P. P., Caliari, V., Dalbó, M. A., Welter, L. J., 2019: Field performance of five white Pilzwiderstandsfahige (PIWI) cultivars in the south of Brazil. Acta Horticulturae, 1248, 115–121.

Brown, M. V, Moore, J. N., Mcnew, R. W., Fenn, P., 1999: Inheritance of Downy Mildew resistance in table grapes. Journal of the American Society for Horticultural Science, 124, 262–267.

Buttaro, D., Rouphael, Y., Rivera, C. M., Colla, G., Gonnella, M., 2015: Simple and accurate allometric model for leaf area estimation in *Vitis vinifera* L. genotypes. Photosynthetica, 53(3), 342–348. DOI: 10.1007/s11099-015-0117-2.

Cankaya, S., Kayaalp, G.T., Sangun, L., Tahtali, Y., Akar, M., 2006: A comparative study of estimation methods for parameters in multiple linear regression model. Journal of Applied Animal Research, 29, 43–47. DOI: 10.1080/09712119.2006.9706568.

Cardoso, G. L., Lomônaco, C., 2003: Variações fenotípicas e potencial plástico de *Eugenia calycina* Cambess. (Myrtaceae) em uma área de transição cerrado-vereda. Revista Brasileira de Botânica, 26(1), 131–140. DOI: 10.1590/S0100-84042003000100014.

Carvalho, L. B., Alves, E. A., Bianco, S., 2017: Modelo não destrutivo para estimar a área foliar de *commelina diffusa*. Planta Daninha, 35. DOI: 10.1590/S0100-83582017350100088.

Cemek, B., Unlukara, A., Kurunc, A., 2011: Nondestructive leaf-area estimation and validation for green pepper (*Capsicum annuum* L.) grown under different stress conditions.

Photosynthetica, 49(1), 98–106. DOI: 10.1007/s11099-011-0010-6.

Chitwood, D. H., Ranjan, A., Martinez, C. C., Headland, L. R., Thiem, T., Kumar, R., Covington, M. F., Hatcher, T., Naylor, D. T., Zimmerman, S., Downs, N., Raymundo, N., Buckler, E. S., Maloof, J. N., Aradhya, M., Prins, B., Li, L., Myles, S., Sinha, N. R., 2014: A modern ampelography: a genetic basis for leaf shape and venation patterning in grape. Plant Physiology, 164(1), 259–72. DOI: 10.1104/pp.113.229708.

Cirillo, C., Pannico, A., Basile, B., Rivera, C. M., Giaccone, M., Colla, G., De Pascale, S., Rouphael, Y., 2017: A simple and accurate allometric model to predict single leaf area of twenty-one european apricot cultivars. European Journal of Horticultural Science, 82(2), 65–71. DOI: 10.17660/eJHS.2017/82.2.1.

Colaizzi, P. D., Evett, S. R., Brauer, D. K., Howell, T. A., Tolk, J. A., Copeland, K. S., 2017: Allometric method to estimate leaf area index for row crops. Agronomy Journal, 109(3), 883–894. DOI: 10.2134/agronj2016.11.0665.

Costanza, P., Tisseyre, B., Hunter, J. J., Deloire, A., 2004: Shoot development and non-destructive determination of grapevine (*Vitis vinifera* L.) leaf area. South African Journal of Enology & Viticulture, 25(2), 43–47. DOI: 10.21548/25-2-2138.

Cui, T. T., He, K. H., Chang, L. G., Zhang, X. H., Xue, J. Q., Liu, J. C., 2017: QTL mapping for leaf area in maize (*Zea mays* L.) under multi-environments. Journal of Integrative Agriculture, 16(4), 800–808. DOI: 10.1016/S2095-3119(16)61524-1.

Demmings, E. M., Williams, B. R., Lee, C. R., Barba, P., Yang, S., Hwang, C. F., Reisch, B. I., Chitwood, D. H., Londo, J. P., 2019: Quantitative trait locus analysis of leaf morphology indicates conserved shape loci in grapevine. Frontiers in Plant Science, 10. DOI: 10.3389/fpls.2019.01373.

Easlon, H. M. Bloom, A. J., 2014: Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. Applications in Plant Sciences, 2(7), 1400033. DOI:10.3732/apps.1400033.

Eibach, R., Zyprian, E., Welter, L., Töpfer, R., 2007: The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis 46(3), 120-124, DOI: 10.5073/vitis.2007.46.120-124.

EUR-LEX, T.E.P.A.T.C.O.T.E.U., 2009, EUR-Lex – 32009R1107.

EUR-LEX, T.E.P.A.T.C.O.T.E.U., 2017, EUR-Lex – 32017R0625.

FAO, 2023: FAOSTAT [online]. Food and Agriculture Organization FAOSTAT. Available: https://www.fao.org/faostat/en/#data/QCL/visualize. [Accessed 12 March 2023].

Fascella, G., Rouphael, Y., Cirillo, C., Mammano, M. M., Pannico, A., De Pascale, S., 2018: Allometric model for leaf area estimation in *Bougainvillea* genotypes. Acta Horticulturae, 1215, 449–452. DOI: 10.17660/ActaHortic.2018.1215.81.

Forrester, D. I., Tachauer, I. H. H., Annighoefer, P., Barbeito, I., Pretzsch, H., Ruiz-Peinado, R., Stark, H., Vacchiano, G., Zlatanov, T., Chakraborty, T., Saha, S., Sileshi, G. W., 2017: Generalized biomass and leaf area allometric equations for European tree species incorporating stand structure, tree age and climate. Forest Ecology and Management, 396, 160–175. DOI: 10.1016/j.foreco.2017.04.011.

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C., Milgroom, M. G., 2012: Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Molecular Plant Pathology, 13(1), 1–16. DOI: 10.1111/j.1364-3703.2011.00728.x.

Gobbin, D., Rumbou, A., Linde, C. C., Gessler, C., 2006: Population genetic structure of *Plasmopara viticola* after 125 years of colonization in European vineyards. Molecular Plant Pathology, 7(6), 519–531. DOI: 10.1111/j.1364-3703.2006.00357.x.

Gupta, S., Rosenthal, D. M., Stinchcombe, J. R., Baucom, R. S., 2020: The remarkable morphological diversity of leaf shape in sweet potato (*Ipomoea batatas*): the influence of genetics, environment, and G×E. New Phytologist, 225(5), 2183–2195. DOI: 10.1111/nph.16286.

Hang, T., Lu, N., Takagaki, M., Mao, H., 2019: Leaf area model based on thermal effectiveness and photosynthetically active radiation in lettuce grown in mini-plant factories under different light cycles. Scientia Horticulturae, 252, 113–120. DOI: 10.1016/j.scienta.2019.03.057.

Hu, Y., Wang, L., Xiang, L., Wu, Q., Jiang, H., 2018: Automatic non-destructive growth measurement of leafy vegetables based on kinect. Sensors, 18(3), 806. DOI: 10.3390/s18030806.

Hussain, W., Baenziger, P. S., Belamkar, V., Guttieri, M. J., Venegas, J. P., Easterly, A., Sallam, A., Poland, J., 2017: Genotyping-by-Sequencing derived High-Density Linkage map and its application to QTL mapping of flag leaf traits in bread wheat. Scientific Reports, 7, 16394. DOI: 10.1038/s41598-017-16006-z.

Jelihovschi, E. G., Faria, J. C., Allaman, I. B., 2014: ScottKnott: A package for performing the Scott-Knott Clustering Algorithm in R. Trends in Computational and Applied Mathematics, 15(1), 003-017. DOI: 10.5540/tema.2014.015.01.0003.

Jiang, N., Yang, W., Duan, L., Chen, G., Fang, W., Xiong, L., Liu, Q., 2015: A nondestructive method for estimating the total green leaf area of individual rice plants using multi-angle color images. Journal of Innovative Optical Health Sciences, 8(2), 1550002. DOI: 10.1142/S1793545815500029.

Kapusta, I., Cebulak, T., Oszmianski, J., 2017: The anthocyanins profile of red grape cultivars growing in south-east Poland (Subcarpathia region). Journal of Food Measurement and Characterization, 11, 1863–1873. DOI: 10.1007/s11694-017-9568-4.

Keramatlou, I., Sharifani, M., Sabouri, H., Alizadeh, M., Kamkar, B., 2015: A simple linear model for leaf area estimation in Persian walnut (*Juglans regia* L.). Scientia Horticulturae, 184, 36–39. DOI: 10.1016/J.SCIENTA.2014.12.017.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R. T., Hoffmann, C., Töpfer, R., Kicherer, A., 2018: Effects of canopy architecture and microclimate on grape-

vine health in two training systems. Vitis, 57(2), 53–60. DOI: 10.5073/vitis.2018.57.53-60.

Lande, R. Shannon, S., 1996: The role of genetic variation in adaptation and population persistence in a changing environment. Evolution, 50(1), 434–437. DOI: 10.1111/j.1558-5646.1996.tb04504.x.

Lane, J. E., Czenze, Z. J., Findlay-Robinson, R., Bayne, E., 2019: Phenotypic plasticity and local adaptation in a wild hibernator evaluated through reciprocal translocation. The American Naturalist, 194(4), 516–528. DOI: 10.1086/702313.

Liu, Z., Zhu, Y., Li, F., Jin, G., 2017: Non-destructively predicting leaf area, leaf mass and specific leaf area based on a linear mixed-effect model for broadleaf species. Ecological Indicators, 78, 340–350. DOI: 10.1016/j.ecolind.2017.03.025.

Malagi, G., Citadin, I., Scariot, S., Reis, L., 2010: Método não destrutivo para determinação da área foliar da videira, cultivar BRS-Violeta. Revista Brasileira de Fruticultura, 32(4), 1250–1254. DOI: 10.1590/S0100-29452011005000005.

Migicovsky, Z., Li, M., Chitwood, D. H., Myles, S., 2018: Morphometrics reveals complex and heritable apple leaf shapes. Frontiers in Plant Science, 8, 2185. DOI: 10.3389/fpls.2017.02185.

Mohimont, L., Alin, F., Rondeau, M., Gaveau, N., Steffenel, L. A., 2022: Computer vision and Deep Learning for precision viticulture. Agronomy, 12(10), 2463. DOI: 10.3390/agronomy12102463.

Montaigne, E., Coelho, A., Khefifi, L., 2016: Economic issues and perspectives on innovation in new resistant grapevine varieties in France. Wine Economics and Policy, 5(2), 73–77. DOI: 10.1016/J.WEP.2016.11.002.

Narduzzi, L., Stanstrup, J., Mattivi, F., 2015: Comparing wild American grapes with *Vitis vinifera*: a metabolomics study of grape composition. Journal of Agricultural and Food Chemistry, 63(30), 6823–6834. DOI: 10.1021/acs.jafc.5b01999.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M. Duchesnay, E., 2011: Scikit-learn: Machine Learning in Python. Journal of Machine Learning Research, 12, 2825-2830.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M. S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V., Anfora, G., 2017: A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Protection, 97, 70–84. DOI: 10.1016/J.CROPRO.2016.11.025.

Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., Peressotti, E., Zulini, L., Stefanini, M., Angeli, G., Masiero, S., Welter, L. J., Hausmann, L., Vezzulli, S., 2019: Emergent ascomycetes in viticulture: An interdisciplinary overview. Frontiers in Plant Science, 10, 1394. DOI: 10.3389/FPLS.2019.01394.

Plesovskaya, E. Ivanov, S., 2021: An empirical analysis of KDE-based generative models on small datasets. Procedia Computer Science, 193, 442–452. DOI: 10.1016/j.procs.2021.10.046.

Pudumalar, S., Muthuramalingam, S., Shanmugapriyan, R., 2020: A review and impact of data mining and image processing techniques for aerial plant pathology. In: Lecture Notes on Data Engineering and Communications Technologies (Springer), 747–754.

Rahman, I. U., Hart, R., Afzal, A., Iqbal, Z., Abd_Allah, E. F., Alqarawi, A. A., Ijaz, F., Ali, N., Kausar, R., Muzammil, S., Alzain, M. N. O., Majid, A., Calixto, E. S., 2019: Phenological plasticity in *Berberis lycium royle* along temporal and altitudinal gradients. Applied Ecology and Environmental Research, 17, 331–341. DOI: 10.15666/aeer/1701_331341.

Rouphael, Y., Mouneimne, A. H., Rivera, C. M., Cardarelli, M., Marucci, A., Colla, G., 2010: Allometric models for non-destructive leaf area estimation in grafted and ungrafted watermelon (*Citrullus lanatus* Thunb). Journal of Food, Agriculture & Environment, 8(1), 161–165.

Scheepens, J. F., Frei, E. S., Stöcklin, J., 2010: Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. Oecologia, 164, 141–150. DOI: 10.1007/s00442-010-1650-0.

Silvestroni, O., Lanari, V., Lattanzi, T., Palliotti, A., 2018: Delaying winter pruning, after pre-pruning, alters budburst, leaf area, photosynthesis, yield and berry composition in Sangiovese (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research, 24(4), 478–486. DOI: 10.1111/ajgw.12361.

Souza, A. L. K. de, Brighenti, A. F., Brighenti, E., Caliari, V., Stefanini, M., Trapp, O., Gardin, J. P. P., Dalbo, M. A., Welter, L. J., Camargo, S. S., 2019: Performance of resistant varieties (PIWI) at two different altitudes in Southern Brazil. BIO Web of Conferences, 12, 01021. DOI: 10.1051/BIO-CONF/20191201021.

Stefanini, M., Tomazetti, T. C., Rossarolla, M. D., Costa, A., Dela Bruna, E., Malinovski, L. I., Silva, A. L., Nodari, R. O., Guerra, M. P., 2019: Phenology and thermal requirements of disease-resistant genotypes (PIWI) grown in the Goethe Grape Valley region of Brazil. Acta Horticulturae 1248, 141–148. DOI: 10.17660/ActaHortic.2019.1248.21.

Tech, A. R. B., Silva, A. L. C. da, Meira, L. A., Oliveira, M. E. de, Pereira, L. E. T., 2018: Methods of image acquisition and software development for leaf area measurements in pastures. Computers and Electronics in Agriculture, 153, 278–284. DOI: 10.1016/j.compag.2018.08.025.

Teobaldelli, M., Basile, B., Giuffrida, F., Romano, D., Toscano, S., Leonardi, C., Rivera, C. M., Colla, G., Rouphael, Y., 2019: Analysis of cultivar-specific variability in size-related leaf traits and modeling of single leaf area in three medicinal and aromatic plants: *Ocimum basilicum* L., *Mentha* Spp., and *Salvia* Spp. Plants, 9(1), 13. DOI: 10.3390/plants9010013.

Tondjo, K., Brancheriau, L., Sabatier, S. A., Kokutse, A. D., Akossou, A., Kokou, K., Fourcaud, T., 2015: Non-destructive measurement of leaf area and dry biomass in *Tectona grandis*. Trees, 29, 1625–1631. DOI: 10.1007/s00468-015-1227-y.

14 | Original Article

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E., Eibach, R., 2011: New Horizons for Grapevine Breeding. In: Flachowsky, H., Hanke, V. M. (Eds): Methods in Temperate Fruit Breeding. Fruit, Vegetable and Cereal Science and Biotechnology 5 (Special Issue 1), pp. 79-100.

Vezzulli, S., Vecchione, A., Stefanini, M., Zulini, L., 2018: Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy). European Journal of Plant Pathology, 150, 485–495. DOI: 10.1007/s10658-017-1298-2.

Vitasse, Y., Hoch, G., Randin, C. F., Lenz, A., Kollas, C., Scheepens, J. F., Körner, C., 2013: Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. Oecologia, 171, 663–678. DOI: 10.1007/s00442-012-2580-9.

Waskom, M. L., 2021: Seaborn: statistical data visualization. The Journal of Open Source Software, 6(60), 3021. DOI: 10.21105/joss. 03021.

Weiner, J., 2004: Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics, 6(4), 207–215. DOI: 10.1078/1433-8319-00083.

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul, E., Eibach, R., Töpfer, R., Zyprian, E. M., 2007: Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). Molecular Breeding, 20, 359–374. DOI: 10.1007/s11032-007-9097-7.

Williams, L., Martinson, T. E., 2003: Nondestructive leaf area estimation of 'Niagara' and 'DeChaunac' grapevines. Scientia Horticulturae, 98(4), 493–498. DOI: 10.1016/S0304-4238(03)00020-7.

Zanghelini, J. A., Bogo, A., Dal Vesco, L. L., Gomes, B. R., Mecabo, C. V., Herpich, C. H., Welter, L. J., 2019: Response of PIWI grapevine cultivars to downy mildew in highland region of southern Brazil. European Journal of Plant Pathology, 154(4), 1051–1058. DOI: 10.1007/s10658-019-01725-y.

Zeist, A. R., Tomazetti, T. C., Rossarolla, M. D., Alberto, C. M., Giacobbo, C. leviso. L., Welter, L. J., 2017: Plastochron index of 'Cabernet Sauvignon' and 'Chardonnay' grapevines in Fronteira Oeste, in the state of Rio Grande do Sul, Brazil. Pesquisa Agropecuaria Brasileira, 52, 244–251. DOI: 10.1590/S0100-204X2017000400004.