Comparison of different methodologies to estimate bunch compactness

Beatrice Carmignani

Dissertation to obtain a Master's Degree in

Engineering of Viticulture and Oenology – Double Degree

President of the jury:
PhD Joaquim Miguel Rangel da Cunha Costa, Assistant Professor at Instituto Superior de Agronomia, Universidade de Lisboa.

Members of the jury:
PhD Carlos Manuel Antunes Lopes, Associate Professor with Habilitation at Instituto Superior de Agronomia, Universidade de Lisboa, supervisor;
PhD José Eduardo Jorge Eiras Dias, Assistant Researcher with Habilitation at Instituto Nacional de Investigação Agrária e Veterinária I.P.

2019

To my Family,

thank you for being my greatest strength

and my greatest weakness.

Acknowledgement

I would like to thank the Higher Institute of Agronomy and, in particular, Professor Carlos M.A. Lopes for the support, the patience and the trust placed on me. In addition, I thank the University of Turin for the opportunity offered and Professor Vittorino Novello for the availability shown.

A special thank goes to the team: to Gonçalo for the great help and to Ruben, Luigi and Giuseppe for sharing this adventure, I could not ask for better colleagues!

Thanks to all those who have been part of the great Lisbon family, new friendships and strengthened friendships: Filippo, Andrea C., Andrea Z., Ciccio, Cico, Gavino, Peppe, Simone, Vittoria, Marta, Anna, Ruben, Luigi, Alessandro, Damiano. It was unique to share with you this year.

I thank my family: my mother, my father, Chiara and Ilaria, for always being my anchor, my certainty in difficult times, my safe haven. Thank you for always being on my side and for encouraging me to always give my best. I can never thank you enough.

Thanks to all the relatives, uncles, aunts, cousins and grandmothers, for their support and infinite love.

Thanks to longtime friends, those who are there and will always be there, despite the kilometers and time zones to separate us, I love you: Tiziano, Chiara, Thomas, Giulia and Lucia.

Thanks to my two crazy friends, Vittoria and Anna, so different and basically so similar. Thank you because without you this experience would not have been the same. Thank you for the sunsets, for the endless talks, for the hugs and for loving me in my imperfection.

Thank you to Francis for made me felt less homesick, for hugging me when I needed it, and for being as it is, a friend.

Thanks to Filippo, because I love him and because he loves me. Have lived a part of this experience with you has made it even more unique. Thank you for the travels, for the laughter, for the fights and for always being able to make peace. Thank you for believing in me even when I didn't believe in myself.

Finally, thanks to myself, for have had the courage to leave, for not giving up, for crying to the point of exhaustion and laughing at my heart, for putting myself on the line, for overcoming my limits and for giving me new ones to face.

Abstract

Bunch compactness (BC) is a key target for wine sector because it affects disease susceptibility, berry ripening among other grapes characteristics. The most common method to estimate BC is the O.I.V. descriptor n°204: manual and subjective. Objective and automated methods are based on indices, using different relations between bunch traits, some obtained manually and other automatically through image analysis (as example: BW – weight; BV – volume; ML – maximum length; A – projected area; MVO – morphological volume; V3 – derived volume; BN – berries number). All the variables were significantly and positively correlated between each other: the highest Pearson correlation coefficient was between BW and BV (r = 0.99) followed by BW and A (r = 0.95). Fourteen compactness indices (CI) were tested (9 published and 5 created) on 61 Syrah bunches. These indices were then correlated with the mode of the O.I.V. descriptor n°204, where 11 were positively correlated and three were negatively correlated (CI-3, CI-3a, CSF). The index CI-10a, which relates bunch weight and maximum length, was the most suitable one to define BC (r = 0.78). In the frame of the EU VINBOT project, to improve BW estimation finding the best explanatory variables, a stepwise regression analysis between BW and the variables considered easy to extract by automated image analysis (A1 – projected area, V3 – volume 3, BN – berries number and CI-10a as index) was performed. The variable which explained best BW was A1 (partial R^2 = 0.905), followed by CI-10a and V3 with a much smaller contribution (partial R^2 <0.06 and partial R^2<0.007, respectively). The variable BN was not selected by the model. We concluded that BC can be estimated in an objective and automatic way using image analysis. Furthermore, such estimations can enhance BW prediction by using BC as one of the explanatory variables which can improve automatic yield estimation methodologies.

Keywords: bunch trait – objective indices – O.I.V. descriptor 204 – bunch volume – bunch weight

Resumo

A compacidade dos cachos (BC) é uma componente da arquitetura dos mesmos com elevada importância para o setor vitivinícola. O método mais comum para estimar a BC é o descritor do O.I.V. n°204, manual e subjetivo. Outros autores propuseram índices como metodologias para estimare BC objectivamente. Tais índices utilizam alguns componentes do cacho avaliados manualmente e outros automaticamente através de metodologias de análise de imagem, tais como BW – peso; BV – volume; ML – comprimento máximo; A – área projetada; MVO – volume morfológico; V3 – volume derivado; BN – número de bagos. Destas variáveis, todas apresentaram uma correlação (coeficiente de correlação de Pearson, r) positiva e significativa entre elas, BW e BV obtendo o maior coeficiente ($r = 0.99$) seguido de BW e A ($r = 0.95$). Foram testados catorze índices de compacidade (CI; 9 publicados e 5 criados) em 61 cachos de Syrah. Estes índices foram então correlacionados com a moda do O.I.V. n°204, onde 11 apresentaram correlações positivas e três negativas (CI-3, CI-3a, CSF). O índice CI-10a, que relaciona o peso do cacho com o comprimento máximo do cacho, foi o que melhor explicou BC ($r=0.78$). Além destes resultados, no âmbito do projeto EU VINBOT, exploraram-se diferentes possíveis variáveis explicativas para estimar o BW com uma regressão linear *step wise* entre BW (variável independente) e outras variáveis consideradas obtíveis via análise de imagem automatizada (A1 –área projetada, V3 – volume 3, BN – número de bagos e o índice de compacidade CI-10a). A variável que explicou melhor o BW foi A1 (R^2 parcial = 0.905), seguida de CI-10a e V3 apresentando uma contribuição muito inferior (R^2 parcial <0.06 e R^2 parcial <0.007, respectivamente). A variável BN não foi selecionada pelo modelo. Os resultados mostram que a BC pode ser estimada de forma objetiva e automática por meio da análise de imagem e pode melhorar a previsão do BW utilizando BC como uma das variáveis explicativas e que pode ajudar futuras metodologias de estimativa automática do rendimento na vinha.

Palavras chaves: compacidade de cachos– índices de compacidade – O.I.V. descritor 204 – volume do cacho – peso do cacho

Resumo alargado

A compacidade dos cachos (BC) é uma componente da arquitetura dos mesmos com elevada importância para o setor vitivinícola pois está relacionado com a susceptibilidade da uva a doenças assim como ao amadurecimento dos bagos, entre outras características das uvas. O método mais comum para estimar a BC é o descritor do O.I.V. n°204, normalmente realizado por um painel de 10 juízes treinados de forma a reduzir a subjectividade associada a este método baseado numa observação a olho nú. Outros autores propuseram índices que relacionam componentes do cacho mensuráveis de forma a desenvolver metodologias de estimativa da BC continuas e mais objectivas. Tais índices utilizam alguns componentes do cacho avaliados manualmente e outros automaticamente através de metodologias de análise de imagem, tais como BW – peso; BV – volume; ML – comprimento máximo; A – área projetada; MVO – volume morfológico; V3 – volumes derivados; BN – número de bagos. Neste trabalho ambos os tipos de índices foram testados e todas as variáveis mencionadas anteriormente calculadas. Destas variáveis, todas apresentaram uma correlação (coeficiente de correlação de Pearson, r) positiva e significativa entre elas, BW e BV obtendo o maior coeficiente (r = 0.99) seguido de BW e A (r = 0.95). Foram testados catorze índices de compacidade (CI; 9 publicados e 5 criados) em 61 cachos de Syrah. Estes índices foram então correlacionados com a moda do O.I.V. n°204, onde 11 apresentaram correlações positivas e três negativas (CI-3, CI-3a, CSF). Os coeficientes de correlação de Pearson entre cada índice de compactidade e o O.I.V. n°204 foram inicialmente calculados para todos os cachos amostrados e então apenas calculados para os cachos sem asas e agrupados consoante a sua forma (de acordo com o descritor O.I.V. n°208) de forma a entender se, criando uma base de dados mais homogénea, os índices apresentam uma maior capacidade de explicar a compacidade do cacho. O índice CI-10a, que relaciona o peso do cacho com o comprimento máximo do cacho, foi o que melhor explicou BC (r=0.78). Além destes resultados, no âmbito do projeto EU VINBOT, uma plataforma robótica que visa prever a produção de uva na vinha, exploraram-se diferentes possíveis variáveis explicativas para estimar o BW. Para tal, uma regressão linear *step wise* foi realizada entre BW (variável independente) e outras variáveis consideradas obtíveis via análise de imagem automatizada (A1 –área projetada, V3 – volume 3, BN – número de bagos e o índice de compacidade CI-10a). O volume do cacho apresentou a maior capacidade de estimar o BW, no entanto não é obtível automaticamente apenas com recurso a imagens 2D. Por esta razão, outros volumes foram obtidos de forma indireta, a partir de análise de imagem (V1, V2, V3). A variável que explicou melhor o BW foi A1 (R^2 parcial = 0.905), seguida de CI-10a e V3 apresentando uma contribuição muito inferior (R^2 parcial <0.06 e R^2 parcial <0.007, respectivamente). A variável BN não foi selecionada pelo modelo. Os resultados atuais mostram que a BC pode ser estimada de forma objetiva e automática por meio da análise de imagem. Para além disso, estas estimativas podem melhorar a previsão do BW utilizando BC como uma das variáveis explicativas que podem potencialmente melhorar futuras metodologias de estimativa automática do rendimento na vinha.

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List of abbreviations

A Mean Area

A1 Area from frontal image

A2 Area from lateral image

ABV Actual bunch volume

ATV All-terrain vehicle

BABA DL-βamino-n-butyric acid

BB Berries per bunch

BL Bunch length

BN Berries number

BV Bunch real volume

BW Bunch weight

C Cluster

CBV Conical bunch volume

CI Compactness index

CIELAB Colour space CIE L*a*b*

CSF Compactness shape factor

h Rachis length

HD Hand defoliation

IL Internode length

IPGRI International Plant Genetic Resources Institute

IPMA Instituto Português do Mar e da Atmosfera

ISA Instituto Superior de Agronomia

ISO International Organization for Standardization

IVV Instituto da vinha e do vinho

LOCI Laboratory for Optical and Computational Instrumentation

M Maturation

MAX Maximum value

MBV Morphological bunch volume

MD Manual defoliation

MEAN Medium value

MIN Minimum value

ML Bunch maximal length

MVO Morphological bunch volume

MW Maximum width

N Nitrogen

O.I.V. Organisation Iternationale de la Vigne et du Vin

P Mean Perimeter

PAR Photosynthetically Active Radiation

PL Pedicel length

Prep Precipitation

r radius

r Pearson correlation coefficent

R Richter

R2 Determination coefficient

RB Ramifications per bunch

RD Bunch roundness

RGB Red-Green-Blue

RU Ruggeri

RL Rachis length

S1 Smart point 1

S2 Smart point 2

S3 Smart point 3

S4 Smart point 4

SB Seeds per berry

SY Syrah variety

Tmean Mean temperature

TMEAN Mean thickness

TMAX Maximum thickness

UPOV International Union for the Protection of New Varieties of Plants

V Volume

Va Volume of the cylinder a

Vb Volume of the cylinder b

Vc Volume of the cylinder c

Vd Volume of the cylinder d

V1 Volume 1

V2 Volume 2

V3 Volume 3

VSP Vertical shoot position

Wi Bunch width

1RL First ramification length

2RL Second ramification length

1. Introduction

Bunch compactness is becoming a key target for grapevine cultural management and for genetic improvement of table and wine grapes (Ibáñez *et al.,* 2015) because this trait affects disease susceptibility, berry ripening and other characteristics of grapes. Grapevine yield can be determined by the yield components: number of bunches, number of berries per bunch and berry weight (Tardaguila *et al.,* 2012). These traits influence bunch compactness, also called bunch density or, considering the opposite attribute, bunch openness. It refers to the arrangement of berries in the bunch and to the portion of free space they leave. It is linked to the morphological volume of the bunch and to its solid component (Tello & Ibáñez, 2018). Indeed, berries in compact bunches are in close contact, hindering the development of the protective waxy cuticle and increasing rot incidence (Vail & Marois, 1991). This incidence is higher when the pressure exerted by growing berries during berry ripening causes berry cracking. The leakage of juice in compact bunches riches in water and nutrients is the most suitable condition for conidia germination and mould development; which might rapidly spread due to berry-to-berry contact until the entire bunch is rotted (Hed *et al.,* 2009). The situation can be aggravated by the retention of senescent flower debris in the inner parts of compact bunches, because it serves as inoculum for berries infection (Molitor *et al.,* 2011). The dense berry distribution in compact bunches also restricts airflow, increasing bunch internal temperature and humidity, allowing the development of different organisms. Moreover, fungicide spraying has low efficacy in compact bunches, confirming that compactness is one of the main factors affecting the epidemiology of *Botrytis cinerea* (Vail & Marois, 1991) that causes large economic loss for wine and grape industry, reducing directly yield and quality. Additionally, grape and wine quality are affected by bunch compactness because berries receive non-uniform solar radiation, influencing berry ripening and composition and this situation increases bunches heterogeneity (Vail & Marois, 1991; Pieri *et al*., 2016). These differences make the market acceptance of table grapes difficult, due to sensory attributes, like berries texture and flavour, chemical composition and, mainly, because the first impression is based on visual attributes, including bunch compactness (Piazzolla *et al*., 2016). Some practices used in fruit industry are hindered if grape bunches are too compact: fruit washing, handling or transportation (Sepahi, 1980) and packaging and shelf-life (Chen *et al*., 2018). For this reason, wine growers prefer cultivars with looser bunches and, as a consequence, grapevine breeders have to pay attention to the select seedlings and new cultivars. It is hard because bunch architecture is a mosaic of different single traits which makes phenotyping labor-intensive and time-consuming (Rist *et al*., 2018). Considering the commercial relevance of quality and sanitary status of bunch compactness for table and wine grapes, numerous strategies have been tested to manipulate this trait. These strategies can be divided in treatments based on agrochemical applications (gibberellins, Prohexadione-calcium, Forchlorfenuron and anti-transpirants; Silvestroni *et al*., 2016) and crop management strategies. These cultural techniques include removal of vegetative organs of the plant: living shoots, buds, leaves, bunches and berries; the use of alternative training systems and different rootstocks (Molitor *et al*., 2011). Considering the impact of bunch compactness on yield estimating methods and the relevant differences among varieties, the objectives of this work are: comparing different methodologies to estimate bunch compactness, including image analysis methods; comparing bunch compactness methods for the variety Syrah; study the impact of bunch compactness in yield estimation methodologies based on image analysis; contribute to the development of yield estimation algorithms associated with the Vinbot platform. This aim is innovative, and the final method will be useful to estimate bunch compactness, not only in one variety but in class of varieties divided for common traits.

2. Literature review

2.1. Definition of bunch compactness

Bunch compactness is defined by the degree of compaction of berries along the rachis that arises from how berries are disposed in the morphological volume of the bunch, which is determined by rachis architecture (Tello & Ibáñez, 2018) as showed in Figure 1. Berries are sparsely distributed in loose bunches, whereas they are densely packed in the compact ones (Tello & Ibáñez, 2018). Berries number and size define the bunch solid component, where the final number of berries depends on flowers number and fruit set rate (Carmona *et al*., 2008). According to Diago *et al*. (2014), flowering and fruit set are two physiological processes that define the number of berries per bunch and, with berry volume, influence bunch compactness. After fruit set, berry development follows a double sigmoidal trend with two peaks of growth (berries formation and ripening) separated by a period called lag phase of slow or no growing. These two peaks of growth define the final berry size (Coombe & McCarthy, 2000).

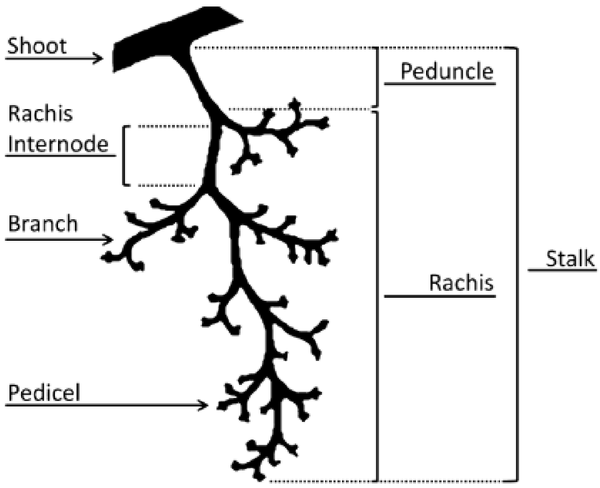


Figure 1: Schematic diagram showing the main section of a grapevine bunch at harvest time, after removing berries (Tello & Ibáñez, 2018)

Predicting the final bunch compactness each season would be helpful to decide vineyard management practices or treatments in advance; in order to have such a predictive capacity. Tello & Ibáñez (2018) stated that the development of models to predict bunch compactness should include variables defined at early stage of inflorescences and bunch development.

2.2. Factors that can influence bunch compactness

Bunch compactness can be affected by a series of factors. First, the number of berries per bunch, which is affected by the number of flowers per inflorescence and is greatly dependent on fruit set rate, defined by the proportion of flowers converted into berries (Eltom *et al.*, 2017).

Inflorescences, bunch architecture and growth as well as the above mentioned fruit set rate are genetic factors that can be highly influenced by environmental conditions and adjusted in the vineyard with different management strategies (Tello & Ibáñez, 2018). One or two weeks after flowering, during fruit set, the final number of berries is established and the proportion of flowers converting into berries is greatly dependent on the number of flowers per inflorescence (Eltom *et al.*, 2017).

2.2.1. Genetic factors

Tello *et al.* (2015) analysed bunch compactness and its genetic variability, evaluating 125 wine and table grape cultivars in three different seasons. The results showed that bunch compactness is mainly defined by differences between morphological/apparent volume and real/solid volume of bunches. Real volume is determined by the total number of berries, while the apparent volume depends on berries spatial arrangement, determined by rachis architecture (the length of bunch main axis) that is highly variable between cultivars. Additionally, flowers number and fruit set rate are under genetic control and influenced by the environment, which hinders the genetic analysis of grape berries number (Tello *et al.*, 2016a). Baby *et al.* (2016) showed fruit set differences between three cultivars: Syrah, Merlot and Cabernet Sauvignon, attributable to differences in pollen variability and different amount of amines (e.g. diamino-propane and phenylethylamine) in flowers. These differences can inhibit pollen tube growth and, consequently, the normal fertilization process.

Another work by Houel *et al.* (2013) studied the genetic variability of berries size in 304 table and wine grape genotypes, concluding that cell division (before and after fruit set) and cell expansion (after fruit set) are the main determinant factors affecting berries size variation and compactness at multi-cultivar level (Eltom *et al.*, 2017).

2.2.2. Environmental factors

It is generally accepted that a combination of sufficient light intensity, short-term exposure to high temperature, absence of water and nutrition stresses is required for an optimum inflorescence initiation (Li-Mallet *et al.,* 2015). These factors influence other critical processes, as the date of budburst and the growth rate of the inflorescence, that have a consequent effect on bunch architecture and compactness (Carmona *et al.*, 2008).

2.2.2.1. Radiation and temperature

Radiation and temperature are two factors that are independent critical signals for inflorescence induction, for the differentiation during season one and for inflorescence development during the following season (Carmona *et al.*, 2008; Li-Mallet *et al.,* 2015). Indeed, the illumination of buds is strongly related to the rate of budburst, the number of bunches per shoot and berries per bunch (Tello & Ibáñez, 2018). It was observed that often illuminated buds outside the canopy are more fruitful than those located inside of it (Perez & Kliewer, 1990). Petrie & Clingeleffer (2005) reported that light seemed to have effects on bunch components, in addition to those caused by temperature: lower temperature during flower formation increased flowers number and inflorescence length; the opposite with high temperature (Petrie & Clingeleffer, 2005; Tello & Ibáñez, 2018). Shading treatment before budburst increased flowers number by 13%, approximately. It has been hypothesised that high temperatures can accelerate the vegetative growth phase, with a reduction on the inflorescence differentiation stage to a shorter period of time, giving a lower number of individual flowers (Pouget, 2016). Consequently, at harvest time there will be a lower berries number and a lower value of bunch compactness, but, as mentioned above, there is a compensating effect between flowers number and berry set rate (Eltom *et al.*, 2017). Differences in berries size are probably consequence of the repression of light-mediated effects on cell division or expansion due to sunlight deprivation during the first stages of berry growth (Tello & Ibáñez, 2018).

2.2.2.2. Water and mineral nutrients

Different cultivars, and clones, have different genetic variability in tolerance to water stress (Tortosa *et al.*, 2016) and, as found by Matthews & Anderson (1988), an early water deficit during the first weeks after flowering in season one can lead to a lower number of individual flowers in the following season and can affect the potential berry growth by hindering cell division processes. Different studies indicate that water deficit reduces berries size, an aspect more sensitive to early water stress than to late one (Niculcea *et al.*, 2014). According to these studies, berries size reduction was exclusively caused by a decrease of pericarp volume, meaning that early water stress can modify the structural properties of the cell components and cell wall extensibility, limiting the subsequent enlargement of pericarp cells and compromising potential berry size (Tello & Ibáñez, 2018). Later water deficit, after veraison, also reduces berries mass (Matthews & Anderson, 1988), mostly reducing the growth of berry mesocarp tissues. In fact, limiting water input at a certain stage of berry development is a common practice to limit berry size and bunch compactness, improving berry composition and diminishing bunch rot incidence (Intrigliolo *et al.*, 2012). Related to water, the mineral nutrients content available for vine is important. For example, nitrogen (N) is the most important macro-nutrient for the optimum growth of vines, with has direct and indirect effects on vegetative growth: low nitrogen (N) availability during season one limits the reserves needed for an optimum inflorescence development in the following season; moreover, it has been suggested that low N reserves on plants can reduce fruit set ratio, which influences the final number of berries per bunch (Duchêne *et al.,* 2001). Keller *et al.* (2001) evaluated the short-term responses to N supply on Müller-Thurgau variety and observed that fertilization increased bunch compactness due to an increment of both berries number (because of a higher fruit set rate) and berries size, with problems related to the appearance of Botrytis bunch rot. In contrast, El-Razek *et al.* (2011) reported that berries size of a seedless cultivar (Crimson Seedless) was the only yield component affected by N fertilisation, without affecting berries number and bunch compactness. Moreover, excessive N fertilisation can lead to denser canopies, hindering sunlight irradiation, varying photosynthesis efficiency and thus carbon availability for optimum bunch development (Tello & Ibáñez, 2018). Phosphorus and potassium, plus different micronutrients (e.g. boron, zinc and molybdenum), have been shown to affect grapevine reproductive efficiency, may modifying bunch architecture and compactness (Li-Mallet *et al.,* 2015). As an example, insufficient zinc can compromise pollen formation and, consequently, pollination; while, boron is needed for pollen germination and pollen tube growth, so it is essential for the ovule fertilisation process. Deficiency of any of these nutrients may lead to reproductive disorders, like an abnormally high rate of flowers fall (*coulure*) or the development of tiny and seedless berries (*millerandage*), leading to low fruit set rates and looser bunches (Keller, 2015).

2.3. Methodologies to estimate bunch compactness

There are visual and qualitative, direct and indirect, destructive and non-destructive methods to estimate bunch compactness.

2.3.1. O.I.V. descriptor

The most common method to evaluate bunch compactness, classifying bunches into predefined categories, is the *Organisation Internationale de la Vigne et du Vin* (O.I.V.) descriptor n°204 for bunch density (O.I.V., 2001). It is equivalent of the descriptor 33 in the International Union for the Protection of New Varieties of Plants (UPOV) list and of the descriptor 6.2.3 in the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) list. The O.I.V. descriptor n°204 classifies bunches into five categories, based on the mobility of the berries and the visibility of the pedicels: very loose (notation 1); loose (3); medium (5); compact (7) and very compact (9) as reported in the Table 1 and Table 5. This visual scale can be simple and cost-saving; as a matter of fact, the viticultural sector finds this method rapid and non-destructive, therefore useful. Nonetheless, its application needs trained evaluators and entails great subjectivity that makes difficult to discriminate the compactness of several bunches. This problem can be overcome with a panel of judges, but some studies required objective and continuous variable and it is limited by the categorical data obtained (Tello & Ibáñez, 2018).

Table 1: O.I.V. descriptor n°204 for bunch compactness evaluation (Moro, 2016)

Notation		Definitions
1	Very loose bunch	Berries clearly separated, many visible pedicels
3	Loose bunch	Berries in loose contact with each other with some visible pedicels
5	Medium bunch	Densely distributed berries, pedicels not visible, berries are movable
7	Dense bunch	Berries not readily movable
9	Very dense bunch	Berries deformed by compression

2.3.2. Objective methods

There are bunch characteristics that vary with compactness; this variation may be used for indirect estimation of bunch compactness. For example, compact bunches are less flexible than loose ones and this trait is used indirectly through the so-called "density index" applied by Molitor *et al.* (2011) and Evers *et al.* (2010). This method classifies bunches in five categories considering the proximity between berries and the bending of the stem: 1 — very loose (no berry contact; bending of the stem to 90° possible); 2 — loose (berry contact; bending of the stem up to 45–90° possible); 3 — dense (berries still flexible; bending of the stem up to 10–45° possible); 4 — compact (berries not flexible; bending of the stem up to 10° possible); and 5 — very compact (berries not flexible; bending of the stem not possible) (Evers *et al.*, 2010). Another characteristic that varies with compactness is the inter-berry space: loose bunches have more space between berries than compact ones. It has been used to evaluate bunch compactness indirectly, determining the existing distance between two randomly chosen berries through the insertion of wedges in the inter-berry space (Zabadal & Dittmer, 1998). A similar approach has been proposed by Vail & Marois (1991) using a firmness tester to measure the required force to separate two contiguous berries by a distance of 2 millimetres and determined qualitatively bunch compactness. Moreover, these authors used another indirect method involving the volumes, morphological/apparent and solid/real. The actual volume occupied by bunch solid elements can be easily measured through the immersion of the bunch in a bucket filled with water and, following the Archimedes' principle, measured the amount of water displaced by the bunch. Nevertheless, the morphological volume is more difficult to determine because any modification in the natural arrangement of berries will modify the apparent volume (Tello & Ibáñez, 2014). A method to estimate the morphological volume used melted paraffin to fill in the empty holes (Sepahi, 1980) but this method provides a rough estimation because it does not consider the irregularities along the bunch and it is not applicable to bunches with other shapes: cylindrical or funnel-shaped bunches (Tello & Ibáñez, 2018). Another method to estimate the morphological volume has been proposed by Ferreira & Marais (2017) using a bucket fitted with an overflow pipe filled with water. After water stopped dripping from the overflow pipe, bunches were separately placed in polyethylene bags with the air removed by suction and completely submerged in the water (one bunch per bucket). The water thus displaced was measured in a measuring cylinder. Bunch mass was determined and bunch compactness was calculated through the density, dividing bunch mass by bunch volume (Ferreira & Marais, 2017).

Tello & Ibáñez (2014) compared bunch compactness of 110 grape bunches from 11 varieties using various indices as showed in Table 2: eleven published in literature (Sepahi, 1980; Pommer *et al.*, 1996; Fermaud, 1998; Shavrukov *et al.,* 2004; Valdés-Gómez *et al.*, 2008; Sternad-Lemut *et al.*, 2011; Ferreira & Marais, 2017) and eight designed in Tello & Ibáñez (2014)'s study; using the following parameters: first ramification length (1RL); second ramification length (2RL); actual bunch volume (ABV); berries per bunch (BB); bunch length (BL); bunch weight (BW); conical bunch volume (CBV); internode length

(IL); morphological bunch volume (MBV); ramifications per bunch (RB); pedicel length (PL); rachis length (RL); seeds per berry (SB).

Table 2: Different bunch compactness indices (CI) (Tello & Ibáñez, 2014).

Index:	Equation:	References:
CI-1	BW (g)/[RL (cm) + 1RL (cm)]	(Fermaud, 1998)
CI-2	BB/[RL (cm) + 1RL (cm)]	(Valdés-Gómez *et al.*, 2008)
CI-3	BB/BL (cm)	(Pommer *et al.*, 1996)
CI-4	[ABV (mL)/MBV (mL)] × 100	(Sepahi, 1980)
CI-5	$\frac{\text{ABV (mL)}}{\text{RL (cm)} + \text{1RL (cm)} + \text{2RL (cm)}}$	(Sepahi, 1980)
CI-6	$\frac{\text{BW (g)}}{\text{RL (cm)} + \text{1RL (cm)} + \text{2RL (cm)}}$	(Sepahi, 1980)
CI-7	$\frac{\text{ABV (mL)} \times \text{RB}}{\text{RL (cm)} + \text{1RL (cm)} + \text{2RL (cm)}}$	(Sepahi, 1980)
CI-8	$\frac{\text{BW (g)} \times \text{RB}}{\text{RL (cm)} + \text{1RL (cm)} + \text{2RL (cm)}}$	(Sepahi, 1980)
CI-9	$\frac{[\text{CBV (mL) - ABV (mL)}]}{\text{ABV mL}} \times 100$	(Shavrukov *et al.,* 2004)
CI-10	BW (g)/BL (cm)	(Sternad-Lemut *et al.*, 2011)
CI-11	BW (g)/MBV (mL)	(Ferreira & Marais, 2017)
CI-12	BW (g)/$[\text{BL (cm)}]^2$	(Tello & Ibáñez, 2014)
CI-13	ABV (mL)/$[\text{BL(cm)}]^2$	(Tello & Ibáñez, 2014)
CI-14	$\frac{\text{BB}}{\text{BL (cm)} + \text{1RL (cm)} + \text{2RL (cm)}}$	(Tello & Ibáñez, 2014)
CI-15	BB/$\sum_{1-6}$ IL (cm)	(Tello & Ibáñez, 2014)
CI-16	10.368 + [0.015 x BW (g)] + (0.002 × BB) [-0.443 × BL (cm)] + (0.018 × 1RL)	(Tello & Ibáñez, 2014)
CI-17	$\frac{\text{BW (g)} \times \text{BB}}{[\text{BL (cm)}]^2 + \text{1RL (cm)}}$	(Tello & Ibáñez, 2014
CI-18	$\frac{\text{BW (g)} \times \text{BB} \times (1 + \text{SB})}{[\text{BL (cm)}]^2 \times \text{1RL (cm)} \times \text{PL (mm)}}$	(Tello & Ibáñez, 2014)
CI-19	$\frac{\text{BW (g)} \times \text{BB} \times (1 + \text{SB})}{[\text{BL (cm)}]^2 \times \text{1RL (cm)} \times \text{RB}}$	(Tello & Ibáñez, 2014)

1RL — first ramification length; **2RL** — second ramification length; **ABV** — actual bunch volume; **BB** — berries per bunch; **BL** — bunch length; **BW** — bunch weight; **CBV** — conical bunch volume; **IL** — internode length; **MBV** — morphological bunch volume; **PL** — pedicel length; **RB** — ramifications per bunch; **RL** — rachis length; **SB** — seeds per berry

These published indices derive from different mathematical combinations of ten morphological parameters of the bunch: five of them (first and second ramification length; first to sixth internode length; bunch length; conical bunch volume) correlated significantly with bunch compactness determined with O.I.V. descriptor n°204 by a panel of experts. A low applicability was observed for the indices derived from literature, probably because they were created to evaluate a low number of varieties with a narrow diversity for bunch morphology; instead, some of the indices designed by Tello & Ibáñez (2014) showed to be more efficient on evaluating this trait. The indices with the highest Pearson coefficient of correlation with bunch compactness, established by the O.I.V. descriptor n°204, were the CI-18 and CI-19 (Tab.2). Also, the index CI-12 (Tab.2) has stood out in all the criteria used to evaluate bunch compactness (Tello & Ibáñez, 2014).

2.3.3. Sensor-based methods

Different new approaches based on the analysis of two-dimensional (2D) and three-dimensional (3D) images have been studied for the automated reconstruction of grape bunch architecture (Tello *et al.*, 2016) with the aim to measure, in an objective and accurate way, bunch morphological volume (Tello & Ibáñez, 2018). Indeed, the natural irregularity of bunches makes difficult the application of the images-based methods mentioned previously. Diago *et al.* (2015) with the evaluation of bunch compactness from image-based technologies have recently shown that the 2D image analysis allows the determination of some compactness-related attributes that cannot be assessed by hand, and this determination can be automated. Additionally, a 3D laser scanner has been proposed for the external analysis of food product, especially with irregular fruits (Siswantoro *et al.,* 2013) and it has been applied for the direct measurement of their volume. Furthermore, image analysis technology opens a new opportunity for the automatic measurement of the morphological volume of the bunches and, thereby, for the estimation of bunch compactness (Moro, 2016). Chen *et al.* (2018) stated that multi-perspective imaging analysis combined with multivariate modelling to predict grape bunch compactness is a method with the potential to be rapid, automated and objective. The main goal of several recent works is to explore the potential of image analysis methodologies for bunch components estimation in a fast, inexpensive and potentially automated way, as an alternative to current manual methods, which are time-consuming, expensive and destructive, like manual destemming of berries from bunches (Diago *et al.*, 2015). Moreover, image analysis is more frequently used to inspect fruit production, allowing the development of systems able to estimate or predict some features (Diago *et al.*, 2015).

In viticulture, some studies on image analysis methods using red–green–blue (RGB) images have been conducted to estimate berries number per bunch at harvest time, based on simple image colour discrimination (Dunn & Martin, 2004). A classifier based on the Mahalanobis distance (Mahalanobis, 1936) has been created to identify and quantify the pixels corresponding to grape bunches in an RGB image of a grapevine canopy and then correlated with the actual grape yield of the plant (Diago *et al.*, 2015). Examples of the use of image analysis in bunch compactness estimation are enunciated below.

Grossetête *et al.* (2012) proposed an application for a low-cost and automatic counting of berries (at pea size) on RGB images taken at night (using a uniform background of black colour) with smartphones and a simple image-processing algorithm by identification of a unique and bright spot in the centre of the berries created by the reflection of the light from the camera flash. The results showed that the developed algorithm to analyse digital images captured by pocket cameras under uncontrolled outdoors conditions was able to provide automatically useful estimations of the number of flowers per inflorescence at early stages of flowering. This could give an automated prediction of fruit set rates and potential yield and help vineyard managers. In a short time, the developed algorithm may be implemented on a mobile device (such as a smartphone) to obtain flower count information at each georeferenced position of a given vineyard for mapping.

Tello *et al.* (2016) studied the automatic evaluation of bunch length, width and elongation with the analysis of 2D images and volume with 3D scanners and compared the results with time-consuming approaches. The resulting images were analysed as described by Cubero *et al.* (2015) to obtain an automatic value for bunch maximal length (ML), maximum width (WI), widths at 25% (WI25), 50% (WI50) and 75% (WI75) of the major axis of the bunch per each image (Figure 2). All this process was carried out with the software Food-Color-Inspector (free available at http://www.cofilab.com).

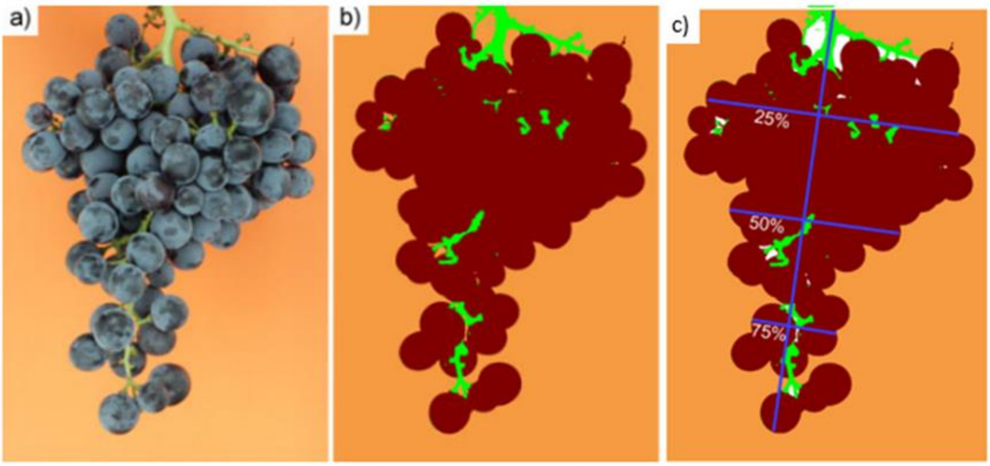


Figure 2: Process followed to segment the bunch image in: background, berries, rachis and holes: (a) original image, (b) segmented image, (c) main axis and width at 25, 50 and 75% of the main axis length in a grapevine bunch (Tello *et al.,* 2015)

Through these variables, a geometric reconstruction of each bunch has been performed to estimate the morphological volume. Practically, the classification of bunches according to their shape can be achieved by evaluating their conicity in different sections of the bunches that were divided into four sections with equal height (a, b, c and d; Figure 3).

Their volumes were estimated according to Equations 1 to 4, and the total morphological volume of the bunch (MVO) was calculated as showed in the Equation 5 (Tello *et al.,* 2016).

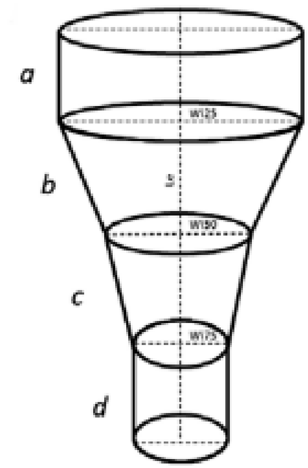


Figure 3: Geometric reconstruction of a bunch divided into four sections (a, b, c and d) (Tello *et al.*, 2016)

Equation 1: $$Va = \pi * \left(\frac{WI25}{2}\right)^2 * \frac{ML}{4}$$ (Tello *et al.*, 2016)

Equation 2: $$Vb = \frac{\pi}{3} * \left[\left(\frac{WI25}{2}\right)^2 + \left(\frac{WI50}{2}\right)^2 + \left(\frac{WI25}{2} * \frac{WI50}{2}\right)\right] * \frac{ML}{4}$$ (Tello *et al.*, 2016)

Equation 3: $$Vc = \frac{\pi}{3} * \left[\left(\frac{WI50}{2}\right)^2 + \left(\frac{WI75}{2}\right)^2 + \left(\frac{WI50}{2} * \frac{WI75}{2}\right)\right] * \frac{ML}{4}$$ (Tello *et al.*, 2016)

Equation 4: $$Vd = \pi * \left(\frac{WI75}{2}\right)^2 * \frac{ML}{4}$$ (Tello *et al.*, 2016)

Equation 5: $$MVO = Va + Vb + Vc + Vd$$ (Tello *et al.,* 2016)

ML — maximal length; **MVO** — morphological volume; **Va** — volume of the section *a*; **Vb** — volume of the section *b*; **Vc** — volume of the section *c*; **Vd** — volume of the section *d*; **WI** — maximal width; **WI25** — width at 25%, **WI50** — width at 50% and **WI75** — width at 75% of the major axis of the bunch

On the other hand, the compactness index CI-13 proposed by Tello & Ibáñez (2014) with the Equation 6, using the values obtained from 2D image analysis, was calculated as the ratio between the bunch morphological volume (which is close to the actual volume in tight bunches) and the squared bunch maximum length.

Equation 6: $$CI - 13 = \frac{MVO}{(ML)^2}$$ (Tello *et al.*, 2016)

CI — compactness index; **ML** — maximum length; **MVO** — morphological volume

Moreover, in their work, Tello *et al*. (2016), showed that CI-13 index increased with bunch compactness increment, because bunches with more volume per centimetres of rachis (high values for CI-13) have usually fewer visible pedicels and empty holes, and vice versa.

Tello *et al.* (2015) used image analysis to estimate the following bunch variables: projected area (A), perimeter (P), compactness shape factor (CSF) and roundness (RD); the resulted indices are showed in Equation 7 and 8.

Equation 7: $$\mathrm{CSF} = (\mathrm{P}^2/\mathrm{A})$$ (Tello *et al*., 2015)

Equation 8: $$\mathrm{RD} = (4.0 * \pi * \mathrm{A})/\mathrm{P}^2$$ (Tello *et al*., 2015)

A — projected area; **CSF** — compactness shape factor; **P** — perimeter; **RD** — roundness

The CSF came from the relation between the projected area and the perimeter; for this reason, bunches with a lower perimeter were more compact. While, RD measured how the shape of any object is related to the shape of a circle.

Chen *et al.* (2018) collected grape bunches from three vineyards in two consecutive seasons and imaged them with a multi-perspective imaging system, sensing mass and collecting images of the bunch surface from three perspectives using mirror reflection. Then, they correlated bunch bulk density with compactness (Pearson correlation coefficient = 0.68); bulk density was calculated as the ratio between bunch mass (weighting sensor) and bulk volume because it is difficult to estimate compactness index by using only bulk density. According to same authors, other factors should be included: bunch mass, region and edge features. They stated that, in order to accurately assess bunch compactness, images from different perspectives are required. The imaging time, costs and complexity of such a system can be reduced using mirror reflection when collecting images. Finally, the authors concluded that combining multi-perspective imaging, image processing and multivariate data analysis, bunch compactness can be accurately assessed.

2.3.4. Possible contribution of bunch compactness for automated yield estimation methods: the case study of Vinbot robot

Due to the fact that grapevine is one of the most profitable crops worldwide, used to produce wine, table grapes, and raisins (O.I.V., 2017), grapevine yield estimation outstands for its economical relevance (Dunn, 2010) mainly because yield variability within a vineyard has been proved to be high (Bramley & Hamilton, 2004) but classical yield estimation methods are difficult and insufficient to obtain representative yield data. Consequently, non-invasive imaging-based methods were being investigated to realise an efficient and continuous capture of detailed information from vines throughout their growing cycle (Li *et al*., 2014). The use of ground robots has been able to automate some other cultural practices in precision viticulture, like mechanical weeding, seeding and pruning (Roure *et al.*, 2018) and the future will be make all the practices automated. Robotics technology is becoming and will remain dominant in the future, according to the EU Strategic Research Agenda For Robotics in Europe 2014-2020 (EUrobotics, 2013).

The EU Vinbot project (Autonomous cloud-computing vineyard robot to optimize yield management and wine quality) (http://www.vinbot.eu/) is a good example of the recent research effort of the EU on this topic. The Vinbot project aimed to develop an all-terrain autonomous mobile robot with a set of sensors capable of capturing and analyzing vineyard images and 3D data by means of cloud computing applications, in order to obtain canopy and yield maps representing the spatial variability of the vineyard plots. This project used the Vinbot robot platform based on a commercial off-the-shelf mobile robot Summit XL HL able to carry up to 65 kg payload.

This robot scans the vines with a sensor composed with a 2D laser rangefinder, a camera and a set of robot navigator sensors with yield estimation as its main objective. Height, volume and exposed leaf area were the canopy features estimated by Vinbot platform. Regarding the yield estimation, the first Vinbot algorithms underestimated the yield with as major explanation the problem of the bunch occlusions (bunch-on-bunch and leaf-on-bunch occlusions) and, moreover, the empirical models used to convert the projected area into kg of grapes could have amplified errors, contributing to reduce the prediction ability of the Vinbot algorithms (Lopes *et al*., 2017).

Due to the fact that this conversion was done using only a linear relationship between the projected bunch area and the corresponding weight and because this relationship depends of several factors related to bunch traits, it has been proposed the possibility to use bunch compactness as an explanatory variable to add to the models contributing to improve the accuracy of the algorithm to convert the projected area obtain with the Vinbot image into weight. Further researches on the algorithms, on data processing, on modelling and calibration are needed to improve the accuracy of the yield estimations by the Vinbot and this is the reason why understand bunch compactness is important to improve the yield accuracy.

2.4. Effect of source-sink balance on bunch compactness

The source-sink balance is the most common practice among the agronomic techniques to control bunch compactness (Tello & Ibáñez, 2018). Usually, the main source of photo-assimilates for the successful development of the inflorescences is leaves photosynthesis (Lebon *et al.*, 2008).
In the developing grapevine, the leaves undergo a gradual transition from importing photosynthetic products to export (Fig. 4). When the leaf is about 1/3 of its full size it exports more food than it uses and begins to contribute to vine growth. When the leaf reaches its full size (about 30 to 40 days after unfolding) it is photosynthesising at its peak (Retallack, 2012).

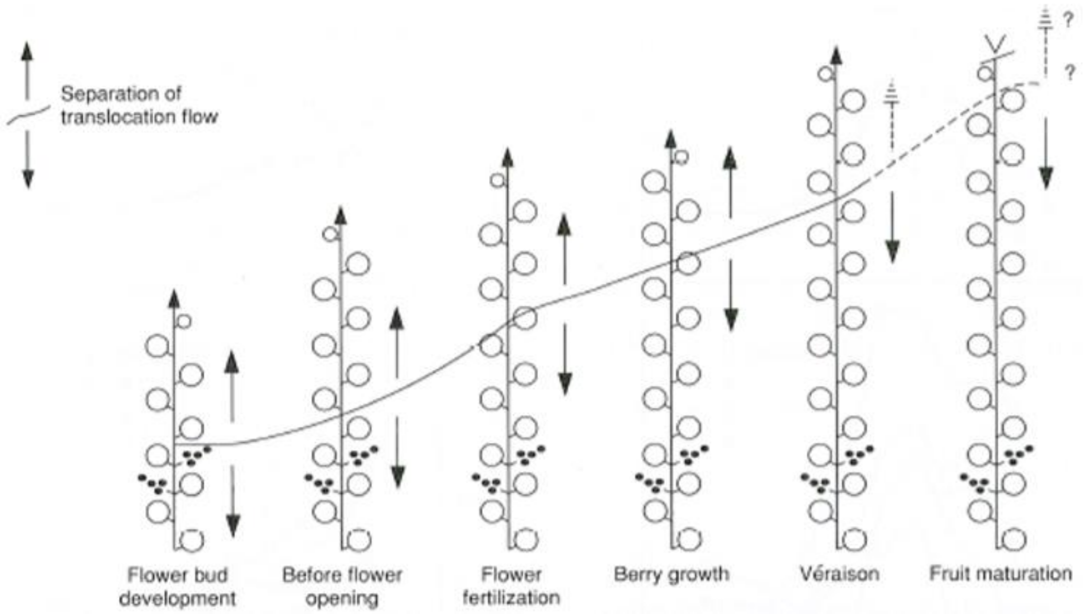


Figure 4: Translocation of photosynthate from leaves during shoot growth (Koblet, 1969)

Following harvest, the majority of photosynthates are directed towards and stored in the roots. Leaf fall or senescence normally begins in late autumn when minerals are translocated back into the canes and trunk (Retallack, 2012).

When basal, and then older, leaves are removed by defoliation, they are also the largest leaves along the shoot and their size can offset lower photosynthetic rates. These defoliated vines at veraison show younger canopies because median and apical shoot leaves at this time are now mature and, additionally, lateral leaves can be present due to a compensating reaction to early leaf removal. The removal of source leaves around bloom causes dynamic changes to shoot photosynthesis and age as well as to source-sink balance of the plant (Poni *et al.,* 2006). This may lead to higher photosynthesis late in the season, helping to explain the better grape composition.

Improved grape composition in defoliated shoots is also relates to the quality of the source, because there is a functional relationship between source availability around bloom and yield (Poni *et al.,* 2006). In fact, the number of set berries, therefore yield per shoot, is primarily a function of the number of source leaves on main shoots at pre-bloom; this is due to the strong physiological principle which makes source availability at pre-bloom the primary regulator of the subsequent fruit set.

Moreover, the alteration of source-sink balance in defoliated vines lead to an increment of the final leaf-to-fruit ratio, indicating that the temporary source limitation caused by defoliation was more than offset by the subsequent lateral regrowth and by the decline in yield per shoot (Poni *et al*., 2009).

Additionally, long-lasting source limitation at any specific phenological stage can also be obtained through the use of anti-transpirants with the potential to significantly reduce both transpiration and photosynthesis. As shown by Palliotti *et al.* (2010), using the anti-transpirant Vapor Gard sprayed twice before flowering on Sangiovese grapevines lead to lighter, less compact bunches and enhanced soluble solids and phenolic concentration in the must (Palliotti *et al*., 2010).

Thus, the yield compensation pattern subsequent to early source limitation would overcome the potential negative side effects of the traditional practice of bunch thinning leading to heavier, more compact bunches with also larger berries and a lower skin-to-pulp ratio (Palliotti *et al*., 2012).

For all these results, this strategy can be profitably applied also in vineyards characterized by high vigour and vegetation density, where shading bunch zone is high and the virulence of fungi disease is very dangerous and difficult to control (Palliotti *et al*., 2012).

After this preamble, wanting to modify bunch compactness, acting on source-sink balance, there are several techniques explained in depth below.

2.4.1. Cultural practices

In general, leaf removal, anti-transpirants and canopy shading have been demonstrated to reduce fruit set when applied pre-flowering or at full-flowering (Tello & Ibáñez, 2018). Moreover, the regulation of bud-load with winter pruning and manual bunch/shoot thinning are also main tools to manage the yield with consequences on bunch compactness (Tardaguila *et al.*, 2012).

2.4.1.1. Defoliation

Leaf removal effects on yield are quite dependent on timing and severity as suggested by Poni *et al.* (2006); in fact, basal leaf removal at trace of blooming reduced significantly bunch compactness and weight, as well as rot incidence (Hed *et al.,* 2009), fruit set, berries number and berries size.

Early defoliation induced source limitation, decreasing the length of the first rachis branch. This had impact on bunch size (Silvestroni *et al*., 2016), while improved grape composition with higher Total

Soluble Solids (°Brix), higher anthocyanins, phenols and skin-to-pulp ratio as compared to non-defoliated shoots (Poni *et al.*, 2006).

Grape composition is influenced by defoliation because promotes the translocation of assimilates towards the bunches, as proved by Candolfi-Vasconcelos *et al.* (1994) with an autoradiograph taken 24 hours after the defoliation in which was evident the photosynthate movement towards bunches.

Instead, pre-bloom basal leaf removal showed interesting results in cultivars with tight bunches due to the effect of the strong reduction of leaf area which limits the potential photo-assimilate uptake by flower bunches and reduces berry set, highly dependent from carbohydrate supply (Intrieri *et al.*, 2008).

Fruit zone early defoliation may be an excellent solution for yield control, replacing manual bunch thinning that is more time consuming (Poni *et al.*, 2006). Focusing the attention to the possibility that, removing all the leaves from the fruit zone, there is an exposition of the bunches to full sun, increased the risk of sunburn and compromised grapes composition, especially in warm climates (Bergqvist *et al.,* 2001), decreased must quantity and increased must pH (Poni *et al.,* 2008). Poni *et al.* (2006) also showed a reduction of fruit set in defoliated vines of Sangiovese and Trebbiano of 5.7% and 19% respectively, less than control.

Additionally, the number of mature leaves removed and the percentage of fruit set did not show a linear correlation. Intrieri *et al.* (2008) tested pre and post bloom hand and mechanical defoliations, respectively hand defoliation and mechanical defoliation, by removing the first six basal leaves. Both treatments reduced fruit set significantly, as well as bunch weight, berries per bunch and bunch compactness; while soluble solid concentration, total anthocyanins and Brix increased more in hand defoliation than in mechanical defoliation.

Additionally, there is a response cultivar dependent on the berry mass change, as reported in contrasting articles (Intrieri *et al.,* 2008; Silvestroni *et al.,* 2016), and on the effects of post-flowering defoliation on bunch architecture (Tello & Ibáñez, 2018).

2.4.1.2. Canopy shading

Tello & Ibáñez (2018) showed that artificial shading can influence bunch architecture, as it caused a reduction in vine photosynthesis and potential over-wintering reserves. It has generated looser bunches because vines carbohydrate reserves influence inflorescence number and flowers number per inflorescence in the following season. Therefore, it can decrease berry mass and diameter. It has been proposed as solution to problems caused by pre-bloom defoliation, testing the hypothesis that shading grapes between pre-anthesis and fruit set may lead to decrease bunch compactness without removing leaves around the bunches, protecting them from sunburn risk and maintaining berry juice acidity at harvest (Basile *et al.*, 2015). This study tested five whole-canopy shadings (10, 30, 50, 75 and 90% reduction of ambient light) and one partial canopy shading using a 30% shade net. Bunch compactness was reduced by shading but only in the range of 50 to 90%, while berry composition was not negatively affected by 50 to 90% shading. So, Basile *et al.* (2015) concluded that early shading may be an efficient alternative practice to decrease bunch compactness without negative effects berry composition and sunburn. The use of shading nets decreased the Photosynthetically Active Radiation by 50 to 90% and appeared to be an effective alternative management practice to decreased fruit set and bunch compactness. Moreover, a partial shading of the canopy appeared to be a promising technique, but more experiments are needed to test different types of netting with different shading qualities and shading different fractions of the canopy (Basile *et al.*, 2015).

2.4.1.3. Other management strategies

Bunch thinning reduced significantly berries number per bunch, bunch compactness, rot incidence and bunch weight due to higher berries weight that change the leaf-to-fruit ratio; while increasing phenolic concentration at harvest (Tardaguila *et al.*, 2008). In fact, bunch thinning did not affect berry size but affect total acidity and pH, they decreased with the application of early bunch thinning while they increased with late bunch thinning (Gatti *et al.,* 2012). Gatti *et al.* (2012) underlined the undesired effect of berry growth compensation by the retained bunches as possible but also influenced by the seasons, whereas data of final leaf-to-fruit ratio support the assumption that bunch removal was a necessary tool to adjust an otherwise source limited vine balance (Gatti *et al.,* 2012).

Shoot thinning at pre-flowering led to a reduction in shoot number, more than 30%, and influenced vine vigor during the growing season. For shoot-thinned vines, there was a significant increase in fruitfulness due to the removal of unfruitful shoots and a higher final number of berries, which led to the higher bunch mass and compactness.

Shoot trimming is another strategy to influence bunch compactness as showed by Bondada *et al.* (2016) analysing the influence of post-veraison trimming on bunch architecture and yield, with three levels of treatment: light trimming (14 nodes), sever trimming (10 nodes) and untrimmed control. The results showed that especially severe trimming reduced bunch weight, compactness, vine productivity and total yield; but there were also effects on grape quality, as lower Brix and pH, minor influence on titratable acidity, anthocyanin content and yeast assimilable nitrogen. This study demonstrated that post-veraison shoot trimming can be a valid practice to reduce bunch compactness without compromising on the whole fruit quality. In fact, shoot trimming reduced yield and sugar levels, maintaining fruit quality due to adaptations by grapevines to maintain homeostasis. This is a possible practice to reduce bunch compactness and sugar level, for example it is recommended to organic viticulturists (Bondada *et al.*, 2016).

2.4.2. Growth regulators

These chemicals were applied to different plant organs, at different stages of development, to modify bunch architecture either by promoting the elongation of the inflorescence or by reducing berry size and/or berry number (Moro, 2016).

2.4.2.1. Gibberellins

One of the most common practices in viticulture to obtain looser bunches is the used of gibberellins; they act as endogenous growth regulator on the principal aspects of plant development with the elongation of bunch stems leading to stem elongation and to a reduction of bunch compactness as well as a reduction of bunch rot severity (Molitor *et al.*, 2012). The efficiency depends on several factors, namely cultivar, timing and quantity of applied product. Pre-flowering application of gibberellins led to looser bunch and lower incidence of rots without compromising crop yield (Tello & Ibáñez, 2018). Full-flowering application proved that it may be an effective strategy to reduce the rate of fruit set, resulting in lower berries number and bunch compactness; sometimes the reduction of yield is unsustainable (Hed *et al.,* 2015). The opposite effect was obtained with post-flowering application which increased compactness by increasing berry size (El-Banna & Weaver, 1979). The use of synthetic gibberellins is not allowed in organic farming (Navarro & Pérez, 2006) but abscission is both a biological and an agronomic challenge that can be induced independently by shade and gibberellic acid sprays (Domingos *et al.*, 2015). Particularly, concerning carbohydrates metabolism: sucrose, glucose and intermediates of the oligosaccharide's pathway were lower in shaded bunches while were higher in gibberellins samples.

2.4.2.2. Anti-transpirants

A possibility to obtain shading is to apply anti-transpirants to plant leaves for stomata occlusion to obstruct transpiration and carbon dioxide absorption, what ultimately hinders the photosynthetic activity of leaves (Intrieri *et al.*, 2013). Anti-transpirants treatments were investigated often in comparison to leaf removal, due to the weather changes of the last years with spring-summer temperature that reached peaks higher than the seasonal averages (Jones *et al.*, 2005). Bunch weight and yield decreased with the application of Pinolene (Vapor Gard), while compactness registered an intermediate value. This application did not modify the natural state of bunches and did not interfere in the growing berries as showed by Intrieri *et al.* (2013). Furthermore, oil substances used as leaf anti-transpirants, like Pinus oil or paraffin, can occlude leaves stomata, hinder their transpiration and influence the adsorption of carbon dioxide, hindering the photosynthetic activity of leaves (Gatti *et al.*, 2016). Applied at pre-flowering and full-flowering reduced the mobilisation of carbohydrates to develop inflorescence, promoting flower drop and reducing berry number and bunch compactness (Tello & Ibáñez, 2018). A lower berry number, lower bunch compactness and rot severity was observed by Hanni *et al.* (2013) after the application of two anti-transpirants (UFO and Vapor Gard) to the entire leaf canopy at full-flowering. While Gatti *et al.* (2016) determined that berry size, fruit set, bunch compactness and rot severity were not significantly modified after the application of Vapor Gard at pre-flowering, pre-veraison or pre-flowering plus pre-veraison. Additional researches are needed to evaluate the efficiency of different anti-transpirants on different grapevine cultivars, with the aim to improve bunch architecture without compromising grape composition.

2.4.2.3. Other chemical regulators

Prohexadione-calcium (3-oxido-4-propionyl-5-oxo-3-cyclo-hexene-carboxylate) application at full-flowering has been proposed as an efficient strategy to reduce bunch compactness (Molitor *et al.*, 2011) inhibiting the biosynthesis of growth active gibberellins, causing an accumulation of its inactive direct precursor (Evans *et al.*, 1999). This imbalance promotes flowers or berries abortion, with a potential reduction in berries number and, consequently, bunch compactness (Molitor *et al.,* 2011).

Forchlorfenuron [N-(2-chloro-4-pyridi- nyl)-N-phenyl-urea] is a synthetic cytokinin-like regulator that, at low concentration, can promote berry-set and development, increasing berry size and number; but the efficacy is strongly cultivar-dependent (Zabadal & Bukovac, 2006).

Ethephon (2- chloroethyl-phosphate acid) has been used to stimulate the spontaneous abscission of mature berries (Rizzuti *et al.*, 2015) and, with abscisic acid, to intensify the colour of red grapes (de Souza Leão *et al.*, 2015).

DL-βamino-n-butyric acid (BABA) was investigated by Kocsis *et al.* (2018) which showed that it affects bunch compactness and, consequently, *Botrytis* bunch rot development. The main effect of BABA is female sterility in grapevine flowers that gives looser bunches. The study revealed that 2.0 g/L of BABA decreased bunch compactness and led to lower disease incidence. Other studies showed that BABA increased the defence capability of plants through a fast hypersensitive response, lignin accumulation, synthesis of pathogenesis related proteins (Cohen *et al.,* 2011), callose formation in the ovules and inhibition of pollen tube guidance in the ovary. These two aspects induced female sterility inhibiting fully or partly the fertilization of flowers, that resulted in a lower number and lighter berries (Kocsis *et al.*, 2018). Kocsis *et al.* (2018) showed that the application of BABA on grapevine inflorescences reduced bunch compactness and *Botrytis* infection, so a protective role through resistance induction; but disease pressure and weather conditions strongly conditioned the results.

2.5. Effect of bunch compactness on pests and diseases

Bunch rot, primarily caused by *Botrytis cinerea* Pers., is an important disease of grapes that typically begins to develop after veraison, when bunches begin to ripen. The cost to control *Botrytis* damage is a major cause of profit reduction in many companies because, even if this effect is more evident in case of table grapes, *Botrytis* bunch rot reduces the quality of wines by generating off-flavours, oxidative damage, ageing problems and difficulties in clarification of wines. For these reasons rotten bunches are often rejected in the wine industry (Negri *et al.*, 2017; Tello & Ibáñez, 2018).

Bunch compactness is strongly related to bunch rot, higher in compact bunches than in loose ones. Control with specific fungicides is often unsatisfactory and the efficacy of spray coverage varies between season, number of applications and decreased as bunches become more compact. Tardaguila *et al.* (2008) showed that bunch loosening reduced rot incidence in cultivars Tempranillo and Grenache in Spain.

Furthermore, by reducing bunch compactness is possible to improve spray penetration, as well as chemical control program. Hed *et al.* (2009) proved that sometimes leaf removal alone can be more efficient than the application of fungicides. Indeed, the several cultural practices that modify bunch compactness may be considered an integrated control of bunch rot. For example, leaf removal at bloom by reducing bunch compactness can, consequently, reduce bunch rot infection (from 60 to 83%) as showed by Hed *et al.* (2009).

Another of these practices is the removal of floral debris, such as necrotic flower caps, anthers, filaments and aborted unfertilised ovaries. It is probably the principal source of primary inoculum because their accumulation is higher in compact bunches and their removal reduce bunch rot of 40% (Hed *et al.,* 2009). According with this work, it has been demonstrated that berries per centimetre of rachis, a way to estimate bunch compactness, was uniformly related to bunch rot incidence and severity, so every additional berry per centimetre intensified bunch compactness and increased the possibilities of a bunch to become infected with bunch rot. Berry cuticle is an important barrier that is reduced at the point of contact between berries and the degree of contact (the higher the degree of contact, the higher the compactness) can affect the susceptibility to bunch rot (Percival *et al.,* 1993; Tello & Ibáñez, 2018).

Hed *et al.* (2009) underlined that bunch compactness can affect the wet duration in bunches after rainfall events; the rainfall amounts during the ripening period can also increase the retained debris and consequently the risk of rot. Bunch compactness is also positively related to the infestation rate of *Lobesia botrana*, the European grapevine moth that causes substantial damage to the yield due to the larval feeding of grape berries (Fermaud, 1998). Larvae of *Lobesia botrana* also increase the severity of grey mould on bunches because act as vector for the transport of conidia from berry to berry (Fermaud & Menn, 1989).

Additionally, Leong *et al.* (2006) reported a greater incidence of the ochratoxigenic fungi *Aspergillus* spp. in compact bunches, and another work pointed out that the humid and warm microclimate of compact bunches may also stimulate the incidence of *Cladosporium* spp. and, consequently, the development of *Cladosporium* rot (Latorre *et al.,* 2011). At the end, high pest or disease levels in vineyard (particularly bunch rot) can force growers to harvest grapes at a stage of incomplete maturity, also affecting the final composition of grapes, musts and wines (Molitor *et al.*, 2016).

3. Material and methods

3.1. Localization and characterization of the vineyards

This work was carried out at the Instituto Superior de Agronomia (ISA) campus, located in Tapada da Ajuda, Lisbon (38° 42’ 27.5’’ N, 9° 10’ 56.3’’ W and 62 m above sea level) in the vineyard called “Vinha Almotivo” (Fig. 5).

Figure 5: Partial map with the red varieties vineyard (B), “Vinha Almotivo”, where this work was set up (adapted from Google Earth)

The vineyard (B) called “Vinha Almotivo” was planted in 1997 with a North-South row orientation and covers 1.0 hectare with the following red grapes varieties: Syrah, Touriga Nacional, Trincadeira and Cabernet Sauvignon. All the vines had the rootstock 140 RU (Ruggeri) used because appropriate in hot and dry regions and because has an active limestone resistance of 17 to 20% (Mottard *et al.,* 1963). There are three different training systems, VSP, Lys, Lira spaced 1.0 x 3.0 m and on a bilateral Royat Cordon system spaced 1.2 x 2.5 m, spur-pruned (18-20 nodes per vine) on a vertical shoot positioning trellis with two pairs of movable wires (Teixeira *et al.,* 2018). The soil of this vineyard is a clay loam with 1.6% organic matter and a pH of 7.8. The red varieties vineyard is not irrigated. The cultural practices performed during the biological cycle in which the experiment took place were those that are annually planned by the ISA technical team, having been performed equally throughout the assay, not being a differentiation factor in this study.

3.1.1. Varieties subject of study

This work took into consideration the International variety Syrah; it is a late ripening variety (second half of September) with a medium-small bunch and a medium-low compactness. The shape of the bunch is cylindrical mainly and sometimes wings are present (Eiras-Dias *et al.,* 2011). The training system adopted in the vineyard is the vertical shoot positioning (VSP), the most extensive trellis system chosen for vineyards over the past two decades, that expose an important proportion of inter-row space (Towers *et al.,* 2019).

3.2. Climatic characterization

The climate in Tapada da Ajuda, following the Thornthwaite characterization based on the global index of humidity (Thornthwaite, 1948), is mesothermic with an accumulated precipitation of around 725 mm/year and a mean annual temperature of 15.4°C (with a maximum annual value of 39°C and a minimum annual value of 5°C). In the Figure 6 and 7 are visible, respectively, the mean temperature and the amount of precipitation of the last 30 years (IPMA, 2019) and of the year of the study 2019, from January to August.

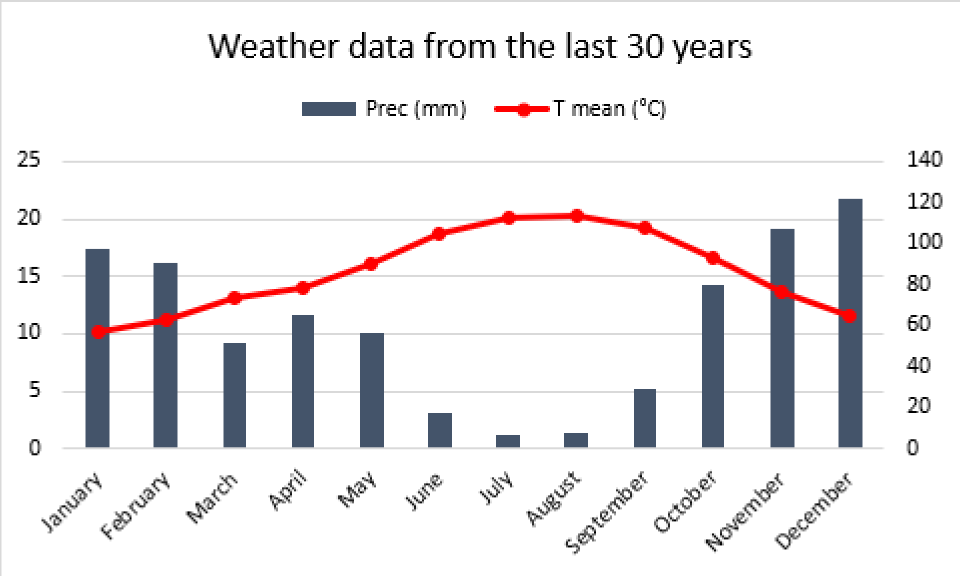


Figure 6: Monthly mean precipitation (mm) in blues and mean temperature (°C) in red of the last 30 years data (adapted from IPMA, 2019).

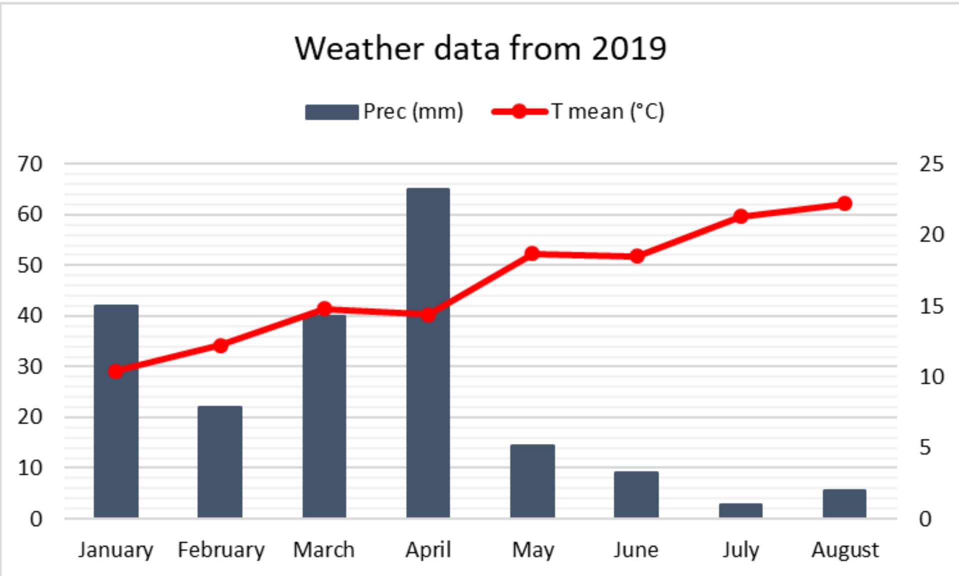


Figure 7: Monthly mean temperature (°C) in red and precipitation (mm) in blue from 2019 (data collected from the weather station located within the white varieties vineyard

3.3. Methodologies

The methods have been applied on Syrah, on the rows number 14, 15, 16 and 18. Each row represented a smart point of 5 linear meters, labelled with a plastic scale (steps of 10 cm), positioned under the cordon of the vine. These 5 meters were used to select the bunches to obtain detailed measurements.

3.3.1. Data collection

The bunches of Syrah were collected in the vineyard at full maturation and analysed at the laboratory at ISA (Instituto Superior de Agronomia).

3.3.1.1. Field

For each smart point, on the five metres destined to detailed measurements, 1 or 2 vines, based on bunches number to achieve from around 50 to 70 bunches for the 4 smart point, were selected. Each selected bunch has been labelled with a plastic label (10x1.5 cm) secured with a wire.

On each label there was written a sequence to identify the bunches and reported the following data with abbreviations:

- Smart point: S1, S2, S3, S4;
- Phenological stage: full maturation (M);
- Bunch number (C): for example, C1 means the first bunch removed for vine;
- Variety: Syrah (SY);
- Meter selected: between one and five.

After these operations the bunches were taken to the laboratory.

3.3.1.2. Laboratory

The laboratory was divided in different sections, each one with a specific task and a sequence:

a) Bunch photo: two blue panels were used as background and positioned perpendicularly each other. The photos were taken for each individual bunch twice, one frontal and one lateral, with a Nikon single-lens reflex (SLR) digital camera (Fig.8), paying attention that the label of each bunch was clearly visible in the frontal photo. The labels were used to organize the bunches on the Excel file and to set the scale (1.5 cm for frontal images) on the program used to analyse the images; while for the lateral images the black clamp was the reference point (2.8 cm).

Figure 8: Nikon D5200, digital camera (User's Manual Nikon D5200, 2012)

The digital camera had the following characteristics (Tab.3):

Table 3: Nikon D2500 features (User's Manual Nikon D5200, 2012)

Lens mount	Nikon F mount
Effective pixels	24.1 million
Image sensor	23.5 x 15.6 mm
Shutter	electronically-controlled vertical.travel focal-plane shutter
Exposure	TTL exposure metering using 2016-puxel RGB sensor
Autofocus	Nikon Multi-CAM 4800DX autofocus sensor module with TTL phase detection, 39 focus point and AF-assist illuminator (autofocus is available with AF-S lenses)

b) Bunch weight: each bunch has been weighted with a digital table scale (max = 30kg, d = 5g) and the data collected in grams have been reported on an Excel file;
c) Bunch real volume: the real volume has been measured using a glass ISO cylinder (1000:10 ± 10) filled with water and measuring the water displaced by the bunch and the data collected in millilitres have been reported on the same Excel file.

After manual bunch destemming:

d) Rachis length: measured using a ruler in cm as showed in Figure 9, without consider the peduncle and paying attention to the eventual presence of wings, measured separately; the final length in presence of wings was given by the sum of rachis length and wings length;

Figure 9: Manually measurement of rachis length (A) and its wing (B) with a ruler.

e) Berries weight: measurement in grams using the same digital scale used for the bunches;
f) Berries photo: berries for each bunch were photographed with a SONY Cyber-shot DSC-H90 digital camera (Fig.11) paying special care on the separation of each berry in order to allow the program ImageJ to count them automatically (e.g. Fig.10, Annex2).

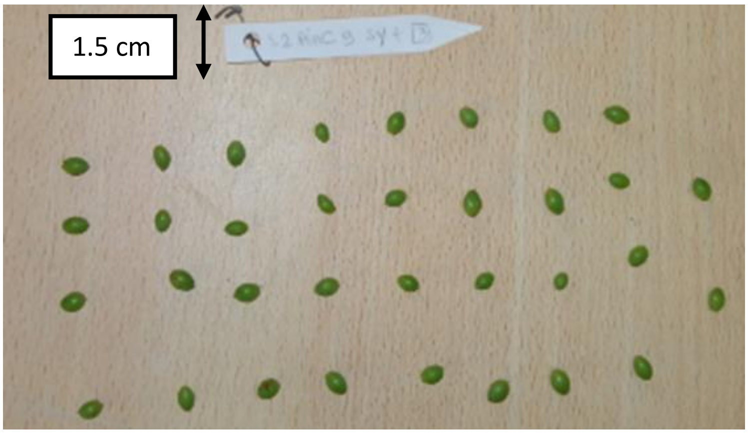


Figure 10: Berries photo with label taken with Sony DSC-H90 digital camera

The digital camera had the characteristics showed in Table 4:

Table 4: Sony DSC-H90 features (Instruction manual Sony DSC-H90, 2012)

Lens mount	Sony G 16xzoom lens
Effective pixels	16.4 megapixels
Exposure	automatic or manual control

Figure 11: SONY Cyber-shot DSC-H90 digital camera (Instruction manual Sony DSC-H90, 2012)

Both digital cameras were used on autofocus that comes automatically; the Nikon D2500 digital camera was used on a tripod in a vertical position, perpendicular to the floor, instead the Sony DSC-H90 was used parallel to the work table and secured at a plastic base.

3.3.1.3. Image processing

Each image of each bunch was analysed with the program ImageJ, an open source image-processing program designed for scientific multidimensional images developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin). The parameters defined with this program were: maximum length of the rachis, maximum width, perimeter and projected area (from the frontal image); maximum thickness, mean thickness, perimeter and projected area (from the lateral image) as showed in Figure 12. Also, width at 25%, 50% and 75% of the maximum length as explained in Figure 13.

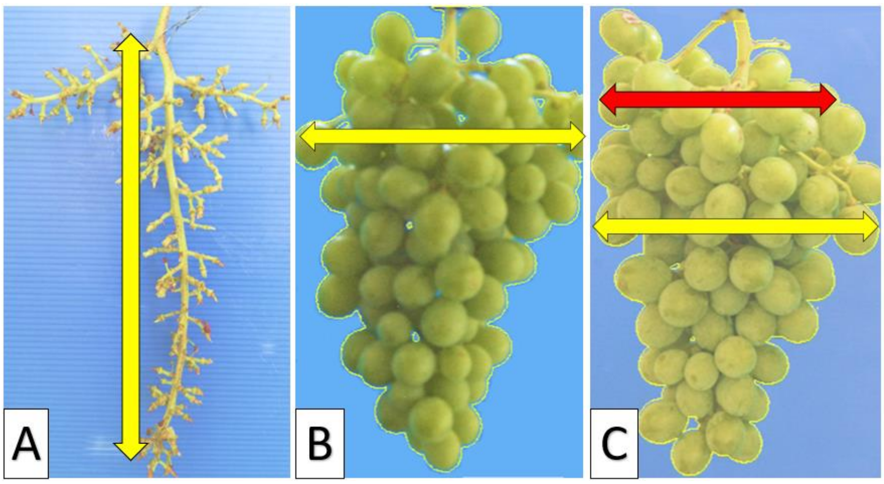


Figure 12: Image of the rachis length (A), frontal image of a bunch (B) with the maximum width (yellow arrow), lateral image of the same bunch (C) with maximum thickness (yellow arrow) and mean thickness (red arrow).

To obtain this values, a protocol (detailed in Annex 1) for bunch detection was created and it included several steps, starting from open the photo on ImageJ; set the global scale; cut the bunch as close as possible to the perimeter; highlight bunch projected area; save the selection as selection (.roi format) and show the results. Copy the needed data on the Excel file. The only parameters that needed to be done manually on ImageJ were the mean thickness and the width at 25%, 50% and 75%; these last three were done using the maximum bunch length, dividing it by four to obtain the distance between each width and then calculating each width with the tool rectangle as shown in Figure 13.

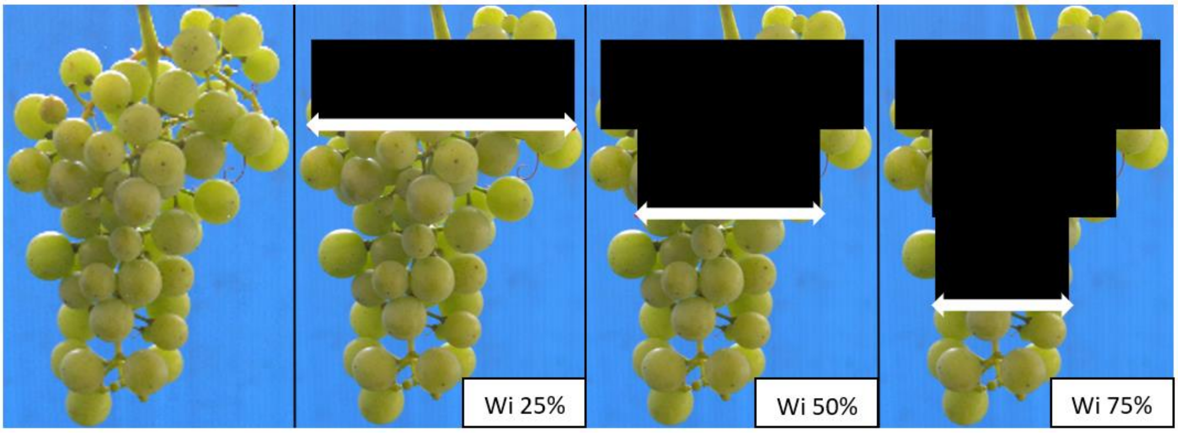


Figure 13: Bunch segmentation into 4 sections to estimate the width at 25%, 50% and 75% of bunch length

3.3.2. Bunch compactness subjective evaluation: *the O.I.V. descriptor number 204*

A panel of 8 judges was formed, aged between 24 and 28, to evaluate a series of bunches images with the O.I.V. descriptor n°204 that classify bunches into five categories, based on the mobility of the berries and the visibility of the pedicels: very loose (notation 1); loose (3); medium (5); compact (7) and very compact (9) (O.I.V., 2001) as explained in Table 5. The data was organized into Excel file with which it was obtained a mode value. This subjective method was correlated with the objective methods, the indices, to evaluate the degree of correlation.

Table 5: O.I.V. descriptor n°204 for bunch compactness (adapted from O.I.V., 2001)

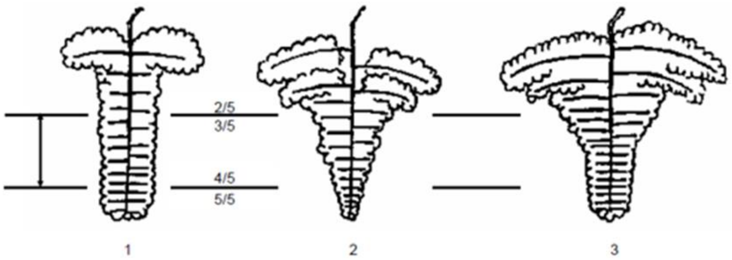
Characteristic	*Bunch compactness*
Code O.I.V. n°	204
Levels of expression	1 – very loose 3 – loose 5 – medium 7 – dense 9 – very dense
Observation at maturity *Example*	Examination of the largest bunches of 10 shoots. 1 – berries clearly separated, mainly visible pedicels 3 – berries in loose contact with each other with some visible pedicels 5 – densely distributed berries, pedicels not visible, berries are movable 7 – berries not readily movable 9 – berries deformed by compression 

The main problem to use this descriptor was related to its subjectivity given by human observation.

3.3.3. Evaluation of morphological volume

Tello *et al.* (2016) explained a method to evaluate the morphological volume of bunches starting with the separation of them in classes based on their shape with the O.I.V. descriptor n°208: class 1 (cylindrical shape), class 2 (conical shape) and class 3 (funnel shape). To discriminate each bunch, it was necessary to observe only the portion between 3/5 and 4/5 of bunch axis as described in Table 6 (O.I.V., 2001) of the frontal image.

Table 6: O.I.V. descriptor n°208 for bunch shape (adapted from O.I.V., 2001)

Characteristic	*Bunch shape*
Code O.I.V. n°	208
Levels of expression	1 – cylindrical 2 – conical 3 – funnel shape
Observation at maturity	Examination of the largest bunches of 10 shoots. Description of the bunch shape between 3/5 and 4/5 of the axis. Wings in the upper part and the tip are excluded from observation.
Example	

After the separation in the three classes, the bunches images from each shape class were analysed with the program ImageJ to obtain value for bunch maximal length (ML), maximal width (WI), widths at 25% (WI25), 50% (WI50) and 75% (WI75) of the maximum length. With these parameters a geometric reconstruction of each bunch was performed dividing bunches into only four sections of equal height (*a, b, c* and *d*). Their volumes were estimated according to Equations 1 to 4 reported before.

The total morphological volume of the bunch (MVO) was calculated as the sum of the four volumes (Tello *et al.*, 2016) as shown in Table 7, and compared with other possible equations (Tab.7) in which Volume 1 and Volume 2 were calculated using the mean projected area (A), obtained by the mean of the two projected areas from frontal and lateral picture respectively, and the maximum or mean thickness (TMAX or TMEAN) that was the width obtained from lateral images.

The last volume in the table consisted in the volume of a cone, the most representative bunch shape, that includes two parameters: *r* as radius (half of the maximum width from frontal image) and *h* as maximum length of the rachis.

Table 7: List of formulas used to calculate bunch volume.

Parameter	Formula	Reference
Morphological volume	$MVO = Va + Vb + Vc + Vd$	(Tello *et al.,* 2016)
Volume 1	$V = A * TMax$	
Volume 2	$V = A * TMean$	
Volume of a cone	$V = (\pi * r^2 * h)/3$	

A — mean projected area; **h** — rachis length; **MVO** — Morphological volume; **r** — radius; **TMAX** — maximum thickness; **TMEAN** — mean thickness; **V** — volume; **Va** — volume of the section a; **Vb** — volume of the section b; **Vc** — volume of the section c; **Vd** —volume of the section d

The main problem of the morphological volume determination was the necessity to exclude bunches with wings, due to an incompatibility with the automatic process of ImageJ and to an excessive time-consuming work needed to divided rachis and wing in two different images and analyse them separately.

3.3.4. Bunch compactness indices

From the study of Tello & Ibáñez (2014), only 5 indices with a positive and significant correlation with the O.I.V. descriptor n°204 and higher feasibility were extrapolated and used to calculate bunch compactness (Table 8). The index CI-3 was included because is the most used to determined bunch compactness. The parameters considered were: actual bunch volume (ABV); bunch length (BL); bunch weight (BW); berries number (BN) and morphological bunch volume (MVO) following the method previous explained. Tello *et al.* (2016a) used the morphological volume (MVO) to calculate this bunch compactness index (CI-13_2) dividing morphological volume with rachis length (ML). Moreover, Tello *et al.* (2015) used the compactness shape factor (CSF) and the roundness index; the first one as index to obtain bunch compactness, showed in Table 8 where P was the perimeter and A the projected area of the bunch and the roundness (RD) of the bunch using area and perimeter (Tab.8).

Table 8: Indices correlated with the mode of the O.I.V. descriptor n°204 given by the panel.

Index:	Equation:	References
CI-3	BN/BL (cm)	(Tello & Ibáñez, 2014)
CI-4	$\frac{ABV}{MVO} * 100$	(Tello & Ibáñez, 2014)
CI-10	BW/BL	(Tello & Ibáñez, 2014)
CI-11	BW/MVO	(Tello & Ibáñez, 2014)
CI-12	BW/BL^2	(Tello & Ibáñez, 2014)
CI-13	ABV/BL^2	(Tello & Ibáñez, 2014)
CI-13_2	MVO/ML	(Tello *et al.,* 2016a)
CSF	P^2/A	(Tello *et al.,* 2015)
RD	$(4 * \pi * A)/P^2$	(Tello *et al.,* 2015)

ABV — actual bunch volume; **BL** — bunch length; **BN** — berries number; **BW** — bunch weight; **CI** — compactness index; **CSF** — compactness shape factor M**L** — maximum rachis length; **MVO** — morphological bunch volume.; **P** — perimeter; **RD** — roundness
Significance: n.s.: non significant, *: significant with $\rho < 0.05$, **: significant with $\rho < 0.01$, ***: significance with $\rho < 0.001$

In addition, 5 indices were designed in this work to establish if it was better to use bunch length given by the sum of rachis length and wing length or maximum length obtained by ImageJ. In Table 9 were reported these indices created by replacing bunch length with maximum length or vice-versa in the case of CI-13_2.

Table 9: New indices created in this work.

Index:	Equation:
CI-3 a	BN/ML (cm)
CI-10 a	BW/ML
CI-12 a	BW/ML^2
CI-13 a	ABV/ML^2
CI-13_2 a	MVO/BL

ABV — actual bunch volume; **BL** — bunch length; BN — berries number; **BW** — bunch weight; **ML** — maximum rachis length; **MVO** — morphological bunch volume

Furthermore, all the indices, from literature and designed in this work, were separated based on bunch shape class and correlated with the results of the O.I.V. descriptor n°204, trying to attribute at each bunch shape a specific index to define the compactness.

3.4. Data analysis

The collected data were organized in Excel files divided by type of use for which they were intended, that means one Excel file for the determination of the indices and one for bunch morphological volume. The statistical analysis was done with the program Statistix 9.0 with correlations in order to study the relationships between the O.I.V. descriptor n°204 and each compactness index and understand which one was the closer one to the visual observations of bunch compactness. For the morphological volume, each calculated volume (MVO, V1, V2 and V3) was correlated with the real volume (measured with the displacement of water) and then with the weight to look for the best explanatory variables to be used to improve the algorithms for yield estimation of the Vinbot project. For this aim, a Stepwise Regression, with weight as dependent variable and the other as non-forced variable, was used to find the parameters that better explain bunch weight.

4. Results and discussion

This section of the work presents all the results obtained on 61 bunches at maturation for the variety Syrah from, at least, 5 different plants.

4.1. O.I.V. descriptor n°204

Bunch compactness was evaluated through the subjective method of the O.I.V. descriptor n°204 (O.I.V., 2001) by a panel of 8 trained judges to reduce the problem related to the subjectivity given by the use of this ordinal and qualitative descriptor (Moro, 2016). Nevertheless, in spite of these limitations and given the absence of other standardized alternatives, this descriptor is the most commonly used method for bunch compactness evaluation (Gatti *et al.,* 2012; Moro, 2016). The percentage of bunches for each compactness class was reported in Figure 14.

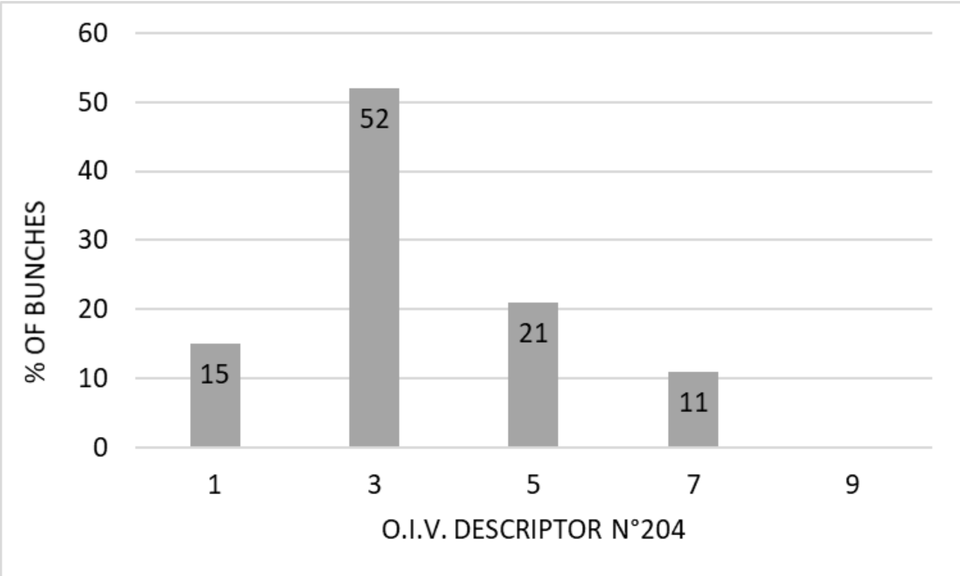


Figure 14: Histogram of the frequencies of the O.I.V. descriptor n°204 for 61 bunches of Syrah.

More than the 50% of the bunches were loose bunches (compactness class 3) with berries in loose contact with each other with some visible pedicel (O.I.V., 2001) and, in fact, the mode obtained from this qualitative method was 3. There were not very dense bunches (compactness class 9) with berries deformed by the compression.

Other authors have developed their own visual scales for the categorization of bunches compactness (Christodoulou *et al.,* 1967, El-Banna & Weaver, 1978, Firoozabady & Olmo, 1987, Miele *et al.,* 1978, Zabadal & Bukovac, 2006, Zabadal & Dittmer, 1998).These scales varied from only three groups of categorization (1: very loose; 2: medium loose; 3: very compact) to scales including up to six different categories (1: rigid, unable to move berries on bunch; 2: some movement of the berries; 3: able to manually separate berries from one another; 4: loose, occasional berries not touching each other; 5: uniformly loose with many berries not touching others, some gaps apparent in bunch; 6: large gaps apparent in bunch; Zabadal & Bukovac, 2006, Zabadal & Dittmer, 1998). This difference increased the difficulty to compare the results obtained in different works.

4.2. Quantitative and objective estimation of bunch compactness

Several studies proposed different relations between different bunch components, to provide a continuous and objective estimation of bunch compactness (Pommer *et al.,* 1996; Sepahi, 1980; Sternad-Lemut *et al.,* 2011; Ferreira & Marais, 2017; Tello & Ibáñez, 2014; Tello *et al.,* 2016; Tello *et al.,* 2015) and to develop new non-destructive methodologies.

4.2.1. Parameters needed to obtain indices

In this work, 61 bunches of Syrah were morphologically described using 11 quantitative components and a summary statistics was reported in Table 9.

Table 9: Maximum, medium and minimum value for 11 quantitative parameters.

PARAMETERS	MAX	MEAN	MIN
BW (g)	239.10	72.07	4.90
BV (ml)	215.00	65.16	5.00
ML (cm)	17.80	11.47	3.40
BL (cm)	31.50	15.70	4.00
A (cm^2)	124.61	48.93	8.83
P (cm)	194.33	70.34	15.18
WI (cm)	13.72	6.99	3.60
TMAX (cm)	11.57	6.91	3.56
TMEAN (cm)	7.88	4.86	2.66
BN	212.00	72.90	10.00
MVO (ml)	361.64	181.13	37.63

A — projected area; **BL** — bunch length; **BN** — berries number; **BV** — bunch real volume; **BW** — bunch weight; **ML** — maximum length; **MVO** = morphological volume; **P** — perimeter; **TMAX** — maximum thickness; **TMEAN** — mean thickness; **WI** — bunch width. n = 61

The difference between the maximum value of bunch length and maximum length was due to the fact that in bunch length was included the wings, if present.

Other parameters were derived from these components, such as width (cm) at 25%, 50% and 75% of the maximum length of the rachis; volumes (ml) a, b, c and d, that were the four bunch sections to obtain the morphological volume; and the radius (cm) as equivalent to half of bunch width. These derived components and the previous 11 ones were required to calculate different bunch volumes and the objective indices.

4.2.2. Bunch compactness: comparison between indices

The different relations between different components of bunches have led to the creation of several indices, an example: number of berries divided by bunch (or rachis) length is the most common estimator of bunch compactness, and it has been used in numerous works (Fawzi *et al.,* 2010, Hed *et al.*, 2009, Hed *et al.,* 2011, Palliotti *et al.,* 2012, Palliotti *et al.,* 2011, Pommer *et al.,* 1996, Vail & Marois 1991, Valdés-Gómez *et al.,* 2008). Moreover, different modifications of this ratio have been proposed, and the value obtained when dividing bunch weight (an easier and faster metric than berries number) by bunch (or rachis) length has been used in different works (Fermaud, 1998, Sternad-Lemut *et al.,* 2010, Sternad-Lemut *et al.,* 2015).

In this work, 9 indices published in literature and 5 new indices were evaluated to determine their usefulness on bunch compactness estimation through an objective way. These compactness indices (CI) were listed in Table 10. Maximum, medium and minimum values were reported, together with the visual evaluation of bunch compactness given by the panel using the O.I.V. descriptor n°204. This descriptor used a code with classes (categoric variable) so the medium value was the one more frequent and it was necessary to calculate a mode instead of a mean.

The 5 designed compactness indices were created to establish if it was better to use bunch length given by the sum of rachis length and wing length or maximum length obtained by ImageJ. The indices with the bunch length inside were done twice with both the alternative lengths, those indicated with the "a" are with the maximum length, the others were with the sum of rachis and wing. Except for the index CI-13_2 created by Tello *et al.* (2016) with the maximum length; so, CI-13_2a used the bunch length.

Table 10: Maximum, medium and minimum values for the compactness indices and the O.I.V. descriptor n°204.

INDICES	MAX	MEAN	MIN
O.I.V. 204	7	3	1
CI-3 (1)	30	6.19	0.59
CI-3 a	35.29	7.95	1.23
CI-4 (2)	52.15	32.12	13.06
CI-10 (3)	9.33	4.52	0.34
CI-10 a	9.61	5.93	0.66
CI-11 (4)	0.59	0.36	0.13
CI-12 (5)	0.77	0.33	0.02
CI-12 a	1.06	0.53	0.09
CI-13 (5)	0.63	0.30	0.02
CI-13 a	0.87	0.48	0.09
CI-13_2 (6)	2.46	1.41	0.69
CI-13_2 a	2.27	1.06	0.18
CSF (7)	548.32	123.23	26.1
RD (7)	0.48	0.15	0.02

(1) Pommer et al., 1996; (2) Sepahi, 1980; (3) Sternad-Lemut et al., 2011; (4) Ferreira & Marais, 2017; (5) Tello & Ibáñez, 2014; (6) Tello et al., 2016; (7) Tello et al., 2015.

CI-3 = BB/BL; **CI-3a** = BB/ML; **CI-4** = (ABV/MVO) ∗100; **CI-10** = BW/BL; **CI-10a** = BW/ML**; CI-11** = BW/MVO; **CI-12** = BW/BL^2; **CI-12a** = BW/ML^2; **CI-13** = ABV/BL^2; **CI-13a** = ABV/ML^2; **CI-13_2**= MVO/ML^2; **CI-13_2a** = MVO/BL^2; **CSF** = (P^2/A); **RD** = (4∗π∗A)/P^2. For the legend see Table 8 and 9. n = 61

All the compactness indices with the maximum length showed higher values than using the sum of rachis and wing, due to the fact that the denominator in the ratio was smaller than using bunch length.

The range for the O.I.V. descriptor n°204 had a maximum of 7 (dense bunch - berries not readily movable) that meant that there were not bunches with the higher score for bunch compactness; while, the medium value was 3 (loose bunch - berries in loose contact with each other with some visible pedicel).

To verify which indices better explained bunch compactness, a correlation between each compactness index and the mode values given by the panel with O.I.V. descriptor n°204 has been done and reported in Figure 15. Additionally, Pearson correlation coefficients (r) were calculated at first for all the bunches sampled and then only for the bunches without wings (removing also the 4 bunches that were too small) to understand if, creating a more homogeneous database, the indices would have been able to better explain the trait of bunch compactness.

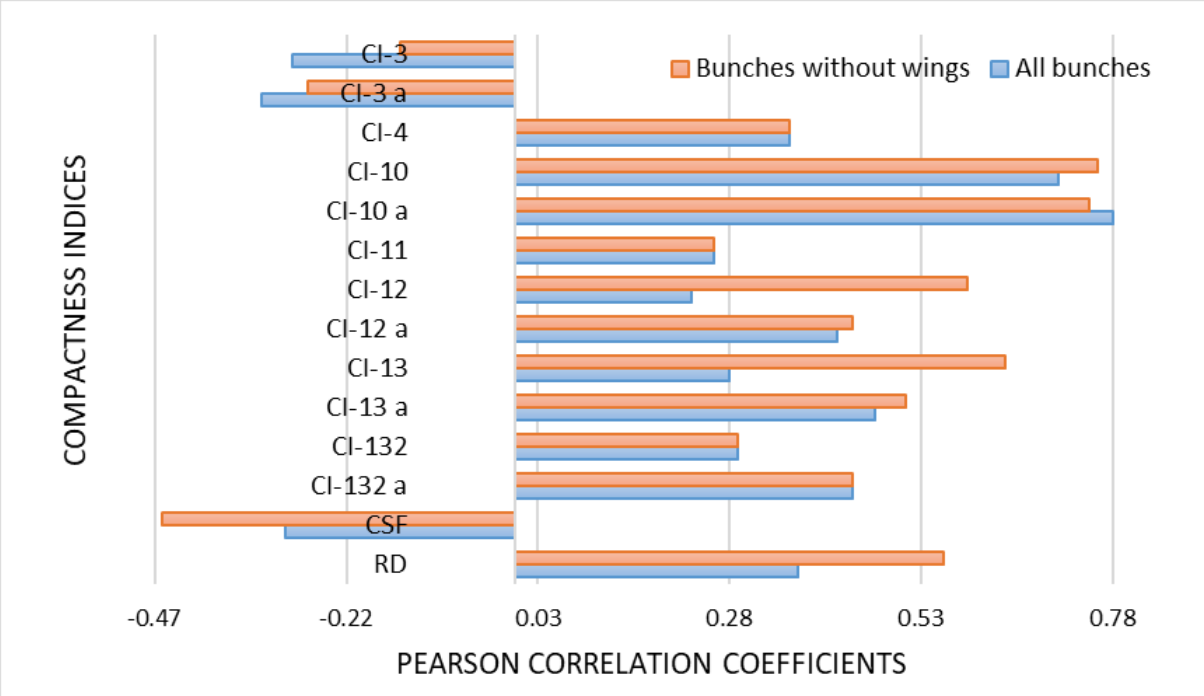


Figure 15: Box plot of the Pearson correlation coefficients between each compactness index and the O.I.V. descriptor n°204 for all the bunches (blue boxes) and for bunches without wings (orange boxes). **CI-3** = BB/BL; **CI-3a** = BB/ML; **CI-4** = (ABV/MVO) ∗100; **CI-10** = BW/BL; **CI-10a** = BW/ML**;** **CI-11** = BW/MVO; **CI-12** = BW/BL^2; **CI-12a** = BW/ML^2; **CI-13** = ABV/BL^2; **CI-13a** = ABV/ML^2; **CI-13$_2$**= MVO/ML^2; **CI-13$_2$a** = MVO/BL^2; **CSF** = (P^2/A); **RD** = $(4*\pi*A)/P^2$. For the legend see Table 8 and 9. All bunches: n = 61; bunches without wings: n = 44

The majority of these indices were positively correlated with the O.I.V. descriptor n°204; only three of them were negatively correlated (CI-3, CI-3a and CSF). The Pearson correlation coefficient was higher using only bunches without wings than using all the sampled bunches. The index CI-10a was the only one that showed a higher Pearson correlation coefficient (r = 0.78) considering all the bunches instead of considering only the bunches without wings (r = 0.75), even if it was a small difference. The indices CI-12, CI-13 and RD (roundness) were the only ones to show grater difference passing from Pearson correlation coefficients of 0.23, 0.28 and 0,37 (for all the bunches) to values of 0.59, 0.64 and 0.56 (for bunches without wings).

The following step consisted in the use of the 44 bunches (no wings and no smallest) to understand which was the best rachis length to be used for the indices. For this reason, the Pearson correlation coefficients (r) were listed in Figure 16 for each couple of indices, the one with the maximum length ("a") and the one with the bunch length given by the sum of rachis plus wing length. Except for the index CI-13_2 created by Tello *et al.* (2016) with the maximum length; so, CI-13_2a used the bunch length.

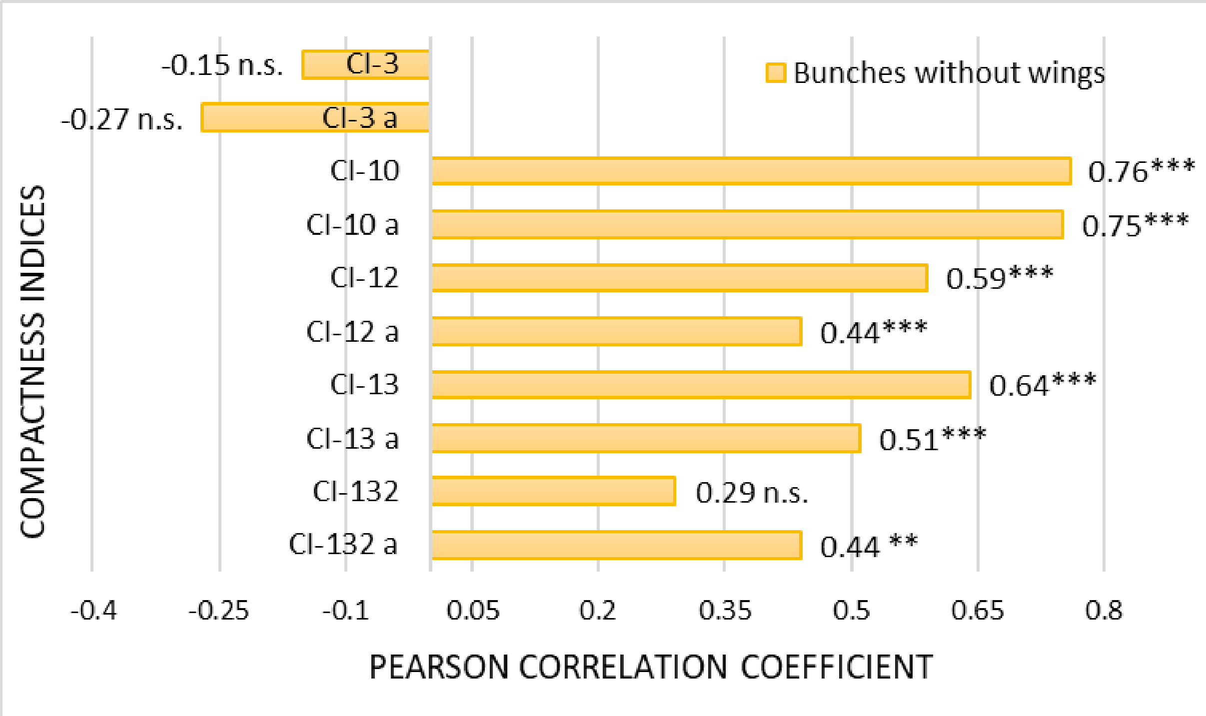


Figure 16: Comparison of Pearson correlation coefficient between the mode of the O.I.V. descriptor n°204 and the indices. **CI-3** = BB/BL; **CI-3a** = BB/ML **CI-10** = BW/BL; **CI-10 a** = BW/ML; **CI-12** = BW/BL^2; **CI-12 a** = BW/ML^2; **CI-13** = ABV/BL^2; **CI-13 a** = ABV/ML^2; **CI-13_2**= MVO/ML^2 and **CI-13_2 a** = MVO/BL^2. For the legend see Table 8 and 9. n = 44
Significance: n.s.: non significant, *: significant with $\rho \leq 0.05$, **: significant with $\rho \leq 0.01$, ***: significant with $\rho \leq 0.001$.

Considering the couple of indices (the one with the maximum length and the other with bunch length) and in particular, for the indices CI-10, CI-12, CI-13 and CI-13_2, the Pearson correlation coefficient (r) was higher using the bunch length as sum of rachis and wing than the maximum length. The index CI-3 had negative value, so a negative correlation with the O.I.V. evaluation for bunch compactness.

These results were in agreement with the one reported by Tello & Ibáñez (2014) for all the indices, except for CI-13_2 studied by Tello *et al.* (2015), for which there were not results to compare with.

To make these indices even more precise, all the bunches sampled were divided according to their shape, using the O.I.V. descriptor n°208 (Tello *et al.,* 2015), with the aim to find different indices for different bunch shapes. For Syrah no bunch with shape 3 (funnel-shape) was found, 16.4% with shape 2 (conical) and 83.6% with shape 1 (cylindrical). In Table 11 were listed all the Pearson correlation coefficients between each index and the mode of the O.I.V. descriptor n°204 for all the bunches at first (left column) and then, dividing by shape. The statistical significance was reported only for the three indices with the best Pearson correlation coefficients compared with the others.

Table 11: Pearson correlation coefficient of compactness indices, divided by shape, and the mode of the O.I.V. descriptor n°204.

Indices	All bunches	O.I.V. 208 shape 1	O.I.V. 208 shape 2
CI-3	-0.29	-0.19	-0.83
CI-3 a	-0.33	-0.29	-0.69
CI-4	0.36	0.37	0.31
CI-10	0.71 ***	0.74 ***	0.47
CI-10 a	0.78 ***	0.73	0.84 ***
CI-11	0.26	0.26	0.39
CI-12	0.23	0.32	-0.24
CI-12 a	0.42	0.32	0.35
CI-13	0.28	0.38	-0.23
CI-13 a	0.47	0.39	0.35
CI-13_2	0.29	0.22	0.17
CI-13_2 a	0.44	0.41	0.28
CSF	-0.30	-0.26	-0.74
RD	0.37	0.27	0.72 *

CI-3 = BB/BL; **CI-3a** = BB/ML; **CI-4** = (ABV/MVO) ∗100; **CI-10** = BW/BL; **CI-10a** = BW/ML; **CI-11** = BW/MVO; **CI-12** = BW/BL^2; **CI-12a** = BW/ML^2; **CI-13** = ABV/BL^2; **CI-13a** = ABV/ML^2; **CI-13_2**= MVO/ML^2; **CI-13_2a** = MVO/BL^2; **CSF** = (P2/A); **RD** = $(4 *\pi *A)/P^2$. For the legend see Table 8 and 9. All bunches: n = 61; shape 1: n = 51; shape 2: n = 10

Significance: n.s.: non significant, *: significant with ρ ≤ 0.05, **: significant with ρ ≤ 0.01, ***: significant with ρ ≤ 0.001.

The most suitable indices proved to be CI-10, CI-10a and RD. In particular, CI-10a can be used for all the bunches and for the ones with a conical shape (class 2). For Syrah bunches having a cylindrical shape (class 1) the Pearson correlation coefficient was not so different between CI-10 (r = 0.74) and CI-10a (r = 0.73). It has allowed to reduce the number of indices available to only three with high and significant Pearson correlation coefficient, with CI-10a as useful for all bunches and for both cylindrical and conical bunches (r = 0.78, r = 0.73 and r = 0.84, respectively).

Trying to go even further, the same operation was done on the bunches without wings, 49 observation of whom 85.7% of cylindrical bunches (shape 1) and 14.3% of conical bunches (shape 2). The results were showed in Table 12 with the statistical significance reported only for the three indices with the better Pearson correlation coefficients than the others. The most suitable indices were still CI-10, CI-10a and RD, with CI-10a that maintained its good representation of bunch compactness for all the cases (bunches without wings, cylindrical bunches and conical bunches). Comparing Table 11 and 12, the Pearson correlation coefficient between CI-10 and the mode value from the panel of judges (O.I.V. descriptor n°204) for cylindrical bunches went from 0.74 to 0.75 and for conical bunches RD went from 0.72 to 0.90, both maintaining the significance level; instead, for cylindrical bunches the correlation between CI-10a or CI-10 and the mode of the descriptor n°204 became non significant, due to the lower number of observations (n=5).

Table 12: Indices divided by shape for the bunches without wings sampled.

Indices	Bunches without wings	O.I.V. 208 shape 1	O.I.V. 208 shape 2
CI-3	-0.15	-0.08	-0.75
CI-3 a	-0.27	-0.22	-0.69
CI-4	0.36	0.37	0.31
CI-10	0.76 ***	0.75***	0.76 n.s.
CI-10 a	0.75 ***	0.72	0.82 n.s.
CI-11	0.26	0.26	0.39
CI-12	0.59	0.53	0.69
CI-12 a	0.44	0.37	0.52
CI-13	0.64	0.59	0.77 n.s.
CI-13 a	0.51	0.46	0.44
CI-13_2	0.29	0.22	0.17
CI-13_2 a	0.44	0.42	0.28
CSF	-0.46	-0.44	-0.84
RD	0.56	0.46	0.90*

CI-3 = BB/BL; **CI-3a** = BB/ML; CI-4 = (ABV/MVO) $*$100; **CI-10** = BW/BL; **CI-10a** = BW/ML; **CI-11** = BW/MVO; **CI-12** = BW/BL^2; **CI-12a** = BW/ML^2; **CI-13** = ABV/BL^2; **CI-13a** = ABV/ML^2; **CI-13_2**= MVO/ML^2; **CI-13_2a** = MVO/BL^2; **CSF** = (P2/A); **RD** = $(4 * \pi * A)/P^2$. For the legend see Table 8 and 9. Bunches without wings n = 44; shape 1 n = 39; shape 2 n = 5
Significance: n.s.: non significant, *: significant with $\rho \leq 0.05$, **: significant with $\rho \leq 0.01$, ***: significant with $\rho \leq 0.001$.

In this way it was possible to confirm the three indices with best results. Between them, CI-10a maintained more constant results by comparing Pearson correlation coefficient for all the bunches and for bunches without wings. For shape 2, the Pearson correlation coefficients became non significant due to the lower number of observations (n=5).

4.3. Contribution to improve bunch weight estimation from image analysis

Table 13 presented a correlation matrix between BW and all the variables measured and calculated on Syrah bunches.

Table 13: Correlation matrix with the parameters obtained at harvest and the significance of the Pearson correlation coefficients.

	BW	BV	ML	BL	A	P	WI	TMAX	TMEAN	BN
BW	1	***	***	***	***	***	***	***	***	***
BV	0.99	1	***	***	***	***	***	***	***	***
ML	0.78	0.79	1	***	***	***	***	***	***	***
BL	0.74	0.75	0.72	1	***	***	***	***	***	***
A	0.95	0.95	0.88	0.77	1	***	***	***	***	***
P	0.42	0.43	0.70	0.75	0.57	1	***	***	***	***
WI	0.75	0.76	0.62	0.78	0.72	0.53	1	***	***	***
TMAX	0.74	0.75	0.77	0.70	0.85	0.65	0.56	1	***	***
TMEAN	0.80	0.80	0.77	0.61	0.89	0.54	0.63	0.85	1	***
BN	0.88	0.89	0.77	0.78	0.84	0.51	0.77	0.72	0.68	1

A — projected area; **BL** — bunch length; **BN** — berries number; **BV** — bunch real volume; **BW** — bunch weight; **ML** — maximum length; **P** — perimeter; **TMAX** — maximum thickness; **TMEAN** — mean thickness; **WI** — bunch width. n = 61
Significance: n.s.: non significant, *: significant at $\rho \leq 0.05$, **: significant at $\rho \leq 0.01$, ***: significant at $\rho \leq 0.001$.

All the variables were significantly and positively correlated between each other with the highest Pearson correlation coefficient (r) presented by the relationship between BW and BV (r = 0.99) followed by BW and A (r = 0.95); while the lowest one (r = 0.42) was observed between BW and P.

The high and significant correlation coefficients obtained indicate that most part of the studied parameters can be used as predictors of bunch weight. In particular, bunch volume was a very powerful estimator of bunch weight with the related problem that was not automatically obtainable. For this reason, other volumes obtained from image analysis were studied in this work. In Table 14 were showed the maximum, medium and minimum values for the four different volumes used to find a more accurate and automatable way to measure bunch volume. The morphological volume (MVO) was determined by the sum of four volumes of four bunch sections; volume 1 (V1) as the product of mean projected area (A) per maximum thickness (TMAX), while volume 2 (V2) as mean projected area per mean thickness (TMEAN) and, the last one was the volume of a cone (V3).

Table 14: Maximum, medium and minimum values of the four calculated volumes.

VOLUMES	MAX	MEAN	MIN
MVO (ml) (1)	361.64	181.13	37.63
V1 (ml)	642.33	371.28	32.92
V2 (ml)	540.91	258.82	24.61
V3 (ml)	290.44	175.01	26.21

(1) Tello *et al*. (2015)

MVO = Va + Vb + Vc + Vd; **V1** = $\mathrm{Area} * \mathrm{TMax}$; **V2** = $\mathrm{A} * \mathrm{TMean}$; **V3** $= (\pi * r^2 * h)/3$. For the legend see Table 7. n = 61

An important aspect, regarding the morphological volume (MVO), was that to determine this volume bunch images must be divided in four sections of the same height and the width of each section was the required value. This operation was not applicable to bunch with wings because these images should be separated in two: the main bunch and the wing, repeating the operation twice; at the end would have been a very time-consuming operation and impossible to automate. For Syrah, the number of bunches with wing was 12 on 61 observations, so a 19.7% of excluded bunches for wings presence. Another selection criterium was to exclude too small bunches (bunches with a maximum length less than 5 cm) due to the difficulty to determine the four sections. For Syrah these bunches were 5 on 61 observation that meant a percentage of 8.2% of excluded bunches on the volume evaluation due to their dimensions.

Additionally, these volumes were correlated with BW for all the bunches, for bunches without wings and then by dividing the bunches by shape. In this last case, the morphological volume was the only one divided by shape using only bunches without wings because it was not possible to calculate it for all the bunches. Instead, the other parameters were evaluated for all the bunches and for only the ones without wings. Then all the bunches divided by shape (O.I.V. descriptor n°208) and only the one without wings divided by shape.

The approach to divide the bunches based on their shape did not show relevant differences on Pearson correlation coefficients (r) between BW and the parameters (Tab.15). Volume 1 showed more suitability for conical bunches (shape 2), while volume 2 for cylindrical bunches (shape 1). More constant values were observed for RV and A1 (projected area from frontal image) which showed the highest values (r) with bunch weight for both the shapes and considering all bunches or bunches without wings.

Table 15: Pearson correlation coefficients between bunch weight and parameters for all bunches, for bunches without wings and for all bunches divided per shape following the O.I.V. descriptor n°208.

Parameters	**All bunches**	**Bunches without wings**	**O.I.V. 208 shape 1**	**O.I.V. 208 shape 2**
RV	0.99 ***	0.99 ***	0.99 ***	0.99 ***
MVO	0.90 ***	0.90 ***	0.89 ***	0.94 *
V1	0.88 ***	0.82 ***	0.89 ***	0.93 ***
V2	0.90 ***	0.85 ***	0.92 ***	0.87 **
V3	0.78 ***	0.74 ***	0.77 ***	0.86 **
A1	0.95 ***	0.94 ***	0.95 ***	0.99 ***
A2	0.86 ***	0.80 ***	0.87 ***	0.88 ***
Mean A	0.95 ***	0.93 ***	0.95 ***	0.97 ***

MVO = Va + Vb + Vc + Vd; **V1** = $A * TMax$; **V2** = $A * TMean$; **V3** $= (\pi * r^2 * h)/3$; For the legend see Table 8 and 9. **A1** — projected area from frontal image; **A2** — projected area from lateral image; **Mean A** — mean between A1 and A2; **RV** — real volume. All bunches n = 61; bunches without wings n = 44; shape 1 n = 51; shape 2 n = 10
Significance: n.s.: non significant, *: significant at $\rho \leq 0.05$, **: significant at $\rho \leq 0.01$, ***: significant at $\rho \leq 0.001$.

In order to find the best explanatory variables to estimate BW, a stepwise regression analysis between BW (dependent variable) and the above variables that are considered easy to extract by automated image analysis (A1, V3, BN and CI-10a) was performed.

The first variable selected was A1 (partial $R^2 = 0.905$). In the second step of the regression, the variable CI-10a was chosen but with a very low contribution for the explanation of BW variance (partial $R^2<0.06$). In the third step the variable V3 was chosen with an even lower contribution (partial $R^2<0.007$). The variable BN did not meet the significance level for entry into the model. The final model obtained was represented in equation 9.

$$BW = -29.4205 + 1.48854 * A1 + 6.62070 * CI - 10a - 0.05570 * V3 \quad \text{(Eq. 9);}$$

Adj. $R^2 = 0.9684$ ($p \leq 0.001$); n = 61; RMSE = 7.6g.

The estimated BW values fitted well with the actual BW as showed in Figure 17.

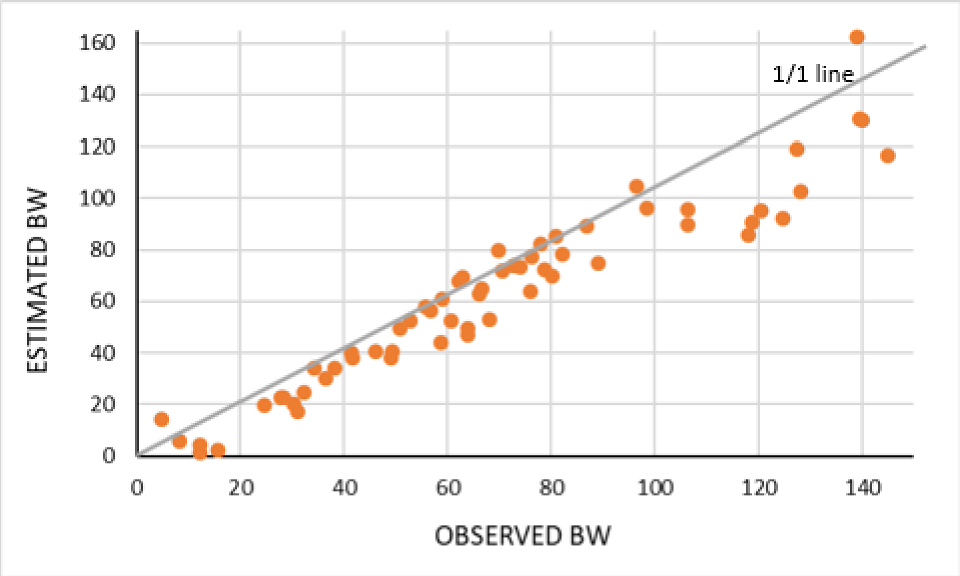


Figure 17: Relationship between observed and estimated bunch weight values (BW) using the model represented in equation 9.

In the model the variable A1 was the first variable selected with a very high partial R^2 showing that this bunch feature is a very important predictor of BW. As demonstrated by several authors (Diago *et al.*, 2012; Nuske *et al.,* 2014; Font *et al.,* 2015; Di Gennaro *et al.,* 2019) bunch projected area in the image was an easy feature to extract with image analysis and machine vision technologies.

CI-10a and V3 were the second and third variables selected, and this confirms their importance as yield estimators (Clingeleffer *et al.,* 2001). However, in the multiple regression model, after the selection of the variable A1, the contribution of CI-10a and V3 to explain BW variance was low.

The variable BN didn´t met the 0.05 significance level for entry into the model indicating that, despite being an important variable, when in the presence of the other variables like A1, its contribution to explain BW became irrelevant.

5. Conclusions

The aim of this work was to compare different methods to estimate bunch compactness on Syrah at maturation. The easiest and the least time-consuming way to define this trait remains the use of the O.I.V. descriptor n°204 but, to be accurate, it requires a panel of judges to reduce the error given by the subjectivity of the evaluation. A vine grower would need time to form and train the panel and time to do the sections for the estimation, so it is not probably the best method, even if in spite of its limitations and given the absence of other standardized alternatives, this descriptor is the most commonly used for bunch compactness evaluation.

The alternative to this method was the use of indices. The different relations between different components of bunches have led to the creation of several indices; they can be more precise, they don't need trained worker and are quickly obtainable. In this work 9 indices already published in literature and 5 new indices were evaluated to determine their usefulness as bunch compactness estimation through an objective way. The majority of these indices were positively correlated with the O.I.V. descriptor n°204. Only three of them were negatively correlated (CI-3, CI-3a and CSF). The Pearson correlation coefficient was higher when using only bunches without wings as compared to the use of all the sampled bunches.
For the estimations of the compactness indices (CI-3; CI-3a; CI-10; CI-10a; CI-12; CI-12a; CI-13; CI-13a; CI-13_2; CI-13_2a) the correlation analysis between each index and the mode of the O.I.V. descriptor n°204 showed that the use of bunch length as sum of rachis and wing was preferable than the maximum length.

To make these indices even more precise, all the bunches sampled were divided according to their shape, using the O.I.V. descriptor n°208, with the aim to find different indices for different bunch shapes. The most suitable indices proved to be CI-10, CI-10a and RD. In particular, CI-10a for all the bunches and for the ones with both shapes (cylindrical and conical). The same procedure was done on the bunches without wings and the results confirmed the index CI-10a as the most suitable one. Further studies are necessary and in-depth analysis between the largest possible number of varieties to achieve a method able to discriminate different bunch shapes.

The last consideration is about the relation of this work with the Vinbot project. In order to improve the accuracy of the algorithms for BW estimation from 2D images, the relationships between bunch components and corresponding bunch weight were explored with the aim to find the best explanatory variables. Most part of the bunch components determined (volume, projected area, berry number, rachis length, bunch width and bunch compactness) were significantly and positively correlated with

bunch weight indicating they can be used to predict bunch weight. Using a multiple stepwise regression approach, we obtained an empirical model for the estimation of the bunch weight based on the following bunch attributes: bunch projected area, compactness index (CI-10a) and volume 3 (V3). The model obtained presented a good fit between estimated and actual bunch weight, indicating that grapevine bunch weight can be estimated with accuracy from 2D images using explanatory variables derived from automated non-intrusive assessment of bunch morphological attributes.
Further studies are necessary to validate this model on other varieties, to figure out the best indices also based on bunch shape; on other sites.

References

Baby, T., Gilliham, M., Tyerman, S. D., & Collins, C. (2016). Differential fruit set between grapevine cultivars is related to differences in pollen viability and amine concentration in flowers. Australian Journal of Grape and Wine Research, 22(1), 149-158.

Basile, B., Caccavello, G., Giaccone, M., & Forlani, M. (2015). Effects of early shading and defoliation on bunch compactness, yield components, and berry composition of Aglianico grapevines under warm climate conditions. American Journal of Enology and Viticulture, 66(2), 234-243.

Bergqvist, J., Dokoozlian, N., & Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. American Journal of Enology and Viticulture, 52(1), 1-7.

Bondada, B., Covarrubias, J. I., Tessarin, P., Boliani, A. C., Marodin, G., & Rombolà, A. D. (2016). Post-veraison shoot trimming reduces cluster compactness without compromising fruit quality attributes in organically grown Sangiovese grapevines. American Journal of Enology and Viticulture, 67(2), 206-211.

Bramley, R. G. V., & Hamilton, R. P. (2004). Understanding variability in winegrape production systems: 1. Within vineyard variation in yield over several vintages. Australian Journal of Grape and Wine Research, 10(1), 32-45.

Candolfi-Vasconcelos, M. C., Koblet, W., Howell, G. S., & Zweifel, W. (1994). Influence of defoliation, rootstock, training system, and leaf position on gas exchange of Pinot noir grapevines. American Journal of Enology and Viticulture, 45(2), 173-180.

Carmona, M. J., Chaïb, J., Martínez-Zapater, J. M., & Thomas, M. R. (2008). A molecular genetic perspective of reproductive development in grapevine. Journal of Experimental Botany, 59(10), 2579-2596.

Chen, X., Ding, H., Yuan, L. M., Cai, J. R., Chen, X., & Lin, Y. (2018). New approach of simultaneous, multi-perspective imaging for quantitative assessment of the compactness of grape bunches. Australian Journal of Grape and Wine Research, 24(4), 413-420.

Christodoulou, A.; Weaver, R. J.; Pool, R. M. (1967). Response of Thompson Seedless grapes to prebloom thinning. Vitis 6, 303-308

Clingeleffer, P. R., Martin, S. R., Dunn, G. M., & Krstic, M. P. (2001). Crop development, crop estimation and crop control to secure quality and production of major wine grape varieties: a national approach: final report to Grape and Wine Research & Development Corporation/principal investigator, Peter Clingeleffer; [prepared and edited by Steve Martin and Gregory Dunn].

Cohen, Y., Rubin, A. E., & Vaknin, M. (2011). Post infection application of DL-3-amino-butyric acid (BABA) induces multiple forms of resistance against Bremia lactucae in lettuce. European Journal of Plant Pathology, 130(1), 13-27.

Coombe, B. G., & McCarthy, M. G. (2000). Dynamics of grape berry growth and physiology of ripening. Australian Journal of Grape and Wine Research, 6(2), 131-135.

Cubero, S., Diago, M. P., Blasco, J., Tardáguila, J., Prats-Montalbán, J. M., Ibáñez, J. & Aleixos, N. (2015). A new method for assessment of bunch compactness using automated image analysis. Australian Journal of Grape and Wine Research, 21(1), 101-109.

De Souza Leão, P. C., Lima, M. A. C., Costa, J. P. D., & da Trindade, D. C. G. (2015). Abscisic acid and ethephon for improving red color and quality of crimson seedless grapes grown in a tropical region. American Journal of Enology and Viticulture, 66(1), 37-45.

Di Gennaro, S.F., Toscano P., Cinat P. , Berton A. and Matese A. (2019). A Low-Cost and Unsupervised Image Recognition Methodology for Yield Estimation in a Vineyard. Frontiers in Plant Science 10, 1-13.

Diago, M. P., Correa, C., Millán, B., Barreiro, P., Valero, C., & Tardaguila, J. (2012). Grapevine yield and leaf area estimation using supervised classification methodology on RGB images taken under field conditions. Sensors, 12(12), 16988-17006.

Diago, M. P., Sanz-Garcia, A., Millan, B., Blasco, J., & Tardaguila, J. (2014). Assessment of flower number per inflorescence in grapevine by image analysis under field conditions. Journal of the Science of Food and Agriculture, 94(10), 1981-1987.

Diago, M. P., Tardaguila, J., Aleixos, N., Millan, B., Prats-Montalban, J. M., Cubero, S., & Blasco, J. (2015). Assessment of cluster yield components by image analysis. Journal of the Science of Food and Agriculture, 95(6), 1274-1282.

Domingos, S., Scafidi, P., Cardoso, V., Leitao, A. E., Di Lorenzo, R., Oliveira, C. M., & Goulao, L. F. (2015). Flower abscission in Vitis vinifera L. triggered by gibberellic acid and shade discloses differences in the underlying metabolic pathways. Frontiers in Plant Science, 6, 457.

Duchêne, E., Schneider, C., & Gaudillere, J. P. (2001). Effects of nitrogen nutrition timing on fruit set of grapevine, cv. Grenache. Vitis-Geilweilerhof-, 40(1), 45-46.

Dunn, G. M. (2010). Yield forecasting. Technical Booklet.

Dunn, G. M., & Martin, S. R. (2004). Yield prediction from digital image analysis: A technique with potential for vineyard assessments prior to harvest. Australian Journal of Grape and Wine Research, 10(3), 196-198.

Eiras-Dias, J. E., Faustino, R., Clímaco, P., Fernandes, P., Cruz, A., Cunha, J. & Castro, R. D. (2011). Catálogo das castas para vinho cultivadas em Portugal. Vol. I, Instituto da Vinha e do Vinho, Lisboa.

El-Banna, G. I., & Weaver, R. J. (1979). Effect of ethephon and gibberellin on maturation of ungirdled Thompson Seedless grapes. American Journal of Enology and Viticulture, 30(1), 11-13.

El-Razek, E. A., Treutter, D., Saleh, M. M. S., El-Shammaa, M., Amera, A. F., & Abdel-Hamid, N. (2011). Effect of nitrogen and potassium fertilization on productivity and fruit quality of 'crimson seedless' grape. Agriculture and Biology Journal of North America, 2(2), 330-340.

Eltom, M., Trought, M. C., Agnew, R., Parker, A., & Winefield, C. S. (2017). Pre-budburst temperature influences the inner and outer arm morphology, phenology, flower number, fruitset, TSS accumulation and variability of Vitis vinifera L. Sauvignon Blanc bunches. Australian Journal of Grape and Wine Research, 23(2), 280-286.

EUROBOTICS AISBL, 2013. Robotics 2010. Strategic Research Agenda For Robotics in Europe 2014-2020. (https://ec.europa.eu/research/industrial_technologies/pdf/robotics-ppp-roadmap_en.pdf. Accessed 12 June 2017).

Evans, J. R., Evans, R. R., Regusci, C. L., & Rademacher, W. (1999). Mode of action, metabolism, and uptake of BAS 125W, prohexadione-calcium. HortScience, 34(7), 1200-1201.

Evers, D., Molitor, D., Rothmeier, M., Behr, M., Fischer, S., & Hoffmann, L. (2010). Efficiency of different strategies for the control of grey mold on grapes including gibberellic acid (Gibb3), leaf removal and/or botrycide treatments. OENO One, 44(3), 151-159.

Fawzi, M.I.F., M.F.M. Shanin, & E.A. Kandil (2010) Effect of bud load on bud behavior, yield, cluster characteristics and some biochemical contents of the cane of Crimson seedless grapevines. Journal of American Science 6, 187-194

Fermaud, M. (1998). Cultivar susceptibility of grape berry clusters to larvae of Lobesia botrana (Lepidoptera: Tortricidae). Journal of Economic Entomology, 91(4), 974-980.

Fermaud, M., & Menn, R. L. (1989). Association of Botrytis cinerea with grape berry moth larvae. Phytopathology, 79(6), 651-656.

Ferreira, J. H. S., & Marais, P. G. (2017). Effect of rootstock cultivar, pruning method and crop load on Botrytis cinerea rot of Vitis vinifera cv. Chenin blanc grapes. South African Journal of Enology and Viticulture, 8(2), 41-44.

Firoozabady, E., & Olmo, H. P. (1987). Heritability and correlation studies of certain quantitative traits in table grapes, Vitis spp. Vitis 26, 132-146.

Font, D., Tresanchez, M., Martínez, D., Moreno, J., Clotet, E., & Palacín, J. (2015). Vineyard yield estimation based on the analysis of high-resolution images obtained with artificial illumination at night. Sensors, 15(4), 8284-8301.

Gatti, M., Bernizzoni, F., Civardi, S., & Poni, S. (2012). Effects of cluster thinning and pre-flowering leaf removal on growth and grape composition in cv. Sangiovese. American Journal of Enology and Viticulture, 63(3), 325-332.

Gatti, M., Galbignani, M., Garavani, A., Bernizzoni, F., Tombesi, S., Palliotti, A., & Poni, S. (2016). Manipulation of ripening via antitranspirants in cv. Barbera (V itis vinifera L.). Australian Journal of Grape and Wine Research, 22(2), 245-255.

Grossetête, M., Berthoumieu, Y., Da Costa, J. P., Germain, C., Lavialle, O., & Grenier, G. (2012). Early estimation of vineyard yield: site specific counting of berries by using a smartphone. In International Conference of Agricultural Engineering—CIGR-AgEng.

Hanni, E., Lardschneider, E., & Kelderer, M. (2012). Alternatives to the use of gibberellins for bunch thinning and bunch compactness reduction on grapevine. In I International Workshop on Vineyard Mechanization and Grape and Wine Quality 978 (pp. 335-345).

Hed, B., H.K. Ngugi, & J.W. Travis (2011) Use of gibberellic acid for management of bunch rot on Chardonnay and Vignoles grape. Plant Disease 95, 269-278

Hed, B., Ngugi, H. K., & Travis, J. W. (2009). Relationship between cluster compactness and bunch rot in Vignoles grapes. Plant Disease, 93(11), 1195-1201.

Houel, C., Martin-Magniette, M. L., Nicolas, S. D., Lacombe, T., Le Cunff, L., Franck, D., Torregrosa, L., Conéjéro, G., Lalet, S. & Adam-Blondon, A. F. (2013). Genetic variability of berry size in the grapevine (V itis vinifera L.). Australian Journal of Grape and Wine Research, 19(2), 208-220.

Ibáñez, J., Carreño, J., Yuste, J., & Martínez-Zapater, J. M. (2015). Grapevine breeding and clonal selection programmes in Spain. In Grapevine breeding programs for the wine industry (pp. 183-209). Woodhead Publishing.

Instituto Português do Mar e da Atmosfera, IPMA (2019). Normais Climatológicas - 1971-2000. http://www.ipma.pt/pt/oclima/normais.clima/1971-2000/001/

Instruction manual Sony DSC-H90 (2012). Instruction operation manual. Sony Corporation, pp. 25.

Intrieri, C., Allegro, G., Valentini, G., Pastore, C., Colucci, E., & Filippetti, I. (2013). Effect of pre-bloom anti-transpirant treatments and leaf removal on “Sangiovese”(Vitis vinifera L.) winegrapes. Vitis—J Grapevine Res, 52, 117-124.

Intrieri, C., Filippetti, I., Allegro, G., Centinari, M., & Poni, S. (2008). Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (Vitis vinifera L.). Australian Journal of Grape and Wine Research, 14(1), 25-32.

Intrigliolo, D. S., Pérez, D., Risco, D., Yeves, A., & Castel, J. R. (2012). Yield components and grape composition responses to seasonal water deficits in Tempranillo grapevines. Irrigation Science, 30(5), 339-349.

Jones, G. V., White, M. A., Cooper, O. R., & Storchmann, K. (2005). Climate change and global wine quality. Climatic change, 73(3), 319-343.

Keller, M. (2015). The science of grapevines: anatomy and physiology. Academic Press.

Keller, M., Kummer, M., & Vasconcelos, M. C. (2001). Reproductive growth of grapevines in response to nitrogen supply and rootstock. Australian Journal of Grape and Wine Research, 7(1), 12-18.

Koblet, W. (1969). Translocation of photosynthate in vine shoots and influence of leaf area on quantity and quality of the grapes. Wein-Wiss, *24*, 277-319.

Kocsis, M., Csikász-Krizsics, A., Szata, B., Kovács, S., Mátai, A., & Jakab, G. (2018). Regulation of cluster compactness and resistance to Botrytis cinerea with β-aminobutyric acid treatment in field-grown grapevine. Vitis-Journal of Grapevine Research, 57(1), 35-40.

Latorre, B. A., Briceño, E. X., & Torres, R. (2011). Increase in Cladosporium spp. populations and rot of wine grapes associated with leaf removal. Crop protection, 30(1), 52-56.

Lebon, G., Wojnarowiez, G., Holzapfel, B., Fontaine, F., Vaillant-Gaveau, N., & Clément, C. (2008). Sugars and flowering in the grapevine (Vitis vinifera L.). Journal of Experimental Botany, 59(10), 2565-2578.

Leong, S. L. L., Hocking, A. D., Pitt, J. I., Kazi, B. A., Emmett, R. W., & Scott, E. S. (2006). Australian research on ochratoxigenic fungi and ochratoxin A. International Journal of Food Microbiology, 111, S10-S17.

Li, L., Zhang, Q., & Huang, D. (2014). A review of imaging techniques for plant phenotyping. Sensors, 14(11), 20078-20111.

Li-Mallet, A., Rabot, A., & Geny, L. (2015). Factors controlling inflorescence primordia formation of grapevine: their role in latent bud fruitfulness? A review. Botany, 94(3), 147-163.

Lopes, C. M., Torres, A., Guzman, R., Graça, J., Reyes, M., Vitorino, G., Braga, R., Monteiro, A. & Barriguinha, A. (2017). Using an unmanned ground vehicle to scout vineyards for non-intrusive estimation of canopy features and grape yield. In GiESCO International Meeting, 20th, Sustainable viticulture and wine making in climate change scenarios, 5-10 November 2017. GiESCO. http://www.vinbot.eu/

Mahalanobis, P. C. (1936). On the generalized distance in statistics. National Institute of Science of India.

Matthews, M. A., & Anderson, M. M. (1988). Fruit ripening in Vitis vinifera L.: responses to seasonal water deficits. American Journal of Enology and Viticulture, 39(4), 313-320.

Miele, A.; Weaver, R. J., & Johnson, J. (1978). Effect of potassium gibberellate on fruit set and development of Thompson Seedless and Zinfandel grapes. Am. J. Enol. Vitic. 29, 79-82.,

Molitor, D., Baus, O., Hoffmann, L., & Beyer, M. (2016). Meteorological conditions determine the thermal-temporal position of the annual Botrytis bunch rot epidemic on Vitis vinifera L. cv. Riesling grapes. Oeno One, 50(4).

Molitor, D., Behr, M., Hoffmann, L., & Evers, D. (2012). Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA3) for stem elongation in Sauvignon blanc. South African Journal of Enology and Viticulture, 33(2), 198-202.

Molitor, D., Rothmeier, M., Behr, M., Fischer, S., Hoffmann, L., & Evers, D. (2011). Crop cultural and chemical methods to control grey mould on grapes. Vitis, 50(2), 81-87.

Moro, J. T. (2016). An integrative genetic study of the bunch compactness trait in grapevine (Doctoral dissertation, Universidad Autónoma de Madrid).

Mottard, G., Nesploulous, J., & Marcout, P. (1963). Cépages et Vignobles de France. Tome I. Les Vignes Americanes. Imprimerie Charles Dehan, Montpellier, 2ª ed.

Navarro, J. A. & Gonzálvez Pérez, V. (2006) 'Cultivo ecológico de la uva de mesa', Hojas divulgadoras, Ministerio de Agricultura, Pesca y alimentaicón.

Negri, S., Lovato, A., Boscaini, F., Salvetti, E., Torriani, S., Commisso, M., Danzi, R., Ugliano, M., Polverari, A., Tornielli, G. B. & Guzzo, F. (2017). The induction of noble rot (Botrytis cinerea) infection during postharvest withering changes the metabolome of grapevine berries (Vitis vinifera L., cv. Garganega). Frontiers in Plant Science, 8, 1002.

Niculcea, M., López, J., Sánchez-Díaz, M., & Carmen Antolín, M. (2014). Involvement of berry hormonal content in the response to pre-and post-veraison water deficit in different grapevine (V itis vinifera L.) cultivars. Australian Journal of Grape and Wine Research, 20(2), 281-291.

Nuske, S., Wilshusen, K., Achar, S., Yoder, L., Narasimhan, S., & Singh, S. (2014). Automated visual yield estimation in vineyards. Journal of Field Robotics, 31(5), 837-860.

O.I.V. Organisation International de la Vigne et du Vin (2001). 2nd Edition of the O.I.V. descriptor list for grape varieties and Vitis species, pp. 138.

O.I.V. Organisation International de la Vigne et du Vin (2017). Statistical Report on World Vitiviniculture.

Palliotti, A., Gardi, T., Berrios, J. G., Civardi, S., & Poni, S. (2012). Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red Vitis vinifera L. cultivar. Scientia Horticulturae, 145, 10-16.

Palliotti, A., M. Gatti, & S. Poni (2011) Early leaf removal to improve vineyard efficiency: gas exchange, source-to-sink balance, and reserve storage responses. American Journal of Enology and Viticulture 62, 219-228.

Palliotti, A., Poni, S., Berrios, J. G., & Bernizzoni, F. (2010). Vine performance and grape composition as affected by early-season source limitation induced with anti-transpirants in two red Vitis vinifera L. cultivars. Australian Journal of Grape and Wine Research, 16(3), 426-433.

Percival, D. C., Sullivan, J. A., & Fisher, K. H. (1993). Effect of cluster exposure, berry contact and cultivar on cuticular membrane formation and occurrence of bunch rot(Botrytis cinerea PERS.: FR.) with 3 Vitis vinifera L. cultivars. Vitis, 32(2), 87-97.

Perez, J., & Kliewer, W. M. (1990). Effect of shading on bud necrosis and bud fruitfulness of Thompson Seedless grapevines. American Journal of Enology and Viticulture, 41(2), 168-175.

Petrie, P. R., & Clingeleffer, P. R. (2005). Effects of temperature and light (before and after budburst) on inflorescence morphology and flower number of Chardonnay grapevines (Vitis vinifera L.). Australian Journal of Grape and Wine Research, 11(1), 59-65.

Piazzolla, F., Pati, S., Amodio, M. L., & Colelli, G. (2016). Effect of harvest time on table grape quality during on-vine storage. Journal of the Science of Food and Agriculture, 96(1), 131-139.

Pieri, P., Zott, K., Gomès, E., & Hilbert, G. (2016). Nested effects of berry half, berry and bunch microclimate on biochemical composition in grape. Oeno One, 50(3), 23-33.

Pommer, C. V., Pires, E. J. P., Terra, M. M., & Passos, I. R. S. (1996). Streptomycin-induced seedlessness in the grape cultivar Rubi (Italia Red). American journal of Enology and viticulture, 47(3), 340-342.

Poni, S., Bernizzoni, F., & Civardi, S. (2008). The effect of early leaf removal on whole-canopy gas exchange and vine performance of Vitis vinifera L. Sangiovese. Vitis-Geilweilerhof-, 47(1), 1.

Poni, S., Bernizzoni, F., Civardi, S., & Libelli, N. (2009). Effects of pre-bloom leaf removal on growth of berry tissues and must composition in two red Vitis vinifera L. cultivars. Australian Journal of Grape and Wine Research, 15(2), 185-193.

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S., & Intrieri, C. (2006). Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. American Journal of Enology and Viticulture, 57(4), 397-407.

Pouget, R. (2016). Action de la temperature sur la differenciation des inflorescences et des fleurs durant les phases de pre-debourrement et de post-debourrement des bourgeons latents de la vigne. OENO One, 15(2), 65-79.

Retallack, M. (2012). Grapevine biology.

Rist, F., Herzog, K., Mack, J., Richter, R., Steinhage, V., & Töpfer, R. (2018). High-precision phenotyping of grape bunch architecture using fast 3D sensor and automation. Sensors, 18(3), 763.

Rizzuti, A., Aguilera-Sáez, L. M., Gallo, V., Cafagna, I., Mastrorilli, P., Latronico, M., Pacifico, A., Matarrese, A. M. S. & Ferrara, G. (2015). On the use of Ethephon as abscising agent in cv. Crimson Seedless table grape production: Combination of Fruit Detachment Force, Fruit Drop and metabolomics. Food Chemistry, 171, 341-350.

Roure, F., Moreno, G., Soler, M., Faconti, D., Serrano, D., Astolfi, P., Bardaro, G., Gabrielli, A., Bascetta, L. & Matteucci, M. (2017). GRAPE: ground robot for vineyard monitoring and protection. In Iberian Robotics Conference (pp. 249-260). Springer, Cham.

Sepahi, A. (1980). Estimating cluster compactness in Yaghouti grapes. Vitis, 19(2), 81-90.

Shavrukov, Y. N., Dry, I. B., & Thomas, M. R. (2004). Inflorescence and bunch architecture development in Vitis vinifera L. Australian Journal of Grape and Wine Research, 10(2), 116-124.

Silvestroni, O., Lanari, V., Lattanzi, T., Palliotti, A., & Sabbatini, P. (2016). Impact of crop control strategies on performance of high-yielding Sangiovese grapevines. American Journal of Enology and Viticulture, 67(4), 407-418.

Siswantoro, J., Prabuwono, A. S., & Abdulah, A. (2013). Volume measurement of food product with irregular shape using computer vision and Monte Carlo method: a framework. Procedia Technology, 11, 764-770.

Sternad-Lemut, M., Sivilotti, P., Butinar, L., & Vrhovsek, U. (2011). Controlling microbial infection by managing grapevine canopy. In 46th Croatian & 6th International Symposium on Agriculture (pp. 984-987). HR.

Sternad-Lemut, M., Silvilotti, P., Butinar, L., & Vrhovsek, U. (2010). Controlling microbial infection by managing grapevine canopy. Proceedings. 46th Croatian and 6th International Symposium on Agriculture. 1, 984-987.

Sternad-Lemut, M., Sivilotti, P., Butinar, L., Laganis, J., & Vrhovsek, U. (2015) Pre-flowering leaf removal alters grape microbial population and offers good potential for a more sustainable and cost- effective management of a Pinot Noir vineyard. Australian Journal of Grape and Wine Research 21, 439-450

Tardaguila, J., Blanco, J. A., Poni, S., & Diago, M. P. (2012). Mechanical yield regulation in winegrapes: comparison of early defoliation and crop thinning. Australian Journal of Grape and Wine Research, 18(3), 344-352.

Tardaguila, J., Petrie, P. R., Poni, S., Diago, M. P., & de Toda, F. M. (2008). Effects of mechanical thinning on yield and fruit composition of Tempranillo and Grenache grapes trained to a vertical shoot-positioned canopy. American Journal of Enology and Viticulture, 59(4), 412-417.

Teixeira, G., Monteiro, A., Santos, C., & Lopes, C. M. (2018). Leaf morphoanatomy traits in white grapevine cultivars with distinct geographical origin. Ciência e Técnica Vitivinícola.

Tello, J., & Ibáñez Marcos, J. (2014). Evaluation of indexes for the quantitative and objective estimation of grapevine bunch compactness.

Tello, J., & Ibáñez, J. (2018). What do we know about grapevine bunch compactness? A state-of-the-art review. Australian Journal of Grape and Wine Research, 24(1), 6-23.

Tello, J., Aguirrezábal, R., Hernáiz, S., Larreina, B., Montemayor, M. I., Vaquero, E., & Ibáñez, J. (2015). Multicultivar and multivariate study of the natural variation for grapevine bunch compactness. Australian Journal of Grape and Wine Research, 21(2), 277-289.

Tello, J., Cubero, S., Blasco, J., Tardaguila, J., Aleixos, N., & Ibáñez, J. (2016). Application of 2D and 3D image technologies to characterise morphological attributes of grapevine clusters. Journal of the Science of Food and Agriculture, 96(13), 4575-4583.

Tello, J., Torres-Pérez, R., Grimplet, J., & Ibáñez, J. (2016a). Association analysis of grapevine bunch traits using a comprehensive approach. Theoretical and Applied Genetics, 129(2), 227-242.

Thornthwaite, C. W. (1948). An approach toward a rational classification of climate. Geographical review, 38(1), 55-94.

Tortosa, I., Escalona, J. M., Bota, J., Tomas, M., Hernandez, E., Escudero, E. G., & Medrano, H. (2016). Exploring the genetic variability in water use efficiency: evaluation of inter and intra cultivar genetic diversity in grapevines. Plant Science, 251, 35-43.

Towers, P. C., Strever, A., & Poblete-Echeverría, C. (2019). Comparison of Vegetation Indices for Leaf Area Index Estimation in Vertical Shoot Positioned Vine Canopies with and without Grenbiule Hail-Protection Netting. Remote Sensing, 11(9), 1073.

User's Manual Nikon D2500 (2012). Downloaded from www.Manualslib.com manuals search engine, pp. 92

Vail, M. E., & Marois, J. J. (1991). Grape cluster architecture and the susceptibility of berries to Botrytis cinerea. Phytopathology, 81(2), 188-191.

Valdés-Gómez, H., Fermaud, M., Roudet, J., Calonnec, A., & Gary, C. (2008). Grey mould incidence is reduced on grapevines with lower vegetative and reproductive growth. Crop Protection, 27(8), 1174-1186.

Zabadal, T. J., & Bukovac, M. J. (2006). Effect of CPPU on fruit development of selected seedless and seeded grape cultivars. HortScience, 41(1), 154-157.

Zabadal, T. J., & Dittmer, T. W. (1998). Vine Management Systems Affect Yield, Fruit Quality, Cluster Compactness, and Fruit Rot of Chardonnay Grape. HortScience, 33(5), 806-809.

Appendix

Annex 1: Protocol for bunch images analysis on ImageJ

Step -1:	Open ImageJ
Step 0:	Load the photo on ImageJ
Step 1: SET SCALE	Using the label height (1.5cm) set the scale and press "global" [IMPORTANT: change scale for the photos with the different prospective using the longer part of the black clamp as scale → 5.0 cm]
Step 2: CUT	Choose the rectangle as tool to cut as close as possible to the bunch perimeter pressing (ctrl + shift + X) to cut
Step 3: Color the label (if necessary)	Using the color picker to copy the background color (blue) and then the paintbrush tool to paint the label is it is remained in the cut image. (double click on the paintbrush tool to enlarge it)
Step 4: RED BUNCH	Click in sequence: Image → Adjust → Color threshold → change from RGB to Lab →move the bar of each color component as follow: L: 0-255 a:0-255 b: around 120-255 (until only the bunch is red)
Step 5: SELECT	Click in sequence: Select → File → Save → Save as selection → Choose the folder and OK
Step 6: RESULTS	To obtain the results press (ctrl + M)
Step 7: EXCEL	Copy the data needed on the pre-set Excel file

Annex 2: Protocol for berries counting with ImageJ

Step -1:	Open ImageJ
Step 0:	Load the photo on ImageJ
Step 1: SET SCALE	Using the label height (1.5cm) set the scale and press "global"
Step 2: CUT	Choose the rectangle as tool to cut as close as possible to the berries and press (ctrl + shift + X) to cut
Step 3:	Click in sequence: Image → Type → 16-bit
Step 4: RED BERRIES	Click in sequence: Image → Adjust → Threshold
Step 5: Eliminate interferences	Click in sequence: Process → Smooth (ctrl + Maiusc + S) (click more time to reduce the interferences as much as possible)
Step 6: Set the maximum and minimum size of the berries	Click in sequence: Analyse → Analyse particles → Size
Step 5: SAVE	Click in sequence: Select → File → Save → Save as Jpeg → Choose the folder and OK
Step 6: RESULTS	Display results + clear results → Show outlines → OK
Step 7: EXCEL	Copy the data needed on the pre-set Excel file