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Master thesis

Empirical Models for Grape vine Leaf Area Estimation on cv. Trincadeira

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B1	Area of biggest primary Leaf (estimated)
B2	Area of biggest lateral Leaf (estimated)
BBCH	Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und CHemische Industrie
D_av.	Average basal shoot diameter
ELLA	Estimation for single lateral leaf area
EMLA	Estimation for single primary leaf area
ESL	Effective Shoot Length
L1	Area of the largest primary leaf of each shoot
L2	Area of the largest lateral leaf of each shoot
LA	Leaf Area
LA1	Area of single primary leaves
LA2	Area of single lateral leaves
LAI	Leaf Area Index
LN	Leaf order
LL2S	Sum of lateral leaves' lateral veins
M1	Mean of the largest and smallest primary leaf
M2	Mean of the largest and smallest lateral leaf
MLA1	Mean primary Leaf Area (multiplied by Number of leaves)
MLA2	Mean lateral Leaf Area (multiplied by Number of leaves)
ML2S	Sum of primary leaves' lateral veins
NI	Number of clusters per shoot
NL1	Number of primary leaves
NL2	Number of lateral leaves
r	Pearson product-moment correlation coefficient
S1	Area of the smallest primary leaf of each shoot
S2	Area of smallest lateral leaf
SLT	Shoot length to the apex
STA	Shoot Area
TLA	Total Leaf Area (primary and lateral)
TLA1	Total primary leaf area per primary shoot
TLA2	Total lateral leaf area per primary shoot
V1	Length of the central vein
V2L	Length of the left lateral vein
V2R	Length of the right lateral vein
V2S	Sum of lateral vein lengths

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Abstract

Estimating a Vineyard's leaf area is of great importance when evaluating the productive and quality potential of a vineyard and for characterizing the light and thermal microenvironments of grapevine plants. The aim of the present work was to validate the Lopes and Pinto method for determining vineyard leaf area in the vineyards of Lisbon's wine growing region in Portugal, with the typical local red grape cultivar Trincadeira, and to improve prediction quality by providing cultivar specific models. The presented models are based on independent datasets of two consecutive years 2015 and 2016. Fruiting shoots were collected and analyzed during all phenological stages. Primary leaf area of shoots is estimated by models using a calculated variable obtained from the average of the largest and smallest primary leaf area multiplied by the number of primary leaves, as presented by Lopes and Pinto (2005). Lateral Leaf area additionally uses the area of the biggest lateral leaf as predictor. Models based on Shoot length and shoot diameter and number of lateral leaves were tested as less laborious alternatives. Although very fast and easy to assess, models based on shoot length and diameter were not able to predict variability of lateral leaf area sufficiently and were susceptible to canopy management. The Lopes and Pinto method is able to explain a very high proportion of variability, both in primary and lateral leaf area, independently of the phenological stage, as well as before and after trimming. They are inexpensive, universal, practical, non-destructive methods which do not require specialized staff or expensive equipment.

Key words: Leaf Area, grapevine, Trincadeira, empirical model, non-destructive methods

Resumo

A estimação da área foliar de uma vinha é de extrema importância quando se pretende avaliar o potencial produtivo e qualitativo da mesma, assim como para caracterizar a temperatura e radiação incidida no microclima da videira. O objetivo do presente trabalho é o de validar a metodologia Lopes e Pinto para determinar a área foliar de videiras em vinhas da região vitivinícola de Lisboa, em Portugal, para a casta Trincadeira. Desta forma melhorando a capacidade de previsão do modelo ao serem providenciados modelos específicos para a casta em estudo. Os modelos apresentados neste trabalho são baseados em dados independentes de dois anos consecutivos: 2015 e 2016. Durante todos os estados fenológicos foram recolhidos e analisados sarmentos com frutificações. A área foliar principal é estimada a partir de modelos que utilizam uma variável calculada a partir das médias da área foliar principal da maior e da menor folha da videira, multiplicada pelo número total de folhas principais, como descrito em Lopes e Pinto (2005). A área foliar das folhas netas utiliza ainda a área da maior folha neta como segundo preditor. Ao longo da dissertação foram também testados modelos baseados no comprimento e diâmetro do sarmento e número total de folhas netas, como metodologias alternativas com menores necessidades laborais. Apesar da facilidade e eficiência de análise, os modelos baseados no comprimento e diâmetro do sarmento não foram suficientemente capazes de prever a variabilidade da área foliar das folhas netas, suscetível à gestão da canópia. A metodologia descrita em Lopes e Pinto (2005) é capaz de explicar uma eleva proporção de variabilidade, quanto à área foliar primária e das folhas netas, independentemente do estado fenológico, assim como antes e depois da desponta. Esta metodologia é pouco dispendiosa, universal, prática, não-destrutiva, que não necessita de recursos especializados nem de equipamento dispendioso.

Palavras-chave: Área foliar, videira, Trincadeira, modelo empírico, métodos não-destrutivos

1 Introduction

1.1. Introduction

The plants leaf area (LA) is a parameter which is of significant importance, as it can provide viticulturists and researchers with important indications regarding the vineyard's condition. Leaves are the organs where sun radiation is converted to carbohydrates through photosynthesis, thus LA is the total area which could intercept light, providing an indication of the plant's photosynthetic capacity and transpiration.

As a measurable parameter, LA can be defined as the one sided area of the leaf surface, flattening it to expand its full surface, including any overlapping lobes. Leaf Area can then be estimated per single leaf, shoot, plant or per meter of canopy. In these cases, it refers to the total area of all leaves belonging to the said sets. Expressed as m^2 LA per m^2 soil surface it gives the dimensionless Leaf Area Index (LAI), which is often used as parameter in viticulture to estimate the possible productivity regarding yield and quality of grapes, as it is well comparable between different training systems and row spacing.

Leaf Area is already widely used to estimate daily dry matter production in annual crops such as rice, wheat, maize, soy beans, sugar beet etc., as it is easy to obtain with indirect methods due to the herbaceous nature of these plants. In woody plants, such as trees and shrubs, and trellised crops such as the grapevine, the use of LA is limited for the moment. For example, the estimation of Leaf Area can be important for the calculation of Evapotranspiration for the implementation of energy balance models, and can be used to calculate irrigation quantities or to adapt volumetric irrigation to canopy characteristics. Difficulties to obtain these parameters are given due to the interference of the plant's woody parts and / or the trellis system with total LA, when indirect methods by light extinction through the canopy are used.

It is understood that with its evident interest, grapevine Leaf Area could be more widely used, when easy, cost effective and precise estimation is possible.

1.2. Objectives

The aim of this work is to review the methods available for the estimation of grapevine Leaf Area and to adjust this methodology to the ampelography of the Portuguese variety Trincadeira. Providing a new and improved empirical model for the direct estimation of the area of a single leaf and the total Leaf Area of a shoot for this cultivar, based on the Lopes and Pinto (2005) methodology. This will allow LA to be used more widely in viticulture and research, as it will be obtainable without the use of special equipment, using nothing but instruments which measure length and an equation provided by the authors.

The importance of LA and the benefits from an easy method for its estimation are described below.

2 Literature Overview

2.1. Importance of Leaf Area

Leaf Area is typically defined as the one sided area of a leaf lamina. As such it can be calculated per single leaf (Carbonneau 1976 a; Lopes and Pinto 2000), a single shoot, divided into primary and lateral leaf area (Carbonneau,1976 b; Barbagallo *et al*., 1996; Lopes and Pinto, 2005), but also for the whole plant or per square meter ground (Watson, 1947) and consequently for the entire vineyard.

The fruiting capacity of grapevines in a given climatic region is largely determined by their total Leaf Area, and the proportion of shaded Leaf Area, provided that other factors are not restrictive (Kliewer and Dokoozlian, 2005). Excessive Leaf Area can indicate high vigor (Champagnol, 1984), while an insufficient Leaf Area may impair the vineyard's productive capability. According to Kliewer and Dokoozlian (2005), there must be an equilibrium between Leaf Area and yield, to achieve the desirable fruit ripeness and thus, wine quality, rendering Leaf Area a basic indicator to determine vine balance. In the same study, they provide the ideal Leaf Area to crop ratios for several cultivars.

Leaf Area can also be used to adjust the amount of dosage of plant protection products, to avoid under dosage which would provide insufficient protection against pests, or over dosage, which has adverse environmental effects and increases costs (Siegfried *et al*., 2006).

It has been established (Bravdo *et al.,* 1984; Hepner *et al.,* 1985), that Crop Load (Ravaz Index), is also strongly correlated to wine quality. Crop Load is calculated as the ratio of yield of grapes, to the pruning weight of the following winter (Ravaz, 1903). However, Cohen *et al.,* (2000), suggest that the Leaf Area, rather than pruning weight, should be utilized for the expression of Crop Load, given that photosynthesis and other metabolic processes in the leaf are responsible for changes in fruit quality, rather than pruning weight per se. The reinvention of Crop Load based on Leaf Area rather than pruning weight can provide a more easily applicable and representative parameter, as it will better reflect the growing conditions of the current, rather than the previous season (Cohen *et al.,* 2000).

2.2. Other parameters related to Leaf Area

2.2.1. Leaf Area Index

The Leaf Area Index (LAI) is a dimensionless quantity defined as the ratio between the estimated area of vine foliage and the vineyard's soil, both expressed in m^2 (Champagnol, 1984; Carbonneau, 1989).

Apart from providing an indication of the photosynthetic surface, LAI is also a fundamental indicator for the understanding of the plant's responses to environmental factors (Lopes *et al.,* 2004) and its quantification allows the evaluation of cultural practices, especially those related to leaf management and the training system (Smart, 1995).

Knowledge of the vineyard's LAI can lead to conclusions regarding water balance (Beslić *et al.,* 2009), the competition with weeds (Guisard *et al.,* 2010), whole-plant assimilation, light interception and bunch exposure (Döring *et al.,* 2013). As these factors affect the plant's microclimate, conditions of moisture and temperature related to disease pressure and fruit quality and quantity (Smart, 1985; Sánchez-de-Miguel *et al.,* 2011), can also be predicted. Changes in LAI could also give indications as to the extent of phytosanitary damages (Borghezan et al., 2010). Based on the LAI, several other parameters can be calculated, such as the Leaf Area per yield ratio, the ratio of exposed Leaf Area per total Leaf Area etc., ratios that are very important for viticultural decision making (Smart and Robinson, 1991), and influencing fruit quality (Petrie *et al.,* 2000 a,b). For example, the estimation of Leaf Area Index can be important for the calculation of Evapotranspiration for the implementation of energy balance models, and can be used to calculate irrigation quantities (Fuchs *et al.,* 1987) and to adapt volumetric irrigation to canopy characteristics (Guisard *et al.,* 2010).

2.2.2. Exposed leaf area

Another useful parameter is exposed leaf area (ELA), which is the Leaf Area of the external leaves, which are exposed to sunlight. This parameter is very important, given the fact that 90% of photosynthesis is carried out by these leaves and, thus, the overall productivity of the vineyard (Smart 1973, Schneider 1992, Sánchez-de-Miguel *et al*. 2010, Baeza *et al*., 2010). It is estimated that around 0.9-1.5 m^2 of ELA are necessary for the ripening of 1kg of grapes (Carbonneau, 1989; Kliewer and Dokoozlian, 2005).

An estimation of the ELA for a given plot can be used before planting a vineyard, to set the desired performance targets per meter of row or hectare. In designing the plantation, ELA estimation will allow the calculation of row spacing and canopy height (Sánchez-de-Miguel *et al*. 2010).

2.3. The evolution of Leaf Area during the growing season

2.3.1. Phyllotaxy

The leaf of the grapevine consists of the petiole (stalk) and the lamina (blade). Trincadeira and most grapevine leaves have 5 lobes and 5 main veins arising from a single point at the junction of the petiole to the lamina (Iland *et al.,* 2011). In grapevines that are not juvenile, the phyllotaxy is distichous. This means that the leaves are produced alternating on the opposite sides of the stem, so that the shoot is bilaterally symmetrical with respect to leaf formation and the angle between successive leaves is 180°. In contrary, in juvenile grapevines, phyllotaxy is spiral and the angle between leaves is 145° (Iland *et al.,* 2011). This juvenile stage ends, when 6-10 leaves have developed (Mullins, et.a., 1992)

2.3.2. Fixed and Free Growth

After budburst, shoots sprout from buds formed during the previous season, containing preformed nodes, inter-nodes and inflorescence primordia. Nodes formed in a latent bud before it goes into dormancy, are called 'fixed' nodes. There are 6 to 10 fixed nodes (Iland *et al.,* 2011), or 6 – 12 (Champagnol, 1984; Sánchez-de-Miguel *et al*., 2010), in a N+2 bud and this implies that the structures found on the first 6 – 10 nodes that occur in a season, including the expanded leaves, are a result of the fixed growth of nodes that were preformed in the bud during the previous year. Fixed growth is a result of cell enlargement of preformed primordial cells and not of the formation of new cells (Sánchez-de-Miguel *et al*., 2010). Leaf and inflorescence primordia can be seen at the shoot tip from the time the shoot emerges from a bud (Iland *et al.,* 2011).

On the other hand, nodes of higher ranks, or free nodes, are the result of free growth of the apical meristem which requires cell division, thus the formation of new cells. Free growth is the result of the elongation and production of new primordia in the apical meristem activity (Sánchez-de-Miguel *et al*., 2010). The apical meristem has two functions: the production of new organs and new tissue. Growth occurs at the tip of the shoot and cell division mainly occurs in the apical meristems (Iland *et al.,* 2011). During the season, shoot growth is a combination between fixed growth and free growth (Phinopoulos, 2014).

2.3.3. Shoot growth and leaf growth

The formation of new nodes at the apex usually stops around flowering (Iland *et al.,* 2011). At this point, there may be up to 30 – 35 nodes. The elongation of a single node may last from 7 to

40 days and internode length may vary from 1 to 25 cm (Iland *et al.,* 2011). According to Champagnol (1984), elongation in both nodes and leaves can last between 15-25 days, while radial expansion may be unlimited in time but is interrupted at the end of each period of growth. While the node reaches its final length after about 25 days, and does not increase further, its width or diameter may continue increasing under favorable conditions (Champagnol 1984).

Young leaves grow for 3 to 5 weeks (Huglin and Schneider, 1998), or until they reach their final dimensions. Leaf development is divided into two phases: a rapid growth phase of about 250 degree days, followed by a plateau in Leaf Area (Wermelinger and Koblet, 1990). Most primary leaves usually grow to reach a similar Leaf Area, while lateral leaves usually do not reach the same size, although they can surpass primary leaves in number (Wermelinger and Koblet, 1990). According to the same authors, the leaves remain in a productive condition for about 650 degree days after they reach their full size. At the age of 900 degree days the leaves become senescent, which is indicated by a sudden decrease of nitrogen and water content.

2.3.4. Lateral shoots

Lateral shoots or summer laterals (order N+1), are shoots that arise from the first bud of the axil of the leaves of a current season shoot. These are prompt buds, in the sense that they start growing the same year when they are formed. The growth of lateral shoots can be strongly stimulated by the removal of the shoot tip by trimming, with the maximal effect occurring when at least 9 nodes are removed (Iland *et al.,* 2011). It seems that the dormancy is caused not only by the continuous development of buds on the primary shoots, but also by young leaves (Champagnol, 1984). Lateral shoots may continue growing even when the growth of the primary shoot has stopped. A greater number and length of lateral shoots is associated to high vigor, well exposed primary shoots and severe pruning (Iland *et al.,* 2011).

Lateral Leaf Area can provide an additional source of leaves for photosynthesis. This is useful when a part of the primary Leaf Area has been lost due to operations such as wire lifting, pests and diseases, abiotic stress (water and heat), or due to other reasons such as hail or frost. Lateral shoots are considered beneficial when they are located on the upper part of the shoot and can intercept sufficient sunlight for photosynthesis and in lower parts of the shoots where they may protect the bunches from intense sunlight. However, extensive lateral shoot growth is considered undesirable, as it is a sign of high vigor, vine growth imbalance and may cause an disadvantageous microclimate and shading, especially in the bunch zone (Smart, 1985).

In general, lateral leaves represent younger tissue, as they emerge at a later stage. While they initially consume resources produced by mature leaves, they later start offering a greater contribution to total photosynthesis. Hale and Weaver (1962), consider that lateral shoots

become sources of photosynthetic products after developing two or more fully expanded leaves. In general, the quantity and the proportion of lateral Leaf Area can vary according to the variety, the growing conditions and the cultural practices, but it usually represents an important part of the total Leaf Area. Lateral Leaf Area can comprise 6 -40% (Iland *et al*., 2011), or 22 – 44% (Paliotti *et al*., 2000) of total Leaf Area and may have, in some cases, an important contribution to fruit ripening. Poni *et al.* (2006) point out that, after defoliation of six primary leaves, lateral shoots can contribute to compensate leaf area loss and lead to improved berry composition. Lateral Leaf Area can also represent an even higher proportion of the total Leaf Area under conditions of high vigor (Huglin and Schneider, 1998). In fact, lateral Leaf Area can be a precise indicator of vigor, as vigorous shoots are characterized by a great development of lateral shoots and a large lateral per total Leaf Area ratio. The possibility to distinguish between primary and lateral Leaf Area is also important for assessing the viticultural potential of the training system and the terroir (Ollat *et al*., 2001).

2.3.5. Rate of leaf emergence

The rate of leaf appearance presents a symmetric pattern in time. It progressively increases during the first weeks and then decreases to zero during the ripening period. If there are no drought conditions, this is done by endogenous stimuli (Palchetti *et al.,* 1995). The speed of leaf appearance and development is strongly related to temperature, for both exposed and shaded leaves. In fact, leaves develop at the same speed, regardless of the canopy zone where they belong, thus leaf development speed is not affected by light. The time required for a leaf to grow, increases along the growing season (Schultz, 1993). Other factors such as pruning severity, growth direction, crop load, light exposure and nutrient availability, may also affect leaf appearance to some extent. Leaf emergence is not affected by the training system, but shoots growing vertically present a much higher vigor than those growing downwards (Palchetti *et al.,* 1995). Shoot growth and leaf appearance rate is highly impacted by soil water deficit, which can contribute to explain the decline in shoot and leaf growth in hot and dry regions, towards end of summer (Lebon *et al.,* 2006) Shoot growth rates seem to be proportional to leaf growth rates, at least until before leaf fall (Wermelinger and Koblet, 1990). In fact, the shoot's growth model is very similar to that of the leaves, because the grapevine is a deciduous plant (Sánchez-de-Miguel *et al*., 2010).

2.3.6. Shoot and Leaf Area growth

The pattern of the vine's canopy development is similar to that of its shoots (Mullins *et al.,* 1992). Environmental factors also affect shoot growth. In areas with lack of water, shoot growth may cease earlier. If there are no restrictive environmental factors the stop of shoot growth is caused by endogenous factors, that is, the alteration of hormonal balance within the plant. As shoot growth proceeds, the ratio of the number of older leaves to that of younger leaves increases. Shoot growth slows down or stops. The organogenic activity of the apex stops and the apex dries and falls off. The proportion of adult leaves that have ceased to grow, to young leaves can be a factor predicting the oncoming cease of shoot growth. This can occur on both short and very long shoots, with small or large leaves (Champagnol, 1984).

The bunches become the most important sinks of photosynthesis products after flowering, but shoots and Leaf Area continue to increase due to the increase in structural components. However, lateral shoots may continue growing even when primary shoot growth has slowed or ceased, especially in conditions of high vigor (Iland *et al.,* 2011).

Shoot growth is slow after budburst but it later becomes exponential, reaching the highest rates around flowering, after which shoot growth rate decreases. In field conditions, the curve becomes sigmoidal (Iland *et al.,* 2011; Mullins *et al.,* 1992). The exponential growth cannot persist in a complex organism such as the grapevine, due to increasing competition for carbohydrates from other organs, which cause a cessation of cell division and enlargement (Mullins *et al.,* 1992).

2.3.7. Factors affecting shoot growth

Shoot growth can be affected by water and nutrient supply, as well as by climatic factors. Water deficit can result in reduced vigor and reduction in Shoot Length and Leaf Area, having a greater effect on lateral Leaf Area (Iland *et al.,* 2011). It seems that node elongation is sensitive to water stress, while the formation of new nodes is not affected to the same extent.

The rate of shoot growth increases with the increase of air and soil temperature. Cool to moderate temperatures favor internode elongation and vigor, while high temperatures favor node production. Lack of light results in longer internodes and exposure to wind may reduce shoot growth.

As far as cultural practices are concerned, high plantation density decreases shoot growth but may leave the Leaf Area per area of soil (LAI) unaffected. Vines with a minimal pruning have a higher Leaf Area than cane pruned vines. Shoot thinning can stimulate the growth of the

remaining shoots. Shoot positioning also affects shoot growth, with vertically positioned shoots being more vigorous. (Iland *et al.,* 2011)

The variations in shoot growth also affect the leaves but the fluctuations in light intensity affect leaves to a greater extent. Leaves that grow in shaded conditions are usually thinner and abscise earlier than those exposed to sunlight. This may also be due to the leave's movement, seeking light. Leaves in vines with lack of water usually have smaller epidermal cells and a lower nitrogen concentration. In this case, leaves are smaller and present a more leathery texture (Iland *et al.,* 2011).

2.4. Estimation of Leaf Area

It is understood from all the above, that a rapid, easy and cheap method to estimate Leaf Area could be of significant use to growers. There are several methods of determining LA, which can be categorized as direct or indirect. Mabrouk and Carbonneau (1996), define as direct methods those where the measurements are done directly on plant organs whereas indirect methods are those where Leaf Area is estimated from the measurements of light. It is possible to further subdivide these methods in destructive or non-destructive, depending on if their application will destroy or maintain the measured leaf area respectively.

2.4.1. Indirect methods

Indirect methods do not measure Leaf Area *per se,* but use equipment to measure other parameters, from which the LAI can be estimated, such as the measurement of light extinction through the canopy (Grantz and Williams, 1993; Sommer and Lang, 1994; Oliveira and Santos, 1995; Ollat *et al.,* 1998; Patakas and Noitsakis, 1999, Cohen at al., 2000). These methods only seem to be valid after the canopy reaches a certain size and the measurements have to be done below a clear sky and a sun declination that precludes the overlapping of shadows (Oliveira and Santos, 1995). Furthermore, the accuracy of Gap fraction Inversion seems to vary according to the variety and does not give accurate estimates for most varieties (Cohen *et al.,* 2000). In other empirical models, Leaf Area is calculated using temperature summation (Schultz, 1992; Bindi *et al.,* 1997), or remote canopy imaging (Dobrowski *et al.,* 2002). These methods are rapid and relatively easy to implement but they have the significant drawback that they require expensive special equipment, which is beyond the budget of most growers.

Grantz and Williams (1993) found the results, obtained with the conventional protocol of the LAI-2000 Plant Analyzer, insufficient and consistently underestimating Leaf Area. Sommer and Lang (1994) compared two devices (LAI-2000 and Demon device) that measure natural light

that can pass through the canopy and have special filters which reduce the effect of scattered light on measurements. They found the results provided by DEMON satisfactory, while the LAI-2000 systematically underestimated Leaf Area. Ollat *et al.* (1998) also tested the LAI-2000 Plant Canopy Analyzer, on Bordeaux vineyards. They found that results for single vines were not satisfactory and that good relationships could only be obtained if five or more consecutive vines were used. Regardless of the protocol used, the device underestimated the Leaf Area of small vines and overestimated that of large vines. In general, the performance of the LAI-2000 was considered mediocre, in accordance with Grantz and Williams (1993) and Sommer and Lang (1994). Furthermore, Tregoat *et al.* (2001), point out that the LAI-2000 is a very expensive device.

2.4.2 Most Recent Development of indirect methods

Newer methods work in a similar way, but use a light source that is part of the equipment, rather than ambient light. One such method of indirect determination of Leaf Area is the Normalized Difference Vegetation Index (NDVI). Drissi *et al.* (2009) tested the GreenSeeker RT100 system on VSP Merlot. This is a portable device, using high intensity light emitting diodes (LED) that measures the light reflected by the canopy. The system seems to be unaffected by background light and temperature. Although the device is usually used to make vertical measurements, the authors used it to measure the canopy horizontally, with a screen placed behind it. It is understood that this complicates the procedure and also limits its applicability to VSP systems. One advantage of the equipment is that is can be connected to GPS systems and yield Leaf Area maps of the vineyard. As far as airborne NDVI is concerned, a recent study by Hall *et al.* (2009), concluded that it was effective in mapping spatial variability in planimetric canopy area, thus contradicting previous studies which claimed that Leaf Area could be predicted by airborne NDVI and attributed this correlation to a proxy relationship between NDVI and canopy area, which could then be a predictor of Leaf Area. They also point out, that the relationship depends on the density and the extent of Leaf Area.

Arnó *et al.* (2012) used ground based light detection and ranging sensors (LiDAR), to estimate grapevine Leaf Area. This system measures the time a laser pulse needs to return, after it has been reflected by the canopy and the angle of the beam to the leaf. Although this system provides a relatively good estimation of Leaf Area, the processing of the data is very complicated. The accuracy of the method varies with the number of scans and several measurements have to be performed to obtain sufficient accuracy. Furthermore, the Leaf Area is given for length of canopy and not per vine or field area. Furthermore, the results seemed to be

different if made from the other side of the canopy, a fact which indicates its lack of accuracy (Arno *et al*., 2012)

There have been some attempts to develop methods of Leaf Area estimation that do not require sophisticated equipment. Ollat *et al.* (2001), obtained disappointing results with the use of pictures taken with a commercial digital camera. In a similar approach, Espinosa *et al.* (2010), used a commercial digital camera to estimate Leaf Area, and analyzed the images with computer software. This method however, required pictures to be taken from a specific distance, using a white fiberglass background. The background diminishes the effect of sky brightness on the measurements, but makes measurements less practical. Furthermore, it has only been tested for single, vertical trellises, with limited applicability to other training systems.

A more recent attempt to eliminate the use of complex and expensive devices has been done by Fuentes *et al.* (2012). They developed an application (Viticanopy®) which can be installed on smartphones and Tablets and can use the camera and GPS features of these devices to estimate Leaf Area. Upwards facing pictures from beneath the canopy are transformed, using thresholds to obtain canopy architectural parameters for grape vines i.e. LAI, Canopy cover, Crown Porosity and Clumping Index, using automated analysis by applying gap size assessment algorithms (Macfarlane *et al*., 2007; Fuentes *et al*., 2008; Fuentes *et al*., 2014).

Recently the app has been validated against different reference methods as Licor LAI-2000 and MatLab and has shown high correlations of $R^2 = 0.96$ and R2 = 0.97 respectively (De Bei et. al., 2016). Although this kind of software might solve the problem of expensive equipment, it still maintains most the disadvantages of the older imaging methods to estimate Leaf Area.

In general, the disadvantages of the imaging methods include, that most devices require frequent calibration and specific sampling protocols (Ollat *et al.,* 1998) a fact that implies that they should be operated by specialized staff. It has also been demonstrated (Grantz and Williams, 1993; Sommer and Lang, 1994; Cohen *et al.,* 2000), that under vigorous conditions or dense canopies these methods often underestimate Leaf Area, as leaves are overlapping. The remote-sensing approach seems to have several limitations, such as leaf clumping (Cohen *et al.,* 2000; Blom and Tarara, 2007; López-Lozano and Casterad, 2013) and the variation in color of the vegetative material within the canopy. It is sometimes difficult to distinguish the Leaf Area of a single plant within a row, as the canopies of neighboring plants are often overlapping (Blom and Tarara, 2007). It must also be pointed out, that the results always include non-leafy elements (Cohen *et al.,* 2000), thus do not reflect Leaf Area alone. In fact, a recent study by López-Lozano and Casterad (2013), demonstrated that not only the sampling protocol, but also the position of the sun during the measurements, strongly influence the results for Leaf Area estimation. The same authors point out, that most optical devices for indirect measurement of

Leaf Area Index (LAI) from canopy-transmitted light are tailored for homogeneous canopies, thus limiting their application to discontinuous canopies such as vertically trained vineyards. In order to obtain non-biased LAI estimates, the homogeneity of the canopy fraction measured along the ceptometer at each individual reading is a major requirement (López-Lozano and Casterad, 2013). In fact, most devices for Leaf Area estimation are designed with a mathematical model assumption that individual leaves are randomly and uniformly distributed. Row crops and trellised vineyards in particular, generally violate these assumptions and for this reason, large systematic errors in Leaf Area estimation can arise (Lang *et al.,* 1985).

Another disadvantage of the above mentioned methods is that they treat Leaf Area as a whole, without distinguishing between primary and lateral leaves (Smart and Robinson, 1991; Lopes and Pinto, 2005). Distinguishing the two Leaf Areas and estimating them separately is important, as their physiological activity is different (Sánchez-de-Miguel *et al.,* 2011).

2.4.3 Direct methods

Contrary to indirect methods, direct methods consist of direct measurements of samples of leaves and shoots. They are considered more accurate but also more laborious (Mabrouk and Carbonneau, 1996). They can be categorized as destructive and non-destructive.

2.4.4 Destructive methods

Destructive methods require the removal of leaves and transporting them to a laboratory. Leaf Area can then be determined by special Leaf Area measuring devices, by planimeters, or by determining the area to weight ratio (Sepúlveda and Kliewer, 1983). The relation between blade dry or fresh weight and Leaf Area has also been confirmed by Tregoat *et al.* (2001), although it seems that ageing leaves become heavier relative to their size (Wermelinger and Koblet, 1990). Although leaf fresh weight was found to have a relatively good relation to Leaf Area and does not require special equipment (Sepúlveda and Kliewer, 1983), its applicability seems to be limited, as it is a destructive method. These methods are considered being easy to implement and produce accurate results (Sommer and Lang, 1994), but most of them also require some sort of equipment. Apart from being laborious, time-consuming and reducing the plant's photosynthetic Leaf Area (Lopes and Pinto, 2005), they also present the disadvantage that the evolution the Leaf Area of a specific plant or shoot cannot be monitored along the growing season, as the leaves are destroyed (Lopes *et al.,* 2004).

2.4.5 Non-destructive methods

Non-destructive direct methods require portable versions of the equipment described above, which could be transported to the field and perform the measurements there. These are even more expensive and difficult to use on the field (Lopes and Pinto, 2005). Another possibility of a non-destructive and direct method to determine Leaf Area is to exploit the empirical relationship found between easily measurable parameters of leaf blades or shoots, and Leaf Area.

2.4.5.1 Estimation of single Leaf Area

There are several statistical models that can estimate the area of a single leaf with relatively good accuracy. Carbonneau (1976 a) found a good relation between the sum of the length of the two lateral veins and the total area of a single leaf. He pointed out, that by measuring the two lateral veins, the non-symmetric effect usual to grapevine leaves is eliminated and that they are usually correlated to either the length, or the width of the leaf. After the study mentioned above, several authors have suggested models for the determination of the area of a single leaf, using empirical models. Many models use the length of primary or lateral veins, while others use maximum leaf length and width. A summary of these studies and the models obtained, is presented in table 1.

Silvestre and Eiras-Dias (2001), also found a good relation between the area of a leaf and the distance from the central vein to the end of the right lateral vein, but they point out that this is a predictor which is difficult to measure. They rejected the use of the central vein, giving a correlation lower than 95%. Manivel and Weaver (1974), found good relations between Leaf Area and the length of the petiole.

Table 1: Summary of studies regarding the prediction of the area of a single leaf, using Leaf dimensions, adapted from Phinopoulos,(2014).

Cultivar	Authors	Predictors	R^2	Equation.	NL
Fernão Pires	Lopes and Pinto (2000)	V2S	0.92	$LA=0.5016*(V2S)^{1.9364}$ For all cultivars (n=800)	200
Vital			0.90		
Periquita			0.90		
Touriga Nacional			0.93		
Syrah	Phinopoulos (2014)		0.97	$LA=0.0016*(V2S)^{2.1670}$ $XLA=0.0032*(XV2S)^{2.0273}$	2560 2108
Cabernet Sauvignon	Borghezan *et al.* (2010)		0.98	$LA=0.3039*(V2S)^{2.1267}$	70(1)
Sauvignon B.			0.95	$LA=0.1732*(V2S)^{2.3616}$	
Riesling	Döring *et al.* (2013)		0.96	$LA=0.3152*(V2S)^{2.1396}$	302
Blaufränkish	Beslić *et al.* (2009)		0.93	LA=-74.7687+17.6594*V2S	100
Merlot	Borghezan *et al.* (2010)	V2S	0.97	LA=-0.001*(V2S)2-13.551	70(1)
Cabernet Franc	Tregoat *et al.* (2001)	V2S(4)	0.90 0.94	LA=0.5351*(V2S)2-4.1596*V2S+33.278 XLA=0.3126*(V2S)2+1.7894*V2S-8.1452	150(4)
	Silvestre and Eiras -Dias (2001)	V2P	0.96	$LA=1.608*(V2P)^{1.002}$	50
			0.95	$LA=1.551*(V2P)^{1.020}$	
Chardonnay			0.94	$LA=1.196*(V2P)^{1.054}$	
Grenache N			0.94	$LA=1.265*(V2P)^{1.060}$	
Merlot			0.98	$LA=1.735*(V2P)^{0.988}$	
Syrah	Phinopoulos (2014)		0.97 0.96	$LA=0.0075*(V2P)^{1.0787}$ XLA=0.0134(XV2P)1.0111	2560 2108
Pinot Blanc	Silvestre and Eiras -Dias (2001)	V2P	0.96	LA=1.762*(V2P)0.988	50
Pinot Noir			0.96	LA=1.410*(V2P)1.025	
Riesling			0.96	LA=1.383*(V2P)1.077	
Sangiovese			0.94	LA=2.481*(V2P)0.863	
Sauvignon Blanc			0.98	LA=1.942*(V2P)0.984	
Trebbiano Toscano			0.94	LA=1.273*(V2P)1.060	70(1)
Trincadeira Preta	Borghezan *et al.* (2010) Manivel and Weaver (1974)	V1	0.92	LA=1.516*(V2P)1.068	
Cabernet Sauvignon			0.94	LA=1.1265*(V1)2.0445	
Sauvignon B			0.93	LA=1.0968*(V1)2.1628	
Grenache N	Borghezan *et al.* (2010) Manivel and Weaver (1974) Borghezan *et al.* (2010)	V1 LU	0.998	LA=1.051-0.802*V1+1.162*(V1)2	N/A(2)
Cabernet Sauvignon			0.97	LA=18.379*LU-151.41	70(1)
Riesling	Schultz (1992)	LU LW	0.93(3)	LA=1.18*(LU-2.6)*(LU+8.75)	112
Niagara	Williams and Martinson (2003)	LU	0.98	LA=0.637*(LW)1.995	814

Cultivar	Authors	Predictors	R^2	Equation.	NL
De Chaunac	Williams and Martinson (2003)	LW	0.96	LA=0.672*(LW)1.963	995
Cabernet Sauvignon	Tsialtas *et al.* (2008)		0.87	LA=19.385*LW-144.59	18
Grenache N	Manivel and Weaver (1974)		0.998	LA=1.051-0.109*LW+0.469*(LW)2	N/A(2)
Cencibel	Montero *et al.* (2000)		0.968	LA=0.647*(LL)1.956	1739
Chenin blanc	Sepúlveda and Kliewer (1983)	LL	0.975	LA=2.49+0.68*(LL*LW)	N/A
Chardonnay		LL*LW	0.969	LA=3.17+0.69(LL*LW)	
	Gutierrez and Lavin (2000)		0.93	LA=2.0857+0.6257(LL*LW)	
Cencibel	Montero *et al.* (2000)		0.987	LA=0.587*(LL*LW)	1739
Concord	Elsner and Jubb (1988)	LW*V1	0.984	LA=-3.01+0.85*(LW*V1)	500
		(LW)2+(V1)2	0.988	LA=-1.41+0.527*$(LW)^2$+0.254*$(V1)^2$	
Asgari	Eftekhari *et al.* (2011)	V1+LW	0.920	LA=0.142*(V1+LW)2+0.796*(V1+LW)	1251
Keshmeshi		V1*LW	0.926	LA=-0.001*(V1*LW)2+0.860(V1*LW)+0.845	1247
Shahroodi					
Khalili					

1)It is not clear whether the sample contained 70 leaves in total, or per cultivar
2)Sample size of 10 shoots at veraison.
3)Equation was calibrated using equations with R-squared values from 0.93 to 0.97
4)Separate models for Primary and Lateral Leaves. Number of primary and lateral leaves not specified
LA = Leaf Area of Primary Leaves
LL = Maximum leaf lamina length usually from tip to lowest point but not to petiolar sinus
LU = Leaf length where the exact method of determination is not exactly specified
LW = Largest leaf lamina width, perpendicular to central vein
V1 = Length of central vein, or leaf lamina length from petiolar sinus to tip
V2P = Product of the lengths of the two lateral veins
V2S = Sum of the lengths of the two lateral veins
XLA = Leaf Area of Lateral Leaves

Montero *et al.* (2000) found that the use of maximum width, leaf length and petiole length were not as closely associated to Leaf Area as the combination of leaf width and leaf length, although they also obtained high determination coefficients. Sepúlveda and Kliewer (1983), consider that the use of maximum leaf length and width as single predictors also have high correlation coefficients, but smaller than the combination of both and they have a bigger standard error and highly negative intercepts. For these reasons, they do not consider them as good estimators of Leaf Area. Smith and Kliewer (1984) found the product of maximal leaf length and maximal leaf width to be the best predictors for the Leaf Area of Thompson Seedless (syn. Sultana), but rather than providing a single model, they presented different equations for bloom, veraison and bloom of the following year. Williams and Martinson (2003) also found a good correlation between central vein length, the square of central vein length, the product of central vein length, leaf width and Leaf Area, but they obtained the best results with leaf width, as it uses only one predictor and had a better accuracy. Eftekhari *et al.* (2011) also found central vein length and leaf width to be good predictors (R-squared =0.917, R-squared =0.881 respectively) but results with the combination of both were better. It seems that in studies with a larger sample size, more predictors have good results.

Studies conducted by Guisard and Birch (2005) and more recently Guisard *et al.* (2010), compared several of these methods, concluding that the most important variables for the estimation of the area of a single leaf are the length and the width of the leaf, with the lengths of veins usually improving the accuracy of the models. Borghezan *et al* (2010) maintain that the models should be adapted to each variety separately.

These methods are very simple and accurate and the area of a single leaf can be rapidly calculated without the use of any special equipment. It is usually easier to find and measure the central vein and/or the maximum width of a leaf, than to measure the lateral veins. Furthermore, the models based on the measurement of the main vein only, present the extra advantage of requiring a single measurement to determine Leaf Area, as opposed to the measurement of lateral veins, which always requires the length of both lateral veins. However, a general conclusion that can be reached from the results of previous works, as described in table 1, is that the use of two predictors for the determination of the area of a single leaf always provides more accurate estimates. In this sense, the use of the two veins, or the combination of the length of the lateral veins and the length of the central vein, usually provide better accuracy than the use of the central vein only. Given that the dependent variable (leaf area) is a two dimensional variable, it is also logical to assume that the product of two linear (one dimensional) measurements would provide better results, than one single linear measurement. Furthermore, measuring two parameters on a leaf (such as the two lateral veins) accounts for anomalies,

asymmetry, or injuries on the Leaves, improving the estimation of Leaf Area. The length of the central vein does not take into account any asymmetries in the shape of the Leaf and it is in a way assuming that all Leaves are symmetrical, which is not always the case in field conditions. However, most methods, even with the use of only one predictor, give satisfactory results with high determination coefficients and seem to be practically applicable. On the other hand, to calculate the total Leaf Area of a shoot or a plant, each leaf should be measured separately, a procedure that would be extremely time-consuming.

2.4.5.2 Estimation of shoot Leaf Area

Upon determining an easy way to calculate the area of a single leaf, research was conducted to discover ways to estimate the area of a whole shoot, without measuring all leaves. Carbonneau (1976 b) suggested that measuring only one leaf in each set of four contiguous leaves, would not significantly impair accuracy. In a similar approach, Barbagallo *et al.* (1996) proposed an empirical model, which reduced the number of leaves that had to be measured per shoot to three, that is the largest leaf, the apical leaf and an intermediate leaf. The above methods have made a significant contribution to reducing the required measurements, but their validity has not been documented as regards to lateral Leaf Area.

It has also been proposed, that total Leaf Area could be linked to the length of the primary, or primary and lateral shoots respectively (Spark and Larsen, 1966; Mabrouk and Carbonneau, 1996; Cohen *et al.,* 2000; Tregoat *et al.,* 2001; Blom and Tarara, 2007, Barajas *et al.,* 2008), although this relationship strongly depends on the cultivar (Mabrouk and Carbonneau, 1996). From a physiological point of view, it is logical to assume that Leaf Area follows a growth similar to that of shoots, given the fact that grapevine is a deciduous plant (Schultz, 1992). This would be an easy method to estimate Leaf Area, as it is easy to perform and requires no special equipment, nor training. However, this ratio is not stable, as it varies along the growing season, as especially after shoot elongation stops, or after trimming, the Leaf Area to Shoot Length ratio grows (Mabrouk and Carbonneau, 1996). Even if this method can be accurate, it has to be calibrated specifically for each growth stage, for each variety and separately for primary and lateral shoots (Tregoat *et al.,* 2001), rendering its use extremely complicated. Furthermore, later works (Lopes and Pinto, 2000; Tregoat *et al.,* 2001, Lopes and Pinto, 2005) did not seem to support a strong correlation between Shoot Length (SL) and Leaf Area, especially for primary shoots. It also seems that even if a good relation between Shoot Length and Leaf Area can be found, this is momentary, as it applies only to the specific phenological stage and the equation is not valid for different stages or years (Di Lorenzo *et al.,* 2005).

Table 2: Summary of studies regarding the prediction of the primary and lateral area of a single shoot (adapted from Phinopoulos, 2014)

Cultivar	Authors	Predictors	(P/L)	R^2	Model	NS
Fernão Pires	Lopes and Pinto (2000)	SL, NL, L1, S1 NL2(1), XLALL(1), XLASL(1)	P L(1) L	0.95 0.97	TLA1=1511.44+1.5*SL+111.00*NL+7.06*L1+4.56*S1 TLA2(1)=-195.01+51.31*NL2(1)+2.36*XLALL(1)+1.21*XLASL(1) TLA2=211.31-23.76*XSN+1.90*TLA2(2)-243.80*TLA2(3)	168
Vital			L	0.96	TLA2=-200.5+38.89*NL2+1.43* TLA2(2)	160
Periquita				0.96	(For all cultivars)	96
Touriga Nacional(4)						96
Jaen(4)	Lopes *et al.* (2004)		P L	0.99 0.99	TLA1=1.0028*LAM1.025*LASL0.037 TLA2=1.8094*MLA21.048*XLASL-0.230	230 143
Aragonez	(6)Sánchez-de-Miguel *et al.* (2011) (5)Lopes *et al.* (2005) (7)Döring *et al.* (2013)	MLA1 MLA2	P S	0.99 0.98	TLA1=1.0871*LAM0.0992 TLA2=1.4134*MLA21.029*XLALL-0.125	180 107
Cabernet Sauvignon(4,5,6)						
Riesling(5,7)						
Cabernet Franc(6)						
Merlot(6)						
Syrah(6)						
Blaufränkish (syn. Limberger, Frankovka, Kékfrankos)	Beslić *et al.* (2009)(8)	NL, L1, S1 NL2, XLALL, XLASL	P L	0.78 0.94	TLA1=-2504.21 +172.684*NL+9.10372*L1+5.2072*S1 TLA2=-1630.7+73.228*NL2+8.2757*XLALL+22.7142XLASL	30

1)The area of a single lateral shoot. Not total lateral Leaf Area
2)The total Leaf Area of the lateral shoot with the largest Leaf Area, belonging to a single primary shoot
3)The total Leaf Area of the lateral shoot with the smallest Leaf Area, belonging to a single primary shoot
4)Lopes and Pinto (2005) model, validated for this variety by the same authors
5)Lopes and Pinto (2005) model, validated for this variety by Lopes *et al.* (2005)
6)Lopes and Pinto (2005) model, validated for this variety by Sánchez-de-Miguel *et al.* (2011)
7)Lopes and Pinto (2005) model, validated for this variety by Döring *et al.* (2013)
8)Based on Lopes and Pinto (2000)
P = Primary Leaf Area
L = Lateral Leaf Area
SL = Length of a primary shoot
NS = Total number of shoots
TLA1 = Total primary Leaf Area of a shoot
L1 = Area of a primary shoot's largest primary leaf
S1 = Area of a primary shoot's smallest primary leaf
TLA2 = Total Leaf Area of all lateral shoots belonging to a single primary shoot
XLALL = Area of the largest lateral leaf belonging to a single primary shoot
XLASL = Area of the smallest lateral leaf belonging to a single primary shoot
XSN = Number of lateral shoots belonging to a single primary shoot
NL2 = Total number of lateral leaves belonging to a single primary shoot
MLA1 = Mean Primary Leaf Area of a shoot multiplied by the total number of primary leaves.
MLA2 = Mean Lateral Leaf Area of a lateral shoot, multiplied by the total number of lateral leaves on a primary shoot.

Very strong correlations initially found between Shoot Length and Leaf Area of shoots without topping, underestimate primary and lateral Leaf Area of vines with canopy management, or overestimate primary Leaf Area and underestimate lateral Leaf Area of trimmed vines, when these equations are applied to other situations (Constanza *et al.,* 2004). This can be explained by the fact that the length of internodes is highly influenced by the cultivar and vigor (Huglin and Schneider, 1998), and that trimming disproportionately decreases Shoot Length while having a lesser effect on Leaf Area, or on the contrary, leaf removal, pests and natural defoliation by leaf senescence, may decrease Leaf Area, while leaving Shoot Length unaffected (Lopes and Pinto, 2005). In conclusion, this method of estimation is fragile, as every factor that could affect surface area or Shoot Length, such as microclimate, hormonal relationships, the distribution of assimilates etc., can impair the validity of any established model.

Cohen *et al.* (2000), and Blom and Tarara (2007), also found a correlation between the number of leaves and the total Leaf Area per shoot. This predictor has in fact been incorporated in the models developed by Lopes and Pinto (2000).

For the estimation of primary Leaf Area, Lopes and Pinto (2000), using data from the cv. Aragonez (syn. Tempranillo) proposed another model, consisting of 4 different variables: Shoot Length, number of primary leaves per shoot and the area of the largest and smallest leaf of each shoot. A similar approach was proposed for lateral Leaf Area, treating each lateral shoot as a composed leaf. Lopes and Pinto (2005) further developed this model, suggesting that the mean of the areas of the largest and the smallest leaf of each shoot, multiplied by the number of leaves per shoot, were sufficient to provide a strong predictor of shoot Leaf Area, thus reducing the number of required variables to three.

Regarding lateral Leaf Area, the same authors (Lopes and Pinto, 2005), suggested a similar model, by correlating lateral Leaf Area to the mean Leaf Area, multiplied by the number of Lateral Leaves per shoot. According to this, only the largest and the smallest of all lateral leaves have to be measured and the total number of lateral leaves have to be counted.

The model developed by Lopes and Pinto (2000) and as modified by the same authors in 2005, is accurate, easy and requires no special equipment. It seems to be valid at all stages of the growing season and unaffected by vigor and growing conditions. Furthermore, as presented on table 2, it has been validated for several cultivars by other authors. A difficulty of the method is determining which is the largest and the smallest leaf, predictors which are necessary to implement the model. The largest and the smallest primary leaf can be found in various positions along the shoot. Between budburst and flowering, the largest leaf is found at lower positions of the shoot and the smallest at the end of the shoot, while between flowering and veraison, (assuming that the shoot has been trimmed) the largest leaf can be at higher positions

and the smallest at the end, at the base, or even at intermediate positions of the shoot. These leaves can be visually identified while counting the leaves and in case of doubt, measuring the lateral veins can help distinguishing the largest and smallest leaf (Lopes *et al.,* 2004). However, we can maintain certainty that in non-trimmed, growing shoots, the smallest primary leaf is always located at the apex (Sánchez-de-Miguel *et al.,* 2011). In the case of non-trimmed shoots, the position of the largest primary leaf still remains uncertain, as described above.

Several authors (Intrieri *et al.,* 1992; Zufferey *et al.,* 2000; Sánchez-de-Miguel *et al.,* 2010), consider that leaves become sources rather than sinks, when their main vein length exceeds 4.5 cm. On non-trimmed shoots, there can be several leaves at the apex, the main vein of which is smaller than 4.5 cm. Due to the fact that these leaves do not contribute significantly to total LA and photosynthesis, including them in the estimate would be time consuming without providing any significant benefits. This fact has been taken into consideration by Lopes and Pinto (2000) and the models for Total Leaf Area which use the smallest leaf as a predictor, only take into consideration leaves with a midvein longer than 3 cm. This makes the methods easier to use, without compromising their accuracy.

The Lopes and Pinto (2000) model treats lateral Leaf Area as a whole, without taking into consideration each lateral shoot separately. When trying to estimate lateral Leaf Area with this model, one should search for the smallest and largest of all lateral leaves and not of each lateral shoot separately. This can present even more difficulties than finding the smallest and largest primary leaf, as there has been no pattern observed as to their usual position. Lateral shoots present an irregularity as to the position of the largest and smallest leaves, similar to that of primary shoots, except that they usually do not have as small leaves as the primary shoots have on their apexes. It is understood that is even more complicated to locate the smallest and largest lateral leaf when all lateral shoots have to be treated as a whole.

3 Material and Methods

3.1 Field conditions and plant material

The study was conducted on the Trincadeira grapevine cultivar (*Vitis vinifera* L.). Shoot sampling was performed destructively at the educational vineyard of the Instituto Superior de Agronomia, Tapada da Ajuda, Lisbon, Portugal, in consecutive years 2015 and 2016. The vineyard is situated at a latitude of 38˚42' N and a longitude of 9˚11' W, at an altitude of approximately 120 meters, has a small inclination and is south facing.

Tapada da Ajuda has an annual average rainfall of 674mm, with maximum monthly rainfall during the winter months (about 113mm) and minimum in the summer months (about 5.5mm). According to the characterization of Thornthwaite, C. W. (1948), the climate of Tapada da Ajuda is mesothermal, with zero or low thermal efficiency in summer (C1B'2s2a '), moderate rainy climate in winter and water deficit in the summer, with an average annual temperature of 16,4ºC and an average annual insolation of 2512.4 hours.

According to the classification of Cardoso (1965), the soil of Tapada is clay loam, characterized as a reddish-brown not limy basalt clay. It has a profile of type Ap (B) C, with a high content of montmorillonite colloids, which provides high plasticity when wet and toughness as it is dry, and there may be cracks when the moisture content is too low. The expandability is high, and so is the field capacity. It has a high available field capacity in the first 50 cm. Its permeability is rapid to moderate (Sarmento, 1969).

The vines were planted in 1998 and grafted on 140Ru (*Vitis berlandieri* x *Vitis rupestris)* rootstocks. The total area of the vineyard is 1ha, of which around 800m^2 were planted with the cultivar Trincadeira, with a double cordon Lyra training system. The trellis was built with wooden posts, with two rows of double movable wires and one fixed wire at the top. The trunk of the vines had a height of around 70cm. The vines have an average of 6-8 spurs with 2-3 buds each and are planted at an interrow spacing of 3.0 meters and 1.0 meters between the plants. Thus, the density of the plantation can be calculated to 3333 plants/ha and around 60,000 buds/ha.

Herbicide was applied beneath the rows, while a cover crop of natural vegetation was left between the rows.

3.2 Phenological development of Trincadeira

Throughout the seasons 2015 and 2016 the vine phenology was monitored, with one to three assessments per week. Therefore three vines were randomly chosen and a total of 77 buds were

evaluated to assess the average phenology using the BBCH-code from Hack et al. (1992), which was adapted to *Vitis Vinifera L.* by Lorenz et al. (1995). These three vines functioned as reference for the fields entity of phenological development. At BBCH 53, shoot thinning to 16-18 shoots per vine was performed and the number of reference buds was reduced to 49, of which 27 were carrying inflorescences.

3.3 Shoot sampling and data analysis

Data used in this work was collected in two seasons (2015 and 2016).

In 2015 on eight sampling dates from 22nd April (BBCH 17-55) until 30th June 2015 (BBCH 19-81), ten fruiting shoots per sampling were analyzed.

Therefore the leaves were numbered and labeled after their node insertion on the shoot. To avoid underestimation, twisted leaves were torn or cut into pieces to obtain the plane LA. Leaves with a central vein smaller than 30mm were excluded. Abnormally shaped or damaged leaves were measured, but the damage or irregular shape was noted in the Excel work book for later testing, as described in **chapter 3.4**. When shoots had a primary leaf arising from a base bud this leaf was excluded as, in general, it was too small and had a very abnormal shape.

In total, 80 primary shoots, 951 primary leaves and 1209 lateral leaves were measured. Of these, 70 shoots were untrimmed, while 10 shoots (30th of June, 2015, BBCH 19-81) were trimmed above the top wire at an approximate length of 95cm.

For all primary shoots, the following observations were made: Number of inflorescences or clusters per shoot, number of primary leaves (NL1), shoot length from the base to the apex (SLT) and shoot length from base to the last measurable leaf (ESL) cm. For each leaf, the length of the central vein (V1), the length of the left and right lateral veins (V2L, V2R) and the leaf area in cm2 (LA1) as measured by image analysis, were also recorded. From the lateral veins (V2L+V2R) the sum was calculated (ML2S).

For the lateral leaves number of lateral leaves (NL2) the length of the central vein (V1l), the length of the left and right lateral veins (V2Ll, V2Rl) and the leaf area in cm2 (LA2) were also recorded and analog to primary leaves, the sum of lateral veins was calculated (LL2S).

The observed variables LA1 and LA2 were used to build the models for single leaf area estimation. In order to distinguish the observed from the fitted values, the estimated single leaf areas of primary and lateral leaves are called EMLA and ELLA respectively.

In 2016 six samplings, with a sample size of 20 fertile shoots each (120 shoots in total), were performed on a ten day schedule, in the period between 03rd of May (BBCH 15-53) and 25th of June (BBCH 19-72). An additional sampling of 30 shoots was performed the 15th of July

(BBCH- 19-77), 2 weeks after trimming above the top wire, at an approximate average shoot length of 100cm.

In total 150 shoots, with 2023 primary and 2876 lateral leaves were analyzed as in 2015. Additionally to the assessed variables mentioned for 2015 methodology, weight and the basal diameter of the shoots were recorded. Therefore, starting from the second sampling date, an electronic digital caliper was used to measure the diameter between the second and third node from the base of the shoot. Two measurements were taken perpendicular to each other and the average diameter was computed with the equation:

$$D\_av. = (D1 + D2)/2 \qquad \textit{Equation 1}$$

From this the new variable cross sectional Shoot Area (STA) was computed by multiplying the average diameter with the effective shoot length (ESL):

$$STA = D\_av * ESL \qquad \textit{Equation 2}$$

Pictures were taken perpendicular to the surface, using a reproduction table, with a ruler of twenty centimeters as reference. Afterwards the taken pictures were analyzed for their LA using ImageJ version 1.50g (Wayne Rasband, National Institute of Health, USA), by using a color threshold as shown in figure 1. All measurements were recorded in an Excel worksheet, using Microsoft Office: Mac 2011.

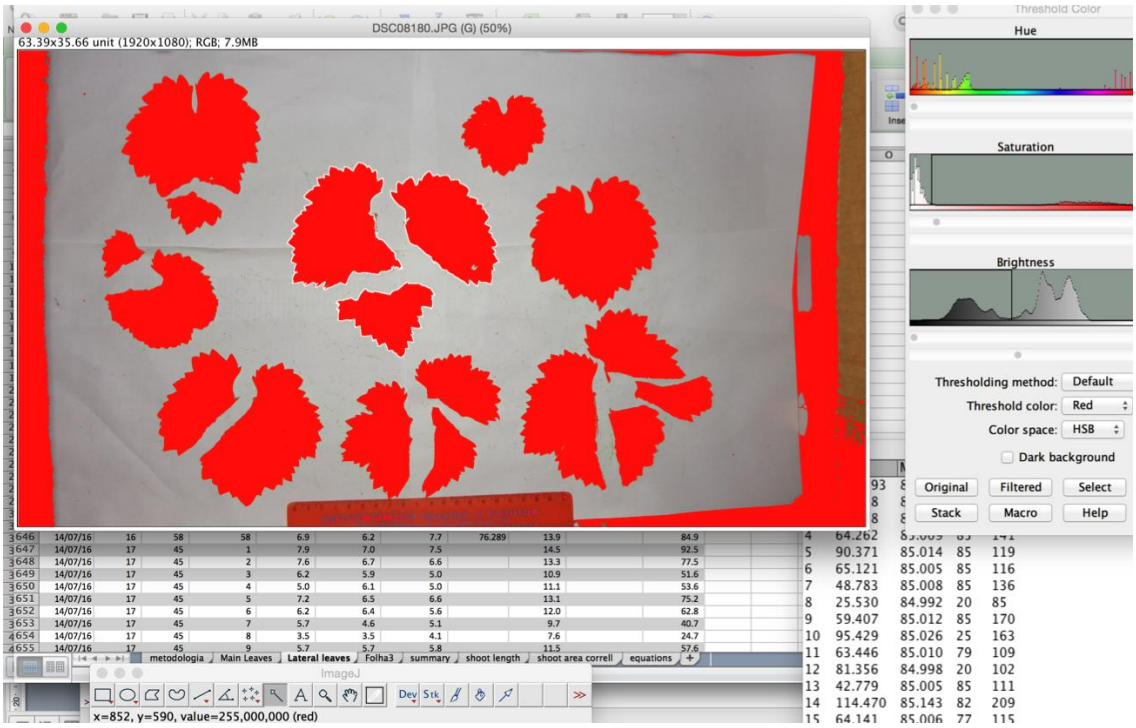

Figure 1: Interface of ImageJ version 1.50g (Wayne Rasband, National Institute of Health, USA), using a hue, saturation and brightness threshold method for Leaf Area assessment. Segmented Leaves are highlighted from the background and LA is measured, using a reference scale (bottom).

A problem that occurred during measuring the leaf area of 2015 leaves, was that the pictures were not always well illuminated, so that in case the leaves were not entirely flat on the paper, shadows could lead to an overestimation of LA. To reduce this overestimation, a correction by manual adjustment aimed for minimizing this error.

Data from the shoots measured were separated into the categories 'primary' and 'lateral'. For each primary shoot the following variables were computed: sum of primary leaf area (TLA1), number of primary leaves (NL1), area of the largest primary leaf (B1) (the highest primary single leaf area), area of the smallest primary leaf (S1) (the lowest single primary leaf area). From these variables two new variables were calculated:

- the mean primary leaf area:

$$\boldsymbol{M1 = (B1 + S1)/2} \qquad \textit{Equation 3}$$

- the mean primary shoot leaf area

$$\boldsymbol{MLA1 = M1 * NL1} \qquad \textit{Equation 4}$$

All lateral leaves were grouped into one set of data, from which the same type of variables were computed per shoot that are reported for primary leaves: sum of lateral leaf area (TLA2), number of lateral leaves (NL2), area of the largest lateral leaf (B2) and area of the smallest lateral leaf (S2). A similar approach was used for calculated variables: the mean lateral leaf area:

$$\boldsymbol{M2 = (B2 + S2)/2} \qquad \textit{Equation 5}$$

the mean lateral shoot leaf area:

$$\boldsymbol{MLA2 = M2 * NL2} \qquad \textit{Equation 6}$$

These variables were then analyzed as described in chapter 3.4.

3.4 Statistical analysis

The recorded data was analyzed using Excel (Microsoft Office: Mac 2011) and R (version 3.3.1, The R Foundation for Statistical Computing) with R commander.

To get an overview of the collected data, correlation matrices were computed. The response, the Pearson product-moment correlation coefficient (r), measures the intensity of a linear correlation of the dependent and the independent variables.

Simple and multiple linear, as well as nonlinear Regression Analyses were performed to model the dependent variables (LA1, LA2, TLA1, TLA2) using several independent variables.

Linear models were fitted using the least squares method and gave results in form of:

$$Y = a + b * x . \qquad \textit{Equation 7}$$

Nonlinear models of the power law family were fitted with least squares method and gave power functions in the form of:

$$Y = a * x^b. \qquad \textit{Equation 8}$$

Another way to fit models of the power law family is by using transformation, which in certain cases can be useful as for example, in order to avoid problems of heteroscedasticity, i.e. to stabilize the variance of the variables. Linearization with natural logarithmic transformation of both variables was performed. Therefore linear models could be fitted with the form:

$$ln(Y) = ln(a) + b * ln(x) \qquad \textit{Equation 9}$$

these equations can then be transformed into:

$$Y = exp(ln(a) + b * ln(x)) \qquad \textit{Equation 10}$$

which then can be transformed back into the power form of equation 8.

The choice of using non linear models as shown in equation 8 and logarithmic transformation as in equations 9 and 10 was supported by the strong curvature in some plots of dependent and independent variables, which seem to disappear when log(y) vs. log(x) are plotted, indicating that the curvature can be described by a power model.

To test for outliers such as damaged or misshaped leaves, the Bonferroni outlier test was performed (Cook and Weisberg, 1982). Afterwards these outliers were observed in the original Excel file or by pictures, they were excluded from the datasets, if the reason for their abnormal values were linked to damaged or severely misshaped foliage, as the purpose of this work is to find a general model with good fit to estimate leaf area of healthy vine leaves. The Akaike Information criterion (AIC) was also used to assess the models' quality.

The following further measures of goodness of fit to the observed data, were also used (Schaeffer 1980):

- mean absolute error:

$$MAE = (\Sigma \mid yi - \hat{y}i \mid)/n \qquad \textit{Equation 11}$$

- mean absolute percentage error:

$$MA\%E = 100\,[\Sigma\,(\mid yi - \hat{y}i \mid/\mid yi \mid)]/n \qquad \textit{Equation 12}$$

where yi represents the observed values, ŷi the fitted values and n the number of pairs.

The modeling efficiency (EF), a dimensionless statistical indicator that relates model predictions to observed data was also determined (Loague and Green, 1991).

$$EF = 1 - \Sigma\,(yi - \hat{y}\,i)^2\,/\Sigma\,(yi - \ddot{y})^2 \qquad \textit{Equation 13}$$

where ÿ represents the mean of observed data. Furthermore a linear regression analysis of observed vs. predicted was performed. For the regression analysis the measured observations

were taken as independent Y-variable and the predicted values as X-variable (Mayer and Butler, 1993; Piñeiro, 2008).

To test for the assumption if separate models should be used to estimate single primary and lateral leaf area an Analysis of Covariance (ANCOVA) was performed. Therefore certain assumptions such as linearity of the regression and homoscedasticity must be fulfilled. Since the elected models were in the form of power laws, linear regressions of logarithmically transformed variables were used as described above. The ANCOVA-model is of the form:

$$\boldsymbol{Y = a + Ip * ap + (b + Ip * bp) * x} \qquad \textit{Equation 14}$$

where Ip is the indicator variable, which equals either 0 or 1 depending on the type of leaves, ap is the additional constant of the intercept, and bp the additional constant of the slope. In this way the Ancova model fits two different models, one for primary and another for lateral leaves. A second model - not differentiating between types (corresponding to ap = bp = 0) - is tested against the ANCOVA-model with a partial F-Test with the hypotheses:

$$H_0: ap = bp = 0 \qquad \text{vs.} \qquad H_1: (ap \neq 0) \text{ or } (bp \neq 0).$$

Type-depending models should be used, when the H_0-hypothesis is rejected, i.e. the models fitted to the types are significantly different to the model fitted to all leaves.

ANCOVA was also used to test if separate models for primary and lateral shoot leaf area (TLA1 and TLA2) estimation should be used.

4 Results and Discussion

4.1 Phenological development of Trincadeira

Figure 2 shows the phenological development of Trincadeira in year 2015 from 10th March to 7th September. Bud burst (BBCH 09) occurred 31st of March, after which followed a stage of

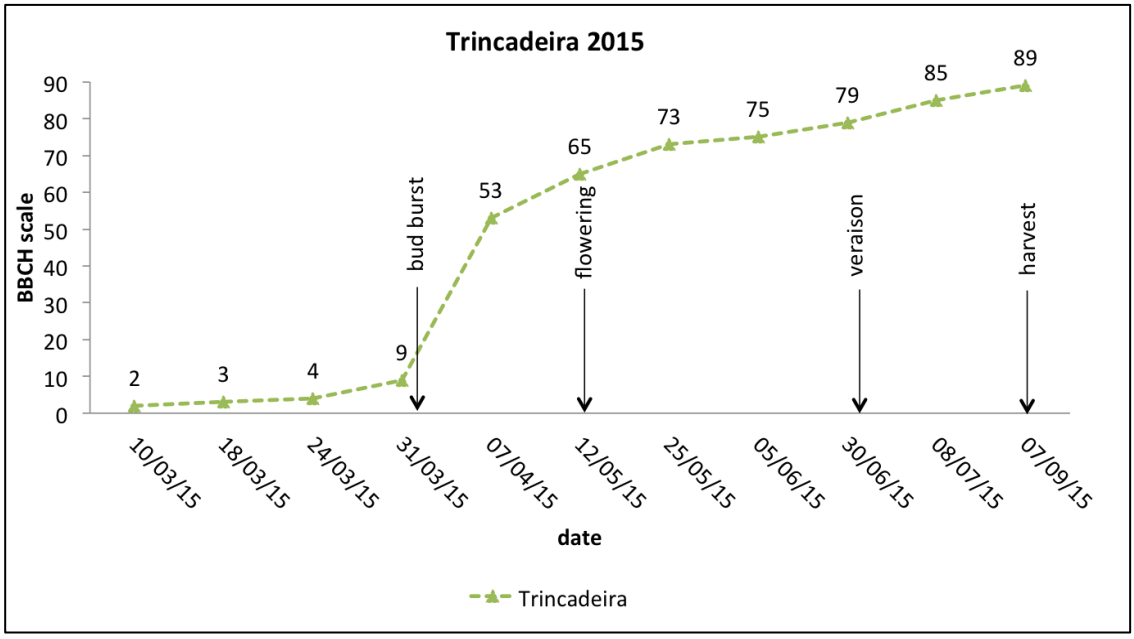

Figure 2: phenological development (BBCH-scale) of Trincadeira during growing season 2015

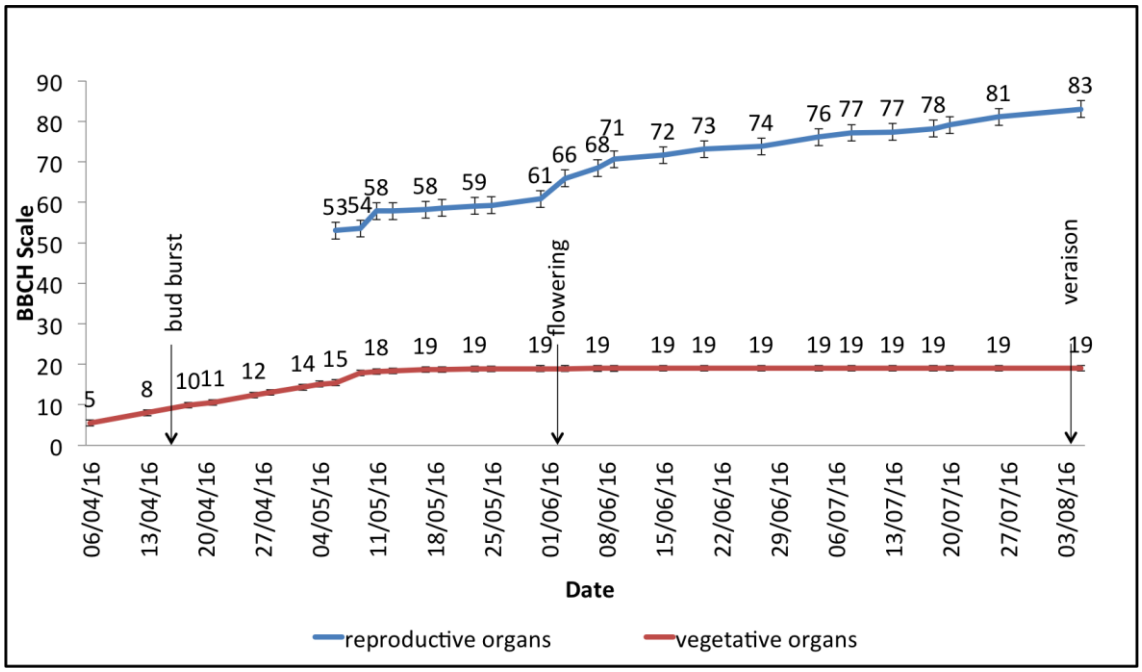

Figure 3: phenological development (BBCH-scale) of Trincadeira during growing season 2016, red line represents vegetative organs, blue line represents reproductive organs

fast shoot and leaf growth during April. Inflorences were visible at 7th April and full flowering occurred at 12th May. Veraison was assessed on 30th June, full ripening and harvest date were 7th of September.

Figure 3 shows the phenological development Trincadeira over the growing season, starting from 6thApril, until 20th June, 2016.

Bud burst occurred two weeks later than in the previous year and very heterogeneously between 6th and 26th of April, with 50% burst on 15th of April. This heterogeneity maintained in all phenological stages during the season. Due to high temperatures in May, shoot development was fast, so that at 17th of May nine or more leaves per shoot were fully unfolded. First Inflorences were visible in beginning of May. The fruitfulness in the reference vines (see chapter 3.3) was low, with 0.56 clusters per shoot in average. At 6th of May, shoot thinning to 16-18 shoots per vine was performed and the number of assessed buds on the reference vines was reduced to 49, of which 27 were carrying inflorescences. This measure led to a sudden increase in assessed phenological development.

First flowers opened on 11th of May, whereas the actual bloom took place between 26th of May and 7th of June. On 18th of July first plants in the plot showed signs of veraison, whereas the reference vines showed veraison (BBCH 83) with 50% colored berries at 3rd of August.

4.2 Leaf area development 2015 and 2016

The growth of Leaf Area (LA) showed regular development in both years 2015 and 2016, with fast growth during May, reaching a plateau end of May. In both years the highest amounts of Shoot Main Leaf Area (TLA1) were naturally observed in the samples before topping (figure 4).

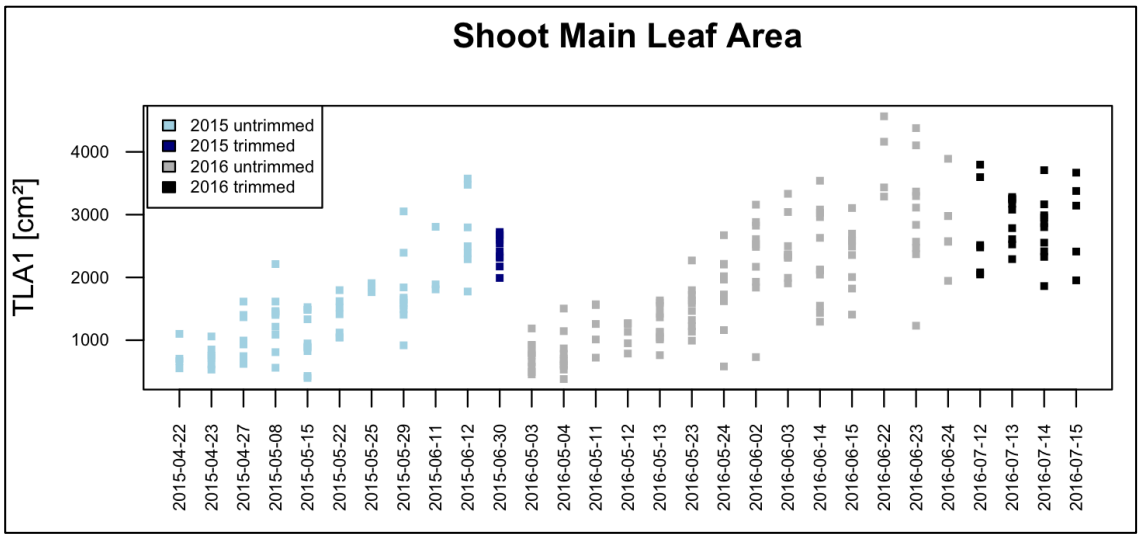

Figure 4: Shoot Main Leaf Area (TLA1) in cm², plotted by sampling date in two consecutive years 2015 (blue boxes) and 2016 (grey boxes)

In 2015 (light blue boxes) before trimming the average TLA1 reached its plateau with 2530.1 cm^2, with an average of 19.9 Leaves per shoot. The final average TLA1 was 2444cm^2 after trimming (blue box) at approximately 95cm shoot length on 30th June with on average 13.5 primary leaves per shoot.

In 2016 (light gray boxes) a similar, but slightly higher development of LA was observed, with highest TLA1 at the last sampling before trimming (22./23./24.06.2016). A mean of 3106.3 cm^2 LA was measured per shoot, with 19 primary leaves in average. The final average TLA1 after trimming (12.-15.07.2016, black boxes) was 2831.9 cm^2 at an approximate shoot length of 100cm and 15 leaves in average.

In both years Lateral Leaf Area (TLA2) development started in a later stage during the growing cycle, around phenological stage BBCH 53 ("Inflorescences visible"). In both years considerable TLA2 started to appear after inflorescence separation (BBCH 19-57) and gained importance after flowering (figure 5).

In 2015 highest amounts of TLA2 were observed during berry development, before trimming, with 2143.6 cm^2 per primary shoot and 38.6 lateral leaves in average (light blue boxes). After shoot topping TLA2 was reduced to 1271.4 cm^2 and 25.8 leaves per primary shoot in average (dark blue boxes).

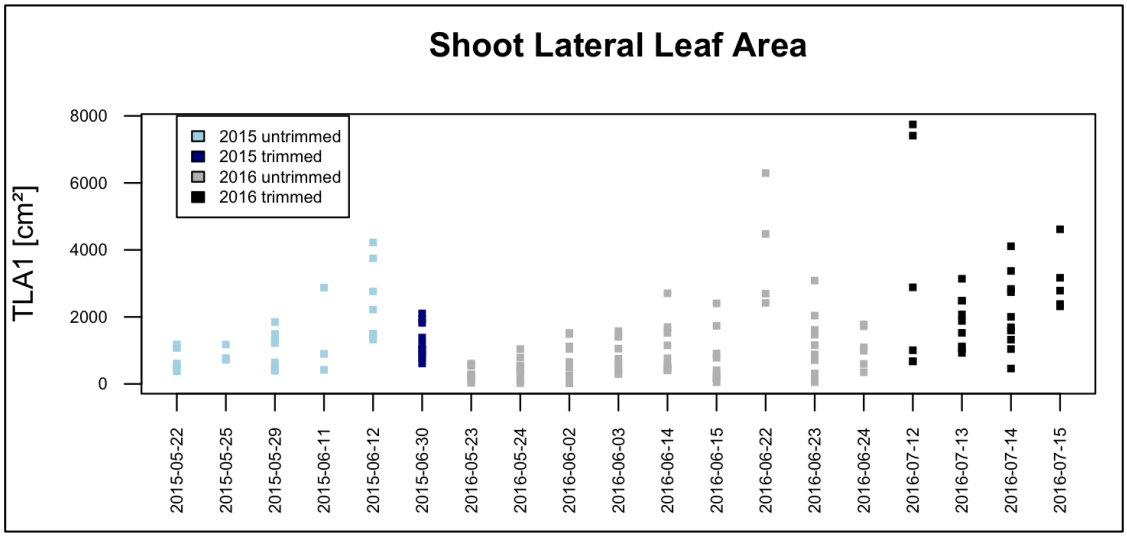

Figure 5: Shoot Lateral Leaf Area (TLA2) in cm2, plotted by sampling date for the consecutive years 2015 (blue boxes) and 2016 (grey boxes)

In 2016 TLA2 development showed a different behavior than in 2015. Lateral Leaf Area continuously gained importance, reaching 1693.6 cm^2 and 29.9 leaves in average before shoot trimming end of June at BBCH 19-75 (light gray boxes). In the last shoot sampling at BBCH 19-77 (12.-15. July), two weeks after shoot trimming, TLA2 showed to be increased to 2451.2 cm^2, with 38.9 lateral leaves per primary shoot in average (dark grey boxes). As shown in figure

5 two anomalies occurred during sampling of shoots. On 22.06.2016 four shoots were sampled, which coincidently showed very high vigor, leading to high average TLA1 of 3862.6 cm^2 (compare with figure 4) and high average TLA2 (3972.1 cm^2, figure 5) for this sampling date.
A similar phenomena occurred on 12th July, when two out of six shoots with high TLA2 led to a high interquartile range.

4.3 Single Main Leaf Area Estimation

The first objective of this work is to analyze different models to estimate single leaf area, therefore the observed variables single primary leaf area (LA1) and single lateral leaf area (LA2) were used as response variables in the models. In order to distinguish the observed from the fitted values, the estimated single leaf areas of primary and lateral leaves are called EMLA and ELLA respectively.

A correlation matrix using the Pearson product-moment was calculated to show possible explanatory variables for the estimation of single primary leaf area (EMLA), as displayed in table 3. All correlations were strong (greater than 0.91).

Table 3: Correlation matrix between actual primary single leaf area (LA1) and the 5 variables: V1 = central vein length in cm; V2L = right lateral vein length in cm; V2R = left lateral vein length in cm; ML2S = sum of lateral veins of the primary leaf; n= 2964 leaves, cv. Trincadeira

Pearson correlations:	LA1	V1	V2L	V2R	ML2S
LA1	1.0000				
V1	0.9158	1.0000			
V2L	0.9269	0.9076	1.0000		
V2R	0.9281	0.9135	0.9223	1.0000	
ML2S	0.9461	0.9288	0.9803	0.9804	1.0000

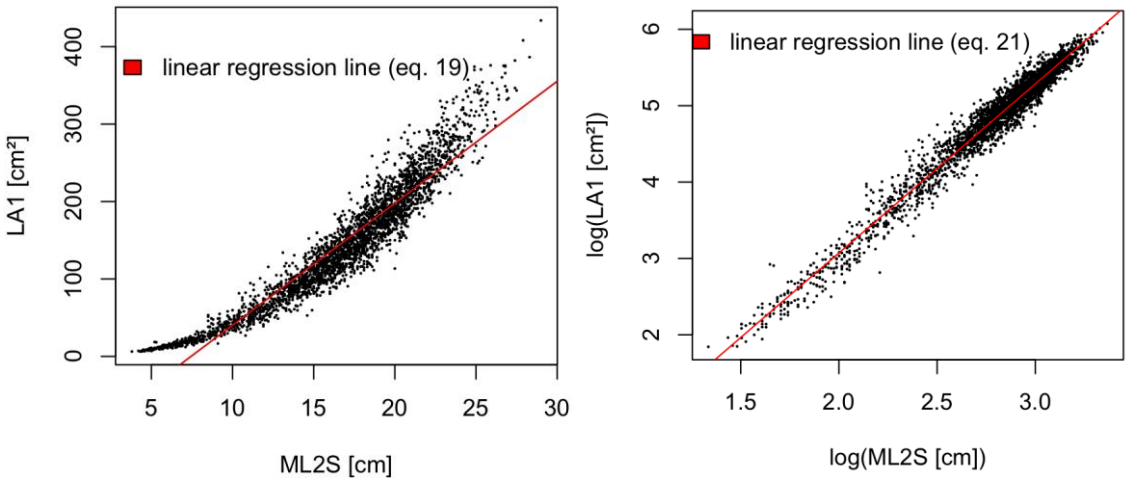

Figure 6: scatterplots of LA1 as response variable and sum of lateral veins of primary leaves (ML2S) as predictor variable; on the left showing strong curvature of untransformed variables, and without curvature on the right, with linear relation of transformed variables

The highest correlations between the measured single primary leaf area (LA1) were found with the sum of the lateral veins (ML2S) (Pearson product-moment correlation coefficient, r = 0.9461), whereas single lateral veins (V2L, V2R) were slightly less but still highly correlated (r = 0.9269 and r = 0.9281 respectively). In this dataset, the central vein (V1) had the least strong correlation with LA1 showed (r= 0.9158).

Plots of response and explanatory variables showed curvature (figure 6) which could be explained by a power law, and therefore this type of nonlinear relation was also studied. Models were built using the combined dataset of two years (2015 and 2016) and linear and nonlinear regressions were performed. Residual plot analyses showed that primary leaf area variability is dependent on the values of the predictor variable. Therefore the constant variance assumption is violated. A logarithmic transformation of both dependent and independent variables were performed, and in general both linearized the relation (suggesting the appropriateness of a power law) and stabilized the variance. As a downside of this methodology, the transformation of the y-scale, influences the possibility of interpretation and comparison with untransformed models.

4.3.1 Central vein as predictor

4.3.1.1 Linear regression with central vein as predictor of single primary leaf area

Linear regression with least squares estimation method of V1 as predictor variable gave the following equation for estimated primary leaf area (EMLA) :

$$EMLA = -88.53 + 24.47 * V1 \qquad \textit{Equation 15}$$

Table 4: test statistics to equation 15: residuals, Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Single primary leaf area (response variable) and central vein length (V1) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (V1); Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-90.924	-20.063	-2.559	17.978	293.167
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-88.5255	1.9899	-44.49	<2e-16 ***
V1		24.4681	0.1973	124.00	<2e-16 ***
AIC= 27883.48		**RSE** = 31.2cm^2		**MAE** = 22.8833	**MA%E**= 23.84666
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-88.5255		-92.42720	-84.62375
V1		24.4681		24.08114	24.85498

The test statistics for equation 15 are shown in table 4. Multiple R^2 = 0.8384, the adjusted R^2 = 0.8384 and the Residual Standard Error (RSE) is 31.2cm^2 and F-statistic is 16220 on 1 and 2948 degrees of Freedom (df) (< 2.2e-16). The Akaike Information criterion is 27883.48, Mean Absolute Error (MAE) = 22.8833 and Mean absolute percentage Error (MA%E) is comparatively high at 23.8466%. The 95% Confidence Interval (CI) indicates that the real value for the intercept is between -92.42720 and -84.62375 and we have 95% confidence, that the true value of the slope lies in the Interval between 24.08114 and 24.85498.

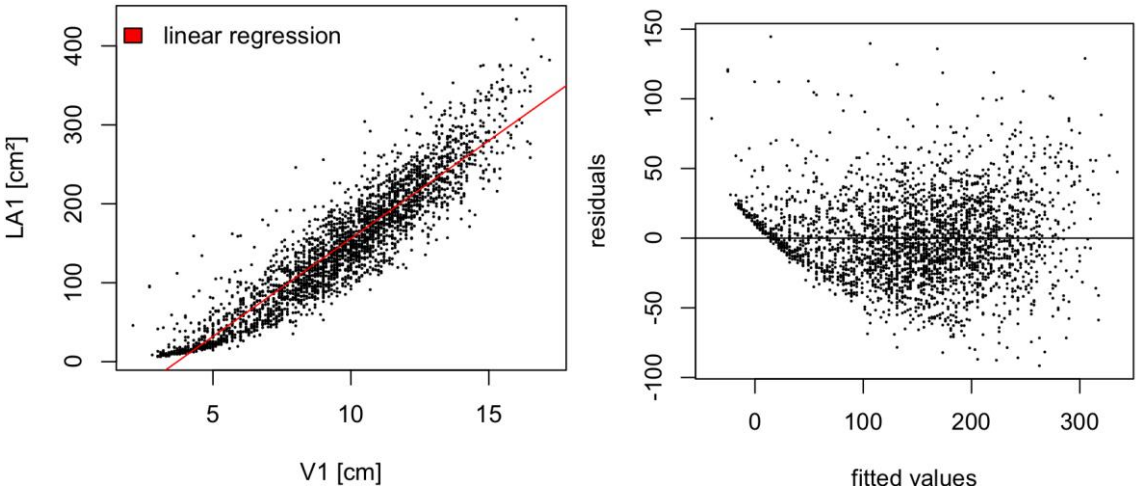

Figure 7: Leaf Area LA1 vs. central vein length (V1) (left), showing curvature; on the right residuals vs. fitted plot, with obvious curvature of residuals, strong underestimation in extreme values and overestimation in medium values

Despite the good relationship, a curvature of the observations in figure 7 was observed, and this is reflected in the fact that extreme LA values (both very high and very low) tend to be above the line. The curvature also implies other drawbacks of the linear model in terms of the validity of inferential results, such as the confidence intervals. This becomes also clear when looking at the residual plots for equation 15 (figure 7, right). A curved pattern can be observed on these plots, where residual vs. fitted values start off being positive, then appear in negative values, and finally appear positive again. This means that equation 15 systematically underestimate predicted high and low LA values.

4.3.1.2 Nonlinear regression with central vein as predictor of single primary leaf area

The nonlinear regression model using V1 as a predictor with least squares method can explain variability in Leaf Area of primary Leaves, with the following equation:

$$EMLA = 2.80671 * V1^{1.72443} \qquad \textit{Equation 16}$$

Residual Standard Error (RSE) is 29.32 cm² on 2948 degrees of Freedom (df) (<2e-16). As shown in table 5, the Akaike Information criterion is very high at 28378.82, Mean Absolute Error (MAE) = 22.67242 and Mean absolute percentage Error (MA%E) is comparatively high at 24.92794%. The 95% Confidence Interval (CI) indicates, for coefficient *a* is between 2.563293 and 2.987240 and for the coefficient *b* is between 1.69925358 and 1.761178. Number of iterations to convergence: 5 Achieved convergence tolerance: 4.416e-06.

Table 5: test statistics to equation 16, residuals, Akaike Information criterion (AIC), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single primary leaf area (EMLA, response variable) and central vein length (V1) in cm as predictor variable; Confidence Interval on 95% level for coefficients (a and b); (1) residuals of linear regression between fitted and observed values

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-106.209	-18.538	-3.474	17.796	146.144
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		2.80671	0.11033	25.09	<2e-16 ***
b		1.72443	0.01613	107.27	<2e-16 ***
AIC= 28378.82		**RSE** = 29.32cm²		**MAE** = 22.67242	**MA%E**= 24.92794
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		2.80671		2.563293	2.987240
b		1.72443		1.699253	1.761178

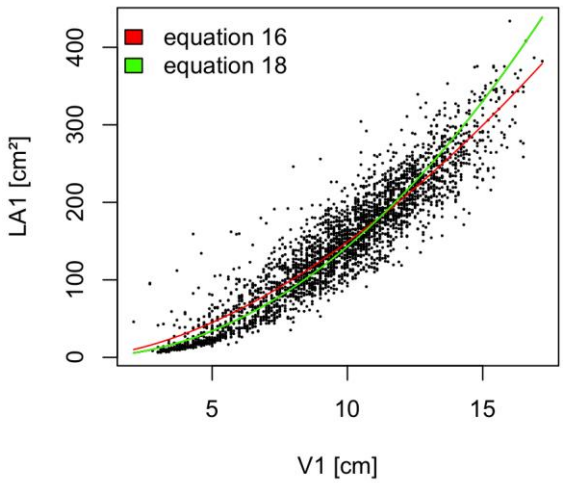

Figure 8: single primary leaf area (LA1) over central vein length (V1) with both power functions, by nonlinear regression (eq. 16) and transformation (eq. 18)

The power model shows no clearly improved estimation of LA1, as MAE and RSE are slightly lower than with the linear fit (Eq. 15), but MA%E and AIC are higher, which leads to no clear preference between these two models. Visual observation of the fit as shown by the curved regression line in figure 8 shows slight overestimation by the model both in the extreme small and big values (red line). Moreover it is shown that a considerable amount of observations is not

well predicted by the model, generally underestimated. This can be – as already described – explained by the inability of the central vein length to account for asymmetries of the leaves. Linear regression between observed and fitted values, showed a multiple and adjusted R^2 of 0.859 and an intercept significantly different from 0 (estimate = -3.084036, p-value = 0.0134 *) and a slope of 1.016947 and F-statistic: 1.796e+04 on 1 and 2948 DF, p-value: < 2.2e-16.

4.3.1.3 Normalized by transformation

Linear regression with least squares estimation method with normalized variance by logarithmic transformation with natural logarithm of both independent and dependent variables gave the following model:

$$\boldsymbol{ln(EMLA) = 0.20499 + 2.06647 * ln(V1)} \qquad \textit{Equation 17}$$

or

$$\boldsymbol{EMLA = 1.227513 * V1^{2.06647}} \qquad \textit{Equation 18}$$

Multiple R^2 is 0.8685, Adjusted R^2 = 0.8685 and the Residual Standard Error (RSE) is 0.2799 and F-statistic is 1.947e+04 on 1 and 2948 DF (< 2.2e-16).

As shown in table 6, the AIC is 862.6198, Mean absolute Error (MAE)= 0.2016413 and Mean absolute percentage Error (MA%E) is 4.788897%. The 95% Confidence Interval (CI) indicates that the value for the intercept is between 0.1399032 and 0.2700674 and the coefficient for log(V1) lies in the interval between 2.0374305 and 2.0955033. It should be noted that this slope in the linearized relation is the power in the corresponding power law. Thus, the confidence intervals for *b* obtained here and in the nonlinear regression do not overlap. The linear regression model of logarithmically transformed variables (equation 17) showed the highest multiple and adjusted R^2 values. RSE, MAE and MA%E are lower than for linear or nonlinear regressions (equations 15 and 16). While these values could imply a better fit of the model than with equation 17 and 18 they need to be seen with caution, as the logarithmic transformation of the response variable makes it impossible to directly compare most of these values with the corresponding ones in a nonlinear regression.

Table 6: Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single primary leaf area (response variable) and sum of lateral vein lengths (log(V1)) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(V1)); Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.95070	-0.15636	-0.00632	0.14444	2.30494
Coefficients:		Estimate	Std. Error	t value	Pr(>|t|)
Intercept		0.20499	0.03319	6.176	7.49e-10 ***
log(V1)		2.06647	0.01481	139.544	<2e-16 ***
AIC= 862.6198		RSE = 0.2799		MAE = 0.2016413	MA%E= 4.788897
Confidence Interval (95%)		Estimate		lower end	upper end
Intercept		0.20499		0.1399032	0.2700674
log(V1)		2.06647		2.0374305	2.0955033

In fact, the AIC for equation 17 seems to be low, when falsely compared to those of equation 15 and 16, but when compared with the AIC of equation 21 (AIC= -3117.888) presented in subsection 4.3.2.3, which is also logarithmically transformed and uses the sum of the lateral veins, it actually appears to be high. Figure 8 (page 33, in subsection 4.3.1.2) shows the regression line of equation 18, and it can be seen, that the fit is comparable to equation 16 for most observations, but differs in the extremes. In fact smaller observations show a better fit with equation 18 (green) than with equation 16 (red).

4.3.2 Sum of lateral vein lengths as predictor

The same approach was used to fit models to estimate single primary leaf area by using the sum of lateral vein lengths as predictor.

4.3.2.1 Linear regression with sum of lateral veins as predictor of single primary leaf area

Linear regression of ML2S as predictor variable gave the following result for estimated primary leaf area (EMLA):

$$EMLA = -118.16999 + 15.79790 * ML2S \qquad \textit{Equation 19}$$

The multiple and adjusted R^2 = 0.9006, the Residual Standard Error (RSE) is 24.49 cm^2 and F-statistic is 2.616e+04 on 1 and 2886 degrees of Freedom (< 2.2e-16).

The test statistics for equation 19 are shown in table 7. Noteworthy are - compared to V1 as predictor of EMLA- the smaller RSE, AIC, MAE, higher R^2, but a comparably high MA%E. It is shown, that the highly negative intercept indicates a strong underestimation of small EMLA.

Table 7: Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of single primary leaf area (response variable) and sum of lateral vein lengths (ML2S) in cm as predictor variable; 95% level CI for intercept and coefficient (ML2S); Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-84.274	-17.154	-3.014	14.221	99.202
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-118.16999	1.9899	-44.49	<2e-16 ***
ML2S		15.79790	0.1973	124.00	<2e-16 ***
AIC= 26673.79		**RSE** = 24.49 cm^2		**MAE** = 19.21231	**MA%E**= 29.96224
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-118.16999		-121.52058	-114.8194
ML2S		15.79790		15.60639	15.9894

This becomes obvious in figure 6 (page 31), where the fit of the linear regression is shown. In fact, the curvature seems even more pronounced than with V1 as predictor of EMLA, indicating the nonlinear relation. On the other hand ML2S explains more variability of single primary leaf area, showing a more compact scattering with less extreme points far away from the regression line.

4.3.2.2 Nonlinear regression with sum of lateral vein lengths as predictor of single primary leaf area

The nonlinear regression model using ML2S as a predictor with least squares method can explain variability in Leaf Area of primary leaves, with the following equation:

$$EMLA = 0.34159 * ML2S^{2.11963} \qquad \textit{Equation 20}$$

Table 8: test statistics for equation 19; Akaike Information criterion (AIC), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single primary leaf area (EMLA, response variable) and sum of lateral vein lengths (ML2S) in cm as predictor variable; Confidence Interval on 95% level for coefficients (a and b); Signif. codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1(1) residuals of linear regression between fitted and observed values**

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-82.186	-11.319	-1.397	10.781	92.141
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		0.34159	0.01409	24.85	<2e-16 ***
b		2.11963	0.01337	157.91	<2e-16 ***
AIC= 25399.71		**RSE** = 20.08 cm^2		**MAE** = 14.65905	**MA%E**= 11.72921
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		0.34159		0.3159976	0.3691116
b		2.11963		1.699253	2.0938935

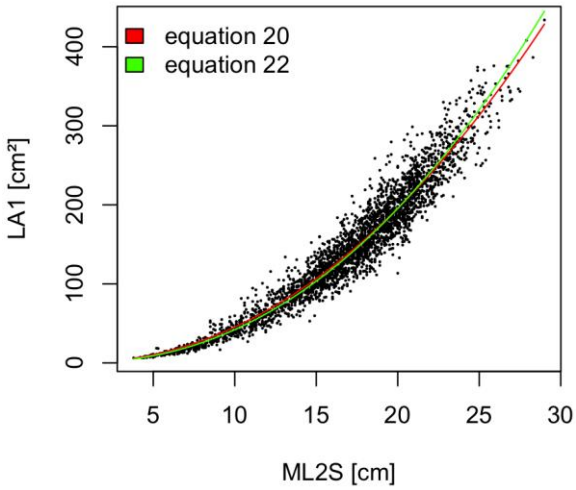

Figure 9: Plot of values of single primary leaf area (LA1) versus sum of lateral vein lengths (ML2S). With both regression lines: equation 20 (red) and equation 22 (green)

Test statistics for equation 19 are shown in table 8. Number of iterations to convergence was 4, with an achieved convergence tolerance of 9.82e-06.

The power model with equation 20 shows the best goodness of fit with the smallest MAE of the non transformed models. The MA%E very close to 10%, which is suggested to be the upper limit of acceptability by Kleijnen (1987). The predicted values vs. predictor values plot (figure 9) shows very similar curves for both power models, fitted by nonlinear regression (equation 20) and by linear regression of the transformed variables (equation 22).

Linear regression of fitted values and single primary leaf area LA1 gave a multiple and adjusted R^2 of 0.9333. The intercept of the linear regression line (-1.103715) is not significantly different from 0 (t-value: -1.345, p-value: 0.179) and the estimate for the slope is 1.005952, with a standard error of 0.004955, t-value: 203.029 and p <2e-16 ***.

4.3.2.3 Normalized model by logarithmic transformation

Linear regression with transformed variables gave the following model:

$$\boldsymbol{ln(EMLA) = -1.384 + 2.223 * ln(ML2S)} \qquad \textit{Equation 21}$$

or

$$\boldsymbol{EMLA = 0.2505742 * ML2S^{2.223}} \qquad \textit{Equation 22}$$

Multiple R^2= 0.9639 and adjusted R^2 equals 0.9638. and the Residual Standard Error (RSE) = 0.1409 and F-statistic is 8.383e+04 on 1 and 2886 DF (<2e-16). The detailed test statistics for equation 20 are shown below (table 9).

Table 9: test statistics for equation 21; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single primary leaf area (response variable) and sum of lateral vein lengths (log(ML2S)) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(ML2S))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.71762	-0.09045	-0.00566	0.09006	0.62989
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-1.384	0.022057	-61.54	<2e-16 ***
ln(ML2S)		2.223	0.007897	280.39	<2e-16 ***
AIC= -3117.888		**RSE** = 0.1409		**MAE** = 0.1089343	**MA%E**= 2.428703
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-1.384		-1.426234	-1.342041
ln(ML2S)		2.223		2.197111	2.228063

Figure 9 and the test statistics show the same difficulties regarding interpretation as the model that uses logarithmically transformed central vein as predictor. Visual observation of the plot shows narrow scattering, i.e. good prediction of LA1 by ML2S. Nevertheless due to transformation the test statistics can be compared with those of equation 17. The AIC of equation 21 is much lower than that of equation 17, indicating a much higher relative likelihood of the model using ML2S as predictor. In the same way RSE, MAE and MA%E are lower, suggesting a better goodness of fit.

4.4 Single lateral leaf area estimation

Analogue to the methodology for primary leaves, models are presented, estimating lateral leaf area. A correlation matrix was calculated to show possible explanatory variables for single lateral leaf area (LA2), as displayed in table 10. Again, all correlations are fairly large, suggesting acceptable linear relations between LA2 and each individual predictor

Table 10: Correlation matrix between actual lateral single leaf area (LA) and the 4 variables: V1 = central vein length ; V2L = left lateral vein length ; V2R = right lateral vein length ; LL2S = sum of lateral veins of the lateral leaf; n= 4072 leaves, cv. Trincadeira

	LA2	**V1**	**V2L**	**V2R**	**LL2S**
LA2	1.0000000				
V1	0.9100099	1.0000000			
V2L	0.9327594	0.9105342	1.0000000		
V2R	0.9354913	0.9099097	0.9107444	1.0000000	
LL2S	0.9556998	0.9312368	0.9772249	0.9776370	1.0000000

The highest correlations (r = 0.9557) between the measured LA2 were found with the sum of the lateral veins (LL2S), whereas single Lateral Veins (V2L, V2R) were slightly less but still

highly correlated (r = 0.9327 and 0.9354 respectively). Least strong correlation in this dataset showed the central vein (V1) as possible predictor for LA2 (r= 0.9100).

4.4.1 Central vein as predictor of single lateral leaf area

For lateral leaf area estimation the same methodology as in section 4.3 was used. Test statistics are shown in the text for the models with the best fits, whereas test statistics and plots of models with lower goodness of fit are shown in the annex in order to increase readability.

4.4.1.1 Linear regression with central vein as predictor of single lateral leaf area

Linear regression with least squares estimation method of V1 as predictor variable gave the following result for estimated lateral leaf area (ELLA):

$$\boldsymbol{ELLA = -49.6831 + 17.5523 * V1} \qquad \textit{Equation 23}$$

Multiple and adjusted R^2 = 0.8369 and the Residual Standard Error (RSE) is 11.69 cm^2 and F-statistic is 2.076e+04 on 1 and 4046 degrees of Freedom (df) ($p<0.001$).

As shown in table 28 (page 69, annex), the AIC is 31400.08, Mean Absolute Error (MAE) = 8.675396 and Mean absolute percentage Error (MA%E) is 20.49631%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between -51.09826 and -48.26784 and we have 95% confidence, that the true value of the slope lies in the interval between 17.31344 and 17.79106. The plot of dependent vs. independent variable (figure 16, page 69, annex) shows the aforementioned curvature as for single primary leaf area.

4.4.1.2 Nonlinear regression with central vein as predictor of single lateral leaf area

The nonlinear regression model using V1 as a predictor with least squares method estimates Leaf Area of lateral leaves, with the following equation:

$$\boldsymbol{ELLA = 1.47006 * V1^{1.99284}} \qquad \textit{Equation 24}$$

Residual Standard Error (RSE) is 10.97cm^2 on 1 and 4046 DF ($p< 2.2e-16$).

Multiple and adjusted R^2 of linear regression with LA2 as dependent and the fitted values (ELLA) as independent variables are comparably low (0.8585 and 0.8584, respectively)

As shown in table 29 (page 70, annex), the AIC is 30879.36, Mean Absolute Error (MAE) = 7.980964 and Mean absolute percentage Error (MA%E) of 18.75328%. The 95% Confidence Interval (CI) is between 1.395843 and 1.54784 and we have 95% confidence, that the true value for the coefficient b lies in the interval (1.966597 , 2.019151), 6 iterations to convergence, with an achieved convergence tolerance of 6.268e-07.

Figure 17 (page 70, annex) shows TLA2 over V1 with both power law functions of equation 24 and 26 in comparison.

4.4.1.3 Normalized linear regression with central vein as predictor of single lateral leaf area

Fitting the power model for ELLA estimation using linear regression of transformed variables gave rise to the following equations:

$$\boldsymbol{ln(ELLA) = 0.23359 + 2.06359 * ln(V1)} \qquad \textit{Equation 25}$$

or

$$\boldsymbol{ELLA = 1.263127 * V1^{2.06359}} \qquad \textit{Equation 26}$$

Multiple R^2 and adjusted R^2 = 0.8519 and the Residual Standard Error (RSE) is 0.2313 and F-statistic is 2.328e+04 on 1 and 4046 degrees of Freedom (df) ($p < 2.2e-16$), with an AIC of -362.222, MAE in transformed scales is 0.1761463 and MA%E = 5.01727. The detailed test statistics are shown in table 30, page 70, annex, and the scatterplot with regression line is can be seen in figure 17 (page 70, annex).

4.4.2. Sum of lateral veins as predictor of lateral leaf area

4.4.2.1 Linear regression with sum of lateral veins as predictor of lateral leaf area

Linear regression with least squares estimation method of LL2S as predictor variable gave the following result for estimated lateral leaf area (ELLA):

$$\boldsymbol{ELLA = -51.37653 + 9.98935 * LL2S} \qquad \textit{Equation 27}$$

The adjusted R^2 = 0.914 and the Residual Standard Error (RSE) is 8.491 cm^2 and F-statistic is 4.3e+04 on 1 and 4070 degrees of Freedom (df) ($p < 2.2e-16$).

As shown in table 31 (page 71, annex), the AIC is 28810, Mean Absolute Error (MAE) = 6.31304 and Mean absolute percentage Error (MA%E) is 17.44316%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between -52.378359 and -50.37471and we have 95% confidence, that the true value for the coefficient for LL2S lies in the interval between 9.894899 and 10.08379.

4.4.2.2 Nonlinear regression with sum of lateral veins as predictor

The nonlinear regression model using LL2S as a predictor with least squares method can explain variability in Leaf Area of lateral leaves, with the following equation:

$$ELLA = 0.379622 * LL2S^{2.072415} \qquad Equation\ 28$$

Residual Standard Error (RSE) is 6.827, F-statistic: 6.88e+04 on 1 and 4070 DF, p-value < 2.2e-16. As shown in table 11, the Akaike Information criterion is 27203.25, Mean Absolute Error (MAE) = 4.90 and Mean absolute percentage Error (MA%E) of 10.50546%. The 95% Confidence Interval (CI) indicates, that the real value for coefficient *a* is between 0.3638524 and 0.3960271 and we have 95% confidence, that the true value for the coefficient *b* lies in the interval between 1.966597 and 2.019151. Iterations to convergence: 6., with an achieved convergence tolerance of 3.345e-07.

Table 10: Akaike Information criterion (AIC), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single lateral leaf area (ELLA, response variable) and sum of lateral vein lengths (LL2S) as predictor variable; Confidence Interval on 95% level for coefficients (a and b)

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-31.982	-3.663	-0.132	3.504	45.690
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		0.379622	0.008208	46.25	<2e-16 ***
b		2.072415	0.008464	244.84	<2e-16 ***
AIC= 27203.25		**RSE** = 6.827		**MAE** = 4.901054	**MA%E**= 10.50546
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		0.379622		0.3638524	0.3960271
b		2.072415		2.0558450	2.0890184

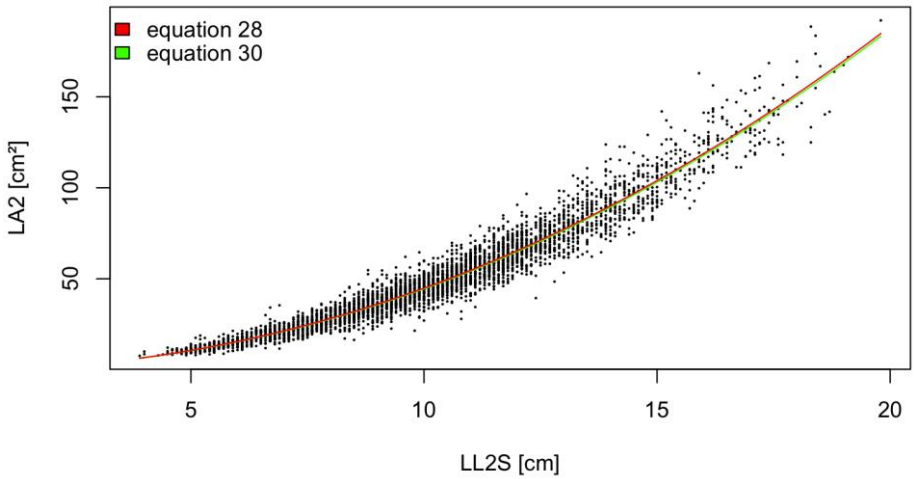

Figure 10: scatterplot of single lateral leaf area (LA2) and sum of lateral vein lengths, with power law functions predicted by nonlinear regression (equation 28) and linear regression of logarithmic transformed values (equation 30)

4.4.2.3 Linearization by logarithmic transformation

Linearization by logarithmic transformation with natural logarithm of both independent and dependent variables of LL2S as predictor variable gave the following result for estimated primary leaf area (ELLA):

$$ln(ELLA) = -0.985879 + 2.076138 * ln(LL2S)$$ *Equation 29*

Or

$$ELLA = 0.3731111 * LL2S^{2.076138}$$ *Equation 30*

The multiple and adjusted R^2 = 0.9523 and the Residual Standard Error (RSE) is 0.1313 and F-statistic is 8.059e+04 on 1 and 4070 degrees of Freedom (df) (<2e-16).

As shown in table 12 the AIC is -4936.81, Mean Absolute Error (MAE) = 0.1024437 and Mean absolute percentage Error (MA%E) is 2.86218%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between -1.018931 and - 0.9528269 and we have 95% confidence, that the true value for the coefficient for log(LL2S) lies in the interval between 2.061801 and 2.0904762. In figure 10 it becomes obvious, that both chosen ways to fit the power models for ELLA with LL2S as predictor are almost indistinguishable. This can already be seen by the coefficients of the equations. Thus it is logical, that both equations 28 and 30 explain a very high amount of observed variability (R^2= 0.9441 and 0.9523, respectively), show low errors and tight CIs. In fact, the AIC of equation 28 (AIC= 27203.25) is considerably lower than compared to those of untransformed linear regression of LL2S (28810), or nonlinear regression with V1 (30879.36), implying a higher likelihood of model 28.

Table 11: test statistics for equation29; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Single lateral leaf area (response variable) and sum of lateral vein lengths (log(LL2S)) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(LL2S))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.57124	-0.08086	0.00485	0.08747	0.56872
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-0.985879	0.016859	-58.48	<2e-16 ***
log(LL2S)		2.076138	0.007313	283.89	<2e-16 ***
AIC= -4936.8		**RSE** = 0.1313		**MAE** = 0.1024437	**MA%E**= 2.86218
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-0.985879		-1.018931	-0.9528269
log(LL2S)		2.076138		2.061801	2.0904762

4.5 Analysis of covariance between single lateral and primary leaf area

Considering the narrow range of yielded coefficients for the sum of lateral veins (V2S) for primary and lateral Leaf area estimation, the assumption was tested, if it is preferable to use

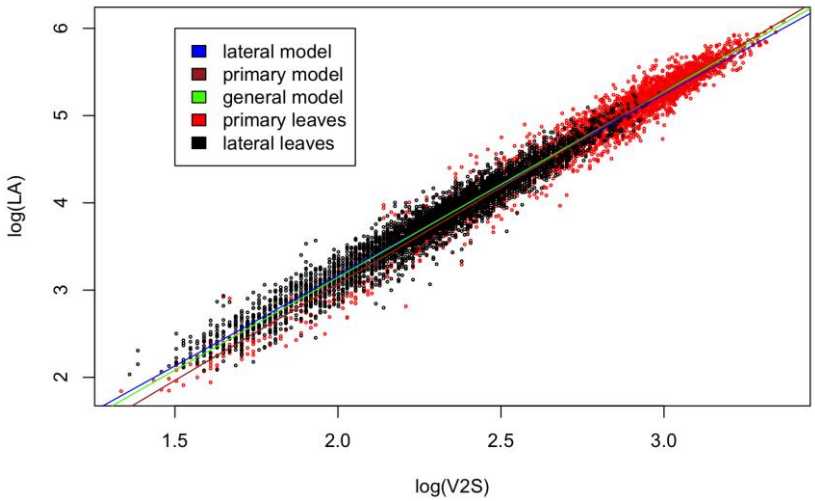

Figure 11: Transformed variables of Leaf area (log(LA)) over sum of lateral vein lengths (log(V2S)), with lateral and primary leaves estimated by general and type-specific models

separate models for primary and lateral leaves, or if both types of leaves can be estimated by a single model. Therefore an Analysis of Covariance (ANCOVA) was performed. A new dataset including both Primary and Lateral Leaves was created and Leaves were categorized as Type "P" (Primary) or "L" (Lateral). A model based on the elected models (equations 21 and 29) was built, relating the log of Leaf Area to the log of the sum of the lengths of the lateral veins, including the variable "Type". A second model was built, without the variable "Type", with the following equation:

$$ELA = 0.3292296 * V2S^{2.128} \quad , \quad \textit{Equation 31}$$

where ELA is the estimated single leaf area, and V2S the sum of lateral vein lengths. Residual standard error: 0.1413 on 7025 degrees of freedom, multiple R^2= 0.9719, adjusted R^2= 0.9719. F-statistic: 2.433e+05 on 1 and 7025 DF, p-value: < 2.2e-16.

The partial F-test to compare the full (ANCOVA) model with the sub-model (without distinction of types), gave an F-value of 100.12 (< 2.2e-16 ***) for the variable "Type". The results of this test are presented in table 13.

Table 12: Results of the ANCOVA test, comparing a full model with the distinction of Primary and Lateral Leaves (Model 1) and a sub model without the interaction of Leaf Type (Model 2)

Model 1: log(LA ~ log(ML2S) Model 2: log(LA~ log(ML2S) * type	Res.Df	RSS	Df	Sum of Sq.	F	Pr(>F)
1	7025	140.28				
2	7023	136.39	2	3.8889	100.12	< 2.2e-16 ***

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The H_0-hypothesis of the partial F-test is rejected, i.e. different models should be used. However comparing the residual sum of squares (RSS), as well as comparing the estimates of the coefficients in the regression equations, the practical use of different models can be seen controversial. The improved precision of using separate models could be a tool for research to gain the most precise prediction possible, however for practical purposes the single model provides just slightly less precision and might be preferred due to being more simple. Figure 11, page 43, presents the plot of the values of ln(V2S) and) against ln(LA) and the regression lines for equations 21, 29 and 31.

It is shown that primary and lateral leaf area are to a certain degree grouped by size and distributed on different parts of the graph. Primary and lateral models estimate leaf area differently in the extremes of very small and very big values, while for the majority of the leaves the difference seems to be minimal. In fact it seems that the factor leaf type has a more pronounced influence on very small leaves. This circumstance, in combination with the results of the ANCOVA test, indicates that primary and lateral leaves should be treated with separate models presented in chapters 4.3 and 4.4, over which an overview is given in table 14.

Table 13: Overview over all presented models for single primary leaf area (EMLA) estimation; equation number (Eq. Nr.), Model equation, Predictor variable, adjusted R^2, n= number of observations.; (1) adjusted R^2 for linear regressions between fitted values of nonlinear regression and original observations;(2) adjusted R^2 for linear regression of transformed variables; Type: p= primary single leaf, l= lateral single leaf

Eq. Nr	Model	Predictor	R^2	n =	Type p/l
15	EMLA = -88.53 + 24 * V1	V1	0.8384	2948	p
16	EMLA = 2.80671 * $V1^{1.72443}$	V1	0.859 (1)	2948	p
18	EMLA = 1.227513 * $V1^{2.06647}$	V1	0.8685(2)	2948	p
19	EMLA= -118.16999 + 15.79790 * ML2S	ML2S	0.9006	2887	p
20	EMLA = 0.34159 * $ML2S^{2.11963}$	ML2S	0.9333(1)	2887	p
22	EMLA = 0.2505742 * $ML2S^{2.223}$	ML2S	0.9638(2)	2887	p
23	ELLA = -49.6831 + 17.5523 * V1	V1	0.8369	4046	l
24	ELLA = 1.47006 * $V1^{1.99284}$	V1	0.8584	4046	l
26	ELLA = 1.263127 * $V1^{2.06359}$	V1	0.8519	4046	l
27	ELLA = -51.37653 + 9.98935 * LL2S	LL2S	0.914	4070	l
28	ELLA = 0.379622 * $LL2S^{2.072415}$	LL2S	0.9441	4070	l
30	ELLA = 0.3731111 * $LL2S^{2.07613}$	LL2S	0.9523	4070	l
31	LA = 0.3293909 * $V2S^{2.128098}$	V2S	0.9719	7029	p + l

Given the high R^2 values and the large sample size, all models seem to be satisfactory and reliable. Most equations using the central vein as a predictor have slightly lower R^2 value than those using the lateral veins, both for Primary and Lateral Leaves. However, the models using the central vein have the advantage of requiring only one variable to be measured, rendering their use more practical. The results are in accordance with previous work presented in table 1, such as Lopes and Pinto, (2000) and Borghezan *et al.* (2010), who also present higher R^2 values for models using sum of lateral vein lengths (V2S) as compared to models using V1, or Beslić *et al.* (2009), Döring *et al.* (2013), who only propose models using V2S.

This is logical, as the length of the central vein does not account for any abnormalities or damage to either side of the leaf. When a Leaf has an abnormal shape on one side, or is damaged with a large part of its area missing, the model using V1 falsely predicts a much larger Leaf Area. The area of these leaves would be more accurately predicted with the use of V2S. It must be pointed out that both damaged and abnormal leaves were used in this study as long as they were not detected by Bonferroni's outlier test and they did not seem to affect the results. In the cases of very large leaves, the increase in V1 is not proportional to the increase in Leaf Area.

The logarithmic transformation of the variables also gave good results, with high R^2 values, low RSE and AIC values and tight confidence intervals. Compared to models without logarithmic transformation, they appear more regular on the graphs, with the data more evenly scattered around the regression line.

Models that were fitted with power laws using nonlinear regressions showed usually the best fit. When looking at the models proposed by previous authors who have worked with single Leaf Area (table 1) it is obvious that several resorted to logarithmic transformation of the variables in order to linearize their data, regardless of the independent variable they have used. A comparison of the results presented in table 14 and the results of other authors presented in table 1, shows several analogies. Although the coefficients are somewhat different, the forms of the equations are similar.

From the several previous works presented in table 1, only Tregoat *et al*. (2001) have proposed separate models for Primary and Lateral leaves. However, all results in the present work yielded different models for Primary and Lateral leaves, a fact that lead to the proposal of a different model for the estimation of the area of lateral leaves. This seems to be logical, even when macroscopically examining leaves. In general, lateral leaves seem to have a more regular shape, compared to primary leaves, and odd-shaped, or disfigured lateral leaves are less frequent. We assume that his can be attributed to the fact that they are younger in age and they have suffered less injuries from pests than primary leaves and that they appear at a later stage of the growing

season, when weather conditions are more favorable. Any damage suffered by primary leaves after budburst will later be apparent when leaves increase in size, giving leaves with more irregular shapes, thus limiting the applicability of any model. This phenomenon is more marked with the first 5-6 primary leaves, which are also usually the largest ones. We have macroscopically observed that if a primary leaf is injured early in the growing season on one side, thus reducing the length of a vein and leaf area, the leaf will later compensate this by non-symmetrical growth. Furthermore, a great part of primary leaves (up to node 10 or 12 as described above) come from preformed nodes and are a product of fixed growth, as opposed to lateral leaves, which are a product of free growth (Iland *et al*., 2011). This can also explain some anomalies in the shape of primary leaves, which could have been caused during the formation of the primordia during the previous growing season.

The adaptation of separate models for primary and lateral leaves can also be explained by their different ranges of sizes, as there are much larger primary than lateral leaves. From the above it is understood that primary and lateral leaves have several differences as far as their morphology and dimensions are concerned, and from this point of view, it seems logical to treat data from Primary and Lateral leaves in separate databases.

The separate use of a model for lateral leaves would more accurately predict their area, as it can be observed on table 14, where the models for Lateral Leaves fit better than the respective models for Primary leaves. It is considered, that a general model for both Primary and Lateral Leaves would be less accurate, as it would have to fit even lower LA values (as lateral leaves are smaller) to the few large LA values observed on Primary Leaves, if this difference in practice is justifying the use of different models, or if the general model is used, is dependent on the required precision.

4.6 Estimation of primary shoot leaf area

The second objective of this work is to find models to estimate primary and lateral shoot Leaf areas. Different approaches are tested and described below.

Table 14: between total primary shoot leaf area (TLA1) and the 5 variables: B1 = Biggest primary leaf area; ESL = effective shoot length; MLA1 = mean primary shoot leaf area; NL1 = Number of primary leaves; STA= Shoot area; n= 230 primary shoots, cv. Trincadeira

	B1	ESL	MLA1	NL1	STA	TLA1
B1	1.000000					
ESL	0.5322592	1.0000000				
MLA1	0.7543015	0.8938830	1.0000000			
NL1	0.1500038	0.8475792	0.6980883	1.0000000		
STA	0.6633520	0.9449442	0.9262573	0.7245497	1.0000000	0.9410674
TLA1	0.6541669	0.9225062	0.9625939	0.7635916	0.9410674	1.0000000

A correlation matrix was performed to show possible explanatory variables for Primary shoot leaf area (TLA1), as displayed in table 15. Most correlations are fairly large, suggesting acceptable linear relations between TLA1 and the individual predictors.

The highest correlations (r = 0.9626) between the measured TLA1 were found with Mean Primary Leaf Area (MLA1), Shoot Area (STA) and Effective Shoot Length also had high correlations(r=0.9411 and 0.9225, respectively). Least strong correlation in this dataset showed the number of primary leaves (NL1) with r = 0.7636 and the biggest primary leaf (B1) as possible predictor for TLA1 (r= 0.6542).

4.6.1 Estimation of shoot leaf area by shoot linked parameters

Estimation of primary and lateral Shoot Leaf Area can be done by using simple empirical models with total shoot length (TSL) or effective shoot length (ESL). The latter measures the distance from the shoots base to the last leaf with a central vein length bigger than 30 mm. As Shoot lengths is already well described to be very closely correlated with primary leaf area (Spark and Larsen, 1966; Mabrouk and Carbonneau, 1996) it was further tested, if the additional variable Shoot diameter (D_av.) could contribute towards a better prediction of shoot leaf area. Therefore the variable Shoot Area (STA) was calculated by multiplying the basal shoot diameter (D_av.) with ESL.

4.6.1.1 Estimation of shoot primary leaf area with models based on shoot length

Linearization by logarithmic transformation with natural logarithm of ESL as predictor variable gave the following result for estimated primary leaf area (TLA1):

$$\boldsymbol{ln(TLA1) = 3.09871 + 1.00825 * ln(ESL)} \qquad \textit{Equation 32}$$

or

$$\boldsymbol{TLA1 = 22.16933 * ESL^{1.00825}} \qquad \textit{Equation 33}$$

The adjusted R^2 = 0.8252 and the Residual Standard Error (RSE) is 1.262508 and F-statistic is 1087 on 1 and 229 degrees of Freedom (df) (<2e-16 ***).

As shown in table 32 (page 71, annex), the AIC is -13.18351, Mean Absolute Error (MAE) 0.1632229 and Mean absolute percentage Error (MA%E) is 2.251494%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between 2.8392707 and 3.358143 and we have 95% confidence, that the true value for the coefficient for log(ESL) lies in the interval between 0.9479882 and 1.068507.

By itself ESL already predicts TLA1 with considerable precision, nevertheless in the context of other presented models R^2 values <0.9 can be considered insufficiently precise.

4.6.1.2 Estimation of shoot primary leaf area with models based on Shoot Area

Linearization by logarithmic transformation with natural logarithm of STA as predictor variable gave the following result for estimated primary leaf area (TLA1):

$$ln(TLA1) = 4.28610 + 0.78548 * ln(STA) \qquad \textit{Equation 34}$$

Or

$$TLA1 = 72.68245 * STA^{0.78548} \qquad \textit{Equation 35}$$

The adjusted R^2 = 0.9152 and the Residual Standard Error (RSE) is 0.1313 and F-statistic is 1404 on 1 and 129 degrees of Freedom (df) (< 2.2e-16).

As shown in table 16, the AIC is -168.7431, MAE = 0.1024437 and MA%E is 2.86218. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between 4.1070019 and 4.4651900 and we have 95% confidence, that the true value for the coefficient for log(STA) lies in the Interval between 0.7440022 and 0.8269565.

Table 15: test statistics for equation 34; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Primary shoot Leaf Area (response variable) and Shoot Area (log(STA)) as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(STA))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.30244	-0.08928	0.00213	0.07137	0.33933
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		4.28610	0.09052	47.35	<2e-16 ***
log(STA)		0.78548	0.02096	37.47	<2e-16 ***
AIC= -168.7431		**RSE** = 0.1313		**MAE** = 0.1024437	**MA%E**= 2.86218
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		4.28610		4.1070019	4.4651900
log(STA)		0.78548		0.7440022	0.8269565

Considering that trimming has a major impact on the shoot length and thus on the predictor variable STA the assumption was tested, if it is necessary to build different models for trimmed and untrimmed shoots, or if both types of shoots can be estimated by a single model. The first mentioned case would render this model less practical, thus shoot trimming as common viticultural practice highly depends on many factors such as training system, climate, vigor etc. Therefore an Analysis of Covariance (ANCOVA) was performed. Shoots were categorized as Type "T" (trimmed) or "U" (untrimmed). A model based on the elected model was built, relating the log of TLA1 to the log SA, including the variable "Treatment". A second model in form of equation 34 was built, without the variable "Treatment".

The partial F-test to compare the full (ANCOVA) model with the sub-model (without distinction of types), gave an F-value of 7.7418 (p= 0.0006722 ***) for the variable "Treatment". The results of this test are presented in table 17.

Table 16: Results of the ANCOVA test, comparing a full model with the distinction of trimmed and untrimmed shoots (Model 2) and a sub model without the interaction of Treatment (Model 1)

Model 1: log(TLA1) ~ log(STA) Model 2: log(TLA1) ~ log(STA) * Treatment	Res.Df	RSS	Df	Sum of Sq.	F	Pr(>F)
Model 1	129	2.0206				
Model 2	127	1.8010	2	0.21958	7.7418	0.0006722 ***

The H_0-hypothesis of the partial F-test is rejected, that is to say the treatment shows significant impact on the observed TLA1, therefore the presented model should not be used to estimate TLA1 on trimmed shoots.

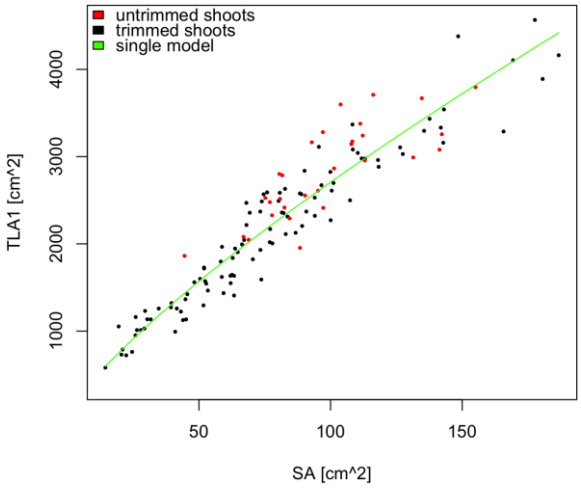

Figure 12: Shoot Primary Leaf Area (TLA1) over Shoot Area (STA), by groups of trimmed and untrimmed shoots, with regression line of equation 35

The models presented in chapters 4.6.1.1 and 4.6.1.2 can explain high amounts of variability in TLA1 by using ESL and STA as predictors. In fact the new variable STA can explain more than 91% of the observed variability, compared to the 82.5% that can be explained by ESL. An explanation for this could be the direct relation between the amount of xylem and the shoot diameter, as the xylem supplies the hydraulic support for transpiration capacity of leaf organs. Considering the very fast and easy way to asses the necessary data these variables are based on – shoot length and basal shoot diameter -, they can provide a concrete tool to estimate TLA1 for practical purposes. Considering the small data pool of 130 observations, of which 100 represent untrimmed shoots and only one sampling of 30 shoots represent trimmed shoots, this matter should be further investigated, as by observation of the scatterplot (figure 12) a slight underestimation of TLA1 on trimmed shoots is visible, but no clear pattern of distribution is obvious.

However it is also shown, that they are susceptible to canopy management and should be used with caution in situations where trimming or leaf removal are common practices or where natural defoliation is expected (Lopes and Pinto, 2005). Canopy management such as trimming,

affects the shoot length/shoot leaf area relationship which is individually depending on variety and vigor (Huglin and Schneider, 1996) and would make individual models necessary for every variety. Further it is known, that after trimming the primary shoot length remains constant but individual leaves can still grow (Lopes and Pinto, 2005). This growth does not seem to be compensated by an increase in shoot diameter. In fact the variable STA does not seem to be equally sensitive to variation in shoot length and diameter

4.6.2 Estimation of shoot primary leaf area with Lopes and Pinto method

First attempt was to test the model suggested by Lopes and Pinto (2005), which was built with cv. Tempranillo:

$$TLA1 = EXP[(0.0835 + 0.992 * ln(MLA1)] \quad \textit{Equation 36}$$

or

$$TLA1 = 1.087085 * MLA1^{0.992} \quad \textit{Equation 37}$$

predicting TLA1 with equation 37 gave a very good result:

Multiple R^2= 0.9473, the adjusted R^2= 0.9471 and the Residual Standard Error (RSE) is 213.8,

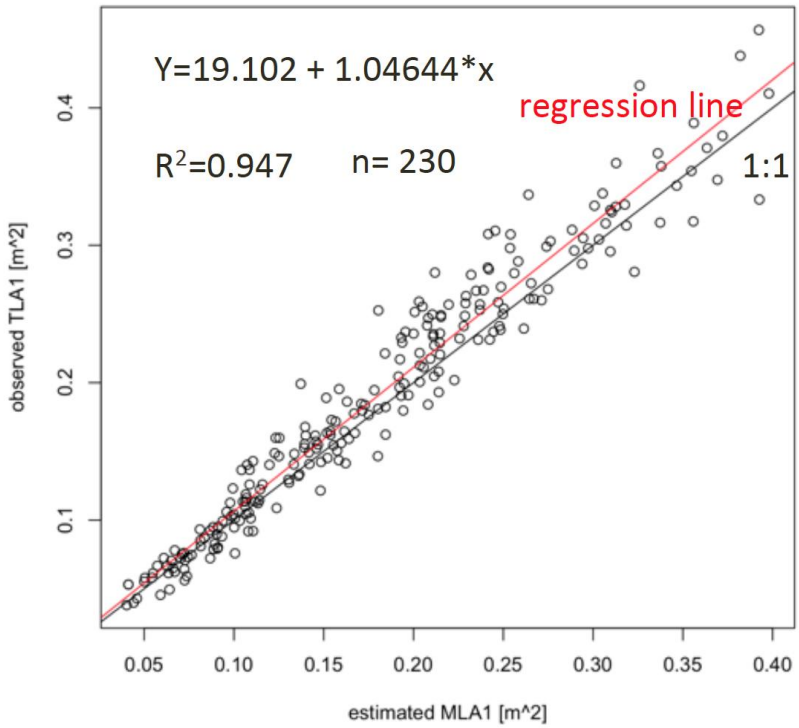

Figure 13: observed vs. predicted Primary Shoot Leaf Area with model provided by Lopes and Pinto (2005), black line: x=y; red line: regression line (Eq. 37)

F-statistic is 4119 on 1 and 229 degrees of Freedom (df) (< 2.2e-16). Modeling efficiency (EF) = 0.997. The AIC is -13.18351, MAE = 0.1632229 and Mean absolute percentage Error

(MA%E) is 2.251494%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between 2.8392707 and 3.358143 and we have 95% confidence, that the true value for the coefficient for log(ESL) lies in the interval between 0.9479882 and 1.068507. Despite the very good fit and very high EF, figure 13 shows, the model based on Lopes and Pinto (2005) with equation 37 systematically overestimates TLA1 for cv. Trincadeira in this dataset (figure 13).

4.6.2.1 Linear regression with Mean Primary Leaf Area as predictor of Shoot Primary leaf area

Linear regression of untransformed variables yielded the model with equation 38:

$$TLA1 = 18.24266 + 1.07309 * MLA1 \qquad \textit{Equation 38}$$

The non-transformed linear regression with MLA1 as predictor variable already gave a very high goodness of fit: With multiple $R^2 = 0.95$, adjusted R^2: 0.9497 and RSE of 207.7 cm^2, F-statistic is 4328 on 1 and 228 DF, p-value: < 2.2e-16. The AIC is 3111.391, MAE = 155.1847 and MA%E is low at 9.028232. The 95% Confidence Interval (CI) for the intercept is between -44.036495 and 80.521819 and for the coefficient for MLA1 it lies in the interval between 1.040953 and 1.105231, more detailed test statistics are shown in table 33, page 71 in the annex.

4.6.2.2 Non-linear regression with Mean Primary Leaf Area as predictor of Shoot Primary leaf area

As in this case the dependent and independent variables already show a linear fit, the fit was not significantly improved by non-linear regression analysis between Shoot Primary leaf area and Mean Primary Leaf Area, giving the following Equation 39:

$$TLA1 = 1.38195 * MLA1^{0.96797} \qquad \textit{Equation 39}$$

multiple $R^2 = 0.9478$, adjusted R^2: 0.9476, and RSE of 213.1 cm^2, F-statistic is 4158 on 1 and 229 DF, p-value: < 2.2e-16. The AIC is 3111.391, MAE = 157.9175 and MA%E is low at 9.293937. The 95% Confidence Interval (CI) for the intercept is between 1.0585818 and 1.796939 and for the coefficient for MLA1 it lies in the interval between 0.9341162 and 1.002295. In fact the coefficient b is not significantly different from 1 (table 18) and thus it is questionable if the underlying relation is in deed a power law. The regression line is shown in figure 14.

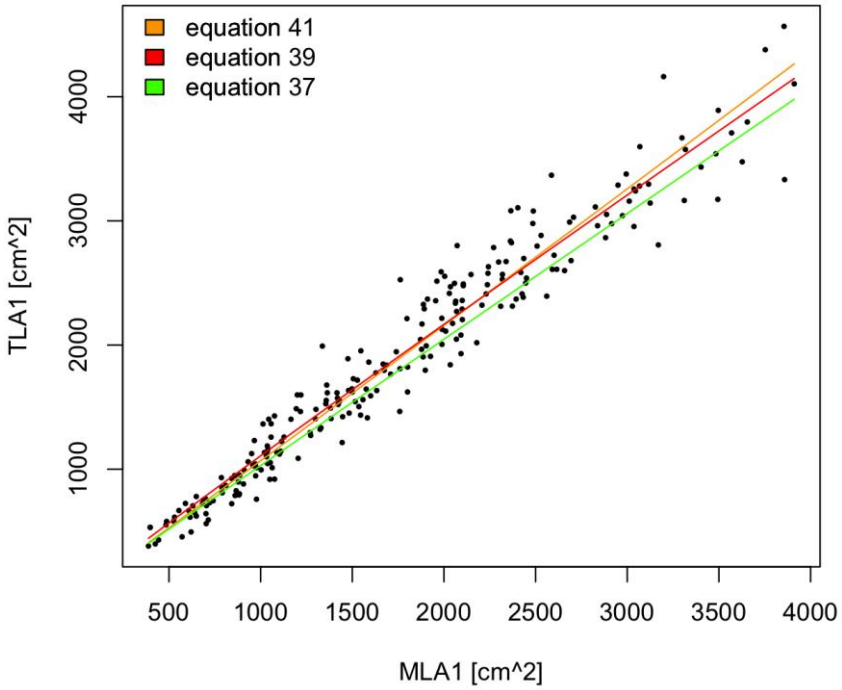

Figure 14: Shoot Primary leaf Area (TLA1) over Mean Primary Leaf Area (MLA1) with regression lines of presented power models (equation 39 and 41) and the power model (equation 37) presented by Lopes and Pinto (2005)

Table 17: test statistics for equation 39; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for non-linear regression of Primary shoot Leaf Area (response variable) and Mean Primary Leaf Area (MLA1) as predictor variable; Confidence Interval on 95% level for coefficients a and b

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-778.46	-126.36	-13.93	102.04	737.58
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		1.3819	0.18860	7.327	3.98e-12 ***
b		0.96797	0.96797	0.01758	55.051
AIC= 3136.59		**RSE** = 213.1		**MAE** = 157.9175	**MA%E**= 9.293937
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		1.3819		1.0585818	1.796939
b		0.96797		0.9341162	1.002295

(1) Residuals for linear regression between observed and fitted values

4.6.2.3 Linear regression with transformation of Mean Primary Leaf Area as predictor of Shoot Primary leaf area

The estimated values of the linear models fit very well with the actual leaf area however, residual plot showed that primary leaf area variation is dependent on the values of the predictor variable. The violation of the constant variance assumption indicated the need of a variable transformation. A logarithmic transformation of both sides of the equation was applied to stabilize the variance and led to the following model for estimated primary leaf area (TLA1):

$$ln(TLA1) = -0.03882 + 1.01515 * ln(MLA1)$$ *Equation 40*

or

$$TLA1 = 0.9619238 * MLA1^{1.01515}$$ *Equation 41*

The adjusted R^2 = 0.9585 and the Residual Standard Error (RSE) is 0.1252 and F-statistic is 7807 on 1 and 228 degrees of Freedom (df) (<2e-16).

As shown in table 19, the AIC is -344.23, Mean Absolute Error (MAE) 0.0885626 and Mean absolute percentage Error (MA%E) is 1.21731%. The 95% Confidence Interval (CI) indicates, that the real value for the intercept is between -0.2411556 and 0.1635177 and we have 95% confidence, that the true value for the coefficient for log(MLA1) lies in the Interval between 0.9876357 and 1.0426620. The regression line is shown in figure 14, page 52.

Table 18: test statistics for equation 40; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for non-linear regression of Primary shoot Leaf Area (response variable) and Mean Primary Leaf Area (MLA1) as predictor variable; Confidence Interval on 95% level for the Intercept and slope of log(MLA1)

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.35706	-0.10283	0.00747	0.10628	0.33825
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-0.03882	0.02688	0.07706	0.349
log(MLA1)		1.01515	0.97829	0.01107	<2e-16 ***9
AIC= -344.23		**RSE** = 0.1252		**MAE** = 0.0885626	**MA%E**= 1.21731
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-0.03882		-0.2411556	0.1635177
log(MLA1)		1.01515		0.9876357	1.0426620

All models presented in section 4.6.2 gave high precision in Primary Shoot Leaf Area prediction. The model presented by Lopes and Pinto (2005) has comparable error terms and R^2 values to the models presented in this work, rendering the original model valid for the prediction of LA for the Trincadeira variety. The provided linear and non linear regressions could improve RSE, MAE, MA%E and thus indicate a slightly better general fit, wich can be explained by a small systematical overestimation by the Lopes and Pinto model. The non linear models show estimates of the exponent for MLA not ignificantly different from 1, indicating, that the underlying relationship can as well be explained by a linear function. In fact, the provided linear regression shows error terms -exept MAE – comparable to those of nonlinear and transformed linear regrssions. All models showed MA%E below 10%, showing their possible acceptability (Kleijnen,1987).

4.7 Estimation of lateral shoot leaf area

The correlation matrix between lateral shoot leaf area, and 4 variables indicates especially two possible predictors with high correlations as displayed in table 20. The best correlation was found with Mean lateral shoot leaf area MLA2 (r = 0.987). Also very high correlation (r = 0.970) was found to be with the number of lateral leaves (NL2).

Table 19: correlation matrix between total lateral leaf area (TLA2) and the 4 variables: B2 = biggest lateral leaf area; MLA2 = mean lateral shoot leaf area; NL2 = number of lateral leaves; S2= smallest lateral leaf; n= 149 primary shoots, cv. Trincadeira

correlation matrix	B2	MLA2	NL2	S2	TLA2
B2	1.00000000				
MLA2	0.84485614	1.00000000			
NL2	0.77935867	0.96312135	1.00000000		
S2	0.01827495	0.06173568	0.03500189	1.00000000	
TLA2	0.79547043	0.98717971	0.96962972	0.05909980	1.0000000

4.7.1 Approaches with number of lateral leaves as predictor

As described before, finding the smallest and biggest lateral leaves for mean lateral leaf area calculation can be time consuming and not easy to accomplish, as those leaves usually can be distributed along all insertions of the shoots. Therefore approaches were tested to build models based only on one predictor: the number of lateral leaves (NL2).

Based on the sum of lateral leaf area (TLA2) of 149 shoots two models were fitted. The first was fitted by multiple linear regression of least squares method with the form:

$$Y = a * x + b * y + c * z + d \qquad \textit{Equation 42}$$

with substitutions of variables y and z by x^2 and x^3, respectively, resulting in the following 3rd degree polynomial equation:

$$TLA2 = 7.4586 * NL2 + 1.6889 * NL2^2 - 0.01 * NL2^3 + 51.254 \qquad \textit{Equation 43}$$

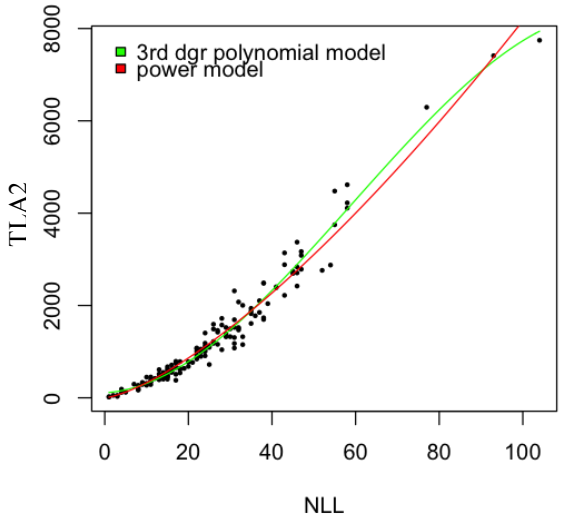

Figure 15: estimation of TLA2 by NL2 with 3rd degree polynomial model (equation 41) and power model (equation 42)

The detailed test statistics are shown in table 21. The polynomial model has a very high multiple R^2 of 0.9701 as well as adjusted R^2 = 0.9695. The RSE is comparably high with 225.3cm^2, so are MAE (148.0483) and MA%E (23.6886). The AIC is 2056.812. The confidence intervals for the coefficients are rather large.

Table 20: test statistics for equation 41; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for multiple linear regression of TLA2 (response variable) and Number of lateral leaves (NLL); Confidence Interval on 95% level for intercept and coefficients

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-805.41	-79.01	-2.98	88.03	763.42
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)		109.326262	71.785912	1.523	0.130
NL2		4.837820	7.051502	0.686	0.494
NL2^2		1.629598	0.188070	8.665	7.83e-15 ***
NL2^3		-0.009154	0.001318	-6.948	1.14e-10 ***
AIC= 2056.812		**RSE** = 225.3cm^2		**MAE** = 148.0483	**MA%E**= 23.6886
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		109.326262		-32.5475125	251.200037478
NL2		4.837820		-9.0983867	18.774025850
NL2^2		1.629598		1.2579075	2.001289149
NL2^3		-0.009154		-0.0117581	-0.006550031

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The power model was fitted by nonlinear regression and gave the following equation:

$$TLA2 = 13.124 * NL2^{1.397} \qquad \textit{Equation 44}$$

Table 22 displays that the goodness of fit is slightly worse than for equation 41, as the RSE AIC and the MAE are slightly higher, whereas only MA%E is considerably lower. Number of

iterations to convergence is 14, with an achieved convergence tolerance of 5.329e-07. Linear regression of the observed and predicted values of equation 42 gave R^2 of 0.9635 and adjusted $R^2 = 0.9632$.

Table 21: test statistics for equation 42; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of TLA2 and Number of lateral leaves (NLL); Confidence Interval on 95% level for coefficients a and b

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-969.02	-93.86	-3.60	73.93	917.59
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		13.12375	1.14190	11.49	<2e-16 ***
b		1.39746	0.02188	63.88	<2e-16 ***
AIC= 2083.783		**RSE** = 248.1 cm^2		**MAE** = 162.9541	**MA%E**= 15.7834
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		13.12375		11.079152	15.509662
b		1.39746		1.355308	1.439858

(1) Residuals for linear regression between observed and fitted values

Both models of this approach explain very high (R^2>0.96) amounts of the shoot lateral leaf area variability. As shown in figure 14 the dataset for this analysis showed to follow a sigmoidal curve rather than an power law, although this observation is based on only three extreme observations with very large TLA2. This behavior however, could be related to a limitation of sources needed for growth, as in general growth relations can initially be explained with power laws, until they reach a saturation point and consequently show a sigmoidal curve. In other words this would mean, that with increasing NL2 the average single LA2 increases up to a plateau and finally decreases. Which in fact seems to be the case with the presented data. In correspondence to this a real sigmoidal model should be tested.

In opposition to this thesis, Lebon et al. (2006) found that with limitation of the growth factor water NLL was particularly affected, so that with increasing water deficit the rate of leaf appearance dropped rather than the leaf size decreased.

4.7.2 Estimation based on shoot linked parameters

Analogue to shoot primary leaf area , it was tested if Shoot area (STA) can be used as predictor for shoot lateral leaf area (TLA2). Linear regression of transformed variables fitted a power model with the following equation:

$$TLA2 = 0.02967056 * STA^{2.299} \qquad \textit{Equation 45}$$

Compared to the other models the low multiple (0.7478) and adjusted R^2 (0.7458) are clearly a drawback of this methodology. F-statistic is 373.7 on 1 and 126 DF, p-value: < 2.2e-16.

Residual standard error (RSE) = 0.6808 on logarithmically transformed y-scale. More detailed test statistics and plots are shown in table 34 and figure 18 in the annex. It seems logical, that effective shoot length (ESL) and primary shoot diameter (D_av.) that give rise to the variable STA, are less correlated to TLA2 than they are to TLA1 (R^2=0.8847). Whereas calculating a corresponding variable for lateral shoots with lateral shoot diameters and lateral shoot lengths would be too laborious to be considered practical.

In fact this approach could render practical, when a very fast and less precise estimation of TLA1 and TLA2 are sufficient and should be done with bigger sample sizes. Nevertheless as all shoot length based models the presented model is susceptible to canopy management such as defoliation and shoot topping. Very strong correlations initially found between Shoot Length and Leaf Area of shoots without topping, underestimate primary and lateral Leaf Area of vines with canopy management, or overestimate primary Leaf Area and underestimate lateral Leaf Area of trimmed vines, when these equations are applied to other situations (Constanza et al., 2004). This can be explained by the fact that the length of internodes is highly influenced by the cultivar and vigor (Huglin and Schneider, 1998), and that trimming disproportionately decreases Shoot Length while having a lesser effect on Leaf Area, or on the contrary, leaf removal, pests and natural defoliation by leaf senescence, may decrease Leaf Area, while leaving Shoot Length unaffected (Lopes and Pinto, 2005).

4.7.3 Estimation of shoot lateral leaf area with models based on the Lopes and Pinto method

Four models were fitted for further analysis, using the Lopes and Pinto (2005) methodology, two of which with simple linear regression using MLA2 as predictor and two with multiple linear regression, including B2 as a second predictor variable. The equations 46 to 49 and their test statistics are shown below.

4.7.3.1 Shoot Mean Lateral leaf area as predictor of Shoot lateral Leaf Area

Untransformed linear regression of Shoot lateral Leaf Area (TLA2) with Shoot Mean Lateral Leaf Area (MLA2) as predictor gave rise to the following equation:

$$TLA2 = 23.60927 + 0.86053 * MLA2 \qquad \textit{Equation 46}$$

Multiple $R^2 = 0.9738$, adjusted $R^2 = 0.9736$; F-statistic: 5493 on 1 and 148 DF, p-value: < 2.2e-16. detailed test statistics are shown in table 23.

Table 22: test statistics for equation 46; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of TLA2 and Mean Lateral Leaf Area (MLA2) Confidence Interval on 95% level for Intercept and slope

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-620.55	-74.76	-11.09	109.64	640.82
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		23.60927	24.61899	0.959	0.339
MLA2		0.86053	0.01161	74.117	<2e-16 ***
AIC= 2033.158		**RSE** = 248.1 cm^2		**MAE** = 145.9846	**MA%E**= 14.21445
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		13.12375		-25.0408756	72.2594073
MLA2		1.39746		0.8375828	0.8834696

Logarithmically transformed linear regression of Shoot lateral Leaf Area (TLA2) with Shoot Mean Lateral Leaf Area (MLA2) as predictor gave rise to the following equation:

$$ln(TLA2) = 0.02688 + 0.97829 * MLA2 \qquad \textit{Equation 47}$$

or

$$TLA2 = 1.027245 * MLA2^{0.97829} \qquad \textit{Equation 48}$$

Multiple $R^2 = 0.9814$, adjusted $R^2 = 0.9813$; F-statistic: 7807 on 1 and 148 DF, p-value < 2.2e-16; detailed test statistics are shown in table 24.

Table 23: test statistics for equation 47; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of TLA2 and Mean Lateral Leaf Area (MLA2) Confidence Interval on 95% level for Intercept and slope

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.35706	-0.10283	0.00747	0.10628	0.33825
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
ln(Intercept)		0.02688	0.07706	0.349	0.728
ln(MLA2)		0.97829	0.01107	88.359	<2e-16 ***
AIC= -149.5698		**RSE** = 0.145		**MAE** = 0.118989	**MA%E**= 0.03023442
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
ln(Intercept)		0.02688		-0.1254088	0.1791653
ln(MLA2)		0.97829		0.9564152	1.0001739

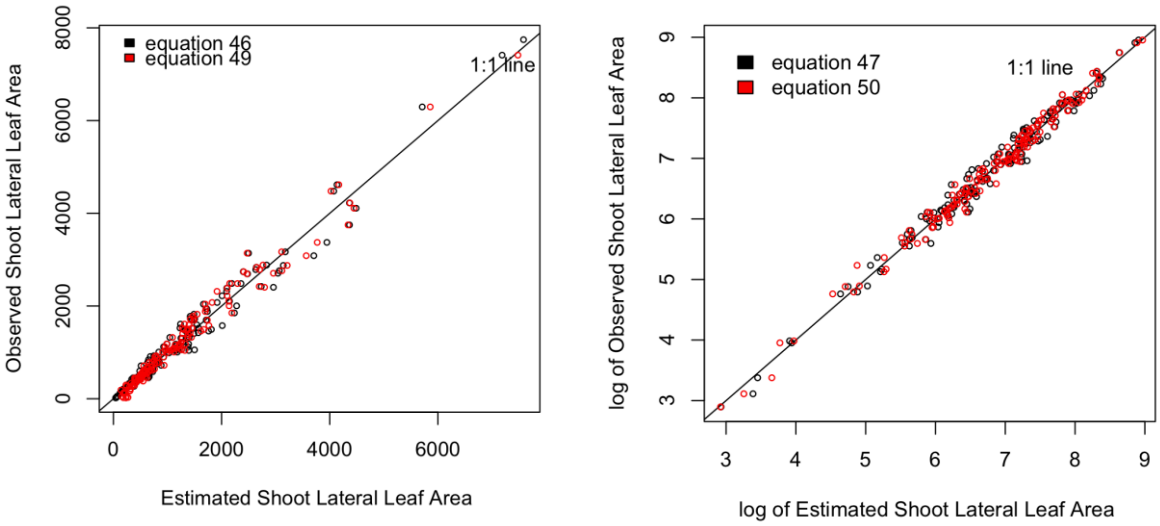

Figure 16: Observed vs. Estimated Shoot Lateral Leaf Area; on the left with simple and multi linear regression of equations 46 and 49, on the right with simple and multi linear regression of transformed variables of equations 47 and 50

4.7.3.2 Shoot Mean Lateral leaf area and biggest lateral Leaf as predictors of Shoot lateral Leaf Area

Multiple linear regression of Shoot lateral Leaf Area (TLA2) with Shoot Mean Lateral Leaf Area (MLA2) and biggest lateral Leaf (B2) as predictors gave rise to the following equation:

$$TLA2 = 309.8331 - 5.0215 * B2 + 0.9606 * MLA2 \qquad \textit{Equation 49}$$

Residual standard error: 188 on 147 degrees of freedom, multiple R^2: 0.979, Adjusted R^2 = 0.9787, F-statistic: 3430 on 2 and 147 DF, p-value: < 2.2e-16, detailed test statistics are shown in table 25.

Table 24: test statistics for equation 49; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for multilple inear regression of TLA2 and Mean Lateral Leaf Area (MLA2) Confidence Interval on 95% level for Intercept and slope

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-589.40	-98.08	-27.22	83.34	666.43
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		309.8331	52.0872	5.948	1.90e-08 ***
B2		-5.0215	0.8276	-6.068	1.05e-08 ***
MLA2		0.9606	0.0195	49.255	< 2e-16 ***
AIC= 2001.631		**RSE** = 188cm^2		**MAE** = 137.1424	**MA%E**= 34.72804
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		309.8331		206.8967112	412.7694137
B2		-5.0215		-6.6569541	-3.3860446
MLA2		0.9606		0.9220202	0.9991003

Multiple linear regression of logarithmically transformed variables Shoot lateral Leaf Area (TLA2) with Shoot Mean Lateral Leaf Area (MLA2) as predictor gave rise to the following equation:

$$ln(TLA2) = 0.81410 - 0.42244 * B2 + 1.0875137 * MLA2 \qquad \textit{Equation 50}$$

or

$$TLA2 = exp(0.81410 - 0.42244 * B2 + 1.0875137 * MLA2) \qquad \textit{Equation 51}$$

Multiple R^2 = 0.9864, adjusted R^2 = 0.9862; F-statistic: 5330 on 2 and 147 DF, p-value < 2.2e-16; Residual standard error: 0.1244 on 147 degrees of freedom. Deviation measures, AIC and detailed test statistics are presented in table 26:

Table 25: test statistics for equation 50; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of TLA2 and Mean Lateral Leaf Area (MLA2) Confidence Interval on 95% level for Intercept and slope

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.33143	-0.08653	-0.01189	0.09344	0.35581
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
log(Intercept)		0.81410	0.12585	6.469	1.38e-09 ***
log(B2)		-0.42244	0.05746	-7.352	1.26e-11 ***
log(MLA2)		1.13319	0.02311	49.032	< 2e-16 ***
AIC= -194.5366		**RSE** = 0.1244		**MAE** = 0.0991214	**MA%E**= 0.0301030
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
log(Intercept)		0.81410		0.5653961	1.0628092
log(B2)		-0.42244		-0.5359914	-0.3088802
log(MLA2)		1.13319		1.0875137	1.1788607

Simple linear regressions presented in chapter 4.6.3.1 explain very high amounts of Shoot Lateral Leaf Area variability. Logarithmic transformation seems to improve the fit by 0.5% to 1%, i.e. most of the observations are better predicted. Multi-linear regressions including the Area of the biggest lateral leaf as second predictor variable seems further to increase the model fit up to R^2 = 0.9862 (equation 50), with a better prediction of medium and big TLA2 values. Anyways visual observation of the plots seems to indicate a slightly higher overestimation of small TLA2 values compared to simple linear regressions. In fact the deviation measure Mean Absolute Percentage Error indicates a much higher error (34.7%) for the multi-linear model compared to 14.2% for the simple linear regression. In contrary, the transformed models show lower error terms and AIC when including B2 as second predictor compared to the simple model.

From a standpoint of prediction precision it is hardly justifiable to elect the more complicated multi-linear models over the simple regression models, however the fact, that B2 is already measured as part of MLA2, does not imply additional work.

4.8 Overview of presented models for Shoot Primary and Lateral Leaf Area

In the following table 27, all models for Shoot Leaf Area estimation, presented in chapters 4.6 and 4.7 are summarized.

Table 26: Overview over all presented models for Shoot Primary and Lateral Leaf Area (TLA1 and TLA2 respectively) estimation; equation number (Eq. Nr.), Model equation, Predictor variable, adjusted R^2, n= number of observations.; (1) adjusted R^2 for linear regressions between fitted values of nonlinear regression and original observations;(2) adjusted R^2 for linear regression of transformed variables; Type: p= primary shoot leaf area, l= lateral shoot leaf area leaf

Eq. Nr	Model	Predictor	R^2	n =	Type p/l
33	$TLA1 = 22.16933 * ESL^{1.00825}$	ESL	0.8252	230	p
35	$TLA1 = 72.68245 * STA^{0.78548}$	STA	0.9152	130	p
37	$TLA1 = 1.087085 * MLA1^{0.992}$	MLA1	0.9471	230	p
38	$TLA1 = 18.24266 + 1.07309 * MLA1$	MLA1	0.9497	230	p
39	$TLA1 = 1.38195 * MLA1^{0.96797}$	MLA1	0.9476	230	p
41	$TLA1 = 0.9619238 * MLA1^{1.01515}$	MLA1	0.9585	230	p
43	$TLA2 = 7.4586 * NL2 + 1.6889 * NL2^2 - 0.01 * NL2^3 + 51.254$	NL2	0.9695	149	l
44	$TLA2 = 13.124 * NL2^{1.397}$	NL2	0.9632	149	l
45	$TLA2 = 0.02967056 * STA^{2.299}$	STA	0.7458	127	l
46	$TLA2 = 23.60927 + 0.86053 * MLA2$	MLA2	0.9736	149	l
48	$TLA2 = 1.027245 * MLA2^{0.97829}$	MLA2	0.9813	149	l
49	$TLA2 = 309.8331 - 5.0215 * B2 + 0.9606 * MLA2$	MLA2 + B2	0.9787	149	l
50	$TLA2 = \exp(0.81410 - 0.42244 * B2 + 1.0875 * MLA2)$	MLA2 + B2	0.9862	149	l

5 Conclusion

This work reviewed previous approaches concerning empirical models for the estimation of grapevine Leaf Area for the cultivar Trincadeira. Several models were found which accurately predict the area of a single Leaf, using the Length of the central vein (V1), the sum of the length of the lateral veins (V2S). The latter were consistently more precise in single leaf area estimation. The logarithmic transformation of the dependent and the independent variables gave more linear relationships corresponding to power law relationships between the original variables. The non-linear model in form of power laws predicted leaf area with the best goodness of fit. A model using the log of V2S as the predictor for the log of the area of a single leaf is proposed, giving a high precision of estimate and avoiding complicated polynomial equations. This is a powerful tool for the estimation of Leaf area, which does not require special equipment, or trained staff. It is also suggested that separate models should be used for the estimation of the areas of Primary and Lateral Leaves.

For the estimation of Total shoot Leaf Area, several approaches were tested. Models based on the Lopes and Pinto (2005) method, are proposed both for primary and lateral Leaf Area. These models use Mean Leaf Area multiplied by the number of Leaves, as predictors. These models

require the Areas of the smallest and the largest leaf and they seem to be applicable to all levels of trimming, throughout the growing season.

Presented models based on primary shoot length and diameter as predictors are accurate in predicting primary leaf area, but less precise in predicting lateral leaf area and measuring lateral shoot length is laborious and very time consuming. Very strong correlations initially found between Shoot Length and Leaf Area of shoots without topping, underestimate primary and lateral Leaf Area of vines with canopy management, or overestimate primary Leaf Area and underestimate lateral Leaf Area of trimmed vines, when these equations are applied to other situations This can be explained by the fact that the length of internodes is highly influenced by the cultivar and vigor and that trimming disproportionately decreases Shoot Length while having a lesser effect on Leaf Area, or on the contrary, leaf removal, pests and natural defoliation by leaf senescence, may decrease Leaf Area, while leaving Shoot Length unaffected)

In conclusion, this method gives a good and fast estimation of primary leaf area for growing shoots, until topped and defoliated, but the estimation is fragile, as every factor that could affect shoot area or shoot length, such as microclimate, hormonal relationships, the distribution of assimilates etc., can impair the validity of any established model. Additionally the estimation of lateral leaf area and thus the total leaf area was rather poor, as these models do not include specific parameters related to lateral shoots.

As the dataset of this work mostly comprised non-trimmed shoots, safe conclusions could not be reached about the global use of all models. For this reason, it is suggested that models using Mean Leaf Area should be used for the prediction of Primary and Lateral Leaf Area and that other models presented here should be further investigated.

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Annex

Table 27: test statistics for equation 23; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Single lateral leaf area (response variable) and central vein length (V1) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (V1)

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-41.705	-7.429	-1.325	5.652	107.013
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-49.6831	0.7218	0.7218	<2e-16 ***
V1		17.5523	0.1218	144.10	<2e-16 ***
AIC= 31400.08		**RSE** = 11.69 cm^2		**MAE** = 8.675396	**MA%E**= 20.49631
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-49.6831		-51.09826	-48.26784
V1		17.5523		17.31344	17.79106

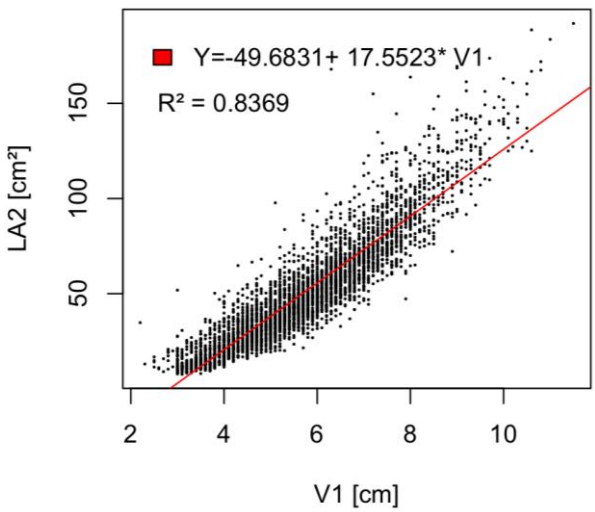

Figure 17: scatterplot of single lateral leaf area (LA2) vs. central vein length (V1), with regression line (equation 23)

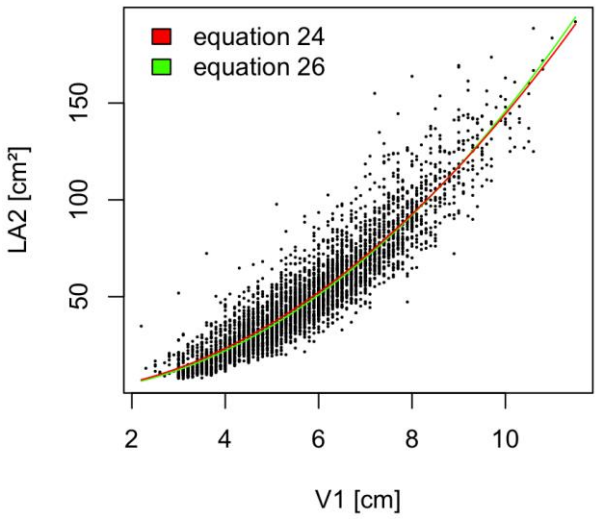

Figure 18: scatterplot od single lateral leaf area (LA2) vs. central vein length (V1) with regression lines of equations 24 (nonlinear regression) and 26 (transformed variables

Table 28: test statistics to equation 24; Akaike Information criterion (AIC), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single primary leaf area (EMLA, response variable) and central vein length (V1) in cm as predictor variable; Confidence Interval on 95% level for coefficients (a and b); (1) residuals from linear regression of fitted values .

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
(1)	-43.110	-6.551	-1.578	5.343	79.862
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
a		1.47006	0.03891	37.78	<2e-16 ***
b		1.99284	0.01346	148.07	<2e-16 ***
AIC= 30879.36		**RSE** = 10.97cm^2		**MAE** = 7.980964	**MA%E**= 18.75328
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
a		1.47006		1.402320	1.553206
b		1.99284		1.964522	2.016485

Table 29:test statistics to equation 25; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for nonlinear regression of Single lateral leaf area (response variable) and central vein lengths (log(V1)) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(V1))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	
Intercept		0.23359	0.02342	9.974	<2e-16 ***
ln(V1)		2.06359	0.01353	152.567	<2e-16 ***
AIC= -362.2223		**RSE** = 0.2313		**MAE** = 0.1761463	**MA%E**= 5.01727
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		0.23359		0.1876774	0.2795106
ln(V1)		2.06359		2.0370691	2.0901051

Table 30: test statistics for equation 27; Residuals, Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Single lateral leaf area (response variable) and sum of lateral vein lengths (ML2S) in cm as predictor variable; Confidence Interval on 95% level for intercept and coefficient ((LL2S)

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-33.086	-5.422	-0.520	4.531	57.258
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-118.16999	1.9899	-44.49	<2e-16 ***
LL2S		15.79790	0.1973	124.00	<2e-16 ***
AIC= 28810		**RSE** = 8.492 cm^2		**MAE** = 6.31304	**MA%E**= 17.44316
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-51.37653		-52.378359	-50.37471
LL2S		9.98935		9.894899	10.08379

Table 31: test statistics to equation 33; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Shoot primary leaf area (response variable) and effective shoot length (ESL) as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(ESL))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-1.14209	-0.09301	0.03614	0.13967	0.54888
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		**3.09871**	0.13167	23.53	<2e-16 ***
log(ESL)		**1.00825**	0.03058	32.97	<2e-16 ***
AIC= -13.18351		**RSE** = 1.262508		**MAE** = 0.1632229	**MA%E**= 2.251494
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		**3.09871**		2.8392707	3.358143
log(ESL)		**1.00825**		0.9479882	1.068507

Table 32: test statistics for equation 34; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of Primary shoot Leaf Area (response variable) and Shoot Area (log(STA)) as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(STA))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-613.00	-129.46	-14.87	102.14	711.67
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		18.24266	31.60700	0.577	0.564
MLA1		1.07309	0.01631	65.791	<2e-16 ***
AIC= 3111.391		**RSE** = 207.7		**MAE** = 155.1847	**MA%E**= 9.028232
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		18.24266		-44.036495	80.521819
MLA1		1.07309		1.040953	1.105231

Table 33: test statistics for equation 43; Akaike Information criterion (AIC), Residual Standard Error (RSE), Mean Absolute Error (MAE) and Mean Absolute Percent Error (MA%E) for linear regression of transformed lateral shoot leaf area (response variable) and Shoot Area (STA) as predictor variable; Confidence Interval on 95% level for intercept and coefficient (log(STA))

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-2.31363	-0.44119	-0.02328	0.52247	1.75395
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept		-3.5176	0.5148	-6.834	3.17e-10 ***
log(STA)		2.2993	0.1189	19.331	< 2e-16 ***
AIC= 268.7966		**RSE** = 0.6808		**MAE** = 0.528353	**MA%E**= 0.3928604
Confidence Interval (95%)		**Estimate**		**lower end**	**upper end**
Intercept		-3.5176		-4.536352	-2.498947
log(STA)		2.2993		2.063880	2.534655

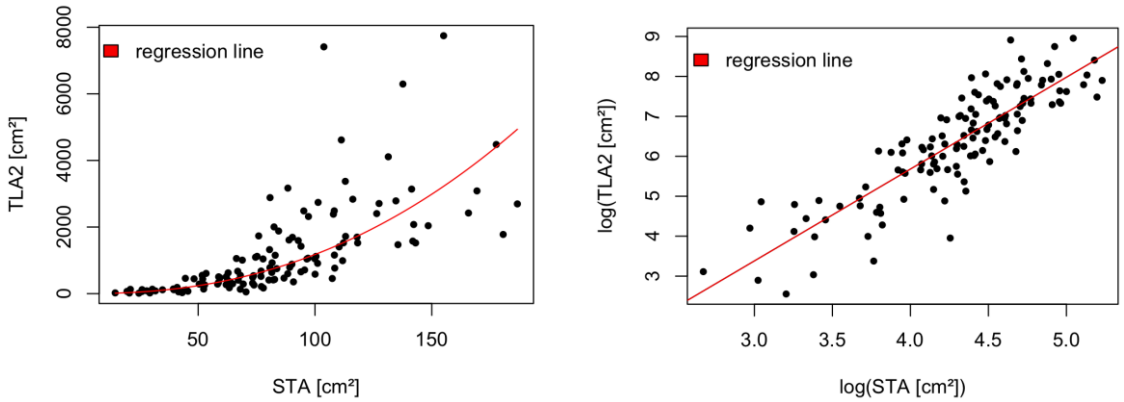

Figure 19: dependent vs. independent variable and regression line with equation 43: TLA= 0.02967*STA^2.2993; left side untransformed scales, right sight logarithmically transformed scales. Well visible the poor prediction of extreme big TLA2