

Methods to assess grapevine water status: a review

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Dissertation to obtain a Master's Degree in
Engineering of Viticulture and Oenology – Double Degree

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Abstract

Viticulture and wine industry are important economic resources for many countries, represented in a wide range of extremely diverse climates all over the world and highly affected by global climate change at different scales. The global warming is the main cause of water sources reduction due to an altered precipitation pattern; this means a reduction in sources of supply and an increase in water demand from crops especially in Mediterranean regions. The high impact of irrigation in grapevine berry quality and yield makes the development of plant water status monitoring systems an essential issue in the context of sustainable viticulture. Knowledge of the physiological responses of the crop and the development of suitable water status monitoring systems are the main prerequisites for proper irrigation management, in order to mitigate climate change effects. This review aims to provide a state-of-the-art summary of the most important literature on grapevine water status assessment for monitoring and adapting vineyard management strategies to production goals in view of global warming. In this work mainly plant-based methods are reviewed, their advantages and drawbacks are discussed. In this work some factors influencing water relations and effects of severe water stress on grapevine are also reported. The main plant-based methods for irrigation scheduling, including those based on direct or indirect measurement of plant water status and those based on plant physiological responses to drought, are outlined and evaluated. New technologies approaches that belong to the field of precision viticulture are also described, which could offer the integration of heterogeneous information collected in the vineyard at different spatial and temporal resolutions. These new approaches offer new synergies to overcome the limitations inherent to plant water status measurement techniques obtained directly or indirectly. The potential of plant-based systems for automated irrigation control using various scheduling techniques is also discussed.

Key words: viticulture, grapevine water status, irrigation, precision irrigation, water potential

Resumo

A viticultura e a indústria do vinho são recursos económicos importantes para muitos países, representados numa ampla gama de climas extremamente diversos em todo o mundo e altamente afetados pelas alterações climáticas globais em diferentes escalas. O aquecimento global é a principal causa da redução dos recursos hídricos devido a um padrão de precipitação alterado; isto significa uma redução nas fontes de abastecimento e um aumento na procura de água pelas culturas, especialmente nas regiões mediterrânicas. O elevado impacto da rega na qualidade e rendimento da videira torna o desenvolvimento de sistemas de monitorização do estado hídrico das plantas uma questão essencial no contexto da viticultura sustentável. O conhecimento das respostas fisiológicas da cultura e o desenvolvimento de sistemas adequados de monitorização do estado hídrico são os principais pré-requisitos para a gestão adequada da rega a fim de mitigar os efeitos das alterações climáticas. Esta revisão tem como objetivo fornecer um resumo do estado da arte da literatura mais importante sobre a avaliação do estado hídrico da videira para monitorizar e adaptar as estratégias de manutenção da vinha aos objectivos de produção face ao aquecimento global. Neste trabalho, faz-se a revisão sobretudo dos métodos baseados na planta, e discute-se as suas vantagens e desvantagens. Neste trabalho também são relatados alguns fatores que influenciam as relações hídricas e os efeitos do stress hídrico severo na videira. Os principais métodos baseados na planta para programação da rega, incluindo aqueles baseados na medição direta ou indireta do estado hídrico da planta e aqueles baseados nas respostas fisiológicas da planta à seca, são descritos e avaliados. São também descritas novas abordagens de tecnologias no âmbito da viticultura de precisão, as quais poderão heterogêneas colhida na vinha em diferentes resoluções espaciais e temporais. Essas novas abordagens oferecem novas sinergias para superar as limitações inerentes às técnicas de medição do estado hídrico da planta obtidas direta ou indiretamente. O potencial de sistemas baseados na planta para controlo e automatizado da rega usando várias técnicas de programação também é discutido.

Palavras-chave: viticultura, estado hídrico da videira, irrigação, irrigação de precisão
potencial hídrico

Resumo alargado

A irrigação é uma prática muito importante para as vinhas destinadas à produção de vinhos de alta qualidade, especialmente nos países de clima árido e quente como as regiões vitícolas mediterrânicas. Na verdade, a rega deficitária influencia positivamente a qualidade da produção e garante maior eficiência dos processos fisiológicos da planta, aumentando a sua longevidade. Hoje, devido ao crescente problema das alterações climáticas, devido ao aumento contínuo da concentração dos gases com efeito de estufa presentes na atmosfera, assistimos a uma diminuição inexorável das disponibilidades hídricas para rega e, ao mesmo tempo, a um aumento do consumo de água devido a uma, cada vez maior, evapotranspiração por parte das culturas. Esta revisão tem como objetivo fornecer um resumo do estado da arte da literatura mais importante sobre a avaliação do estado hídrico da videira para monitorizar e adaptar as estratégias de manutenção da vinha aos objectivos de produção face ao aquecimento global. Existem diferentes métodos de monitorização do estado hídrico da videira, neste trabalho iremos discutir os métodos que fazem uso de indicadores fisiológicos, que se baseiam no princípio de que a fisiologia da videira é modificada pelo défice hídrico. As abordagens mais comuns são o potencial hídrico foliar (Ψ_w) e o potencial hídrico do caule (Ψ_{stem}), pois garantem um bom compromisso entre acessibilidade, tempo de execução e confiabilidade do método. Na verdade, estes potenciais hídricos estão bem correlacionados com o estado hídrico da planta e o conteúdo de água do solo (SWC). A condutância estomática (g_s) é um método preciso para determinar o estado da água da planta, pois também leva em consideração os diferentes comportamentos estomáticos da videira, no entanto requer conhecimentos na gestão da instrumentação e interpretação dos resultados. As variações no diâmetro do tronco não são um método muito fiável visto que a estimativa do estado hídrico é efectuada através da taxa de crescimento do tronco (TGR) e índices máximos diários de contração do caule (MDS), passíveis de determinar o stress hídrico apenas em determinados estágios fenológicos. A medição do fluxo de seiva é uma prática muito interessante para a estimativa da transpiração das plantas através da medição indireta da taxa de fluxo do xilema. Esta abordagem é difícil de aplicar em vinhas comerciais, porque a manutenção da ferramenta é cara devido ao alto limite de durabilidade. A discriminação isotópica é um método utilizado para a avaliação da Eficiência do Uso da Água (WUE) e gestão da irrigação durante a estação, é um método bem correlacionado com o Ψ_{stem} . As câmaras térmicas têm um excelente potencial graças à sua rápida velocidade de execução e confiabilidade, utilizando um índice de stress da cultura (CSWI) e um índice de condutância estomática (I_g). Porém, é necessário facilitar gestão da instrumentação e a interpretação dos dados. Neste trabalho também é feita uma breve menção à medição integrada da espessura da folha e da capacitância elétrica (CAP).

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LIST OF ABBREVIATIONS

ABA	Abscisic Acid
CAP	Leaf Electrical Capacitance
CSWI	Crop Water Stress Index
gs	Stomatal Conductance
lg	Stomatal conductance index
IRGA	Infrared Gas Analyser
IR	Infrared Radiation
IRMS	Isotope Ratio Mass Spectrometry
MDS	Maximum Daily Stem Shrinkage
MXDT	Maximum Value of trunk diameter during the day
MNDT	Minimum Value of trunk diameter during the day
PAR	Photosynthetically Active Radiation
RTL	Relative Leaf Thickness
SF	Sap Flow
SWC	Soil Water Content
TDV	Trunk Diameter Variation
TGR	Trunk Growth Rate
UAV	Unharmful Automatically Vehicle
WAtm	Radiations emitted by the atmosphere
WBackground	Radiation emitted by the object's surroundings and further reflected by the object's surface
WUE	Water Use Efficiency
$\delta^{13}C$	Carbone Isotope Ratio
Ψ_{MD}	Midday Leaf Water Potential
Ψ_{stem}	Stem water potential
Ψ_{PD}	Pre-dawn Leaf Water Potential
Ψ_w	Leaf Water Potential

1. INTRODUCTION

Viticulture and the wine industry are an important economic branch for many countries, represented in a wide range of extremely diverse climates all over the world and highly affected by global climate change at different scales. Nowadays water usage has become a critical point for the vineyard management, mainly due to climate change that caused a reduction of water sources, which implies a reduction in sources of supply and an increase in water demand from crops thanks to the increase of intense heat waves during the vine biological cycle, especially in Mediterranean regions (González-Flor *et al.* 2019). This represents a major limitation for the viticulture of these regions because it limits grapevine longevity, yield and berry quality and can influence its socio-economical sustainability (Costa *et al.* 2014).

A grapevine needs between 300 and 600 mm of water in cool climates (Williams, 2014) and between 400 and 800 mm in hot climates (Williams & Baeza, 2007) during the vegetative cycle, which is highly dependent on cultivar, rootstock, training system, planting density, yield and seasonal temperature patterns. In viticulture, irrigation has a direct impact on vine yield and grape quality, as a result, irrigation is being widely adopted in order to ensure more regular and predictable yields (Chaves *et al.* 2007; Chaves *et al.* 2010; Cifre *et al.* 2005). Concurrently, due to the increasing water scarcity, and the rising competition between water users, deficit irrigation techniques emerged as a potential strategy to improve the efficiency of water, so the implementation of precision watering systems could be considered as useful tools for the precise application of water regimes based on water status reports of the vineyard plot, in order to preserve water sources and increase irrigation efficiency (Keller, 2015). These techniques allow to regulate the relationship between the vegetative and reproductive cycle of the grapevine which is considered the key to improving quality, because the excess of vegetative vigour can negatively influence the berry composition through competition phenomena and microclimate effects. Today's vineyard with controlled deficit irrigation require constant and periodical monitoring of the plant water status, very often this practice is laborious, expensive and sometimes destructive. One important consideration is that often vineyards are characterized by an high heterogeneity due to structural and dynamic factors such as pedo-morphological characteristics, cultural practices and seasonal weather (Bramley, 2003). This variability causes different vine physiological responses, with direct consequences on grape quality (Matese & Di Gennaro, 2015). In order to overcome this issue, recently with the advent of new technologies a modern approach to viticulture is developing, namely precision viticulture, to manage the vineyard system in an increasingly efficient way by making use of new technologies, supporting growers decisions. Thanks to these new technologies, it is possible to think about the development of different approaches for fast and accurate remote diagnosis of a wide range of plant stresses (Costa *et al.*, 2010). Remote and proximal sensing sensors have become alternative tools to monitor crop water status, to evaluate plant

conditions, plant health and/or soil conditions. Precision viticulture aims to exploit the use of remote sensing tools to describe vineyards spatial variability with high resolution, and provide recommendations to improve management efficiency in terms of quality, production, and sustainability (Matese & Di Gennaro, 2015). Despite all this, in most European winegrowing regions, grapevines are still cultivated under rain-fed conditions without any water supply, called “dry farming”, this is often an obligatory choice as a water source is not always available (Rienth & Scholasch, 2019).

2. AIM OF THE WORK

The growing need to adopt irrigation on crops, such as grapevine, traditionally conducted only with natural water supplies is due, on the one hand, to climate change and a reduction in rainfalls, on the other hand, to the need to direct production towards obtaining quality products. Knowledge of the physiological responses of the crop and the development of suitable water status monitoring systems are the main prerequisites for proper irrigation management. Many studies that involved the irrigation management in vineyards and their relationship with other cultural practices have been carried out in the last years, for this purpose different methods to assess grapevine water status were developed along the time. The aim of this work is to review the various techniques to assess grapevine water status and analyse their positive factors, constraints and applicability in the open field. In this work many factors that influence water relations and the consequences of excessive water stress on the grapevine and berries composition are also described.

3. LITERATURE REVIEW

3.1 WATER STRESS

3.1.1 Factors influencing water relations

Irrigation is an agronomic practice that is commonly used in many hot - arid areas around the world, where it becomes indispensable for climatic and soil conditions, in order to obtain good yield and grape quality (Fregoni, 2013). In general, the vine, based on its genetic and morpho - physiological characteristics, regulates the growth and accumulation processes of the products of photosynthesis and the biosynthesis of secondary products through the processes of photosynthesis, respiration and transpiration in response to different environmental stimuli (Behboudian & Singh, 2001). In the world of viticulture exists a large morphological, physiological and molecular diversity in grapevines that affects water needs, these factors can be divided into intrinsic and extrinsic. Genotype is an intrinsic factor that influences grapevine responses to drought and heat stress via different ways, it affects leaf gas exchange behaviour (Chaves *et al.*, 2010; Costa *et al.*, 2012; Tomás *et al.*, 2014) , and the adaptability of rootstocks to drought conditions (Fregoni, 2013). From a genetic point of view, the adaptability of the rootstocks to drought conditions is a determining aspect considering the location of the main wine-growing areas and future developments regarding the availability of water on a global level. Thanks to genetic improvement, rootstocks with a different degree of resistance were selected, which was assessed taking into account different parameters such as the angle between the petiole and the leaf edge of plants subjected to stress, the abscissic acid content of the leaves, density and size of stomata, water potentials, etc. (Fregoni, 2013). Through hybridization, different rootstocks have been obtained with a certain resistance starting from species such as *Vitis monticola*, *Vitis berlandieri*, *Vitis rupestris* and *Vitis vinifera* that possess this characteristic. The rootstocks can be classified as very resistant such as (140 Ru, 1103 P, 110 R, etc.), medium resistant (41B, 420 A, 99 R etc.) and poor resistance (K5BB, SO4, Riparia, etc.) to drought (Fregoni, 2013). Regarding the leaf gas exchange behaviour it influences water use efficiency and leaves temperature, in fact stomatal regulation plays a central role in plant response to drought (Chaves *et al.*, 2003), some authors divided grapevines behaviour in two categories: isohydric and anisohydric. Isohydric plants regulate transpiration to maintain a relatively constant midday leaf water potential (Ψ_{MD}) as soil water potential and predawn leaf water potential (Ψ_{PD}) decrease. Anisohydric varieties, by contrast, respond to drought by allowing Ψ_{MD} to decline nearly in parallel with Ψ_{PD} (Fu *et al.*, 2018), in other words the first limit the stomatal opening to the first sign of stress, in order to contain an excessive drop in water potential, while the latter cultivars tend to accept strong variation in water potential by only partially limiting stomatal closure. However, other authors suggested that this classification is not always respected, because genotypes might present a different

behaviour according to the degree of stress and growing conditions (Chaves *et al.*, 2010; Lovisolo *et al.*, 2010). Extrinsic factors which influence vine water needs interacting with its genetic and morpho-physiological characteristics to determine the overall response to water stress, are mainly the soil (structure, texture, composition, depth), training system and climate (temperature, relative humidity, light, wind, rainfall) (Fregoni, 2013). The characteristics of the soil and climate have a direct influence on the amount of water that roots can have available (Fregoni, 2013). The texture and structure of the soil determine its ability to retain available water against the percolation forces (water capacity), and its surface tension, which contends water for the absorption power of the roots (Fregoni, 2013). The greater or lesser uniformity of the texture and structure, within the layers of the soil hosting the roots, determines the speed of the capillary movements of the water and therefore the volume of soil that each root can exploit for water absorption (Fregoni, 2013). The physiological phenomenon most dependent on the water state of the plant is the transpiration. At the foliar level, the stomatal resistance varies greatly throughout the day and with changes in the water state, thanks to the efficient regulation of the opening of the stomata which in case of insufficient water availability are the first mechanisms to respond to stress, favouring or impairing transpiration. Water consumption is linked to the production of grapes per hectare, therefore the breathable leaf surface increases parallel to the production (Fregoni, 2013). All this is also determined by the training system, generally the most expanded and productive training system are the most demanding in water (Fregoni, 2013). The climate is a very important factor that can influence the water needs of the plant, some of its components are sunlight, CO₂ amount in the atmosphere and rainfall. Solar radiation is the source of energy used by leaves for the photosynthesis process and has a direct effect on the stomatal opening and leaf temperature, only a fraction of the sunlight is used by the plant for photosynthesis named PAR or photosynthetically active radiation (400 –700 nm); the excessive amount of sunlight can induce a reduction of leaf gas exchange due to the increase of leaf temperature that leads to stomatal closure in order to preserve the water contained in the tissues. Due to the constant increase in the emission of greenhouse gases, the CO₂ presents in the atmosphere is greater than in the past, it plays a very important role in the photosynthesis and in the energy balance of plants (Shultz, 2000). The stomatal opening and therefore the transpiratory activity are influenced by the concentration of CO₂ inside and outside the leaf, an environment with high CO₂ concentration could cause a partial stomatal closure and this probably can lead to an increase of leaf temperature as result of reducing cooling (Shultz, 2000). The non-optimal leaf temperature can lead to a reduction in the net photosynthetic activity and to structural and chemical alterations of the leaf, for instance an efficiency decrease of rubisco, one of the most important enzymes of photosynthesis (Shultz, 2000).

3.1.2 Effects of water stress on grapevine

Water stress is a situation in which water becomes a limiting factor for the normal functions of the plant, impacting on its biological cycle and compromising its yield and longevity. Water status of grapevines is a key factor for yield, grape composition, and wine quality (Suter *et al.*, 2019). Several physiological processes of the plant are affected by water stress (Fregoni, 2013; Scholash & Rienth 2019). Water stress can also be due to an excess of water, but it is generally attributed as such when there is a water scarcity.

Grapevine is a plant species able to tolerate drought, generally does not tend to immediately show signs of stress, but shows symptoms of repeated stress. The deficit occurs when the plant has an absorption rate lower than the loss of water through transpiration and is characterized by a decrease in the water content, turgor and water potential, by a partial or complete closure of the stomata and by a decrease in cellular relaxation and plant growth.

Depending on the phenological stage in which the plant is affected by stress, the repercussions that this has on its growth, development and physiology can vary considerably (Wample & Smithyman, 2002; Scholash & Rienth 2019). Water stress during the bud break period can lead to a reduced shoots development, or in the worst case, a reduction of the bud burst percentage (Wample & Smithyman, 2002; Scholash & Rienth 2019), in this case cellular expansion is mainly influenced, as it is normally necessary for optimal cell turgor to be accomplished (Fregoni, 2013). More severe and prolonged water stress may result in poor flower numbers, development and reduced pistil and pollen viability and subsequently berry set (Fregoni, 2013). Furthermore, during this period, due to the scarcity of water, a reduced absorption of the nutrients present in the soil can occur and can lead to nutrients deficiency symptoms in the following season, because during the vegetative awakening the plant uses the nutrients and assimilates absorbed and produced during the previous year (Wample & Smithyman, 2002; Scholash & Rienth 2019). Following during fruit set stage water stress can cause flower abortions and abscissions of flowers or inflorescences, probably associated with hormone changes (Düring *et al.*, 1986). Prolonged water stress in this moment can lead to a reduced canopy development, and consequently to inadequate leaf area that cannot support berries development and ripening (Wample & Smithyman, 2002; Scholash & Rienth 2019). Moreover during this phase the production of the following year may be compromised, since a good water supply is a necessary condition for a good differentiation of buds, this results in a loss of potential fertility; generally this process starts about two weeks before full bloom (Wample & Smithyman, 2002; Scholash & Rienth 2019). Immediately after fruit set, water stress may reduce berry cell division and enlargement, resulting in smaller fruit and lower yield. Early water deficit during the first growth phase has the highest impact on final berry size, it slows down cell expansion in the berry without impacting cell division rate (Ojeda *et al.*, 2001). Limited canopy development during this time will tend to limit the photosynthetic capacity of

the vine with a reduction of fruit development and quality. In some dry climate wine regions, for instance Mediterranean regions, where plants are subjected to a combined effect of light, drought and heat stress early senescence of leaves (often basal leaves) can also occur, in extreme cases the percentage of leaves lost can reach 70% and can have negative effects in vine health and longevity (Wample & Smithyman, 2002; Lopes *et al.*, 2014). The loss of basal leaves can result in an increased exposure of bunches, this could involve a risk of berry sunburn of red and white varieties as a consequence of sudden exposure (Lopes *et al.*, 2014). A strong stress after veraison worsens the organoleptic characteristics of the berries, due to the slowing down or interruption of the production processes of the assimilated substances and the early aging of the leaves (Fregoni, 2013); the result is a reduction of soluble-solids accumulation, higher pH, decrease of total acidity, and less production of anthocyanins in red varieties (Wample & Smithyman, 2002). Water deficit during the ripening phase is less affecting on final berry size, probably due to a switch from symplastic to apoplastic - osmotically driven sugar unloading, via the phloem (Zhang *et al.*, 2006). After the harvest, the water shortage leads to a reduction in the development of the root system, with a consequent reduction in the absorption of mineral elements in the following season (Fregoni, 2013). Santesteban *et al.* (2011) showed that other factors, apart from climatic conditions, such as relations between crop load and vegetative growth, can modify daily plant water dynamics. Vines with higher fruit load showed a greater decrease in water potential which indicates an increase in daily vine water consumption, which seems reasonable given that the presence of fruit promotes stomatal opening to increase carbon fixation. Similar to fruit load, vines with higher shoot growth showed a greater decline in stem water potential during the day, probably as a result of higher water use caused by greater canopy light interception and a larger transpiring surface. In vines for the production of wine grapes, the achievement of a balance between vegetative and reproductive growth by controlling the mechanisms of distribution of resources between vegetative and reproductive sinks is possible thanks to irrigation scheduling. Reducing productivity to increase assimilates accumulation into the berries, can allow to obtain qualitatively higher productions compared to more favourable cultivation conditions and with an optimal water supply (Ojeda *et al.*, 2002). According to this reasoning, moderate stress induces a reduction in vegetative growth favouring the ripening of bunches, formation of aromas and phenolic compounds. Ojeda *et al.* (2002) evaluated the effects of the water deficit on the synthesis and concentration of phenols (flavan-3-ols, anthocyanins, and flavonols) in Shiraz skin. It emerged that the phenols biosynthesis seems to vary according to the level of deficit and the phenological phase in which it occurs. The biosynthesis of flavan-3-ols is reduced from the first manifestations of deficit and that of proanthocyanins and anthocyanins increases only between veraison and maturation in concomitance with moderate stress. At the same time, if the plant is subjected to strong water stress between anthesis and veraison and

between veraison and ripening an overall reduction in the volume of the berries was observed. The reduction in size and weight of the berry is caused by a reduction in cell division, a decrease in the volume of the pericarp occur. Its decrease is mainly due to a first water deficit from flowering to veraison, the phenomenon is irreversible and demonstrates that the first deficits can affect the structural properties of cellular components by inducing less cellular distension from veraison, thus limiting the subsequent pericarp cell growth (Ojeda et al., 2001; Ojeda et al., 2002). Reducing the berry size increases the skin-to-pulp ratio and, consequently, the phenolic concentration is higher in stressed berries.

3.2 METHODS TO EVALUATE GRAPEVINE WATER STATUS

Irrigation planning is one of the most important practices in viticulture, in fact it influences the yield and quality of irrigated vineyards (Scholash & Rienth 2019). For a correct modulation of the water supply, it is necessary to use the right methods of monitoring the plants water status, in order to reduce waste as much as possible and at the same time increase the efficiency of the irrigation intervention. As a general concept, it can be said that it must be irrigated when the water balance of the soil-plant system is no longer in balance, as the system itself cannot compensate for the loss of water by evapotranspiration (Fregoni, 2013). Measurements of vine water condition can be grouped according to three different approaches:

- Measurements of soil water
- Physiological indicators
- Water balance modelling

Some of these methods are more suitable for research purposes, others for vineyard management (Van Leeuwen *et al.* 2010). Grapevine water status can be monitored by the use of physiological indicators (Cifre *et al.*, 2005). These indicators are based on the principle that vine physiology is modified by water deficit. Some indicators based on direct plant measurements will be described below, currently they represent the most used approaches that allow better management of irrigation interventions thanks to accurate estimates and being less time consuming (Scholash & Rienth 2019). However, it is not possible to identify in absolute sense an indicator that is better than others for the determination of the plant water status. The use of one indicator in respect to another one must take into account the cultivated varieties, the environmental conditions and the practical problems, first of all the cost and the possibility of use by unskilled staff (Cifre *et al.*, 2005).

3.2.1 Visual analysis

The manifestation of water stress symptoms usually occurs when the physiology of the plant has been seriously altered and timely intervention is no longer possible. The slowing down of vegetative growth is the earliest responses of a plant sensing a limiting water supply, therefore a slowdown in the shoots growth follows (Rienth & Scholasch 2019). An empirical method to identify the water stress of the plant is to evaluate the state of the vegetative apex (Rienth and Scholasch 2019). Visual analysis can be performed through observation of 30 – 50 apexes per plot and classed according to the apex state (Rienth & Scholasch 2019).

- 1) A growing apex, where the first expanded leave is well beneath the apex
- 2) A slowing down of growth with the first expanded leave covering the apex
- 3) Detachment of the apex that means shoot growth has completely ceased

After the veraison, this is normal, while in other phenological phases it is a symptom of severe stress. A further indicator are tendrils that in non-water stressed grapevines are turgid and expand well beyond the shoot tips, moderate water deficit leads to their wilting and subsequent abscission when water deficit becomes severe (Rienth & Scholasch 2019).

In the case of the vine, visual analysis, although being an extremely practical and easy method, is not much used due to the characteristics of great adaptability of the vine cultivated in drought environments, as the symptoms of severe water stress tend to occur after several successive situations of water scarcity, this therefore does not allow an intervention with the right timing. For these reasons the identification of stress symptoms by visual analysis is considered an approximate method, moreover it can be easily conditioned by the subjectivity of the operator that carried out the evaluation (Rienth & Scholasch 2019).

3.2.2 Leaf water potential

Leaf water potential (Ψ_w) is a good indicator to determine plant water status. The main tools used in leaf potential monitoring are the psychrometer and the Scholander pressure chamber, the latter being widely used as the monitoring of the water potential results less laborious than with the psychrometer. The pressure chamber (Figure 1) evaluates the negative hydrostatic pressure called tension, present in the xylem, which is believed to be close enough to that of the entire organ, the value of the pressure used to make the xylem water appear on the cutting area of the petiole corresponds in absolute value to that of the leaf and is assumed as the value of the tissue water potential under examination (Scholander *et al.* 1965), the pressure inside the chamber is indicated with a manometer and can be expressed in MPa or Bar (1 MPa = 10 bar). Before the measurement the leaf is cut from the vine and the petiole is re-cut with a sharp cutter, subsequently the leaf is introduced into the pressure chamber with the petiole coming out of a hole in the cap.



Figure 1: Scholander pressure chamber

The Scholander pressure chamber, provides a relatively quick, flexible and accurate estimation of plant water status through the measurement of leaf water potential (Santesteban *et al.*, 2011). Water potentials represent instantaneous grapevine water status and several measurements must be carried out with regular intervals to follow the evolution of grapevine water condition during the day and/or season (Van Leeuwen *et al.* 2010). The leaf water state is strongly influenced by the overall water condition of the plant, so it is possible to measure that of the plant by measuring the leaf water potential; it is also important to remember that water moves from high (less negative) to low (more negative) water potential. The Ψ_w allows to identify when the plant enters in water stress condition and therefore intervene with irrigation, in practice we can say that when the plant has reached water stress we will have a certain value of leaf potential, which varies from crop to crop (Fregoni, 2013). Several applications of water potentials have been developed (Begg & Turner 1970). There are different types of leaf

water potential that differ according to the moment when the measurement is performed, the most important are midday and pre-dawn potentials. The midday leaf water potential (Ψ_{MD}) is performed in well exposed adult leaves at midday (Rienth & Scholasch 2019); few disadvantages of this assessment is that the measurement is highly influenced by microclimatic environment conditions of each leaf (Jones, 2004) and stomatal regulation behaviour of the vines (Scholasch & Rienth, 2019). Grapevines may have an isohydric behaviour and they limit variations in water potential of their leaves by stomatal regulation. For these two reasons, midday leaf water potential is not the most accurate indicator of vine water status. The pre-dawn leaf water potential (Ψ_{PD}) is measured just before dawn in adult leaves, when the plant is considered to be in balance with the soil water status, (Chonè *et al.*, 2001; Rienth & Scholasch 2019), so it reflects the soil moisture level and can be utilized as a measure of the static water deficit in the vines (Rienth & Scholasch 2019). At dawn the plant has the maximum water content and maximum leaf potential of the day, while in the hottest hours (midday) the plant has the minimum water content and minimum leaf potential (Scholasch & Rienth, 2019), microclimatic conditions are homogeneous among leaves and grapevines are not transpiring. At this time of day, each single leaf of a grapevine has a similar water potential (Van Leeuwen *et al.*, 2009). However, the main disadvantages for the use of Ψ_{PD} are the time of its measurement (Schultz & Stoll, 2010) and the fact that it may come into equilibrium with the wettest portion of the soil profile which would limit its use in drip irrigated vineyards (Williams & Trout, 2005). Some authors have shown that, when comparing vineyards with high water availability Ψ_{PD} cannot discriminate as well as stem water potential (Williams & Araujo 2002, Williams & Trout 2005, Baeza *et al.* 2007) but it perform better under deficitary conditions. Table I shows different water stress ranges in relation to the pre-dawn leaf water potential (Ψ_{PD}) measurement, more negative is the water potential in the leaf, the greater will be the water deficit in the plant. However, these values presented are average thresholds which can vary from plot to plot depending on root distribution, grapevine vigour and yield (Van Leeuwen *et al.*, 2009).

Table I: Relationship between leaf water potential measured at dawn (Ψ_{PD}) expressed in Mega Pascal (MPa) and vine water status (Adapted from Carbonneau, 1998).

Ψ_{PD} (MPa)	Grapevine water status
$0 \text{ MPa} > \Psi_{PD} > -0,2 \text{ MPa}$	No water stress
$-0,2 \text{ MPa} > \Psi_{PD} > -0,4 \text{ MPa}$	Light to medium water stress
$-0,4 \text{ MPa} > \Psi_{PD} > -0,6 \text{ MPa}$	Medium to high water stress
$-0,6 \text{ MPa} > \Psi_{PD}$	Severe water stress

3.2.3 Stem water potential

Another type of measurement of the plant water status using the Scholander pressure chamber is the stem water potential (Ψ_{stem}), which can be assessed at any hour of the day; generally measured in the early morning, at midday or in the early afternoon (Intrigliolo & Castel, 2010). It is measured on non-transpiring leaves that have been previously enclosed with a plastic bag and surrounded with aluminium foil at least 1 hour before measurement using Scholander chamber (Rienth & Scholasch 2019). This operation permits to stop transpiration enabling the leaf to come into equilibrium with the water potential of the stem (Begg & Turner 1970; Chonè *et al.*, 2001). Stem water potential values reach a minimum value in the early afternoon and generally this moment is chosen for comparing measurements among sites (Van Leeuwen *et al.*, 2009). Six to ten measurements on separate grapevines are necessary to represent water status in a vineyard, according to the size and the intra-variability of the block (Van Leeuwen *et al.* 2010). Stem water potential values reflect soil water availability, but they also depend on climatic parameters. In order to assess the impact of soil water availability and climatic conditions on Ψ_{stem} values, Van Leeuwen *et al.* (2010) measured it two days in a row, in cool and cloudy conditions (first day), warm and sunny conditions (second day). They concluded that the effect of climate on stem water potential is limited compared to the effect of soil water availability, as it possible to observe in the graph of figure 2, Ψ_{stem} values are dependent of soil type in which water availability is different. However, it's important to remember that comparisons of soil water availability through Ψ_{stem} measurements should be carried out in similar climatic conditions, for example on days without extreme temperatures (Van Leeuwen *et al.*, 2009).

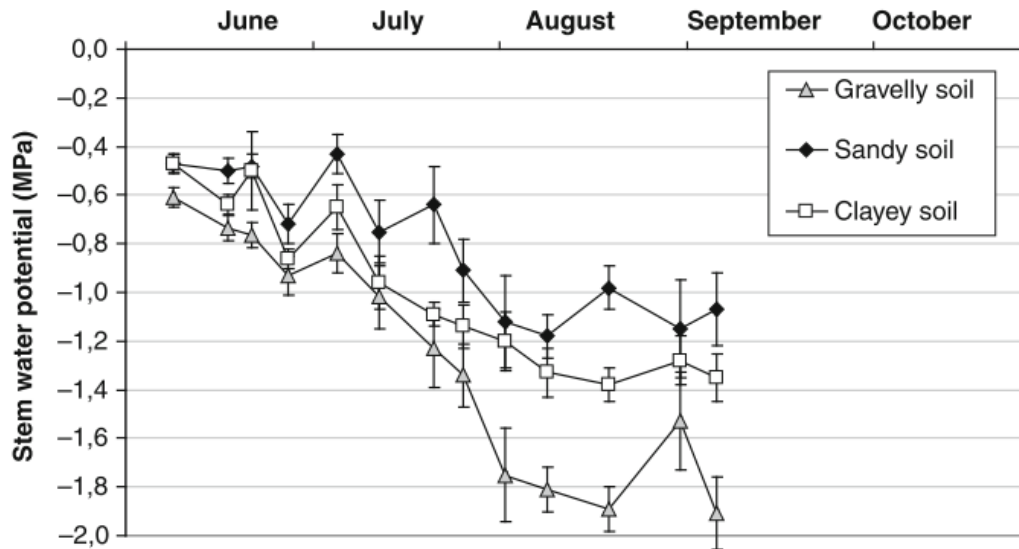


Figure 2: Comparison of seasonal stem water potential on three soils with various soil water holding capacities in the Saint-Emilion region in 2005 (*Vitis vinifera* L. cv. Merlot, Bordeaux). (Van Leeuwen et al. 2010).

The sensitivity of the Ψ_{PD} and the Ψ_{stem} , as tools to estimate the plant water status, depends on the physiological characteristics of the grapevine variety, the environmental conditions and the measurement methods. The isohydric behaviour of some vine cultivars probably makes ineffective the use of leaf potential, especially to highlight medium or moderate stresses, in which a reduction in photosynthesis and production can occur without there being any effects on the water relations of the canopy (Cifre *et al.*, 2005). There are conflicting opinions regarding the use of the leaf and stem water potential to evaluate vine water status, in fact they have been compared in different studies to determine which is the best.

Some authors have concluded that Ψ_{stem} is a better estimator of plant water status than Ψ_{PD} and Ψ_{MD} because there is a better correlation with stomatal conductance; in particular the latter is more dependent on climatic conditions (Chonè *et al.*, 2001). Chonè *et al.* (2001) showed that, after heavy rainfall, only the stem water potential showed significant differences with respect to Ψ_{PD} and Ψ_{MD} , therefore it can be considered a more sensitive indicator than the other two. Shackel, 2006 sustains that Ψ_{stem} accurately represents vine water status, even if soil water content is heterogeneous, which is the case of irrigated vineyards. Williams & Araujo (2002) compared Ψ_{PD} , Ψ_{MD} and Ψ_{stem} to determine vine water status in the field. These three methods were compared with each other, with soil water content (SWC). The regression analysis suggested that all three measurements of grapevine water status were highly correlated with one to another. Furthermore all three methods for the estimation of grapevine water status were significantly correlated with SWC (Table II). According to these results the authors sustain that all three methods are equally viable techniques to assess the grapevine water status.

Table II: Regression equations of the method of measuring vine water status as a function of soil water content and the coefficient of determination and its significance level for of “Chardonnay” grapevines (Williams & Araujo, 2002)

Ψ measurement	Regression	r^2
Predawn leaf (Ψ_{PD})	$y = -3.81 + 0.099x$	0.69**
Midday leaf (Ψ_l)	$y = -5.86 + 0.129x$	0.68**
Midday stem (Ψ_{stem})	$y = -5.77 + 0.134x$	0.63**

**Significant at $P < 0.01$.

In contrast, others authors believe that the leaf potential measured at dawn (Ψ_{PD}) is better, due to the strong influence that environmental conditions have on the potential during the day (Remorini & Massai, 2003). Suter et al. (2019), on the contrary, sustain that a drawback of Ψ_{stem} is that it does not allow for temporal comparisons, because the measured value is impacted both by soil water availability and climatic conditions on the day of measurement. When measurements are carried out on several leaves of the same vine, the coefficient of variation (%) of stem water potential is consistently lower compared to pre-dawn leaf water potentials or leaf water potentials (Van Leeuwen *et al.*, 2007). Ψ_{PD} , and Ψ_{stem} are the most widely used water potentials in ecophysiological studies and industry (Dayer *et al.*, 2017), for practical reasons (less laborious, simple, fast, convenient time of the day) growers use often Ψ_{PD} , which gives satisfying results to assess grapevine water status (Rienth & Scholasch 2019). However, leaf and stem water potential indices cannot be used for the automation of irrigation systems (Jones, 2004), because it is a slow, time consuming practice and invasive for the plant. In order to be suitable for automatic monitoring the method must be non-destructive (Afzal *et al.*, 2017). Table III shows a proposal from Van Leeuwen et al., (2009) for stem water potential ranges and the corresponding vine water stress levels.

Table III: Relationship between stem water potential (Ψ_{stem}) expressed in Mega Pascal (MPa) and grapevine water status (Van Leeuwen et al., 2009)

Ψ_{stem} (MPa)	Grapevine water status
$0 > \Psi_{stem} > -0.6$	No water stress
$-0.6 > \Psi_{stem} > -0.9$	Weak water stress
$-0.9 > \Psi_{stem} > -1.1$	Moderate to weak water stress
$-1.1 > \Psi_{stem} > -1.4$	Moderate to severe water stress
$-1.4 > \Psi_{stem}$	Severe water stress

3.2.4 Stomatal conductance

Plants need to fix CO₂ from the atmosphere and, at the same time, limit water loss from leaves in order to avoid desiccation, therefore regulation of stomatal aperture is a fundamental mechanism for plants survival (Chaves *et al.*, 2016). Stomata are microscopic pores located in the epidermis and play a central role in the pathways for both carbon uptake and water loss by plants (Chaves *et al.*, 2016). *Vitis vinifera* L. is considered well-adapted to dry and hot areas and it has been classified as a “drought avoiding” species due to the good control of water loss by stomata (Schultz 2003). The level of stomatal opening is affected by environmental signals, such as drought and leaf temperature that regulate stomatal pore opening and closure. Stomatal closure is among the most relevant and earliest physiological responses of the plant to water stress. In response to these stresses plants synthesize abscisic acid (ABA), the phytohormone that is mainly responsible for stomatal closure, produced in the roots and transported to the leaves, it causes a series of physiological responses influencing the behaviour of the stomata guard cells (Lovisolo *et al.*, 2002); ABA perception by the guard cells is due to ABA receptors consisting of ABA-binding proteins that regulate the guard cells behaviour (Chaves *et al.*, 2011). Stomatal conductance (gs) is a parameter directly connected with the stomatal opening that can be utilized to assess the degree of grapevine water deficit (Cifre *et al.*, 2005). It is measured by evaluating either the water vapour diffusion from the leaf to a humidity sensor using a porometer, or by measuring both water and CO₂ diffusion from the leaf according to their infrared absorption wavelength using an infrared gas analyser (IRGA) (Rienth & Scholasch 2019). The porometer consists of a leaf chamber equipped with humidity sensors which, fixed to the surface of the leaf, allows to determine the time required to increase, within a cuvette of known volume, the humidity between two predetermined levels or the variation of humidity in a certain range (Rienth & Scholasch 2019), this time interval is then used to determine transpiration rate and stomatal conductance. Porometers, however, require regular calibration procedures, because measurements are affected by differences between leaf and atmospheric temperature (Pearcy *et al.*, 2000), besides they are rather expensive instruments, so to be used it is necessary the presence of skilled staff with knowledge in their use and management (Rienth & Scholasch 2019). Stomatal conductance takes into account the different stomatal behaviour of the grapevine and the readiness with which it regulates the stomatal opening and the transpiration flow (Cifre *et al.*, 2005). These aspects can influence the assessing of plant water status by using water potential, so it could be theoretically the best method to determine grapevine water status. For these reasons gs is often used for research purposes to assess plants water status. Due to practical reasons explained before, its application in commercial vineyards is complicated. Table IV shows different, water stress ranges in relation to the stomatal conductance (gs) measurement.

Table IV: Relationship between stomatal conductance (gs) and grapevine water status
(Reworked from Cifre et al., 2005).

gs (mol H ₂ O m ⁻² s ⁻¹)	Grapevine water status
0,5-0,7 > gs > 0,15	Mild water stress
0,15 > gs > 0,05	Moderate water stress
0,05 > gs	Severe water stress

3.2.5 Dendrometry

Soil water deficit affects some physiological traits of plants that can be used as water status indicators (Intrigliolo & Castel, 2007), an example is the trunk diameter variation (TDV). TDV consists in the micrometric measurements of wood organs, carried out at short intervals of time and this phenomenon can be related to the water status of the plant (Huguet, 1985). Trunk diameter variation depends on the water state of the tissues, on the growth rate of the organ and on thermal variations (Intrigliolo & Castel, 2007). Daily dilations and contractions are mainly due to changes in the thickness of the cortical tissues from which the plant derives water reserves to compensate for a transient imbalance during the growing phase of transpiratory demand; Trunk diameter daily variations occur almost entirely at the phloem level, also leading to a slight elastic deformation of the xylem (Molz & Klepper, 1973), due to a radial water transfer from the cortical tissues to the xylem (Parlange *et al.* 1975). This transferring leads to a reduction of hydration level of phloem tissues into a change in cell size; most of diurnal trunk diameter fluctuations involve phloem tissues (Irvine & Grace, 1997). Along the day the leaf water potential tends to assume more negative values in order to support leaf transpiration, during the late afternoon, plant water uptake exceeds water losses by transpiration and there is an increase of leaf water potential. This leads to a shift of the radial water flow, in which the water comes back to the phloem tissues from xylem (Intrigliolo & Castel, 2007). During the night, the trunk returns to the maximum size of the previous day. The growth of plant organs is characterized by an increase in the number or size of cells, in particular cell expansion is a phenomenon strictly dependent on cell turgor which is strictly influenced by tissues water state, therefore it occurs in moments of the day when tissues are well hydrated, that is late afternoon and during the night. It is therefore possible to determine a limit value of the diurnal contraction that indicates the need for irrigation, according to species and organ categories considered during the monitoring of plant water stress (Huguet, 1985).

In order to monitor TDV, it is possible to employ two indices as plant water status indicators: trunk growth rate (TGR) and maximum daily stem shrinkage (MDS) that means the difference between maximum trunk diameter early in the morning and minimum at early afternoon (Intrigliolo & Castel, 2007). Trunk diameter varies during the day reaching a maximum value (MXDT) just before sunrise and a minimum value (MNDT) in the afternoon, the difference between these two values determines the MDS (Goldhamer and Fereres, 2001). The evolution of these two values can provide several informations, for instance the difference between two consecutive MXDT values provides a measure of plant growth rate (Goldhamer and Fereres, 2001). MNDT reflects the effects of evapotranspiration phenomena, while MXDT reflects the rehydration process efficiency (Goldhamer and Fereres, 2001). TGR is obtained by the difference between the MXTD of two or more consecutive days (Intrigliolo & Castel 2007). The ability of both indices to detect plant water stress was evaluated by Intrigliolo and Castel (2007). It varied according to the phenological stage, MDS and TGR are only able to detect vine water stress during a short period before veraison. After veraison no relationship exists between plant water status and these indices (Intrigliolo & Castel, 2007) due to the elasticity loss of the tissues. The sensors used for this scheduling technique are very cheap, so is possible to install an high number of them per field (Cifre *et al.*, 2005).

3.2.6 Carbon isotope discrimination

Ambient atmospheric CO₂ contains 98.9% of ¹²C isotope and 1.1% of ¹³C isotope, carbon in plant tissues comes from CO₂ molecules present in the atmosphere, fixed through photosynthesis (Santesteban *et al.*, 2015). ¹²C is the lighter form and thus the more easily used by the enzymes of photosynthesis for hexose production and the sugar produced contains an higher proportion of the ¹²C isotope than ambient CO₂ (Van Leeuwen *et al.*, 2010), this process performed by the plant is called isotope discrimination. When plants are under water deficit conditions, the isotope rate tends to reduce due to stomatal closure (Farquhar *et al.* 1989). The carbon isotope ratio of plants dry matter ($\delta^{13}\text{C}$) is determined during photosynthesis by the differential diffusion of the two carbon isotopes ¹³C and ¹²C between the atmosphere and the chloroplast, mainly due to the important discrimination against ¹³C in the reaction center of Ribulose Bisfosphate carboxylase/oxydase (Rubisco), due to its intrinsically lower reactivity to ¹³C compared with ¹²C (Van Leeuwen *et al.* 2010; Santesteban *et al.*, 2015). This Rubisco discrimination becomes less intense when CO₂ is scarce in the chloroplast, due to stomatal closure during water stress conditions (Bchir *et al.*, 2016). In other words, under water stress conditions, sugars produced during water deficit situations contain more ¹³C compared to those produced when plant water status is not limiting (Rienth &

Scholasch, 2019). The $^{13}\text{C}/^{12}\text{C}$ ratio in photo assimilates provides a signature of plant water status over the period in which they were synthesised. The $^{13}\text{C}/^{12}\text{C}$ ratio index measured on grape sugar at ripeness, indicates average vine water status during grape ripening (Van Leeuwen *et al.* 2001; Gaudillère *et al.* 2002). The discrimination against ^{13}C is therefore a widely used indicator of WUE (Water Use Efficiency) and water stress in different plants becoming also an interesting parameter for different purposes in relation with water use in grapevines (Santesteban *et al.*, 2015). To apply this methodology grape juice is obtained from grapes sampled at ripeness or close to ripeness, the juice is subsequently centrifuged and $\delta^{13}\text{C}$ is measured using an isotope ratio mass spectrometry (IRMS) in specialized laboratories (Van Leeuwen *et al.*, 2010). IRMS separates charged atoms or molecules according to their mass-to-charge ratio (Santesteban *et al.*, 2015), measurements can be delayed and samples can be frozen before analyses (Herrero-Langreo *et al.*, 2013). The $^{13}\text{C}/^{12}\text{C}$ ratio in the sample is compared to that in an international accepted standard, the PDB or Pee Dee Belemnite standard which is a rock in which this ratio is particularly stable (Equation 1) (Santesteban *et al.*, 2015).

$$\delta (\text{‰}) = (\text{R}_{\text{sample}} / \text{R}_{\text{standard}} - 1) * 1000 \quad (\text{Eq. 1})$$

$\delta (\text{‰})$ = Isotopic ratio

R_{sample} = isotope ratio of the sample

$\text{R}_{\text{standard}}$ = isotope ratio of the standard

It is usually expressed as per mill or parts per thousand deviation from that standard and varies from $-20 \text{ p. } 1000$ (severe water deficit stress) to $-27 \text{ p. } 1000$ (no water deficit stress) (Gaudillere *et al.*, 2002) (Table V). Repeatability is excellent and it is very accurate, $\delta^{13}\text{C}$ is well correlated Ψ_{stem} values measured between veraison and ripeness (Figure 3).

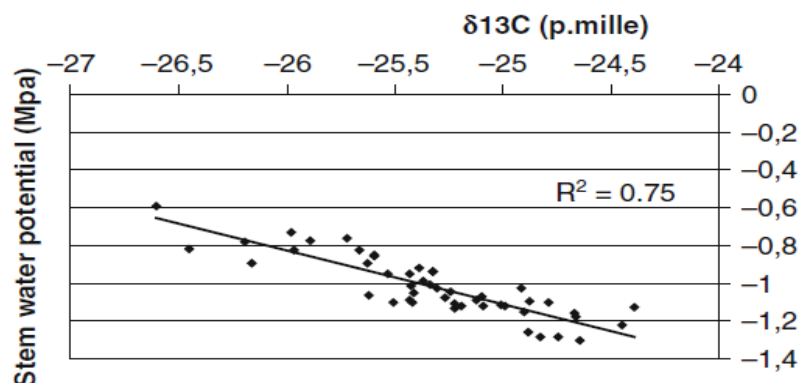


Figure 3: Correlation between stem water potential, measured one week before harvest, and $\delta^{13}\text{C}$ measured on grape sugar at ripeness in *Vitis vinifera* cv. Merlot in 2004 located in the Bordeaux area (Van Leeuwen *et al.*, 2010).

Different plant tissues can be used as samples for ^{13}C discrimination, leaves and berries are the most common ones. For leaf samples, the correspondence can be quite high (Tomás *et al.*, 2012), but variable depending on the environmental conditions (Medrano *et al.*, 2015) and the leaves age (Bchir *et al.*, 2016). Berries usually show better correspondence than leaves with respect to the variations in soil water availability as shown by Chaves *et al.* (2007). Bchir *et al.* (2016) show that there is an important effect of leaf age on the $\delta^{13}\text{C}$, so a careful procedure of leaf sampling is necessary to avoid contradictory conclusions in respect to the representativeness of the plant water status, in particular $\delta^{13}\text{C}$ of young leaves placed in the upper-middle part of the canopy were more significantly correlated with plant water status than older leaves located in the middle-basal part of the stems. However, these results have demonstrated that when sampling is carried out on berries, the estimation of leaf water status based on $\delta^{13}\text{C}$ is much more reliable. The lower correspondence showed by leaves is related to the fact that carbon stored in the leaves is mainly taken up before summer water stress, meanwhile fruit sugar accumulation occurs later in the growing season (De Souza *et al.*, 2005; Chaves *et al.*, 2007; Santesteban *et al.*, 2015). The advantage of the carbon isotope discrimination method is that it requires only the grape sampling at ripeness (Van Leeuwen *et al.*, 2010), this is not the case of the pressure chamber, which is a more time consuming and labour intensive tool. When a dry-farming grower wants to know if some of his vineyards block would benefit from irrigation, $\delta^{13}\text{C}$ can be used as an objective tool to assess the level of vine water deficit stress on each vineyard block. However, because $\delta^{13}\text{C}$ is measured on grape sugar at ripeness, the isotopic composition allows assessment of the water deficit experienced by the grapevines throughout the growing season (De Souza *et al.*, 2005). This is a very important aspect because the water status has an effect on photosynthesis activity. This influence is particularly important in deciduous woody species, such as grapevine, where stored organic compounds are the dominant carbon sources for leaf growth in the early spring (De Souza *et al.*, 2005). De Souza *et al.* (2005) evaluated $\delta^{13}\text{C}$ of commercial vineyards submitted to different watering treatments on leaf and berries. They observed that the carbon isotope composition was different in leaves and berries among treatments, in particular berries $\delta^{13}\text{C}$ was better correlated with stem water potential. These results suggested that berries isotopic composition can be a good indicator to evaluate the WUE of grapevines. Contrary to leaf and stem water potential, this technique cannot be used for day-to-day irrigation management but it represents however a useful and reliable tool to evaluate irrigation strategies adopted in the past season and can help to optimize future irrigation strategies (Van Leeuwen *et al.*, 2010; Rienth & Scholasch, 2019). The only limiting factors can be the number of measurements because the sampling phase of berries takes time and it also influences the costs to be incurred to analyse all samples (Herrero-Langreo *et al.*, 2013).

Table V: Relationship between $\delta^{13}\text{C}$ values expressed in p.mille and grapevine water status
(Van Leeuwen et al., 2010)

$\delta^{13}\text{C}$ values (‰)	Grapevine water status
$\delta^{13}\text{C} < -26$	No water deficit
$-24.5 < \delta^{13}\text{C} < -26$	Weak water deficit
$-23 < \delta^{13}\text{C} < -24.5$	Moderate to weak water deficit
$-21.5 < \delta^{13}\text{C} < -23$	Moderate to severe water deficit
$\delta^{13}\text{C} > -21.5$	Severe water deficit

3.2.7 Sap flow based measurements

Sap flow (SF) is the movement of fluid in the roots, stems and branches of plants, it is typically measured in the xylem of plants, more specifically, it is measured in the sapwood portion of xylem, or the conducting wood, which differs to the other portion of the xylem known as heartwood (Smith & Allen, 1999). SF is synonymous with water movement in plants, however, it is not referred to as water flow, or water velocity, as the fluid in a plant's stem is not pure water. The fluid contains other elements, such as nutrients and hormones, and therefore it is called sap (Lemeur *et al.* 2009). The SF should not be confused with the sugary sap in the phloem of plants. Monitoring sap flow dynamics of plants can thus provide fundamental information of plant hydraulic function or dysfunction in a given environment and provide an estimation of plant transpiration (Steppe *et al.*, 2015). There are different sap flow based methods that can be applied. The heat pulse, Granier, and heat balance methods, which have been used to determine sap flow in the research of grapevine water status. With these methods, without any alteration of the leaf microclimate, a realistic and direct estimate of the water losses of the plant or of a sprout is provided, given that the SF is directly related to the transpiratory activity (Smith & Allen, 1999; Jones *et al.* 2004). The heat-pulse technique consists in inserting inside two holes made in the stem of the plant, in the lower one, a heat source and in the upper one, a thermocouple, generating an impulse lasting 1-2 seconds, the time taken by the heat wave to reach the thermocouple corresponds to that taken by the lymph to overcome the set space (Smith & Allen, 1999). The heat pulse method is semi-continuous in that only the measurements taken in the first few minutes of a 15-30 minute interval are used to calculate sap flow velocity (Braun & Schmid, 1999). Measurements can only occur in this interval because is necessary to dissipate the developed heat in order to bring the

instrument back to initial condition, after this a new measurement can start. The major limitations of this approach are that it only measures sap velocity that is different from the stream velocity of water in a trunk or stem of plants (Sakuratani, 1981). SF velocity multiplied by the cross sectional conducting wood area gives the volume flow per unit time, furthermore the calculation of sap flux from the sap velocity requires knowledge of the active conducting area of the xylem, a figure that is difficult to obtain, because it is not constant over time (Reader & Carlone, 2003). Thermal dissipation probes method, invented by Granier (1985), involves the use of two probes that are inserted into the stem of the plant. The system consists of a continuous heated needle and a reference needle, both contain a thermocouple, this approach is based on the temperature difference between heated and reference probes decrease when sap flow increases. The Granier system differs from the heat pulse method in that the supply of heat is constant versus a pulse of heat as in the heat pulse method. The Granier method measures temperature difference between the probes whereas the heat pulse method measures the rate at which the pulse of heat travels upstream from the heat source. The Granier method for calculating sap flow uses different equations well explained in Granier (1987), however Vergeynst et al. (2014) showed that circumferential and radial variation of sap flux density can lead to both under- and overestimations of sap flow. Furthermore, sap flux density can be underestimated when the heated needle is in contact with non-conducting tissues, for example dead biomass from pruning wounds. Therefore, the thermal dissipation probe method is not suitable for commercial use. As explained previously one of the major limitations of these approaches is the correct insertion of the needles into the wood that affects the measurement of sap velocity. To overcome this point the stem heat balance method was developed as non – invasive technique. The heat balance method involves encircling a plant stem with a flexible heater band to apply a known amount of energy to the stem, and then accounting for the dissipation of that energy within the system (Lascano *et al.*, 2016). This technique is based on the principles of conservation of energy and mass, where all inputs and outputs are considered and works by applying a known amount of heat to a small segment of the stem. It consists of using a heated sleeve wrapped around the stem as described by Lascano et al. (2016), heat is provided uniformly and radially across the stem section. The sleeve is very flexible so is possible to apply it also on slightly bent stems. The heat balance method can be applied even if sap flow trajectory through the stem is tortuous (Lascano *et al.*, 2016). Different series of thermocouples are thus used to quantify the heat flow transferred from the heating element upwards, downwards and radially. By evaluating the difference between the heat supplied and that lost, the heat dissipated by convection is obtained with the flow of lymph along the stem which can be directly correlated with the flow of water (Sakuratani, 1981). Cancela et. al (2017) studied advantages and disadvantages of different techniques for monitoring grapevine water status in economic terms, they showed that the equipment

required for sap flow measurements have high costs compared to others techniques, this is also due to the short equipment durability of about two years, so the application and maintenance of SF instrumentation are not sustainable for every vine grower.



Figure 4: A sap flow sensor installed in the vineyard (Rienth & Scholasch 2019)

3.2.8 Thermography

Nowadays the most common approaches used to assess vine water status are mainly destructive methods, which require high labour, time and significant costs (Rienth & Scholasch 2019). In recent decades, several studies were carried out in order to develop new and efficient possibilities for fast and accurate remote diagnosis of a wide range of plant stresses. One of these innovative possibilities is thermal imaging as a fast and non – destructive method to assess indirectly the water condition of grapevine (Costa *et al.*, 2010). Remote sensing of vegetation is a non-invasive methodology to monitor physical and physiological characteristics of plants and to evaluate the effects of environmental stresses on plant performance (Jones & Vaughan, 2010). It allows observations at different scales, in order to collect data from single leaves to entire canopy and fields (Costa *et al.*, 2013). It includes several imaging techniques such as visible imaging, near-IR and thermal IR imaging (Costa *et al.*, 2013), thermal imaging or thermography is one of the most used in agronomic and environmental sciences (Jones & Vaughan, 2010; Maes & Steppe, 2012). Thermal imaging is based on the use of specific electromagnetic radiations located in the region of the spectrum between 0.75 and 1000 μm (Figure 5) called Infrared Radiations (IR). Every object with a temperature above the absolute zero (0 K) emits infrared radiations (Costa *et al.*, 2010). Every electromagnetic radiations is characterized by a wavelength (μm) and a frequency (Hz), these determine the amount of energy possessed by that specific radiation.



Figure 5: Visible and thermal infrared spectrum (adapted from Gaussorgues, 1999)

One of the most important concept related with thermal imaging is the emissivity (ϵ), which describes the capacity of a material to radiate energy, it represents the amount of radiation emitted from an object as a fraction of that emitted by a blackbody and varies according to the type of material (Costa *et al.*, 2010). A blackbody is a theoretical object, totally black that absorbs all radiations that hit it, so its emissivity is considered $\epsilon = 1$ (Costa *et al.*, 2010). Real objects are not blackbody objects, this means that they absorb a certain fraction of radiations and the rest is reflected or transmitted, so their emissivity correspond to $0 < \epsilon < 1$, (Costa *et al.*, 2010). Plant material has high ϵ values, varying between 0.91 and 0.97 (Jones & Vaughan, 2010). The amount of radiation emitted by an object depends on the absolute temperature of the object, its emissivity and type of material (Costa *et al.*, 2010). According to the Stefan – Boltzmann law (Equation 2) it is possible to determine the amount of IR energy emitted by the object (Costa *et al.*, 2010), according to the relation:

$$W = \epsilon * \sigma * T^4 \text{ (W cm}^{-2}\text{)} \quad (\text{Eq. 2})$$

W = spectral exiting radiation

ϵ = Emissivity (dimensionless)

σ = Stefan Boltzmann Constant ($5.67 \times 10^{-12} \text{ W cm}^{-2} \text{ K}^{-4}$)

T = Temperature (K)

When the total radiation emitted by an object and its emissivity are known, the temperature of that object can be calculated (Costa *et al.*, 2010). Leaves interact with the environment through energy exchange processes. Plants need to maintain their energy balance in equilibrium in order to optimize their metabolic functioning, when this equilibrium is unbalanced the leaf temperature changes (Jones 1992, Lambers *et al.* 1998). Through the transpiration process plants regulate the temperature of the leaves, the water evaporation causes the leaf cooling and the heat loss correspond to the energy needed for the evaporative process (Costa *et al.*, 2010).

In the use of thermal imaging to detect temperature, we must consider three major IR radiation streams (Costa *et al.*, 2013):

- 1) The radiation leaving the object's surface (Costa *et al.*, 2013)
- 2) The radiation emitted by the object's surroundings and further reflected by the object's surface, commonly named background radiation ($W_{background}$) (the first two fractions of radiation are modified by transmission through the atmosphere) (Costa *et al.*, 2013)
- 3) Any radiation emitted by the atmosphere (W_{atm}) (Costa *et al.*, 2013)

Therefore, the total radiation is given by the relation below (Equation 3):

$$W = \tau [\epsilon \sigma (T_s)^4 + (1 - \epsilon) W_{background}] + W_{atm} \quad (\text{Eq. 3})$$

W = spectral exiting radiation

τ = atmospheric transmissivity (dimensionless)

ϵ = Emissivity

σ = Stefan Boltzmann Constant ($5.67 \times 10^{-12} \text{ W cm}^{-2} \text{ K}^{-4}$)

T = Temperature (K)

$W_{background}$ = background radiation (W m^{-2})

W_{atm} = the radiance emitted by the atmosphere (W m^{-2}).

Thermal sensors are used to remotely measure leaf temperature, which increases when water stress conditions occur. During stress conditions stomatal closure occurs, it reduces the water loss and at the same time interrupts the cooling effect of evapotranspiration (Matese & Di Gennaro, 2015). The sensors used for thermal measurements are IR sensors that operate in the 3 – 5 μm and 7 – 14 μm regions. The early IR sensors needed to be cooled by cryogenic fluids or gasses, these tools result very heavy and expensive, and they operate in the 3 – 5 μm region which provides extremely sensitive and accurate measurements (Costa *et al.*, 2010). Today, the most common thermal device are uncooled detectors (Kaplan, 2007), less heavy with better handling which operate in the 7–14 μm IR region (Costa *et al.*, 2010). The development of miniature uncooled thermal sensors has now been installed on small aircrafts to estimate crops surface temperature (Gago *et al.*, 2013). One of the main problems of thermal cameras is the reduced resolution due to the temperature of background pixels that don't belong to the canopy, causing quality reduction of data (Jones & Sirault, 2014). Another important consideration concerning the use of thermal camera is that the calibration, must be performed using blackbodies varying target at room temperature for the development of calibration algorithms (Zarco-Tejada *et al.*, 2012).

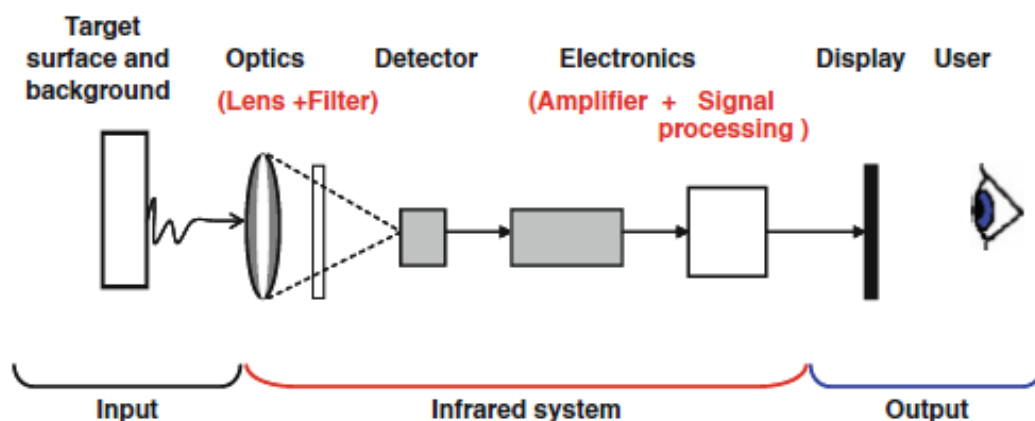


Figure 6: Representation of the components of an IR thermal imaging device (Costa et al., 2010).

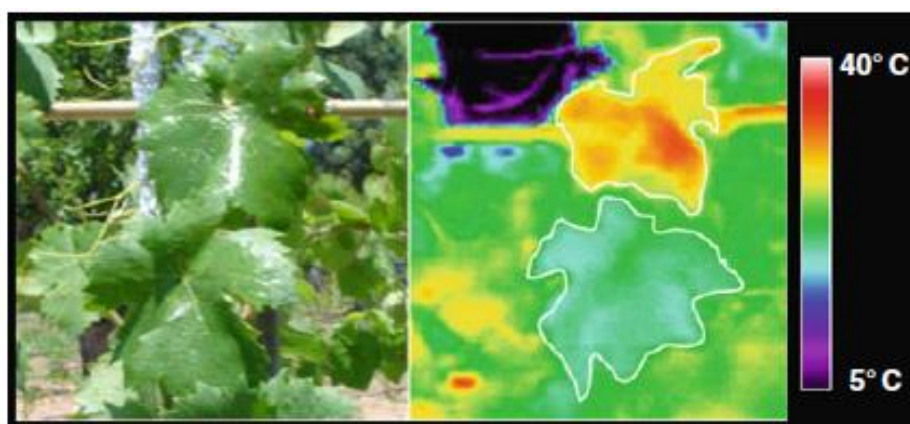


Figure 7: Example of a thermal imaging measurement result (Costa et al., 2010).

The most widespread application of thermal imaging measurements is the estimation of leaf stomatal conductance (g_s), the main advantages of this method are reduced time consumption and the capacity to assess the g_s in large crop areas (Costa *et al.*, 2010). In order to assess grapevine water status through thermal imaging the crop water stress index (CWSI) is used (Equation 4), ranging from 0 to 1 (values close to one are related to high levels of stress) (Idso *et al.*, 1981; Idso *et al.*, 1982) and the stomatal conductance index (I_g) (Equation 5) (Jones *et al.*, 2002). Both indices are calculated from leaf/canopy temperatures relative to dry and wet

reference surfaces that are completely wet or dry to simulate maximum and minimal leaf transpiration under the exposed environmental conditions. (Costa *et al.*, 2010; Gago *et al.* 2015). Thermal indices have been developed in order to reduce the influence of environmental fluctuations in the canopy temperature (Gutierrez *et al.*, 2017).

$$CWSI = (T_{plant} - T_{wet}) / (T_{dry} - T_{wet}) \quad (Eq. 4)$$

T_{plant} = canopy temperature

T_{dry} = temperature of an high stressed canopy

T_{wet} = temperature of a well irrigated canopy

$$lg = (T_{dry} - T_{leaf}) / (T_{leaf} - T_{wet}) \quad (Eq. 5)$$

T_{dry} = temperature of a leaf with closed stomata

T_{leaf} = leaf temperature of the plant of interest

T_{wet} = temperature of a leaf with fully open stomata

In recent years, several studies were carried out in order to determine the correlation between water potential and thermal indices and their capacity to estimate grapevine water status. Gutierrez *et al.* (2017) conducted an experiment in a commercial vineyard located in Tudelilla, La Rioja, Spain, during late August 2016, on Tempranillo grapevine variety. Three different water treatments were deployed in order to induce variability within the vineyard water status, midday stem water potential (Ψ_{stem}) was used as reference method of the plant water stress. The acquisition of the on-the-go thermal images was performed using a FLIR A35, uncooled thermal camera mounted in the front part of an all-terrain-vehicle (quad). Crop water stress index (CWSI) and stomatal conductance index (lg) were calculated according to previously relations reported. Due to the North-South vine rows orientation, both east and west sides of the canopy were monitored at the same time.

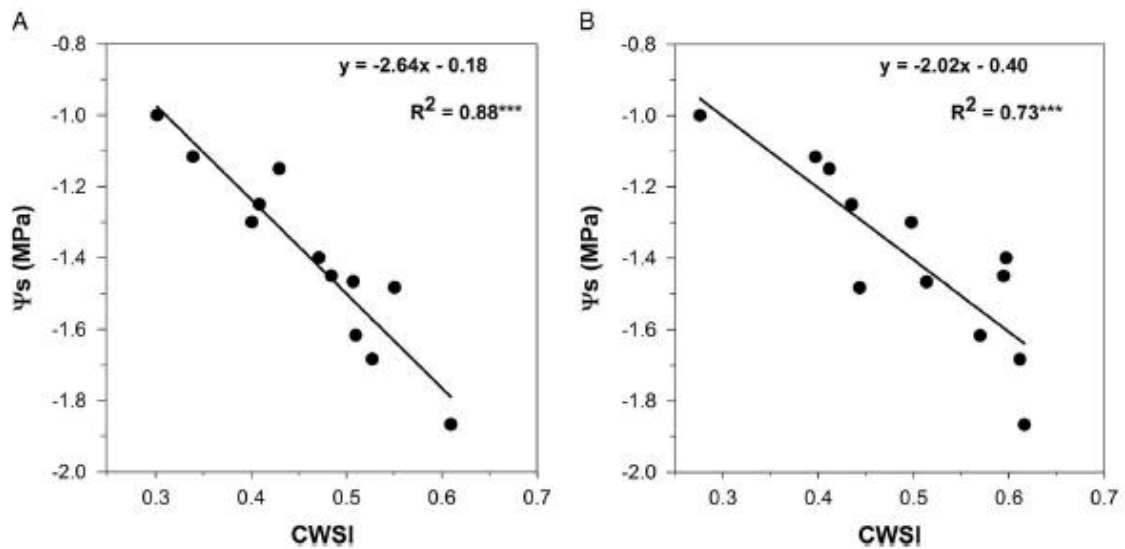


Figure 8: Graphs that show linear correlation between CWSI and Ψ_{stem} in east (A) and west (B) sides of the canopy (Gutierrez et al. 2017).

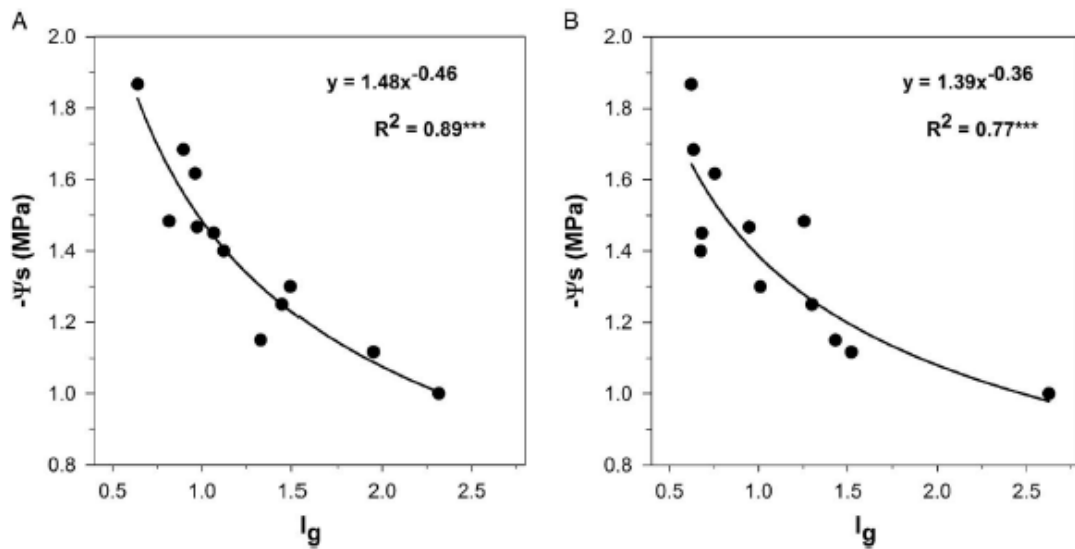


Figure 9: Graphs that show a good correlation between I_g and Ψ_{stem} in east (A) and west (B) sides of the canopy (Gutierrez et al. 2017).

Graphs show a good correlation between stem water potential and thermal indices, in particular the CWSI shows a linear correlation. The evaluation of vine water status using on-the-go thermal imaging on the side of the canopy, could give the possibility to evaluate water stress during an in-field work at the same time (Gutierrez *et al.* 2017), so it is necessary to install a thermal capturing system in agricultural vehicles.

High resolution thermal cameras have been successfully mounted also on aircraft platforms (Sepulcre-Canto *et al.*, 2006), on unmanned aerial (UAV) and terrestrial vehicles, increasingly using higher performance sensors in terms of lower size and weight, and of greater spectral and spatial resolutions. Technological development in the field of automation has provided precision viticulture with a new solution for remote monitoring, UAVs. Fixed or rotary wing platforms able to fly autonomously to a user defined set of waypoints, or can be remote controlled at visual range by a pilot on the ground (Matese & Di Gennaro, 2015). These platforms can be equipped with a series of sensors, which allow a wide range of monitoring operations to be performed. The peculiarity of UAV application in remote sensing is the high spatial ground resolution, and the possibility of highly flexible and timely monitoring, due to reduced planning time. These features make it ideal in vineyards of medium to small size (1–10 ha), especially in areas characterized by elevated heterogeneity (Matese & Di Gennaro, 2015).

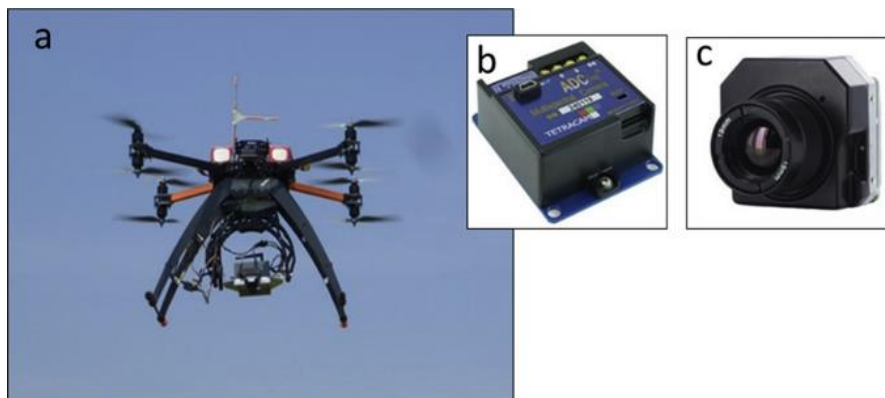


Figure 10: UAV platform (a), detailed view of multispectral (b) and thermal (c) cameras mounted on it (Santesteban *et al.*, 2017).

In this approach a thermal camera is directly used to get a lateral view, or mounted on a shaft or a crane, to get a zenithal view (Santesteban *et al.*, 2017). On the contrary, the implementation of UAV-based thermal imaging solutions has not been well explored yet in viticulture, since the resolution obtained must be sufficient to enable targeting pure canopy pixels, avoiding mixed soil/vegetation pixels (Gonzalez-Dugo *et al.*, 2015), which is particularly complicated in most vineyards due to the structure of the crop, trellised in narrow rows.

Gonzalez-Dugo et al. (2013) suggested that the requirements to achieve the water stress monitoring using aerial platforms are:

- 1) Establish a strong correlation between stress indices and actual water stress in the field
- 2) The spatial resolution must be sufficient to enable targeting pure canopy pixels, avoiding mixed soil/vegetation pixel
- 3) The capability to evaluate entire fields in individual flight
- 4) Faster turn-around acquisition times and processing in order to provide quasi-real time water status maps helping the farmer decision-making process

Santesteban et al. 2017 estimated plant water status within Tempranillo vineyard of 7.5 ha, using high resolution UAV – based thermal imaging (9 cm pixel⁻¹) in order to avoid pixels that don't belong to plants canopy, where part of the temperature of the pixel comes from the background and not from the pure canopy, considerably reducing the quality of the data (Jones & Sirault, 2014). Leaf data acquired in the thermal infrared spectral region allowed the computation of water stress related to leaf temperature, through the estimation of CWSI (Eq.4). Thermal index values were compared to stem water potential (Ψ_s) and stomatal conductance (gs) (Figure 11 – 12). CWSI values obtained from thermal images showed a relatively good correspondence with both Ψ_s and gs.

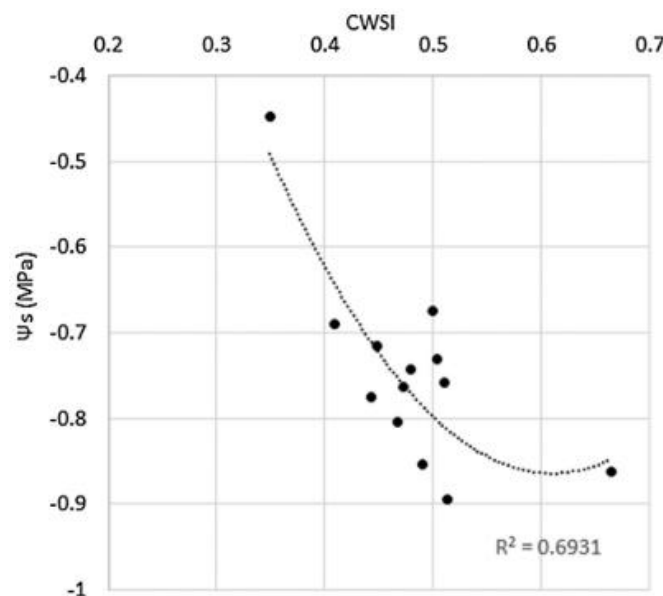


Figure 11: Comparison of Crop Water Stress Index (CWSI) values calculated from UAV-acquired thermal images and stem potential measured at the same time (Santesteban et al., 2017)

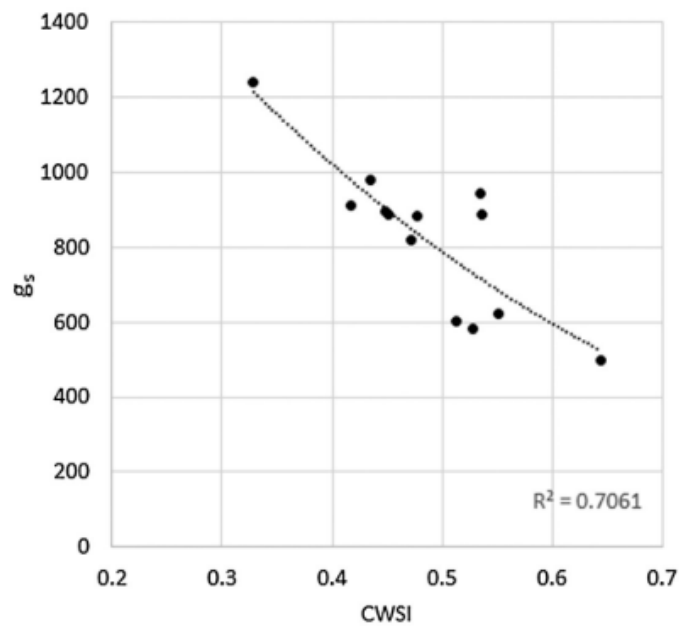


Figure 12: Comparison of Crop Water Stress Index (CWSI) values calculated from UAV-acquired thermal images and stomatal conductance (gs) measured at the same time (Santesteban et al., 2017)

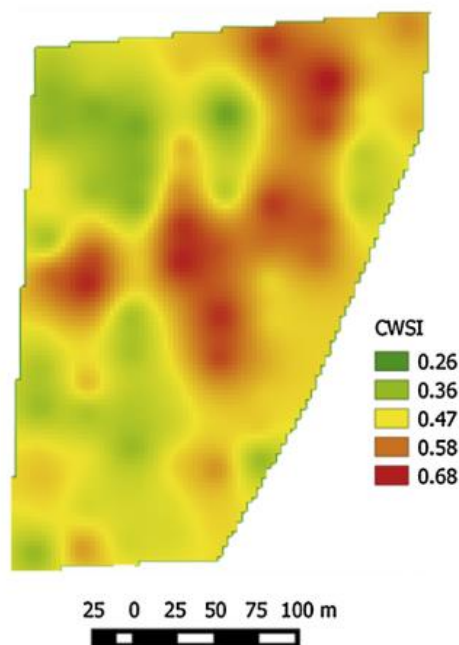


Figure 13: Map showing variation in Crop Water Stress Index (CWSI) (Santesteban et al., 2017)

As it is possible to observe, the graphs show a good correlation between CWSI and reference methods. Moreover it is possible to combine UAV- based thermal imaging with GPS technology to build stress maps (Figure 13) that show seasonal variability of plant water stress in the vineyard. Thank to this methodology it is possible to view the plant water status trend along the time and set stress thresholds beyond which irrigation will be appropriate. High-resolution UAV-based thermal imaging has shown a high potentiality for precision vineyard management applications, and could be a complementary tool for the implementation of precision irrigation systems that, in the near future, would help grape growers to manage water resources in a sounder and more sustainable way. Compared to conventional platforms (such as manned aircrafts and satellites), UAVs present several advantages: they fly at lower altitudes, better images spatial resolution and they cost less, allowing for higher monitoring frequencies (Gago *et al.*, 2015). The use of remote sensing, including thermal imaging, can contribute to the introduction of new crop management strategies in order to optimise the use of inputs and reduce the impact on the environment, it's important to underline that is required skilled staff for the management of these tools because periodically calibrations are necessary for good acquisition and interpretation of data. However, it is clear that these new approaches that rely on new technologies will increasingly take hold over time, and will be able to contribute considerably to irrigation automation.

3.2.9 Leaf sensors to measure leaf thickness and electrical capacitance

Today an important goal of research is to develop non-destructive systems that allow continuous and reliable monitoring of the plant water status in order to intervene more promptly (Afzal *et al.*, 2017). The challenge is to develop non - destructive plant-based devices able to automatically and continuously estimate plant water status directly from the plant, offering more reliable results than current methodologies, for instance evapotranspiration models or soil moisture measurements (Jones, 2004). A new trend as non- invasive and direct method could be the monitoring of leaf thickness and electric capacitance. The mechanism behind the relationship between leaf electrical capacitance and water status is based on the fact that the leaf electrical capacitance changes in response to variation in plant water status and ambient light (Afzal *et al.*, 2017). The analysis of leaf thickness and capacitance variations indicate plant water status - well-watered versus stressed (Afzal *et al.*, 2017). Leaf thickness was sometimes used as an indicator for water stress, several researchers have reported a strong positive relationship between leaf thickness and leaf water content (Burquez, 1987; McBurney, 1992). These studies suggested leaf thickness variations as a potential plant-based method for monitoring water status, but they did not provide details about the variations in leaf thickness and its relationship to soil moisture content or plant water status.

Leaf thickness monitoring devices are commercially available and nowadays, there are different probes that provide this type of measurements. They are non-invasive sensors but have the disadvantage that changes in water status are frequently not reflected sensitively in changes of leaf thickness. Furthermore in addition the sensors used to apply this method are bulky and not appropriate for practical applications (Afzal *et al.*, 2017). Variations in the dielectric properties of a leaf can be an alternative approach to estimate plant water status, this method is based on the leaf electrical capacitance (CAP) (Equation 6) that means the ability of a substance to store electrical energy in an electric field (Jones *et al.*, 2006; Afzal *et al.*, 2017). The CAP can be used to determine the electrical capacitance of a material and it varies according to leaf dielectric constant.

$$CAP = \epsilon_r \epsilon_0 A / d \quad (\text{Eq. 6})$$

CAP = electrical capacitance (Farad, F)

ϵ_r = leaf dielectric constant (dimensionless)

ϵ_0 = permittivity of vacuum (8.854×10^{-12} F m⁻¹)

A = area of a capacitor sheet (m²),

d = the distance between the sheets (m)

ϵ_r depends on leaf chemical composition, leaf temperature, water content, type of leaf tissues and frequency of the electric field (Jones *et al.*, 2006; Afzal *et al.*, 2010), this means that the variations in the water content and the dielectric constant of the leaf are linked. The dielectric constant of water is significantly higher than that of many other substances present in nature, due to the polar properties of the water molecules (Jones *et al.*, 2006). The same authors observed that the biomass water content has a strong positive correlation with the dielectric constant suggesting that dielectric constant measurement of a leaf may be an applicable technique for estimating plant water status. In order to investigate the potential use of leaf thickness and leaf electrical capacitance (CAP), Afzal *et al.* (2017) integrated into a leaf sensor (Figure 14) the capability to simultaneously measure leaf thickness and CAP, which has never been done before. This sensor was applied in tomato plants, where measurements of CAP were made, together with plants relative leaf thickness (RLT), which was calculated by normalizing (the measured leaf thickness divided by the initial leaf thickness). This RLT approach was used to reduce the variability of the leaf thickness measurements. These results showed that plant-based sensors that measure RLT and CAP can be used to measure water stress directly on plants instead of indirectly using soil moisture content or evapotranspiration models. The results also showed that the patterns of RLT and CAP dynamics could be divided into three phases, which correspond to three plant water conditions: well-watered (non-

sensible water stress), water stress, and desiccated. The results suggested that detection of the transition from well-watered to water- stressed conditions can be used as a threshold for irrigation scheduling in field applications. Currently, this research group is developing an algorithm to translate the leaf thickness and electrical capacitance variations to meaningful information about plant water status. It is important to underline that during the bibliographic research no case studies on the application of this approach on the grapevine were found. This is only a brief mention about a possible new trend for the application of non-invasive methods for determining the water status of the plants; it cannot be excluded that, in the near future, it may also be applied to the grapevine.

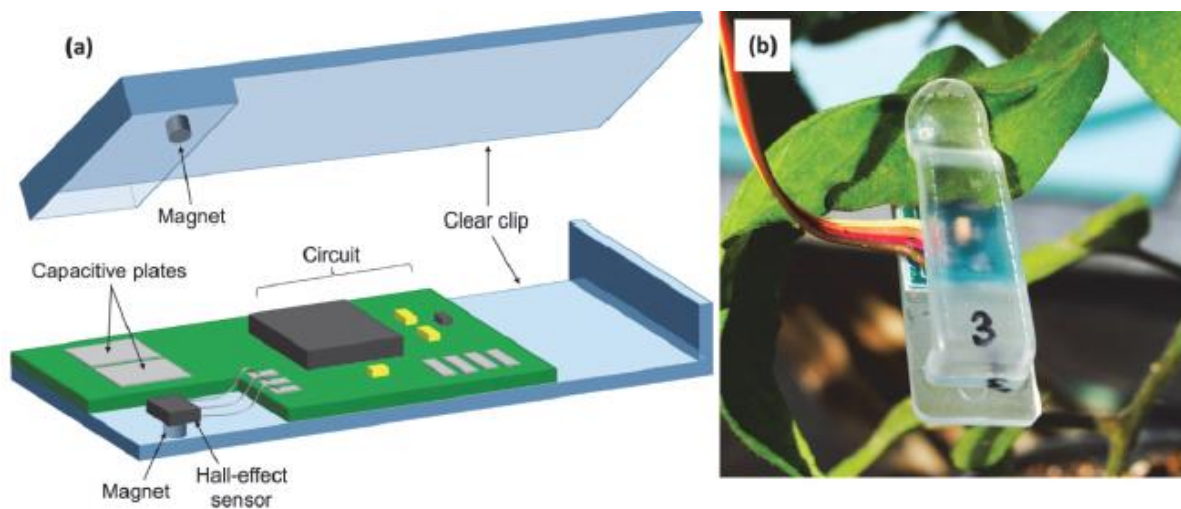


Figure 14: (a) Diagram of the leaf sensor and (b) photo of the integrated leaf sensor used to measure leaf thickness and CAP on a tomato leaf (Afzal et al., 2017)

4. Conclusions

Global warming will increase the risks of drought periods and threatens a commercially sustainable wine production in many growing regions. Several mitigation strategies, such as irrigation exist to sustain a sustainable and quality-oriented viticulture even under very dry conditions. The basic idea of this work was to review the main techniques to estimate water status using some representative studies, in order to help the understanding and provide knowledge of the current methods to assess grapevine water status. These methods will help the growers to properly manage irrigation, in order to mitigate climate change effects. In this work the relationships between the vine and the water during the course of its development cycle were explored and different irrigation techniques that can be applied were described, as showed in Table VI. The different stomatal behaviours of the grapevine, the readiness with which vine regulates the stomatal opening and the transpiration flow have led to define the g_s

as one of the best indicators for determining the water status of the plant, as it takes into account both the behaviour of the variety and the environmental fluctuation conditions. However instruments are not easy to use and the presence of skilled staff is required for the application. Ψ_{PD} and Ψ_{stem} are also very robust methods that can be applied easily in grapevine, where a certain level of stress must be maintained and where irrigation should be carried out when an alteration in the water balance is highlighted. Vine growers, in fact, tend to prefer techniques that are relatively easy to apply and which, at the same time, provide the most reliable result possible. Trunk diameter fluctuations is considered particularly beneficial for its monitoring ability, especially if the stress is moderate or high. The sensors also have a low cost and this could allow continuous measurements to be made, especially in those emerging countries that systematically resort to irrigation of the vineyard to obtain optimal productions both in terms of quantity and quality. However the ability of this approach to detect water stress depends on the phenological stage of the plant. $\delta^{13}C$ turns out to be a very interesting technique, as it allows to further deepen the physiology of the plant, but it cannot be applied as a day-to-day technique for monitoring the plant water status. However, it is a very useful and reliable tool for an assessment of water management adopted to the vineyard during the season and can help to optimize future irrigation strategies. Sap Flow based measurements can be also an important tool for estimate the rate of transpiration, up to now the most applicable methodology is the stem heat balance method as a non-invasive approach. The application of non-intrusive sap flow sensors has been successfully adopted as a practice to drive irrigation strategies, however the high cost of the instrumentation and the complexity of interpreting the data make this technique difficult to use for practical purposes. A relatively new approach that could spread more and more in the coming years is the use of thermal cameras installed in terrestrial platforms and UAVs. In fact thermography is very interesting and robust method thanks to the use of the CWSI and I_g indices which allow a reliable estimation of the plant water status. Moreover thermography could lead to the reduction of time spent and to the automation of the monitoring of the vineyard's water status, however, it is necessary to improve the instrumentation, making it easier to use and manage. In order to ensure an increasingly efficient water management in the vineyard and to obtain quality productions, it would therefore be desirable to develop new low-cost and easy-to-use devices for continuous monitoring of the grapevine water status, and at the same time, improve existing techniques making them more reliable, easy to use and accessible for winegrowers in order to easily achieve the automation of irrigation management.

Table VI: Summary of the reviewed techniques to assess the grapevine water status

Method	Accuracy	Destructive/Non destructive	Possibility of irrigation management	Cost/special equipment	Better suited for vine growers/researchers/vine growers through subcontracting
Visual analysis	Not accurate	Non destructive	No	No equipment requested	Vine growers
Leaf water potential	Accurate	Destructive	Yes	Sustainable cost - Scholander pressure chamber	Vine growers
Stem water potential	Accurate	Destructive	Yes	Sustainable cost - Scholander pressure chamber	Vine growers
Stomatal conductance	Highly accurate	Non destructive	Yes, only for research purpose	High cost - Porometer	Researchers
Dendrometry	Not very accurate	Non destructive	Yes, only in specific phenological stages	Sustainable cost - Devices for micrometric trunk measurements	Researchers/Vine growers
Carbon isotope discrimination	Highly accurate	Destructive	No	Variable cost according to the number of measurements	Researchers/vine growers through subcontracting
Sap flow	Not very accurate	Non destructive	Yes, difficult to apply	High management costs of sap flow devices (probes and heat source)	Researchers
Thermography	Accurate	Non destructive	Yes	Variable cost according to the thermal camera accuracy	Vine growers through subcontracting
Leaf thickness and electrical capacitance	Accurate	Non destructive	Yes	High cost	Researchers

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