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# Physiological and spectral characterization of leaf demography development on resistant grapevine varieties

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# Abstract

This thesis aims to underline the evolution of physiological characteristics and spectral properties during leaf development on the grapevine. In particular, the study was carried out on resistant grapevine varieties known as PIWI. Spectroscopy in viticulture has often been used during the past decades to characterize the spectral properties of vegetation. The quantity of radiation that is reflected or adsorbed from a plant is closely related to the water content and color of its leaves, thus to their chlorophyll content, which depends on the nitrogen content. Therefore, reflectance measurements at specific wavelengths, are useful in assessing the physiological state of the leaf and the plant in general. The study has also considered the physiological aspects related to leaf gas exchange and photosynthetic capacity and how they vary during the vegetative cycle of the leaf. The Plastochron index was used to calculate the biological age of leaves and the entire sprout, to detach the calendar time, and to express this physiological leaf evolution with the biological time.

## Riassunto

Questa tesi intende sottolineare l'evoluzione delle caratteristiche fisiologiche e delle proprietà spettrali durante lo sviluppo fogliare sulla vite. In particolare, lo studio è stato condotto su varietà resistenti di vite note come PIWI. La spettroscopia in viticoltura è stata spesso utilizzata negli ultimi decenni per caratterizzare le proprietà spettrali della vegetazione. La quantità di radiazione che viene riflessa o adsorbita da una pianta è strettamente correlata al contenuto di acqua e al colore delle sue foglie, quindi al loro contenuto di clorofilla, che dipende dal contenuto di azoto. Pertanto, le misure di riflettanza a specifiche lunghezze d'onda sono utili per valutare lo stato fisiologico della foglia e della pianta in generale. Lo studio ha anche preso in considerazione gli aspetti fisiologici legati allo scambio di gas, alla capacità fotosintetica e come questi variano durante il ciclo vegetativo della foglia. L'indice Plastochron è stato utilizzato per calcolare l'età biologica delle foglie e l'intero germoglio, per staccare il tempo di calendario e per esprimere questa evoluzione fogliare fisiologica con il tempo biologico.

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# CHAPTER 1: Introduction and project aim

The aim of this thesis is to highlight the evolution of the transpirative capacity and the spectral properties of the leaves of different resistant varieties during growth.

It has been analyzed the physiological aspects of different shoot leaves related to transpiration during their seasonal evolution. In order to better evaluate the evolutionary aspect of the leaf it has been relied on the Plastochron index that allows to measure the biological age of the leaf and the entire shoot. It is therefore able to express the physiological evolution with biological time and not the chronological one.

The exploratory part of the thesis concerns the evaluation of the spectral characteristics of vegetation and in particular considering the spectrum of reflectance from which some vegetative indices can be derived. By combining these different data it can be possible to have indications of the variation and maturation of the photosynthetic apparatus and evaluate whether these data varies in the different varieties under consideration.

# CHAPTER 2: Leaf morphology and phenology

## 2.1 Leaf phenology

Phenology is a branch of science dealing with periodic biological phenomena correlated with climatic conditions and seasonal changes. Therefore, it studies the correlation between the environment and plant development by considering weather indices and dates of particular growth events. The main aim of this science is to predict plant behavior in different habitats compared to the native one. In viticulture, phenology is used to track, recognize, understand, and describe the developmental stages of the grapevine in the annual cycle. Such knowledge provides the basis for decision-making in the vineyard like site evaluation, selection of cultivars and rootstock; planning of fertilization and equipment requirements, and organization of vineyard management in general (Keller, 2010).

Keeping track of the phenological phases of grapevine, also allows for improved efficiency of vineyard management, resulting in reduced costs and improved plant health. An additional advantage is also the ability to intervene with management practices thanks to a more timely forecast given by the prediction of the beginning of the different phenological phases. The most commonly mentioned growth stages include bud break, bloom, fruit set, cluster closure, veraison, and fruit maturity (harvest) (Westover,2018).

At a seasonal level, each developmental phase is mainly influenced by temperature and photoperiodism, even though also stress factors may modulate the plant response. The raise of temperature due to climate change is influencing also grapevine phenology accelerating plant development (Keller, 2010).

The efficiency of the foliage depends not only on the total quantity of the foliar surface but above all, on the type and spatial and demographic distribution of the set of leaves that make up the canopy (Poni et al., 2007).



The senescence of the leaves begins from the leaf margins, in the cells of the mesophyll, and gradually proceeds inward. This happens because there is a transition from a phase of photosynthetic assimilation of carbon, to the breakdown of the fundamental components of the leaf (chlorophyll, lipids, nucleic acids) that will subsequently be exported to other parts of the plant. Senescence is a really high-cost process, that implies the loss of 50% of carbon leaf composition due to the generation of energy that is required for the degradation of cell membranes and export metabolism. Chlorophyll, in absence of photosynthesis, is considered a phytotoxin by the plant due to its light-absorbing properties. This is the main reason why the degradation of chlorophyll is necessary at the end of the leaf life (Keller, 2010).

## 2.2 Leaf structure

The leaves are one of the main protagonists of each plant because of their fundamental role in producing organic substances thanks to the chemical process of chlorophyll photosynthesis. Especially in viticulture, the leaves are fundamental organs for identifying and classifying different vine cultivars.

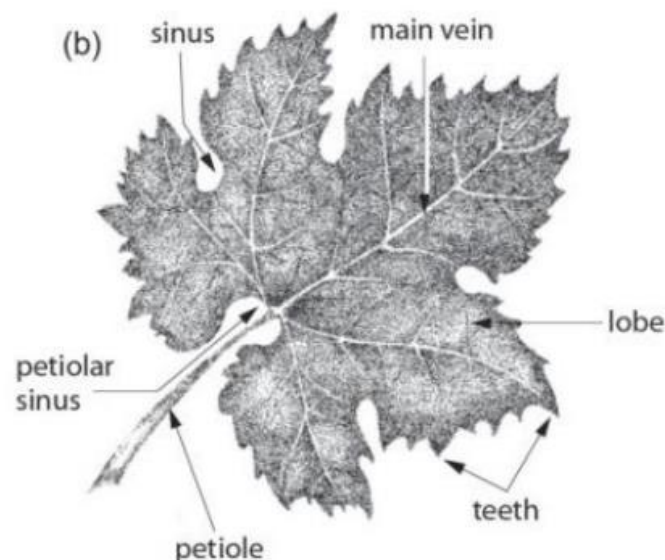


Figure 1: Leaf structure (Meggio<sup>1</sup>,2021)

Phyllotaxy is the term used to describe the arrangement of leaves around the stem, which will differ depending on the species. Phyllotaxy can be classified as alternate, spiral, opposite, or whorled. For

a grown vine the phyllotaxy is opposite, which means that leaves are produced on the opposite side of the stem. The angle between the successive leaves is  $180^\circ$  and the shoot is bilaterally symmetrical when it comes to leaf formation (Meggio<sup>1</sup>, 2021).

The fundamental elements of the leaf are the petiole, which is the point of attachment to the branch, two stipules, which are two small green appendices that are at the base of the petiole and finally the leaf lamina (Strever, 2012).

Petioles can be used for green tissue analysis to determine nutrient status. The lamina of the vine leaf is generally formed by 5 lobes, each crossed by a vein that starts from a single point of junction between the lamina and the petiole (*Figure1*)(Meggio<sup>1</sup>, 2021).

The upper and underside of the leaf blade have different characteristics that vary with the cultivar, but in general, the adaxial side has almost no stomata and no hair, while the abaxial side is quite the opposite being hairy and with a large number of stomata (Strever, 2012).

The upper epidermis is an uninterrupted single-cell layer covered by a coat of wax that prevents water from entering. Underneath the epidermis, there is the mesophyll which can be distinguished in palisade and spongy parenchyma. Chloroplasts are abundant in both types of mesophyll. Around 50% of the leaf thickness is made by the single layer of elongated cells of the palisade mesophyll. Directly under the latter, there is the spongy mesophyll which is made up of 4-6 layers of cell and is characterized by having many intercellular spaces (*Figure 2*). The intercellular spaces in the mesophyll have the important function to make possible the diffusion of gas in the leaf (Strever,2012).

The main functions of leaves are the production of sugars that are used for growth and the metabolism of the plant and transpiration of water via the stomata. The stomata provide the pathway for the diffusion of water vapor out of the leaf, and of carbon dioxide into the leaf acting as a cooling mechanism protecting the leaf from heat damage. Almost 98% of the water acquired by the plant is used for transpiration and thermo-regulation (Meggio<sup>1</sup>, 2021).

Water is one of the main components of the leaf, being more than 60 % of the leaf's fresh weight. Instead, considering the dry matter there are hemicellulose, lignin, protein, starch, minerals, lipids, soluble sugars, amino acids, and other secondary metabolites (Jacquemoud et al, 1996).

According to Jacquemoud et al. (1996), total chlorophyll and total carotenoid content (mainly  $\beta$ -carotene and lutein) range from 0.7 to 2.5  $\text{mg}\cdot\text{g}^{-1}$  fresh mass and 0.3 to 1.0  $\text{mg}\cdot\text{g}^{-1}$  fresh mass respectively and on average leaf carbon constituents are about 47  $\text{g}\cdot\text{g}^{-1}$  of dry matter.

The reason why leaves develop as wide and slender as possible is that this conformation can maximize gas exchange in shade conditions. However, on the other hand, this shape leads to susceptibility to dehydration and photo-degradation (Strever, 2012).

The environment plays an important role when talking about leaf size and thickness. Limitation of nutrients or other unfavorable climatic conditions, especially during cell division, may lead to a consistent limitation of leaf size development (Keller, 2010).

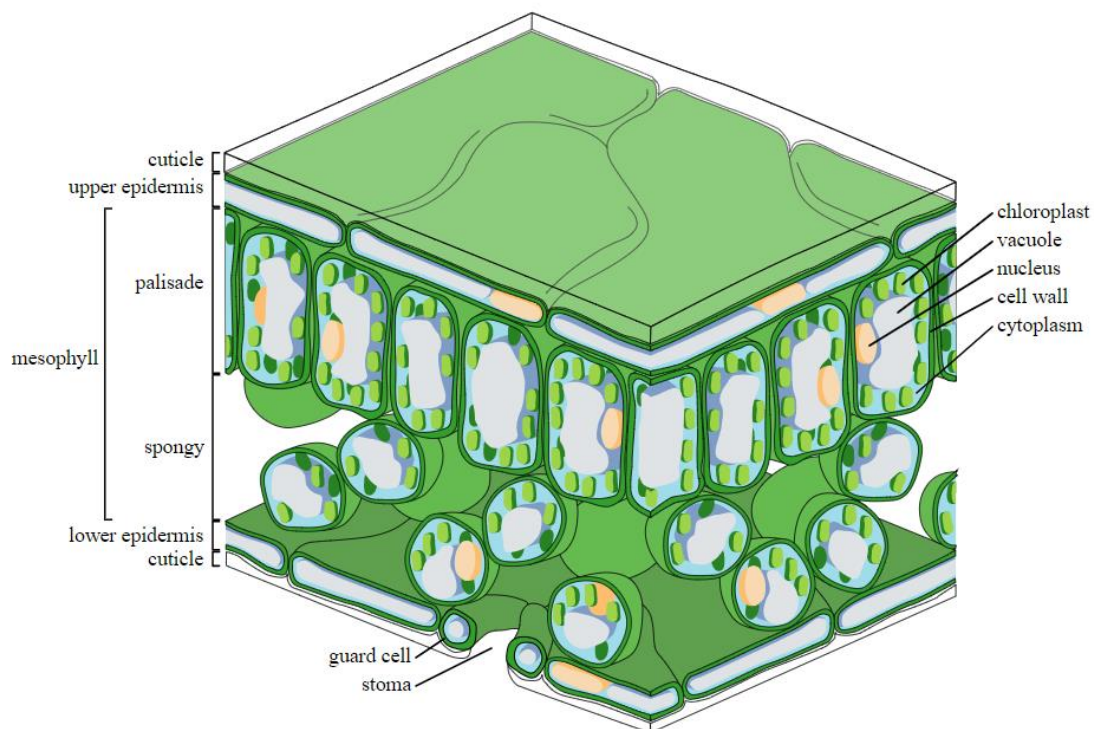


Figure 2: Leaf tissue structure (en.wikipedia.org)

Leaf cells take about two weeks to reach full size and cell division ends when the leaf reaches about half its definitive size. An average leaf requires about 30-40 days after unfolding to reach its full size (Kriedemann et al., 1970).

According to Kriedemann et al. (1970), grapevine foliage's maximum photosynthetic activity occurred at the time when the leaf reaches its full size. After this point, there is a gradual decline in photosynthesis. The increasing chlorophyll concentration and the rise in photosynthetic capacity during leaf expansion are two unrelated phenomena. On the other hand, the anatomical change during leaf growth may be linked to the increase in photosynthesis. In fact, with leaf expansion, there is the creation of more intercellular spaces in the mesophyll structure, which encourage a greater inward flux of CO<sub>2</sub>.

The age of the leaves and the level of sun exposure depend on different foliar characteristics such as thickness, structure and water content. Specific leaf mass doesn't remain constant over time, but varies and increases during the season moving from the lower part of the canopy to the higher one, also considering the secondary branches. This happens because as the season progresses, the leaves of the secondary branches contribute more photosynthetically (Kriedemann et al,1970).

## 2.3 Leaf physiology

The definition of physiology is "the science of the function of living organisms and their parts" and the main aim of plant physiology is the study of causality of biochemistry and development functions. Assimilation is the ability of plants to build their biomass from inorganic compounds and not just carbon compounds, as in the case of photosynthesis. Respiration in the mitochondria, on the other hand, is an example of a dissimilative process, that is, the breakdown of substrates, such as carbohydrates, for energy metabolism or for the creation of monomers for the synthesis of macromolecules, such as proteins and lipids (Lüttge, 2008).

The growth, development and survival of the plant depend on the conditions of the environment in which they live, which in turn are given by abiotic and biotic factors. Light, carbon dioxide, water availability, temperature, nutrients and salinity are the main abiotic factors that, by acting in an interconnected way, affect all the main processes of plant physiology. Light in particular plays a very important role and influences many fundamental processes for the plant: it guides the photosynthetic fixation of CO<sub>2</sub>, photorespiration and can also act as a signal. The photoperiod, and in particular the wavelengths of the red and blue influence the development of the leaves. The

opening and closing of the stomata are also influenced by light, regulating the absorption of carbon dioxide and perspiration (Lüttige, 2008).

As for biotic factors, the competition between plants for light is certainly an element of particular importance. Shading and filtering of certain wavelengths affect plant development (Lüttige, 2008).

The photo-assimilative behavior of vine leaves is very versatile and quickly adapts to different climatic conditions, even the most limiting ones. The physiology of the leaf and the carbon balance are extremely influenced by several factors that concern both the intrinsic properties of the plant (age and type of leaves) and the external conditions (light, temperature, water availability). The cultivation technique can have important implications on the photosynthetic efficiency of the plant in relation to endogenous and exogenous factors. The knowledge of limiting factors for the plant is the starting point to optimize the environmental resources and obtain a good balance between the vegetative and reproductive activity of the vine (Poni et al.,2007).

In the vine, it is particularly evident an uneven photosynthetic response of the canopy, due to a lack of preferential orientation of the leaves. In general, there is a particular diversity between radiant energy perceived by the inner leaves and those outside the canopy. The radiation values perceived by the inner leaves are in fact similar to each other, as they perceive purely diffused light, both transmitted and reflected (Poni et al.,2007).

Often in the foliage, there are several sectors where the leaves benefit only from diffused light for most of the day. It has been shown that even these inner leaves retain their functionality by actively contributing to the carbon balance (Poni et al.,2007).

The photosynthetic response varies from the page below to the page above, and it is more efficient in the latter. The level of photosynthetic response is not homogeneous in all leaves and is affected by the light conditions present during the formation of the leaf lamina. For this reason, the outer leaves have a greater photosynthetic capacity than the inner ones that have developed with less radiant energy. There is also a point of luminous saturation, beyond which there are no changes in net photosynthesis as the light increases (Poni et al.,2007).

Excess radiant energy, usually in combination with high temperatures, can lead to photosynthetic dysfunctions of various levels up to chronic photoinhibition. This last case happens when the capacity of assimilation of carbon dioxide is lower than the excitation energy perceived by the leaf. To protect themselves against this type of problem, the leaves have developed strategies of photo-

protection diverting their metabolism in the activation of mechanisms able to cool the tissues and avoid photo-inhibitions. Among the most known mechanisms is the formation of active oxygenated species, called ROS and the role of stomata in regulating the relationship between carbon dioxide assimilation and water loss (Poni et al.,2007).

### 2.3.1 Photosynthesis and the role of pigments

The leaves are sites for photosynthesis, which produces carbohydrates for vine growth and metabolism. Photosynthesis is the process by which sunlight, water and carbon dioxide are used by plants to produce oxygen and energy in form of sugar.



Water is oxygenized and transformed into oxygen that is released back into the atmosphere, while carbon dioxide is reduced and converted into energy. Chloroplasts are the organelles that have the function to convert sunlight energy into sugar that can be used by the cell. Chloroplasts are oval-shaped organelles composed of inner and outer membranes. The thylakoid system is a complex of the internal membrane and it is the place where light-dependent reactions take place thanks to the presence of a light-absorbing pigment called chlorophyll (cd-genomics, 2019). Chlorophyll molecules absorb photons (red and blue wavelengths) that aim to excite electrons. The movement of electrons along the transport chain of internal membranes creates the chemical energy needed to assimilate carbon dioxide (Lüttige, 2008).

Photosynthesis can be divided into two major processes: light and dark phases. In light-dependent reactions, the energy absorbed by chlorophyll in the thylakoid membrane is converted into chemical energy in form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). The dark phase also called Calvin cycle, doesn't require light and takes place in the stroma. The Calvin cycle consists to assemble carbohydrates molecules using the ATP and NADPH produced into the light phase (cd-genomics, 2019). Calvin's cycle involves processes of carboxylation, reduction and generation of Ribulose 1.5 -Bisphosphate and depends on the presence of ATP and NADPH

produced by dependent light reactions. RuBP (Ribulose Bisphosphate) is a very unstable product that instantly divides into two molecules of 3-phosphoglyceric (3PG) (Strever, 2012).

Pairs of hydrogen ions create an electrochemical gradient through the complex of thylakoid membranes, generating ATP and NADPH. Electron transfer occurs thanks to the action of chlorophyll and carotenoids, pigments that are able to absorb light and transfer energy. Electron pairs are transferred between two photoreaction centers, the PSI, which preferably absorbs far infrared frequencies (700 nm-740 nm), and the PSII, which absorbs near-infrared in the frequency of 680 nm. Photons are transported by pigments proceeding with decreasing energy levels, because photons lose energy in the form of heat in the process. This system ensures that the transfer is always one-way (Strever, 2012).

The optical properties of the leaves are greatly influenced by the presence of pigments and their ability to interact with light, playing a leading role in photosynthesis reactions. Each pigment absorbs a different region of the spectrum. Chlorophyll has a dominant role in light harvesting and preferably absorbs the frequencies of red (650-700nm) and blue (400-500nm) (Ollinger,2010).

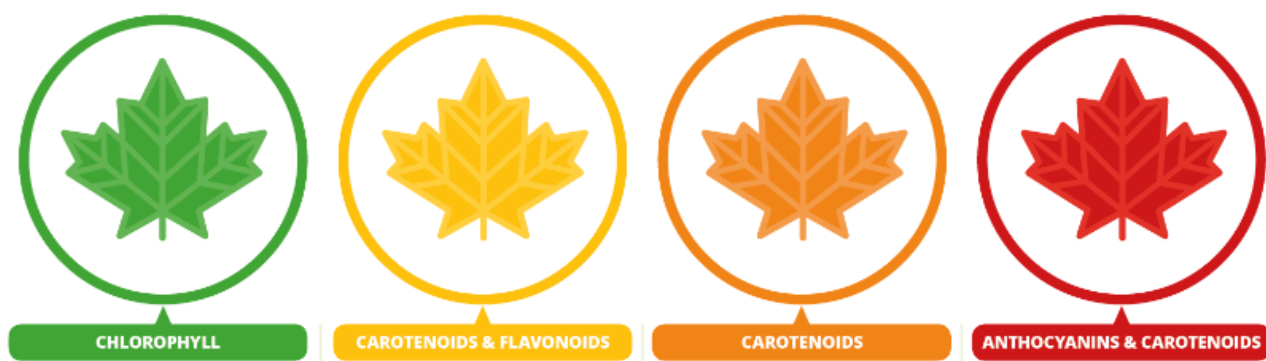


Figure 3 Chemistry of autumn leaf colours. ([www.compoundchem.com](http://www.compoundchem.com))

Carotenoids are lipid-soluble pigments present in the chloroplast membranes and they represent  $\frac{1}{4}$  of the total pigments in the leaves. Their typical colors, yellow, orange and red, are for the most of the time covered by the color green due to the presence of chlorophyll (Strever,2012). Only during plant senescence, in autumn, leaves change color from green to yellow-orange. This phenomenon occurs as a result of the degradation of green chlorophylls that leave carotenoids at the scene, in fact, carotenoids and flavonoids are constantly present in le leaves (Keller, 2010). Carotenoids play

several important functions in the photosynthetic process such as light harvesting, photo-protection and they have a crucial role in the maintenance of the structural integrity in pigment-protein complexes (Strever,2012)

Carotenoids are divided into carotenes and xanthophylls, they absorb in the blue region and play different roles in the plant. Xanthophylls, in particular, have the task of avoiding damage caused by excesses of light absorbed by dissipating energy. Lutein is one of the major xanthophylls and it also contributes giving the leaves the yellow egg yolks colour. The spectral overlap of chlorophyll and carotenoids frequencies makes the estimation of pigments challenging (Ollinger,2010). The xanthophyll cycle is the main method used by the leaves of *Vitis vinifera* for the dissipation of excess energy (Strever,2012).

Anthocyanins are red pigments absent in white grape varieties but characteristic of dark-skinned cultivars (Keller, 2010). They are also called “stress pigments” thanks to their capacity to provide protection from plant stress such as temperature, excess light etc. Photo-protection implemented by carotenoids consists of a series of enzymatic reactions aimed at the thermal dissipation of excess energy in the photosystems. In fact, excessive amounts of thermal energy can lead to the formation of toxic elements, such as singlet oxygen (Strever, 2012).

The study of the spectral properties of pigments, and in particular of reflectance, led to the development of spectral detection indices. These analyze certain combinations of bands in the visible part of the spectrum to give information about the state of the plant analyzed. To eliminate the indirect effect of pigments in the detection of chlorophyll, certain indices use reflection at wavelengths of 800 nm or more (Strever, 2012).

The complexity of a grapevine canopy, which can generate contrasted microclimatic conditions, combined with the effect of leaf aging can generate a huge heterogeneity in photosynthetic activity (Prieto et al, 2012).



## 2.3.2 Chlorophyll structure and functions

Chlorophyll is an effective indicator of photosynthetic capacity that can also be used for estimating the plant's health status. There is a proven correlation between nitrogen content and photosynthetic activity (Du et al, 2018).

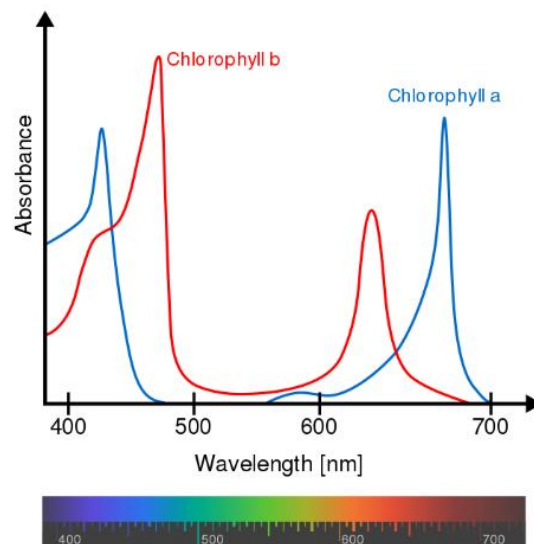


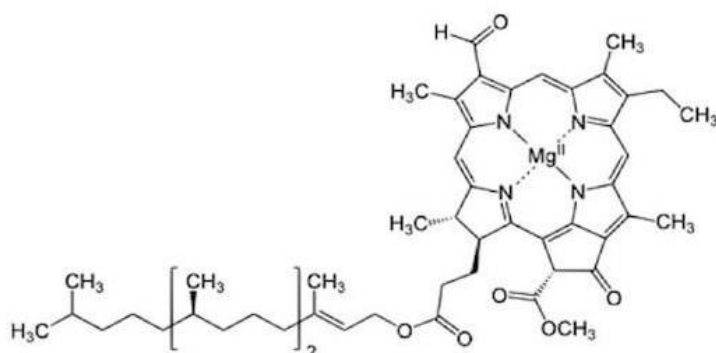
Figure 4: Chlorophyll a and chlorophyll b absorbance spectra ([www.en. Wikipedia.org](http://www.en.wikipedia.org))-

The green color of chlorophyll is given by its ability to absorb in the regions of red (600-700 nm) and blue (400-500 nm), this causes the reflected and transmitted light to appear green (500-600 nm) (Figure 3). There are several forms of chlorophyll, the two main ones being chlorophyll a and chlorophyll b. Chlorophyll a plays a more important role in the collection of light than chlorophyll b, and these pigments are generally present in the ratio of 2:1 and 4:1 in higher plants (Strever,2012).

The chlorophyll molecule consists of a central magnesium atom surrounded by a porphyrin ring, a cyclic structure containing nitrogen. To the latter is attached a side chain of phytol, consisting of carbon and hydrogen atoms. The different forms of chlorophyll are distinguished by variations in the lateral groups: the methyl group present in chlorophyll a is replaced by an aldehyde group in chlorophyll b ([www.ch.ic.ac.uk](http://www.ch.ic.ac.uk)). This structural difference makes chlorophyll b more efficient in light absorption in the 400 to 500 nm spectrum regions (Strever, 2012).

The chlorophyll molecule structure is remarkably similar to that found in hemoglobin, the oxygen-carrying pigment found in the red blood cells of vertebrates, in which the central atom is iron (Guidi et al,2017).

Figure 5: Molecular structure of chlorophyll (www.chimica-online.it)



Light-harvesting complex (LHC) is the complex of subunit proteins dedicated to the absorption of light. The presence of other pigment-proteins in addition to chlorophyll is necessary in order to optimize the absorption of photons and their transfer. Chlorophyll a molecules present in reaction centers are unable to drive energy into chloroplast membranes without the support of other pigment-protein. The energy is transported first between the molecules in the same complex and then from one LCH to another until it is channeled into the reaction centers (RC). There are several LCHs that differ in their composition, structure and number of pigments, in fact, the position of proteins affects their function within the complex. Carotenoids and molecules of chlorophylls a and b together are called antenna complex. The role of the antenna complex is the absorption of sunlight's photons and their transportation to the RC (Reaction center). There are two different RCs, PSII (P680) and PSI(P700) whose chlorophyll absorbing maxima are respectively 680 nm and 700 nm (Guidi et al,2017).

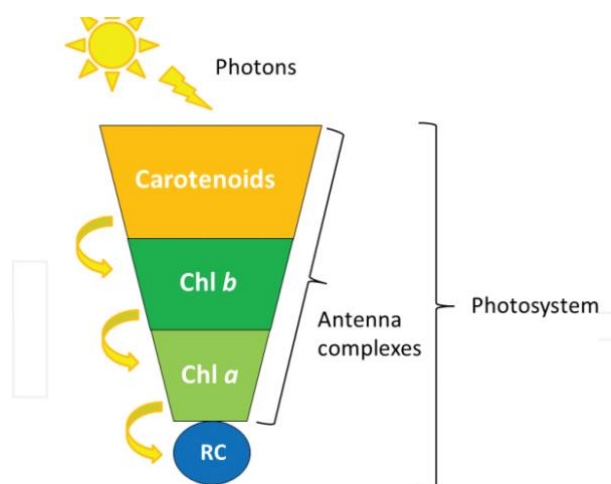


Figure 6: Absorption process of chloroplasts. A photosystem is formed by an antenna complex and RC. (Guidi et al, 2017)

### 2.3.3 Chlorophyll fluorescence

The light that is intercepted by the chlorophyll can follow 3 different paths that are competitive with each other: (1) the radiation can follow the photochemical route and be used for photosynthesis, (2) Excess absorbed energy can be dissipated in the form of heat without causing damage to the plant or (3) radiation can be re-emitted causing chlorophyll fluorescence (Bhagooli et al, 2021). The peak of this emission occurs in the red region, specifically at 685 nm, and extends in the infrared region up to 800 nm ([www.gmrstrumenti.com](http://www.gmrstrumenti.com)).

$$\text{Absorbed energy} = \text{photochemical activity} + \text{heat loss} + \text{loss by fluorescence}$$

Fluorescence is therefore a form of dissipation of the radiation perceived by the leaf. At the time when photosynthesis takes place regularly, fluorescence represents only a small percentage of the entire energy dissipated, about 3-5 % (Bussotti et al, 2012).

The measurement scale of fluorescence can be divided into different levels of cellular organization according to the purpose of the research and the instrument used is called a fluorometer (Lüttge, 2008).

In general, an increase in fluorescence has been observed when there is simultaneously a low level of photosynthesis and heat dissipation. As fluorescence decreases with an increased photosynthetic rate or increased heat dissipation. An increase in fluorescence will then cause a decrease in the yield of the other two processes (Bhagooli et al, 2021).

Fluorescence analysis can therefore be used to assess stress and photosynthetic efficiency. It is therefore possible to evaluate the efficiency of chlorophyll molecules and photosystems and to understand the photo-damage caused by excessive heat, to evaluate the speed of electron transport and the photoinactivation associated with the extinction of fluorescence (Bhagooli et al, 2021).

A short period (1 s) is required to lead to a sudden increase in chlorophyll fluorescence. The transfer of the photosynthetic material from the dark to the light leads to the closure of the reaction centers of photosystem II, which has as a consequence the decrease of the photosynthetic process and therefore the increase of the fluorescence. The decrease in fluorescence takes a few minutes and this process is called "fluorescence extinction". The measurement of fluorescence extinction is linked to the conversion of photochemical energy and can therefore be a good indicator of the percentage of open PSII reaction centers (Bhagooli et al, 2021).

The fluorescence parameters are not constant, but have natural fluctuations that are determined by changes in light during the day, the temperature of the environment and the species analyzed. These external and internal factors affect the photochemical performance and therefore also the measurement results. For this reason, it is important to consider the environmental conditions when carrying out the measurements in such a way that the results can be contextualized. Chlorophyll fluorescence measurements alone are not sufficient to have complete information on gross photosynthesis due to all factors that may affect their value; they must therefore be supplemented by conventional methods (Bhagooli et al, 2021).

## 2.4 Leaf gas exchange

The water vapor and carbon dioxide spread and move in the leaf through the cell walls and stomatic openings. The driving force behind this movement is differences in H<sub>2</sub>O and CO<sub>2</sub> concentrations between different compartments. The first studies on gas flow date back to 1900 when the concept of resistance was used to quantitatively describe this mechanism. Nowadays the term conductance is used to describe transpiration, which is the flow of carbon dioxide and water vapor outside and inside the leaf ([www.galenotech.org](http://www.galenotech.org)).

The diffusion gradient and the stomatic conductance are the main factors influencing the diffusion of CO<sub>2</sub> inside the leaf. The diffusion gradient is expressed in ppm and represents the difference in carbon dioxide concentration between the inside of the leaf and the air present near the leaf surface ([www.galenotech.org](http://www.galenotech.org)).

Stomatic conductance depends on the number of stomata present on the leaf surface and their opening, which is strongly influenced by environmental and biochemical factors (Manes et al, 2019). Environmental factors are highly dynamic over time and interconnected with each other, it is difficult to clearly isolate the effects of a single factor, especially in analyses carried out over long periods of time (Garcia et al, 1990).

Diffusion can be described as a movement of molecules driven by a chemical potential gradient. This concept is explained by the first law of diffusion of Fick, cited below:

*Fick's law states that...The rate of diffusion of a substance across unit area (such as a surface or membrane) is proportional to the concentration gradient... (Davis et al, 1995)*

This law, however, applies only if we consider single homogeneous phases. When considering gas transfers in solution, that is, between two different phases, the law is slightly modified and becomes:

*...The rate of diffusion of a gas across a membrane (or surface area) is proportional to the tension gradient...(Davis et al, 1995)*

$$\text{Flux} = \frac{dq}{dt} = -DA \frac{dc}{dx} \quad [\text{cm}^{-2}\text{s}^{-1}]$$

Where:

q= quantity of solute

A= membrane surface area

c= concentration

D= diffusion coefficient

dx= membrane thickness

dc/dx= concentration gradient

The relation between flux and forces can be also expressed in different forms considering the proportional coefficient of flux density, that is conductance, and the reciprocal of conductivity: resistance.

*Flux density = conductance \* force*

$$\text{Flux density} = \frac{\text{force}}{\text{resistance}}$$

Conductance and resistance terminology are differently used to describe plant behavior. Conductance is commonly used in the description of the responses of plants to environmental changes since it ranges from zero to a maximum value. On the other hand, resistance doesn't have

an upper limit value and is more convenient to use when talking about CO<sub>2</sub> diffusion across the cell wall. In general, both terminologies are widely used in the scientific literature: conductance when talking about transpiration and resistance for photosynthesis (Nobel,2020).

## 2.4.1 Stomatal conductance

During the transpiration of the leaf, the main way out of the water vapor are the stomata. Stomata are openings on the leaf surface surrounded by kidney-shaped cells called guard cells. The latter are distinguished from other cells of the epidermis, in addition to the shape, also for the presence of chloroplasts. The guard cells regulate the opening and closing of the stomatic pore, increasing or decreasing their turgor. Another very important component are the subsidiary cells that are in the immediate vicinity of the guard cells. The subsidiary cells have an important relationship with water as they help to balance the displacements of water and ions in the guard cells, helping in the regulation of the stomatal opening. The turgor of the guard cells is regulated by the change in concentration of K<sup>+</sup>. The absorption of K ions has the effect of spontaneous entry of water, coming from nearby epidermal cells, into the guard cells. The increase in internal hydrostatic pressure due to the entry of water expands the guard cells by opening the stomatic pore (Nobel, 2020).

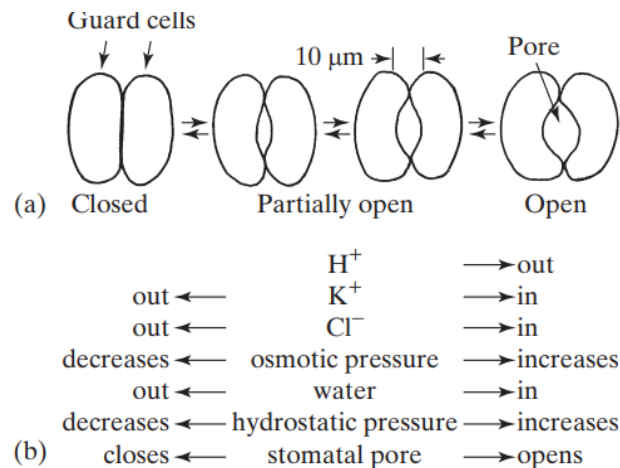


Figure 7: (a) representation of guard cell's movement. (b) cellular events that promote the opening and closing of stomatal pores represented in chronological order from the top to the bottom (Nobel, 2020)

The control of the stomatal opening depends on several factors that also act simultaneously. The first change in electrical potential is due to the ejection of H<sup>+</sup> by the guard cells. The lowering of the membrane potential caused by the increase of internal pH, makes passive the passage of K<sup>+</sup> through K<sup>+</sup> channels. This entry of K<sup>+</sup> ions into the guard cells, thanks to which the opening of the stomatic

pores is possible, is then balanced by the exit of  $\text{Cl}^-$  and malate ions. The degree of stomatal opening depends mainly on two factors: the concentration of  $\text{CO}_2$  in the guard cells and how much  $\text{CO}_2$  is present in the air in intracellular spaces (Nobel, 2020).

Photosynthesis, which occurs in light conditions, triggers the stomatal opening as there is a reduction of both  $\text{CO}_2$  inside the leaf, both in the guard cells. In dark conditions, instead, there is the stomatic closure triggered by the high levels of  $\text{CO}_2$  given by the breathing process. In some cases, the stomatal opening response may be independent of  $\text{CO}_2$  concentrations and depend only on the presence of light. Light in fact starts the process of extruding  $\text{H}^+$  from guard cells (Nobel, 2020).

Although a good stomatal opening is essential from the photosynthetic point of view, it is important that it is not excessive in order to avoid too much water loss. The regulation of the stomatal opening depends not only on external factors (temperature, light, humidity) but also on intrinsic hormonal factors. Some hormones produced by roots and leaves, such as abscissic acid (ABA) inhibits stomatal opening (Nobel, 2020).

The regulatory response of the leaves that leads to the closure of the stomata is extremely important as it prevents the loss of water vapor through the process called transpiration. On the other hand, prolonged closure of stomata can lead to several problems. One of these is the decrease in photosynthesis due to the lack of  $\text{CO}_2$  as an acceptor. Another side effect is the difficulty in cooling the leaf in case of excessive light radiation (Lüttuge, 2008).

In general, understanding the functioning of stomatal openings can be interesting to make accurate predictions of transpiration, even modulating stomatal conductance ( $g_s$ ). But why is the study of the process of transpiration important? Miner et al (2017) explain how almost half of the rainfall reaching the Earth's surface then returns to the atmosphere thanks to the transpiration process operated by plants.

The diffusion of gases,  $\text{CO}_2$  and water vapor, through the stomatic openings, can be described by Fick's first law. Adapting the formula to the situation under consideration we will have the following situation:

$$J_j A = D_j \frac{\Delta c_j^{st}}{\delta_{st}} A^{st}$$

$$J_j = D_j \frac{\Delta c_j^{st}}{\delta^{st}} \frac{A^{st}}{A} = \frac{n a^{st} D_j}{\delta^{st}} \Delta c_j^{st}$$

In this case we will consider:

$\delta^{st}$  = pore depth,

$\Delta c_j^{st}$  = concentration of a substance j varying in the distance  $\delta^{st}$ .

$A^{st}$  = the area occupied by stomata pore (usually ranging from 0.2% to 2% of the total leaf area)

$J_j$  = the amount of j moving per unit of time toward or away from the leaf area A.

$D_j \frac{\Delta c_j^{st}}{\delta^{st}}$  = flux density within the stomata

n = number of stomata per unit area of a leaf

$a^{st}$  = average area per stomatal pore

(Nobel, 2020)

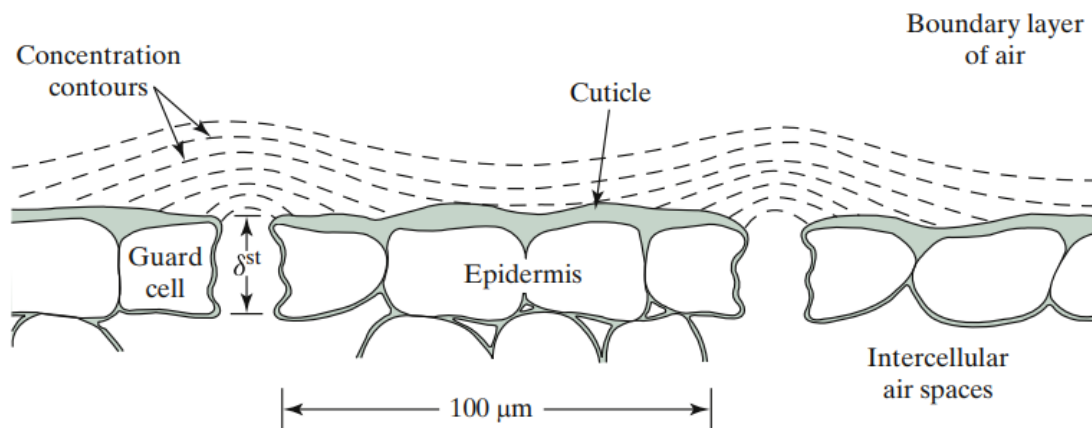


Figure 8: Schematic representation of leaf surface and stomata and the concentration contours of water vapor outside the open stomata (Nobel, 2020)



# CHAPTER 3: Spectroradiometry

## 3.1 Spectral properties characterization

Spectroscopy is the study of the behavior of light and other radiation when they interact with matter (atascientific.com.au).

Electromagnetic energy is not a single body but consists of different wavelengths that can be absorbed or emitted by matter. Different types of spectrometry can be distinguished by considering the interaction between different types of material and distinct portions of the spectrum. Each chemical compound has particular absorption characteristics. For this reason, it is possible to determine the chemical composition of a given object by detecting its spectral characteristics through a spectrometer (Meer, 2018). In our specific case we will consider the relationship between the typical plant composition and certain absorption characteristics. Many biochemical constituents of the leaves are studied at a spectrometric level such as cellulose, lignin, starch, various proteins and in particular chlorophyll. Spectrometric analysis of these components can indirectly give information about the health of the plant that can be useful to monitor and predict the yield (Meer, 2018).

Radiant energy can interact with matter in different ways and can be emitted, reflected, transmitted or absorbed. The type of interaction depends on several factors: the direction of the incident radiation, the wavelength of the radiation and the nature of the material. You can then define 3 type of interactions:

- Reflectance: ratio of radiant flow reflected by a medium to incident radiant flow
- Absorbance: ratio of radiant flow absorbed by a medium to incident radiant flow
- Transmittance: ratio of radiant flux transmitted by a medium to incident radiant flux

Radiant flux [SI unit: watt, W] is defined as the energy reflected, absorbed or transmitted by a source on a surface per unit time (Chessa, 2022).

The electromagnetic radiation emitted by the sun, also called solar radiation has a range of 200 nm to 2500 nm. Earth's surface doesn't receive a homogenous amount of solar radiation, because it varies according to space and time. Not all solar radiation reaches the earth's surface undisturbed

(direct solar radiation), a discrete amount of sunlight passing through the atmosphere is absorbed, scattered and reflected by air molecules, vapor, dust and pollutants (diffuse solar radiation). Also atmospheric conditions can limit and vary the amount of direct sunlight from a 10% to a 100% less (Ollinger,2010).

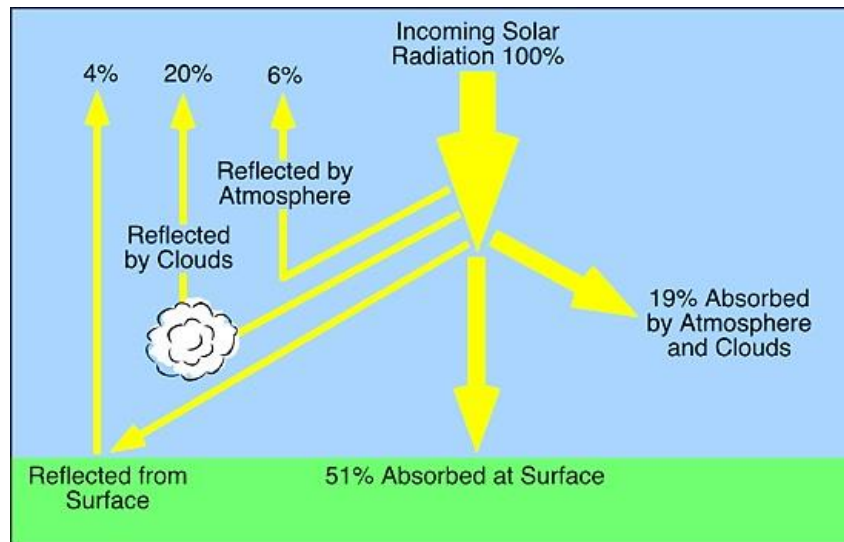


Figure 9: Type of solar radiation: direct, diffuse and reflected. (Alaa et al, 2020)

A large part of the ultraviolet energy that passes through the atmosphere is absorbed by  $O_2$  and  $O_3$ . Only about half of the incident solar energy that reaches the Earth's surface belongs to the visible spectrum, the remaining percentage is divided into near and medium infrared. (Ollinger, 2010).

Light is formed by photons that carry energy, which means quantum of the energy of electromagnetic radiation that travels at the speed of light ( $c= 300,000$  km/s). Light has a double nature: wave-like, like an electromagnetic, and corpuscular wave, referring to light as a set of photons. Electromagnetic waves consist of an electric field and a magnetic field perpendicular that oscillate in a coordinated way and perpendicular to the direction in which they propagate (Chessa,2022).

There is an inversely proportional relationship between wavelength ( $\lambda$ ) and oscillation frequency ( $\gamma$ ) decrypted by the formula:  $c=\lambda\gamma$  (Chessa,2022).

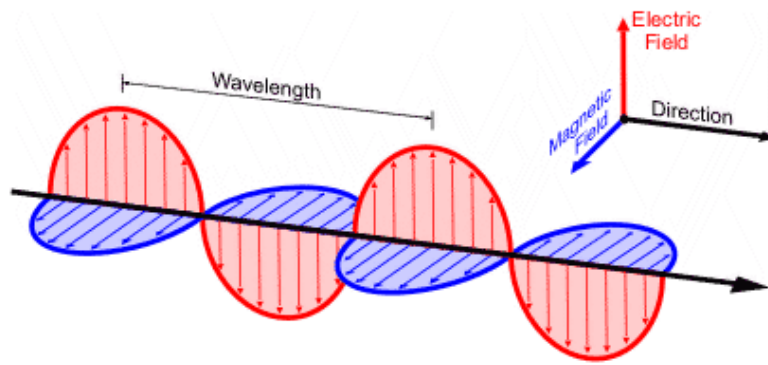


Figure 10: Electromagnetic waves (www.weather.gov)

The interaction of photons with the atoms of matter results in a change in the vibrational energy of electrons. This clash is manifested by the emission or absorption of radiation at certain frequencies of the electromagnetic spectrum (Chessa, 2022).

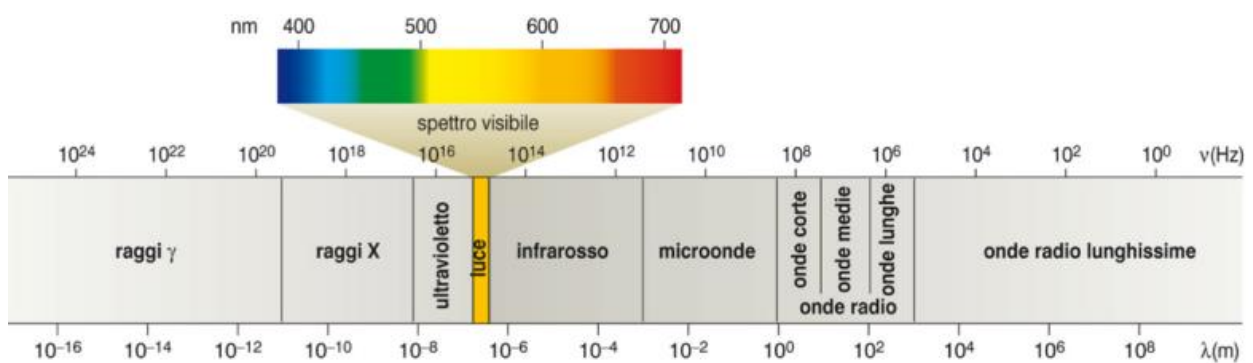


Figure 11: Electromagnetic spectrum (Chessa, 2022)

The electromagnetic spectrum is the set of all possible frequencies of electromagnetic radiation, which can be divided into bands depending on how photons interact with matter. The most important bands considering the interaction of radiation with the biosphere are: ultraviolet, visible light and near infrared bands. The wavelengths of the photons increase from left to right, following this order the first wavelength is called x-rays, to follow the gamma rays and then meet the frequencies of the ultraviolet. The frequencies between 400 and 700 nm correspond to the visible light that can be superimposed on the so-called PAR, or the portion of the photosynthetically active

spectrum. Beyond the frequencies of 700 nm, there are longer wavelengths: infrared, microwave and radio waves. The infrared band can be divided into several sections and the most important is called near infrared (NIR). NIR is often used to assess the physiological state of plants and is a parameter that is therefore often involved in calculating vegetation indices (Chessa, 2022)

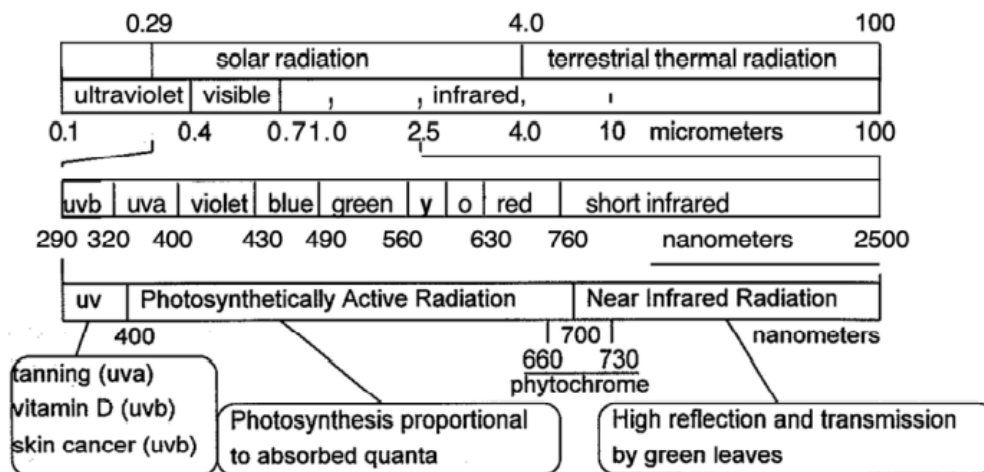


Figure 12: biological interaction of the three main region of the electromagnetic spectra (UV, PAR, NIR) (Chessa, 2022)

### 3.2 Leaf spectral properties and interaction with radiation

While the plant world is presented in many forms and compositions, on the other hand there is a biophysical homogeneity that makes it easier to study the interaction between plants and light.

A relationship has been observed between the reflection of certain wavelength combinations and specific biophysical characteristics of the leaves. By means of vegetation indices based on reflectance, in fact, it is possible to estimate different parameters such as the amount of chlorophyll in the leaf, vegetable biomass, leaf surface, yield, photosynthetic activity, etc... The use of vegetation indices can be useful for monitoring changes over time in a field, so it is possible to intervene in a targeted and preventive manner in case of anomalies are detected during the growing season (Hatfield et al, 2019).

The interaction of matter with radiation can be divided into two categories: absorbance and scattering. This last category can in turn be divided into reflectance and transmittance (Ollinger, 2010).

Absorbance is a parameter that remains rather constant over time and in discrete portions of the spectrum as it analyzes the absorption characteristics of pigments, liquid water and other plant constituents. Scattering, on the other hand, depends on the refractive index and therefore occurs whenever there is a difference in this index between two substances. The refraction index is own and different for each substance, for example for water is 1.33, for air is 1 while that of cells varies from 1.4 to 1.5 depending on the level of hydration of the cell itself (Ollinger, 2010).

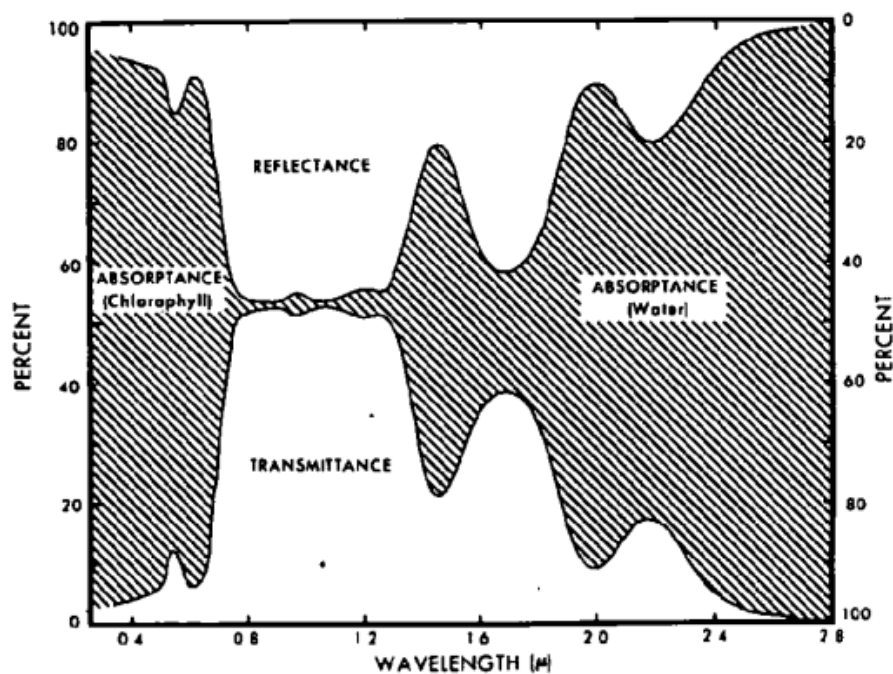


Figure 13: Absorbance, reflectance and transmittance spectra of a leaf (Knipflig, 1970)

The spectral signature of scattering is affected by the selective behavior of the absorbers and thus affects only specific bands of the spectrum. Pigments and water are the main absorbers in plants, the former operates in the visible band, while water absorbs in the near and medium infrared (Ollinger, 2010).

In the NIR region, it has been observed that the direction of incoming photons is very different from the output direction, which results in high leaf reflectance in this particular area of the spectrum (Ollinger, 2010).

The cellular structure of the leaf and its composition greatly affects the behavior of light, especially with regard to reflectance. The incident light is reflected from the surface of the leaf because of the waxes, the latter in fact are almost transparent to visible and infrared radiation (Ollinger, 2010). Most of the visible radiation used for photosynthesis is generally absorbed by the chlorophyll present in the palisade mesophyll tissue (Figure 11).

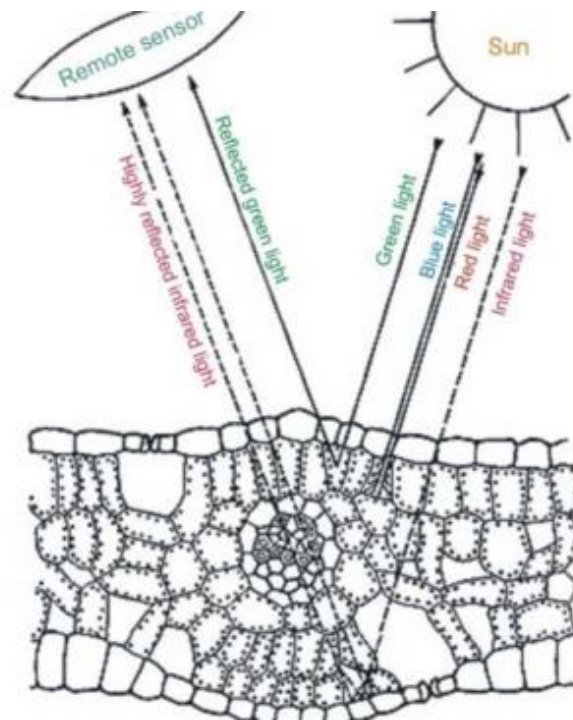


Figure 14: Representation of the interaction of a leaf cell structure with solar radiation (Srtever,2012)

Hydrated cell walls and the presence of intercellular air spaces can cause a discontinuity of the refractive index that vary the reflectance, especially the infrared one (Strever, 2012). The intercellular air spaces are present both in the spongy mesophyll and in the palisade one, what distinguishes the two types of tissue is the distribution and the quantity of these areas that contain air. It is known that the spongy mesophyll has a greater amount of air cavities than the cell content. In palisade tissue, however, the air pockets are arranged between adjacent palisade cells. Light dispersion and consequently the efficiency of chlorophyll in absorption are more advantageous in the palisade tissue region (Knipling et al, 1970).

Infrared reflectance increases in dehydrated leaves and during senescence. This is because despite the substantial loss of water, the structure of the air cavities in the mesophyll is maintained and

there is an increase in the diffusion capacity of the radiation that results in increased leaf reflectance (Knipling et al, 1970).

The interactions of light with the leaf surface are not homogeneous and can vary considering the different climatic conditions in which the plant grows, the age and also the cultivar (Ollinger,2010).

Although these variables can be generalized by saying that 10 % of the incident radiation (I) is reflected from the leaf surface, about 9 % is transmitted (T) and the remaining 81 % is absorbed by the leaf (A). (Figure 12) (Strever, 2012)

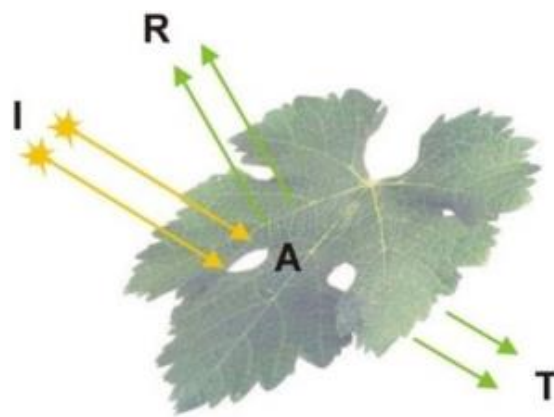


Figure 15: Graphic representation of the subdivision of the incident radiation in radiation absorbed (A), reflected (R) and transmitted (T) on a grapevine leaf (Strever, 2012)

It is interesting to point out that the percentage of light absorbed is in turn divided into radiation emitted (about 20%), energy used for the transpiration process (more than 60%) leaving only 1% for the photosynthetic process (Strever, 2012)

Chlorophyll in palisade cells absorbs in the region of red (about 650 nm) and blue. This last region, and in particular 350-500 nm, is also the part of the spectrum most absorbed by carotenoids. Generally, there is a high reflectance in the NIR region between 700-1300 nm. Pigments do not absorb significantly in the NIR region, for this reason the reflectance in this region is determined by other aspects such as leaf thickness, water content and light dispersion index. It follows that in healthy plants the absorbance is not significant between 780-900 nm. It is therefore able to identify when a plant is damaged or has browning and necrosis when there are increases in absorbance in the region between 800-900 nm (Strever, 2012).

The interaction of the leaf with radiation can be affected by several factors, including the microclimate. It is known that the leaves that are most exposed to solar radiation are usually thicker, which results in longer parenchymal cells or additions of cellular layers. The shaded leaves, especially those of *V. vinifera* have a higher efficiency in the use of diffuse light and a better ability to adapt to changes in exposure. Usually, the leaves that are in the shade have a greater transmittance and reflectance of the visible and near-infrared than the exposed leaves. This phenomenon is explained by considering that in the shaded leaves, there is a greater percentage of intercellular space, while the exposed leaves present a greater amount of intercepting tissue (Strever, 2012).

Another factor that must be taken into account is the structure of the canopy and the aggregation of leaves that affect the percentage and intensity of radiation perceived by the leaves (Ollinger, 2010).

### 3.3 Reflectance

Vegetation reflectance is one of the most commonly used parameters for obtaining information about plants. The quantification of the biochemical and biophysical characteristics of the leaves may concern the use of large areas of the solar electromagnetic spectrum or in other cases, particular spectral regions may be selected. This allows the selection of portions of the spectrum that are more specific for the description of certain properties of the leaf under consideration (Feilhauer et al, 2015).

Using carefully selected spectral regions can be interesting as it allows to clarify any relationship between spectral signatures with molecular activity of the leaf, as well as simplify the adaptation of spectral information in statistical models, making them more precise (Feilhauer et al, 2015).

A practical example can be the correlation between N concentration and chlorophyll in the leaf. It is known that the content of chlorophyll can affect the reflectance of leaves in the wavelengths of green and NIR, so going to monitor these wavelengths you can trace information about the health of plants (Chessa, 2022; Du and Yang, 2018).



In a leaf we can distinguish three regions of variation of the reflectance: in the visible band there is a low reflectance as a consequence of the high absorption made by the chlorophylls, in the near-infrared region the reflectance increases as there is no absorption by pigments, finally, above 1300 nm the reflectance decreases as a result of the absorption of radiation by water (Knipling, 1970). It is important to keep in mind that spectral information also depends on external factors. In fact, the environmental conditions under which reflectance measurements are made can influence the results of the data (Chessa, 2022).

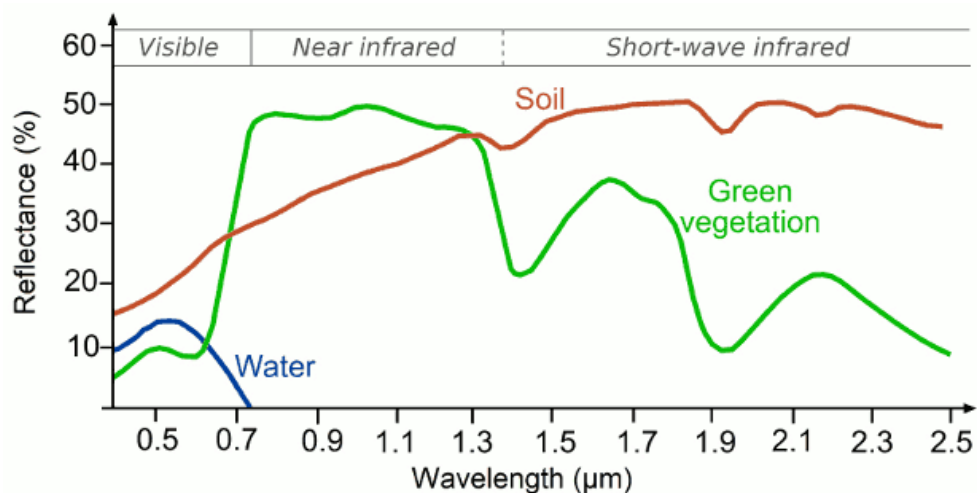


Figure 16: Spectral signatures of different elements: soil, green vegetation and water [www.alspergis.altervista.org](http://www.alspergis.altervista.org)

It has been observed that when a plant is subjected to environmental stress there is an increase in reflectance, especially at the wavelength of 700 nm and the consequent narrowing of the absorption spectrum. This phenomenon is often associated with a reduction in chlorophyll concentration (Carter and Knapp, 2001). As for the variability of the infrared reflectance, chlorophyll concentration varies over time according to the phenological phase, in advanced stages of senescence in fact it tends to decrease (Knipling, 1970).

We have seen how the spectral characteristics of the leaves are influenced by the structure, the phenological stage of the plant and the environmental conditions; an additional factor that must be considered is the water status of the plant. Water deficiencies in fact can have structural consequences for the leaf, which in turn can lead to changes in the bands of water absorption (Ollinger, 2010).

Water is the dominant absorber in the NIR region and is transparent at PAR wavelengths, but has a high potential for visible radiation transmission. In a state of water stress, the water contained in the leaves is lost and replaced by air. The refractive index of air and water are different and this leads to a different interaction with light. This will result in decreased absorption in the NIR region and increased reflectance in this region (Strever, 2012).

Some studies have shown that with the use of spectral signatures in the visible regions and in the NIR it is possible to distinguish the different species of Vitis (Maimaitiyiming et al, 2016).

# CHAPTER 4: Grapevine leaf aging

## 4.1 Leaf development and structural changes

During its development, the leaf undergoes several changes both morphological, functional and biochemical. There are 5 precise stages of development: appearance, development, rapid laminar expansion, senescence and abscission. The average life cycle of a leaf is variable, but it has been observed that to reach the maximum expansion and activity are necessary from 30 to 40 days. From 50-55 days a gradual decline in the rate of assimilation begins. While a decrease in photosynthetic activity has been noted with age, it has been observed that chlorophyll concentrations continue to increase beyond leaf maturity (Strever, 2012).

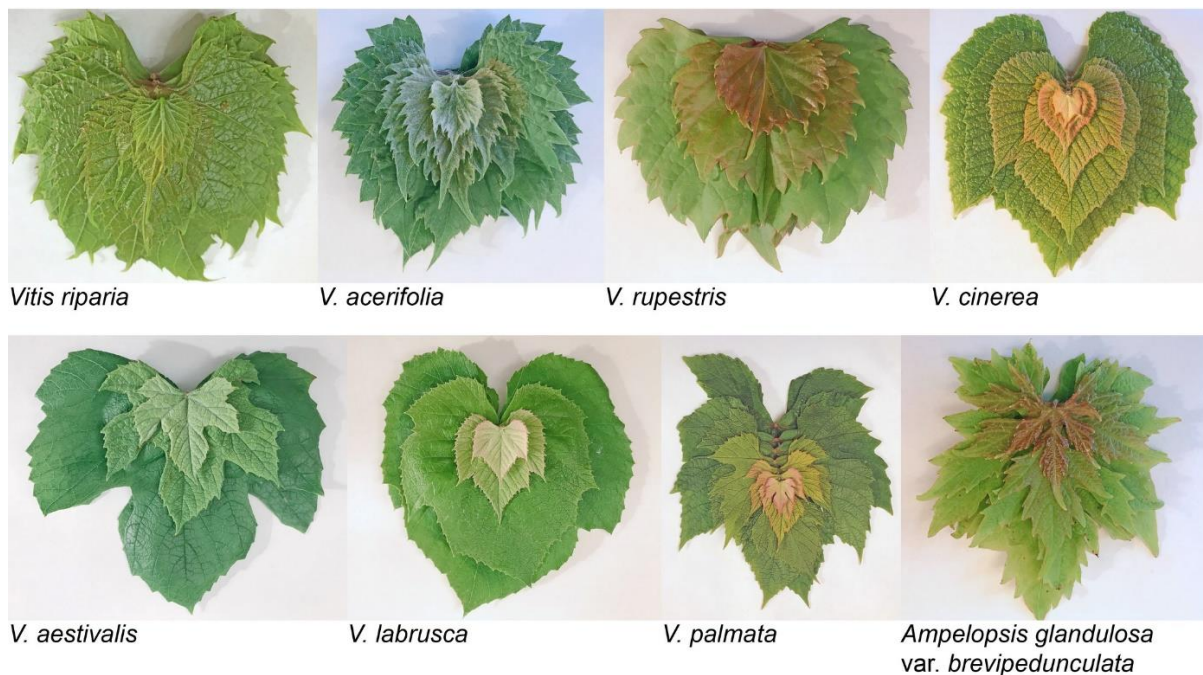


Figure 17: Different developmental stages in different grapevine species (Bryson et al., 2020).

The relationship between photosynthetic activity and chlorophyll content does not remain constant over time and also varies with the position of the leaves. In the early stages of leaf expansion, there is a rapid increase in photosynthesis while the chlorophyll content remains almost constant. This

phenomenon is due to the increase in intracellular air spaces that give less resistance to carbon dioxide entry. In the specific case of the vine, the peak of chlorophyll content coincides with the moment of harvest, and then undergoes a rapid decline (Strever, 2012).

From the point of view of the arrangement in the space instead, it has been observed that in the early stages of development, the content of chlorophyll in the foliar area is significantly lower in the apical leaves than in the basal and median ones. This is until the phenological phase called veraison, when there is a sudden increase in the content of chlorophyll in the more mature apical leaves, which reaches its peak at the end of the season. The ratio of chlorophyll a:b remains constant over time, this means that already at the initial stages the leaves have a developed photosynthetic apparatus (Strever, 2012). The leaves near the bunches, during the ripening period, are photosynthetically weak and are already oriented towards senescence. The apical leaves, on the other hand, maintain a constant photosynthetic performance throughout the season, while the basal ones, after a photosynthetic decline in the post-veraison period, keep contributing to photosynthesis with constant levels (Strever, 2012).

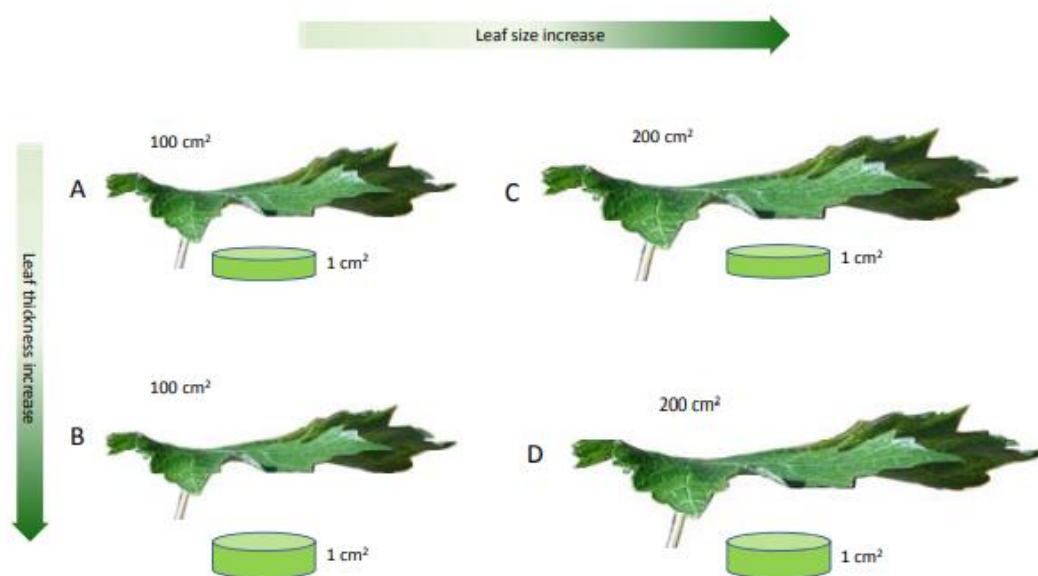


Figure 18: Leaf size and thickness increase during growth (Strever, 2012)

Senescence is a process that involves a series of metabolic changes including the degradation of proteins, chlorophyll until death. There are several causes that can accelerate senescence: low light conditions, nutritional deficiencies and water stress are the most impacting. During these stress situations, the symptoms of senescence in the older leaves and the remobilization of nutrients

towards the younger organs progress (Strever, 2012). The degradation of chlorophyll is considered to be a detoxification mechanism and can occur both chemically and enzymatically. In particular, light, heat, oxygen and weak acids are compounds that accelerate the degradation process (Strever, 2012).

During the expansion of the grapevine leaf, there is a proportional increase in the leaf size and thickness (*Figure 15*), with also the formation of more intercellular spaces (Strever, 2012).

In the visible region, it has been observed that there is a positive correlation between absorbance, quantities of intercepted leaf tissues and absorbing pigments. The younger leaves therefore have a lower capacity of absorption than the mature, but not senescent leaves (Schultz, 1992). While leaf reflectance increases with an increasing number of palisade cells and intercellular spaces and/or when there is a decrease in absorbing leaf tissue. The reflectance is greater in the apical leaves at the beginning of the season and in the basal senescent at the end of the season (Schultz, 1992). As a result of the degradation of chlorophyll due to senescence pathways there is an increase in reflectance and transmittance, which is not related to the thickness of the leaf (Strever, 2012).

If we consider the relationship between leaf age and NIR radiation, as the leaf develops there is an increase of reflectance while the transmittance decrease (Strever, 2012).

## 4.2 Plastochron index

The Plastochron index was proposed by Erickson and Michelini (1957) as a method to measure the morphological age of leaves in a precise way. A plastochron is defined as the period between any identical points in the development of successive leaves (Chen et al, 2009). It records organogenesis rather than an increase in mass or length (Strever, 2012).

In other words, PI gives us information on the occurrence of critical developmental events and it is applicable among a growing population giving the same measure of time among all the organisms of such a population. The use of chronological time becomes problematic when analyzing plant growth and development because it will lead to measures with extremely wide variances (Meicenheimer, 2014). In fact, plants of the same chronological age might not be at the same

phenological stage and this makes problematic and not equal the comparison between them (Ford, 1982)

The plastochron index (PI) is calculated following the equation (Strever,2012):

$$PI = n + \frac{\ln L_n - \ln R}{\ln L_n - \ln L_{n+1}}$$

n = the number of leaves, numbering acropetally, equal to or longer than the reference length

R= the reference length (30 mm),

Ln = the length of leaf n

Ln+1= the length of the leaf smaller than leaf n.

The calculation of PI incorporates leaves lamina length and in order to be a significant measure it has to be determined during the active growth period of the shoot (Strever, 2012). The lamina length has to be recorded from the leaf petiole attachment to the tip of the leaf, having as a reference length 30 mm (Strever, 2012).

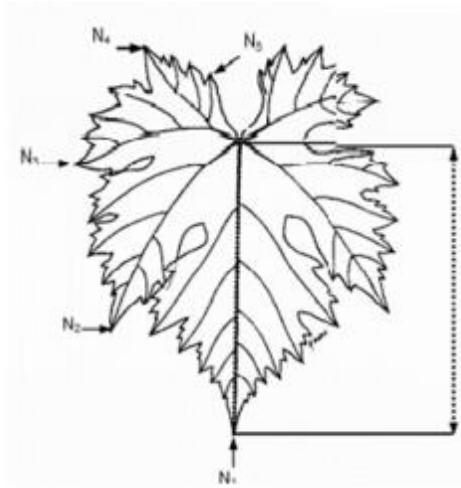


Figure 19: Leaf vein measurement (Meggio, 2022)

Leaf plastochron index (LPI) can be calculated by the subtraction of leaves from the PI, and has become a fundamental tool for the study of the morphological and physiological development of leaves and plant organs in general (Chen et al, 2009). In order to use LPI to quantify shoot development, successive PI have to be of equal duration (Strever, 2012).

Leaf plastochron index (LPI) is calculated according to the following equation (Strever,2012):

$$LPI_i = PI - i$$

Where  $i$  is the serial number (node number) of the leaf in question.

LPI is 0 when  $i$  equal the reference length ( $i=R$ ), negative when  $i$  is smaller than  $R$  ( $i<R$ ) and positive when  $i$  is greater than  $R$  ( $i>R$ ) (Meicenheimer, 2014).

A limitation of this index is the misestimating of the leaves' growth curve when the plastochrons are not of the same duration (Chen et al, 2009).

*Table 1: Chronological and Physiological leaf age representation. (Strever,2012).*

<b>Class</b>	<b>Category</b>	<b>Leaf age (main)</b>	<b>LPI range</b>
<b>1</b>	Young	<20 d	<5
<b>2</b>	Approaching max	20-50 d	5-10
<b>3</b>	Max	50-90 d	10-15
<b>4</b>	Declining	90-120	15-20
<b>5</b>	old	>120 d	>20

PI expresses the morphological age of a plant in terms of the total plastochron that meets the length requirements while LPI expresses the morphological age of any organs of a plant relative to the reference length (Meicenheimer, 2014).

In order to create valid data, the measured organs have to meet some specific criteria: firstly, the growth of the organ has to be exponential. Secondly, successive organs must have the same relative rate, and lastly, the PI has to be constant for a particular plant (Meicenheimer, 2014). This particular point is not always possible in field conditions and can be prone to high variance between plants (Strever, 2012). For example, low temperatures and water scarcity might lengthen the plastochron (Meicenheimer, 2014).

Usually in the vine, the decrease in physiological activity and the presence of limiting growth conditions is the main reason for PI stabilization. On the other hand, these conditions are the main

limitation of the expression of leaf age with LPI. The limited growth would result in a stable value of LPI over time when instead the physiological age of the leaf evolves (Strever, 2012).



# CHAPTER 5:

## 5.1 Materials and methods

Measurements were conducted during the growing season of 12 resistant varieties of grapevine in an experimental vineyard of the University of Padua, located in Legnaro.



Figure 20: Location ([www.tesaf.unipd.it](http://www.tesaf.unipd.it))

The period chosen for data collection was between May 2022, which coincides with the budbreak, until the maximum leaf development in July 2022, for a total of 7 sampling dates. The first 3 measurements were made weekly and with the advance of the growing season, the samples were taken about 10-15 days apart.

The study was conducted on 12 different resistant varieties called PIWI. It has been taken into account 3 rows having about 10 vines for each variety. Then 3 vines were selected for each variety and for each of these, a vine spur was chosen for measurements on the leaves. For each instrument, the measurements were carried out starting from the first basal leaf and then proceeding to all the other leaves.

The PIWI varieties examined are: Merlot Khorus, Regent, Merlot Khantus, Sauvignon Nepis, Solaris FR 360, Sauvignon Kretos, Fleurtai, Aromera, Cadernet Cortis, Cabernet Carbon, Bronner, Prior.

Data were collected using 3 instruments: spectroradiometer, porometer and a ruler for measuring the length of the main leaf vein. For each leaf, there are therefore 3 types of data: spectral data, data of stomatic conductance and data of plastochron.

Leaf porometer measurements were acquired between 10:00 to 12:00 a.m. local time. The instrument needs calibration with the sunlight present on-site at the time of data collection. For this reason, it was decided to keep the time for the porometer measurements unchanged in order to have a calibration as comparable as possible. No precise time has been used for the other instruments as the external light was not required for calibration.

### 5.1.1 PIWI

Little is known about these vineyards, although they are beginning to expand.

Lately, the reduction of the use of pesticides has been one of the main concerns when talking about the wine-making sector. A solution to this problem is the creation of new varieties through the use of plant breeding. The aim of a plant breeding program is the creation of new grape varieties which have to maintain the same yield performance of the predominant grape varieties (Montaigne et al., 2016).

There are different approaches to achieving the creation of resistant varieties and these also vary according to the State in which the project is carried out. In France, for example, inter-specific crossing is not allowed and is then carried out through intraspecific hybridization. This choice, adopted by France at the end of the 1950s, has, however, prevented the possibility of incorporating resistance genes present in species of *Vitis* other than *vinifera*.

Other lines of research have been adopted by Germany and Switzerland, which have led to interspecific crossings followed by "Backcrossing". The backcrossing consists in reintroducing genes of *V. vinifera* in order to obtain plants that maintain very high percentages of viniferous genes and at the same time have the resistance factors of other *Vitis* species (Montaigne et al., 2016). Backcrossing allows transferring of useful genes from a donor to a variety that is lacking from some advantageous characteristics. The donor with the selected gene is used only in the first crossing, while the other parent is constant in the subsequent backcrossing (Lucchin, 2020).

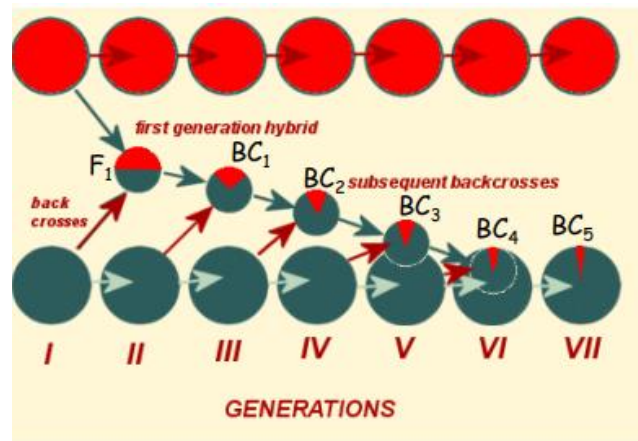


Figure 21: Backcrossing (Lucchin, 2020).

In recent years the European Union is committed to developing new strategies for reducing the use of pesticides, including copper. One of the ways we are going is to prevent the development of fungal diseases through the development of new varieties resistant to fungi. This approach consists in the creation of interspecific crossings between the species *V. vinifera* typical of the Mediterranean environment and species of *Vitis* originating from North America and Asia. The latter, including *V. riparia*, *V. rupestris* and *V. amurensis*, in fact have a higher percentage of genes of resistance to fungal diseases, than *V. vinifera*, which is particularly susceptible to these pathogens. Early attempts at resistant strains had a small percentage of genetics derived from *V. vinifera* and were considered "interspecific hybrids". With the development of new technologies and methodologies, such as marker-assisted selection in combination with multiple crossing of *V. vinifera* varieties, has come to the creation of varieties with a percentage of *V. vinifera* greater than 85% and at the same time with the presence of resistant genes (Pedneault and Provost, 2016). One of the major limitations of resistant varieties is that they are not included in the current labeling system for geographic indication wines or designation of origin wines, which concept is based on local fair and constant practices (Montaigne et al., 2016).

In order to promote these pioneering new varieties, however, new brands such as the PIWI labelling system have been created. PIWI comes from the word "Pilzwiderstandsfähige" of German origin, which means "resistant to fungal diseases" (Montaigne et al., 2016).



Figure 22: PIWI logo ([www.piwi-international.org/it](http://www.piwi-international.org/it))

Fungal pathogens are one of the main problems affecting the vine globally. *Vitis vinifera* is undoubtedly the species most widely present in the agricultural world, but at the same time it is also the most susceptible to different diseases. The most dangerous and widespread fungal infections are powdery mildew and downy mildew. Their effect affects the vitality of the plant and consequently also affects the productivity of the same and the quality of the wine (Ferreiral et al, 2004). The main control method used for the control of fungal diseases is the application of fungicides, which, while they can solve the problem in the short term, on the other hand, require considerable costs and produce significant environmental problems. These are the main reasons why in recent years it has been looking for alternative solutions and in particular it has been aiming at the development of strategies that exploit and strengthen the mechanisms of plant defense (Pedneault and Provost, 2016).

The introduction of resistant varieties could therefore bring several advantages in the management of fungal diseases in both conventional and biological viticulture. There would be a reduction in production costs and a lower environmental impact especially with regard to the accumulation of Cu in soils, and in particular in organic vineyards. Unlike conventional viticulture in which there is a wide range of fungicides, in the biological one the main methods of defense against fungal diseases are copper and sulfur. Copper, being an element with low mobility, tends to accumulate in the soil, especially in conjunction with perennial crops such as vines. It has been observed, in fact, that the concentration of copper present in the vineyards varies between 20-665 mg/kg, unlike other cultivable soils where there is a range between 5 and 30 mg/kg. The climate, the percentage of soil moisture and the type of vineyard management are other factors that influence the presence of copper. In fact, a higher concentration of copper was observed in conventional vineyards located in

wetlands of Northern Italy, compared to organic vineyards located in Mediterranean areas with dry climates (Pedneault and Provost, 2016)

The costs for disease control is very high, for example it has been estimated that for the control of downy mildew in vineyards of *V. vinifera* managed under conventional regime, with an average disease pressure are needed between 8 and 16 million euros per year, for a total of 12 seasonal treatments. Studies have instead shown that for more than 180 varieties of resistant vines, managed with biological regime, less than 4 treatments per season are sufficient. This suggests that the transition to the use of resistant varieties could lead to a substantial and significant reduction of costs (Pedneault and Provost, 2016).

The first steps towards intrerpecific hybridization led to poor results, due to the development of unwanted flavors in the final product. Subsequently *V. lambrusca* was identified as the reason for these failures and was eliminated from the bred species. The identification of markers linked to agronomic characteristics and disease resistance has made it possible to act more accurately in the creation of resistant hybrids (Pedneault and Provost, 2016)

A plant is defined as resistant when it has the ability to defend itself independently from the attacks of pathogens. A plant that has genes linked to resistance is able to activate specific pathogenic-defense mechanisms. This results in the recognition of specific proteins of the pathogen, called proteins Avirulente (Av), which lead the plant to react with a hypersensitive response. However, some pathogens have developed mechanisms to bypass plant defenses. However, there are methods to make the resistance of plants longer lasting, one of them is the pyramidization of resistant haplotypes from different species of vine (Pedneault and Provost, 2016).

During the growth and development of leaves and berries, the plant produces proteins (PR) structurally and functionally unrelated to each other but which play a protective role against fungal attacks and other stresses (Ferreiral et al, 2004). Their intrinsic properties such as acidity and resistance to proteolytic degradation and acidic environments make it possible to damage the structures of the fungus. Among the PR proteins in fact there are  $\beta$ -1,3-glucanase (PR-3) and chitinase (PR-4) which are specific enzymes that hydrolyze glucans and chitin, which are the main structural components of fungi (Ferreiral et al, 2004). Generally, wines contain concentrations of proteins between 15 and 230 mg/L, and despite their quantities are negligible, they have a decisive role for the quality of the final product (Ferreiral et al, 2004).

Wines produced from resistant vineyards have some negative sides, including color instability and flavor alteration. These problems arise from the lack of tannins (200 mg/L EC eq) and the abundance of anthocyanins (200-1200 mg/L M3G eq). These values differ quite substantially from the values found in wines produced by *V. vinifera*, which contain between 150 and 600 mg/L eq CE of tannins and between 200 and 400 mg/L of anthocyanin. The low levels of tannins and their low retention in resistant wines were related to the high presence of PR proteins that contribute to the precipitation of tannins during vinification. (Pedneault and Provost, 2016).

Clarity and stability of wine in fact are directly related to the presence of these nitrogenous polymers that can denature and settle creating turbidity in bottled wine and substantially reducing its commercial value. The over-expression of PR typical of genetically modified plants will therefore lead to more resistant plants, but with increased problems of instability of the resulting wines (Ferreiral et al, 2004). Currently, to try to buffer this problem, is proceeding with clarification with betonite, which however, not being specific for proteins also leads to the removal of many aromatic components (Ferreiral et al, 2004). The amount of PR proteins present in wines also depends on the disease pressure present in the vineyard, being tendentially higher in organic wines than in non-organic ones (Pedneault and Provost, 2016).

Another fundamental problem for marketing wine produced from resistant grapes is the lack of knowledge of the consumer of their existence. (Pedneault and Provost, 2016)

The current situation therefore allows to take two paths: the first that guarantees the cultivation of vineyards with the ability to resist attacks by fungal pathogens and the other we have the cultivation of vines sensitive to fungal diseases, which require continuous applications of fungicides, but which allow the production of stable wines (Ferreiral et al, 2004).

## 5.1.2 Spectroradiometer

In order to obtain detailed information on the reflectance characteristics of vegetation, we often rely on instruments capable of reading the spectral characteristics called spectroradiometers. The solar radiation that is emitted by matter in fact can give information on the biochemical and biophysical characteristics of the object under consideration (Feilhauer et al., 2015)

The selection of significant spectral regions may allow a more in-depth study of the relationship between spectral signatures and leaf traits (Feilhauer et al., 2015).

The instrument used is a portable spectroradiometer that has a spectral resolution that includes all the spectrum of solar irradiance (350 -2500 nm) ([www.directindustry.it](http://www.directindustry.it)).

A spectroradiometer is a particular kind of spectrometer used for measuring radiant energy. The FieldSpec was the spectroradiometer selected for this thesis for collecting data. FieldSpec is designed for outdoor remote sensing using a fixed fiber optic cable. The data collection it's extremely rapid, at about 0.1 seconds per spectrum with a spectral range of 350-2500 nm. Spectral radiance is usually expressed as Watts per square meter per steradian per nanometer (W/m<sup>2</sup>/sr/nm) and is defined as the amount of radiance per unit wavelength (FieldSpec® 3 User Manual, 2010)

The spectral resolution is (FieldSpec® 3 User Manual, 2010):

- 3 nm (Full-Width-Half-Maximum) at 700 nm.
- 10 nm (Full-Width-Half-Maximum) at 1400 nm.
- 10 nm (Full-Width-Half-Maximum) at 2100 nm.

The sampling interval is (FieldSpec® 3 User Manual, 2010):

- 1.4 nm for the spectral region 350-1000 nm.
- 2 nm for the spectral region 1000-2500 nm

<b>Wavelength Name</b>	<b>Wavelength Range</b>
VNIR-SWIR1-SWIR2	350 - 2500 nm
VNIR only	350 - 1050 nm
VNIR-SWIR1	350 - 1800 nm
SWIR1 only	1000 - 1800 nm
SWIR1-SWIR2	1000 - 2500 nm
SWIR2 only	1800 - 2500 nm
VNIR & SWIR2	350 - 1050 nm and 1800 - 2500 nm

Figure 23: FiledSpec wavelength (FieldSpec 3 User Manual, 2010)

A laptop computer is used as the instrument controller, in order to manage the instrument, store the data and process the results (FieldSpec® 3 User Manual, 2010).

FieldSpec spectroradiometer is able to measure: spectral reflectance, spectral transmittance, spectral absorbance, spectral radiance, and spectral irradiance (FieldSpec® 3 User Manual, 2010).

The optical energy is efficiently collected by a bundle of optical fibers that provide the maximum transmission available across the wavelength range of the instrument. Once the optical energy is collected by the fiber cable it is then projected onto a holographic diffraction grating. At this point, the different wavelength components are separated by the grating and are measured independently by the instrument detectors. There are 3 different detectors to different portions of the spectrum (FieldSpec® 3 User Manual, 2010).

- VNIR (Visible/Near infrared): 350-1000 nm
- SWIR1 (Short-Wave Infrared): 1000-1830 nm
- SWIR2 (Short-Wave Infrared): 1830-2500 nm

Each detector converts incident photons into electrons, that are subsequently converted into digital data. The digitalized spectral data are then processed and analyzed by the instrument controller (FieldSpec® 3 User Manual, 2010).

The sample and the light source are variables that influence the measurement made by the spectroradiometer. For this reason, in order to properly calculate the reflectance of the sample an independent measure of the light source illumination on a reference of known reflectance is required. Usually for this purpose and to simplify the calculation a white reference panel is used. It is a material with nearly 100% reflectance across the entire spectrum (FieldSpec® 3 User Manual, 2010).

It is important to underline that reflectance and transmittance are independent of the light source and are inherent properties from a sample.





*Figure 24: FiledSpec front-view (FieldSpec® 3 User Manual, 2010)*

The accuracy of the collected data can be influenced by many factors such as the sources of illumination, the atmospheric characteristics and stability while collecting data, the presence of winds, and the time needed for the instrument for scanning the object. The spatial and temporal variability of the sample characteristics is also a source of variability when talking about data accuracy (FieldSpec® 3 User Manual, 2010).

The spectral response of the target material alone does not give a valid and acceptable data. It is also necessary to have the spectral response of a reference sample. The spectral parameters are then calculated by dividing the spectral response of the target material with that of a reference sample. However, it is necessary that the lighting characteristics remain unchanged during both measurements in order to avoid errors in the resulting spectra (FieldSpec® 3 User Manual, 2010).

Usually for field measurements, the lighting source is sunlight. In the specific case of this thesis work, the lighting source was provided with the instrument. With this method, several problems have been avoided including the surrounding scattered light of the person and the instrument that are making the measurement.

As has already been pointed out, the raw reflectance measurements of an object are affected by the characteristics of the sample itself and by the light source at the time of detection. In order to calculate the reflectance of the sample, therefore, it is necessary to have a measure that may not depend on the characteristics of the light source on a known reflectance reference. Using a white reference panel, also called a reference standard, the ASD application software is able to calculate the reflectance or transmittance ratios of the sampled material. The main feature of a white reference panel is to have a reflectance that is close to 100%. In this specific case, the reference

standard is Spectralon by Labsphere, made of tetrafluorortilene polyethylene and cintered halon. This material is able to diffuse light evenly in all directions within the 350 nm and 2500 nm range (FieldSpec® 3 User Manual, 2010).

The use of artificial illumination incorporated into the field spectrometer has quite a few strength points: it allows to have more control over illumination and viewing geometry. Moreover, it makes it possible to collect data also during non-optimal atmospheric conditions (FieldSpec® 3 User Manual, 2010).

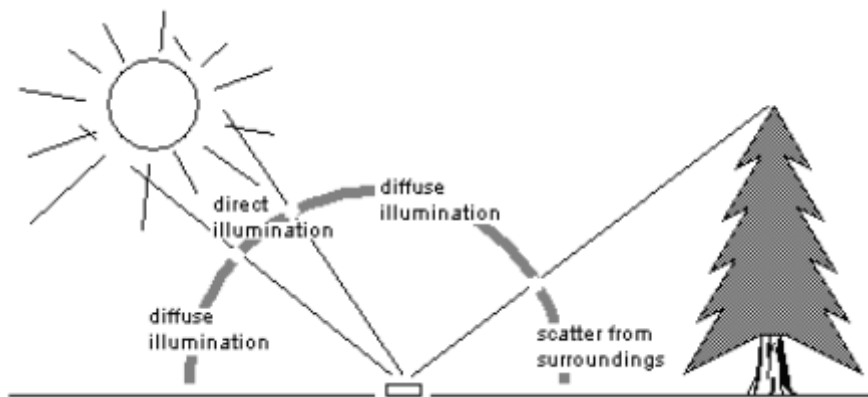


Figure 25: Source of illumination (FieldSpec® 3 User Manual, 2010)

The organic composition of the leaves remains relatively constant in all plant species and is the factor influencing the spectral characteristics of vegetation.

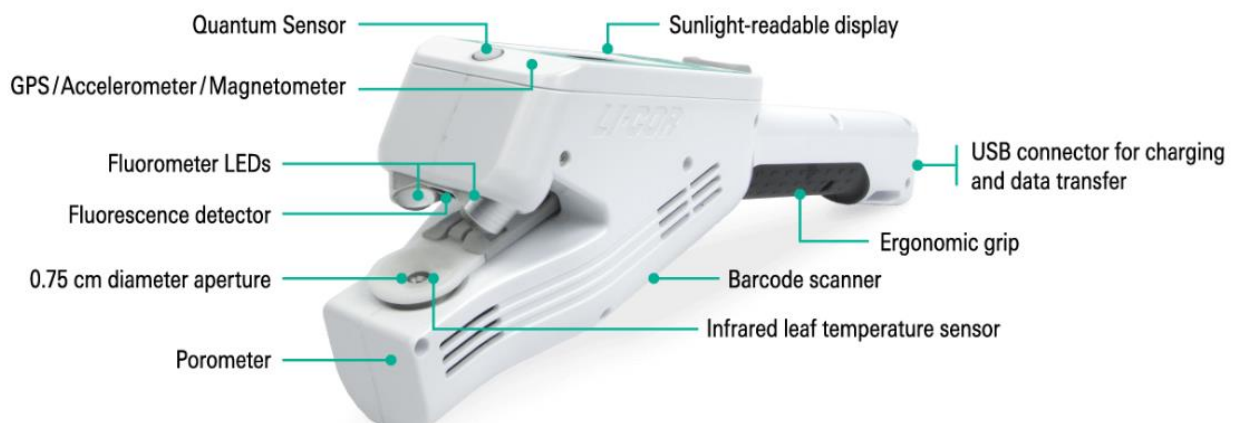
Given the uniformity of the organic components, the information about the leaf's will be given by the intensity of the various features of absorption and in particular of water and vegetable pigments (chlorophyll, carotenoids). Other non-photosynthetic components that may affect spectral characteristics are lignin and cellulose.

The optimization of the instrument should be taken when some changes in the environment.

### 5.1.3 Leaf porometer and fluorometer

The instrument selected for this study is the LI-600 Porometer/Fluorometer. This instrument is able to take both stomatal conductance and PAM chlorophyll a fluorescence measurements of a leaf in seconds. It also has the possibility to track and map the sample location thanks to the presence of a GPS receiver. In order to ensure a good accuracy and quality of the measurement it is important to maintain both the original orientation of the leaf and the light condition of the leaf under examination. Another important rule to follow is to avoid clamping onto the veins of the leaf (Using the LI-600 Porometer/Fluorometer Instruction Manual, 2022).

Figure 26: LI-600 structure and main elements. ([www.licor.com/env/products/LI-600/#how-it-works](http://www.licor.com/env/products/LI-600/#how-it-works))



#### 5.1.3.1. Leaf porometer

LI-600 is an open system that derives the stomatal conductance ( $g_{sw}$ ) by measuring transpiration ( $E$ ) in a leaf cuvette. Transpiration is calculated from the difference in water in an air-steam flowing through a leaf cuvette. Also leaf temperature ( $T_{leaf}$ ) is measured by a non-contact infrared sensor in the cuvette (Using the LI-600 Porometer/Fluorometer Instruction Manual, 2022).

The total conductance to water vapor is given by:

$$g_{tw} = \frac{E \left(1 - \frac{(W_{leaf} + W_{sam})}{2}\right)}{(W_{leaf} - W_{sam})}$$

Where  $E$  is transpiration ( $\text{mol m}^{-2}\text{s}^{-1}$ ),  $W_{\text{leaf}}$  and  $W_{\text{sam}}$  are water vapor mole fraction into and out the leaf cuvette ( $\text{mol H}_2\text{O mol air}^{-1}$ ).  $W_{\text{leaf}}$  derives from  $T$  leaf and pressure measurements and can be expressed as the molar concentration of water vapor within the air space present in the leaf cells. In order to calculate the stomatal conductance ( $g_{sw}$ ) it is necessary the removal of the boundary layer ( $g_{bw}$ ), like is shown in the equation [ $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ] (Using the LI-600 Porometer/Fluorometer Instruction Manual, 2022):

$$g_{sw} = \frac{1}{\frac{1}{g_{tw}} - \frac{1}{g_{bw}}}$$

The air steam that passes through the leaf is measured by the Relative humidity (RH) sensor before and after it interacts with the leaf.

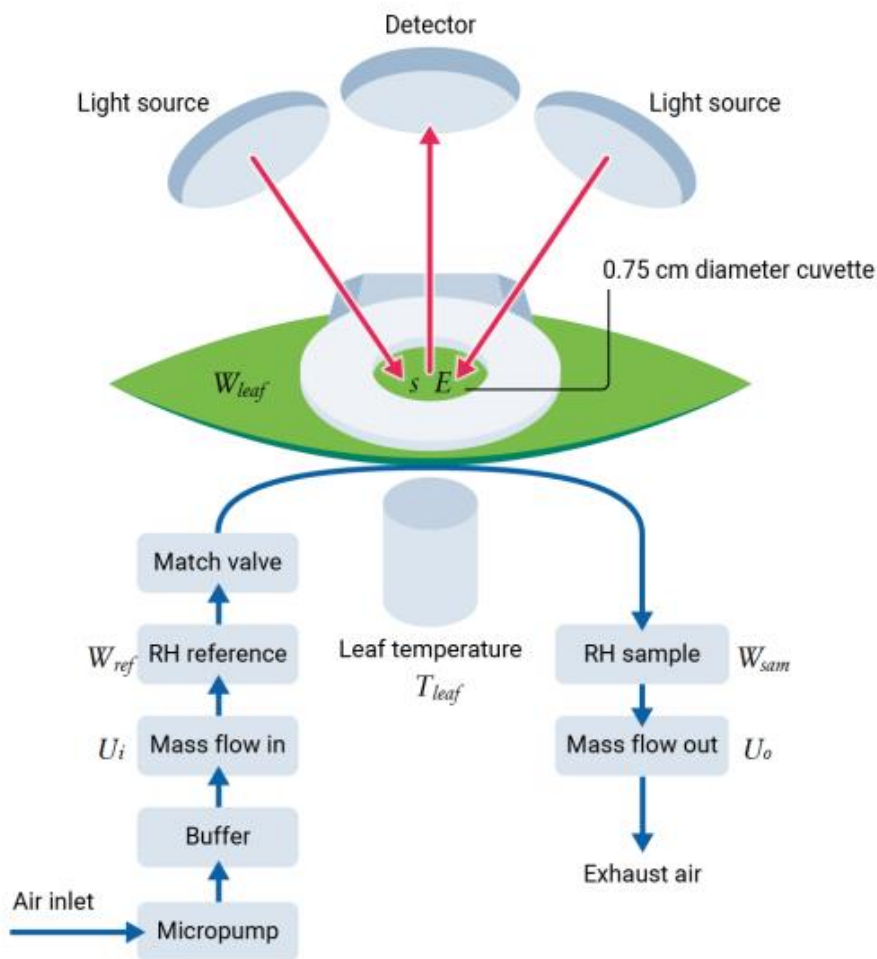


Figure 27: schematic representation of LI-600 internal function ([www.licor.com/env/products/LI-600/#how-it-works](http://www.licor.com/env/products/LI-600/#how-it-works))

### 5.1.3.2. Leaf fluorometer

LI-600 is also able to provide insights into photosynthesis, by using an optical sensor measuring chlorophyll a fluorescence. LI-600 in fact is a Pulse-Amplitude Modulated fluorometer that is able to detect fluorescence via a single detector located between 2 LED measuring beams and filtered by a  $750 \pm 40$  nm band-pass filter. The majority of the fluorescence detected comes from the PSII because the fluorometer detects from 700 to 780 nm (Using the LI-600 Porometer/Fluorometer Instruction Manual, 2022).

The actinic light source is not provided by the instrument but by external sources, and in this case of study was the natural sunlight (Using the LI-600 Porometer/Fluorometer Instruction Manual, 2022).



Figure 28: LI-600 while measuring ([www.licor.com/env/products/LI-600/#how-it-works](http://www.licor.com/env/products/LI-600/#how-it-works))

### 5.1.3.2. Vegetation Index

Vegetation Indices derive from mathematical functions that relate specific wavelengths. The resulting number is then related to a specific phenomenon or characteristic of the object under consideration (Pirotti, 2019).

There are different types of indices, one of them is the index linked to the state of vegetation. These indices highlight vegetation information in a direct or indirect way as they are linked to specific and measurable phenomena such as LAI, biomass, etc (Pirotti, 2019).

Table 2 Vegetational Index

Index	Formula
Normalized Difference Vegetation Index	$NDVI = \frac{R_{800} - R_{670}}{R_{800} + R_{670}}$
Green Normalized Difference Vegetation Index	$GNDVI = \frac{R_{800} - R_{550}}{R_{800} + R_{550}}$
Modified Simple Ratio	$MSR = \frac{\frac{R_{800}}{R_{670}} - 1}{\sqrt{\frac{R_{800}}{R_{670}} + 1}}$
Transformed Chlorophyll Absorption Ratio Index	$TCARI = 3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550}) * \left(\frac{R_{700}}{R_{670}}\right)]$
TCARI/Optimized Soil-Adjusted Vegetation Index	$\frac{3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550}) * \left(\frac{R_{700}}{R_{670}}\right)]}{(1 + 0.16) * (R_{800} - R_{670}) / (R_{700} + R_{670} + 0.16)}$
Green Index	$GI = \frac{R_{554}}{R_{667}}$
Simple Ratio Index 800/550	$SRI = \frac{R_{800}}{R_{550}}$
Visible Atmospherically Resistant Index	$VARI = \frac{R_{550} - R_{670}}{R_{550} + R_{670} - R_{470}}$
Normalized Difference Greenness Vegetation Index	$NDGI = \frac{R_{550} - R_{670}}{R_{550} + R_{670}}$
Photochemical Reflectance Index	$PRI = \frac{R_{530} - R_{550}}{R_{530} + R_{550}}$
Enhanced Vegetation Index	$EVI = 2.5 * \frac{R_{800} - R_{670}}{R_{800} + 6 * R_{670} + 6 * R_{470}}$
Water Band Index	$WBI = \frac{R_{950}}{R_{900}}$

One of the most widely used indices for vegetative stress problems is NDVI.

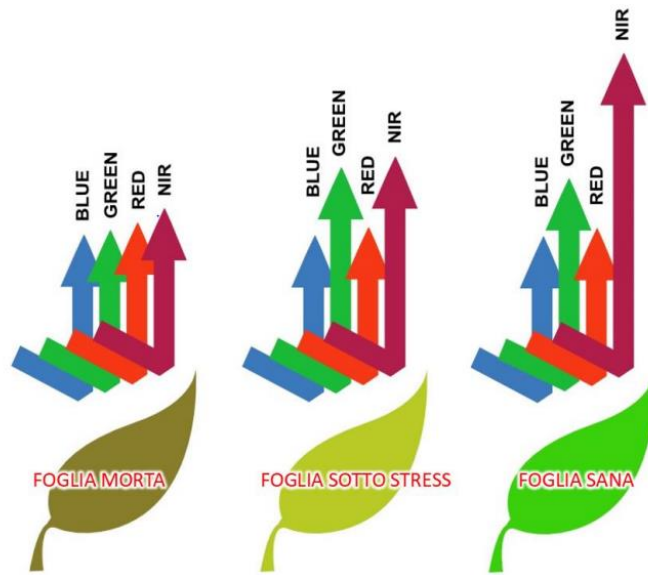


Figure 28 : reflectance of VIS and NIR in dead leaf, stressed leaf and healthy leaf (Pirotti,2019)

For the purposes of this study, some of these vegetation indices measuring greenness were selected, including: NDVI, GNDVI, MSR, TCAR, GI, SRI, VARI, NDGI, EVI. The VI called PRI instead measures the efficiency of the use of incident light used for photosynthesis by the plant. The PRI is linked to the radiation dissipation capacity of the leaves and is based on the cycle of xanthophylls. The ratio between TCARI and OSAVI has been selected as it makes it possible to minimize variations in the results that can be given by the soil in the background and the leaf area index. WBI instead gives us an idea of the variation in the relative water content of crops (Cogato et al., 2021).

## 5.2 Results and Discussion

In the present analysis, to allow a better observation of the effects of the evolution of the leaf demography along the shoot on the spectral responses of different varieties, only one replicate (i.e. one shoot out of the three variety) was selected. The chosen replicate has been selected based on completeness in the number of leaves present and homogeneity among cultivars, to allow the most complete comparison possible.

It has been observed that in all varieties a consistent behavior is maintained that can be divided according to the position of the leaves on the shoot.

The leaf classes can be divided: i) basal leaves: from leaf 1<sup>st</sup>-4<sup>th</sup> node ii) median leaves: 5<sup>th</sup>-7<sup>th</sup> node  
3) apical leaves: from 8<sup>th</sup>-10<sup>th</sup>.

In the basal leaves it is worth to notice a decrease in the reflectance over time in the portion of the spectrum between 800 and 1400 nm. Indeed, reflectance is inversely proportional to the amount of absorbed radiation in the near-infrared portion. As the biomass increases and the leaf mesophyll thickens, the fraction of absorbed radiation in the NIR portion increases, resulting in a decrease in reflectance. As reported for the NIR portion of the spectrum also in the PAR region (400-700 nm) a decrease of the reflectance that is concentrated more in the green bands (500-570nm) can be observed over time. As leaf age increases, the trend is reversed, especially from the last two dates (06/07 and 20/07).

Reflectance values in NIR and PAR begins gradually to increase with leaf age. The senescence of the leaf which results in the degradation of chlorophylls, and the loss of turgor, result in an increase in reflectance in the infrared region. The contents of water and chlorophyll influence the interaction of the leaf with the radiation. Water that is lost with leaf ageing is replaced by air and this affects its spectral characteristics (Strever, 2012).

It has been observed that in conjunction with the median leaves, usually leaf 6-7, a decrease in reflectance over time is observed, but poor differences are evident. In general, the trend of decrease of reflectance is maintained with the leaf age, but this difference does not differ markedly between the different dates.

Studies have shown that in the initial stages of leaf growth, the amount of chlorophyll present in the apical leaves is lower than the amount present in the basal and median leaves. This situation remains stable until the phenological phase called veraison. From veraison onwards a sudden increase in the amount of chlorophyll of the apical leaves occurs (Strever, 2012). This almost opposite trend compared to that observed in the basal and median leaves could explain why in the apical leaves the reflectance in NIR and PAR increases over time.



Aromera

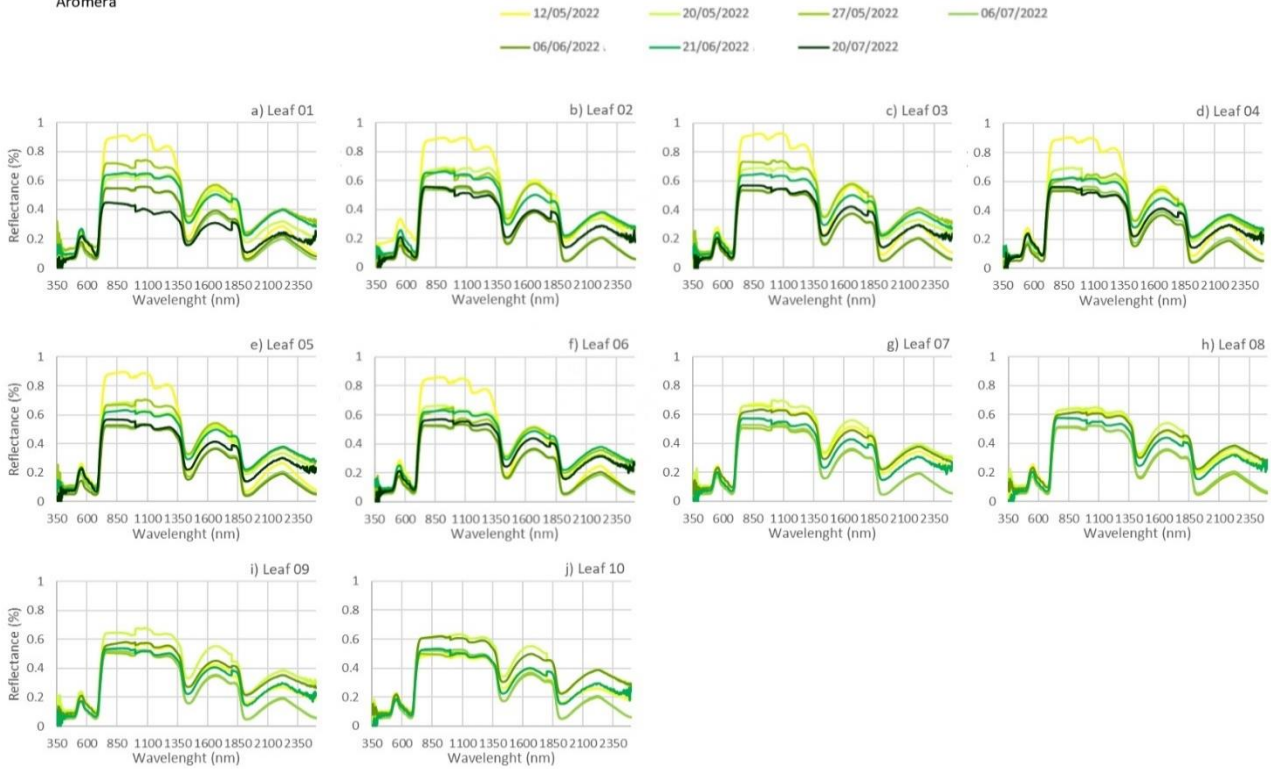


Figure 29: Aromera spectral behavior

C. Carbon

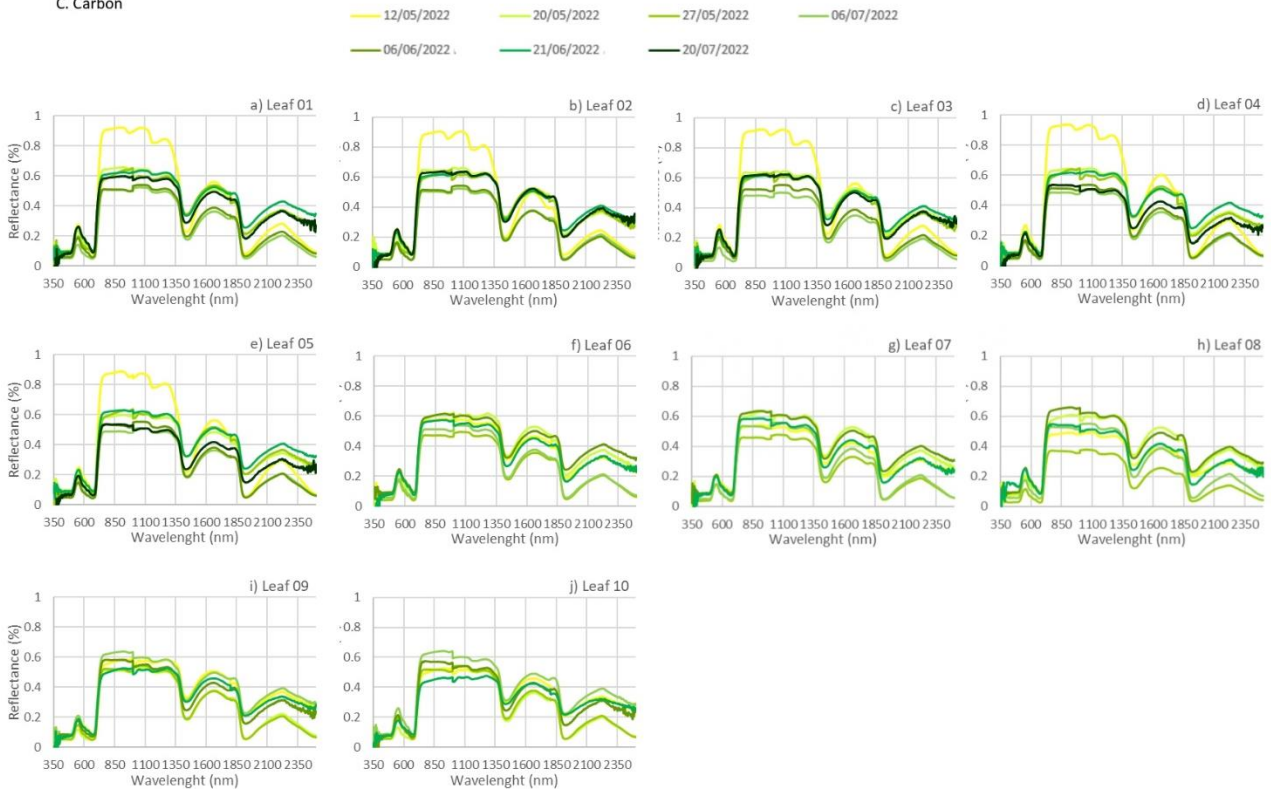


Figure 30: C. Carbon spectral behavior

**Bronner**

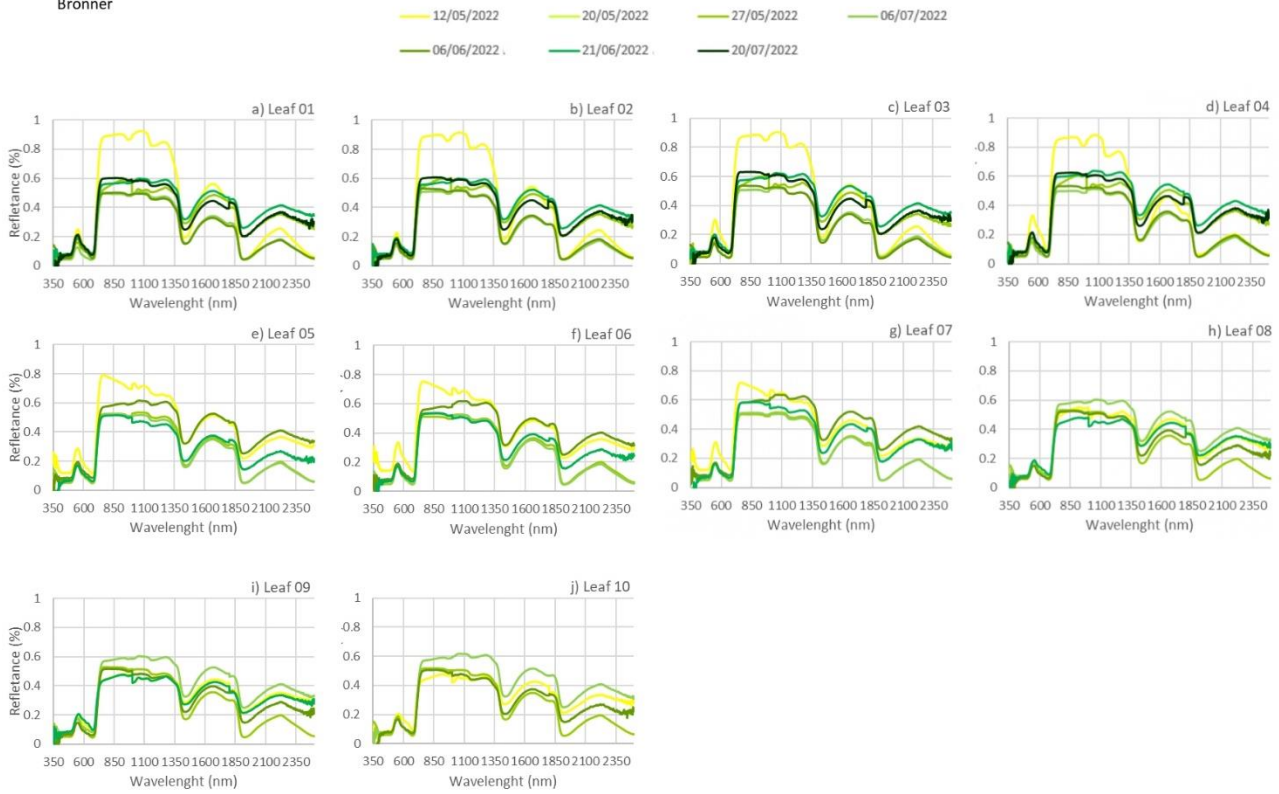


Figure 31: Bronner spectral behavior

**Fleurtaï**

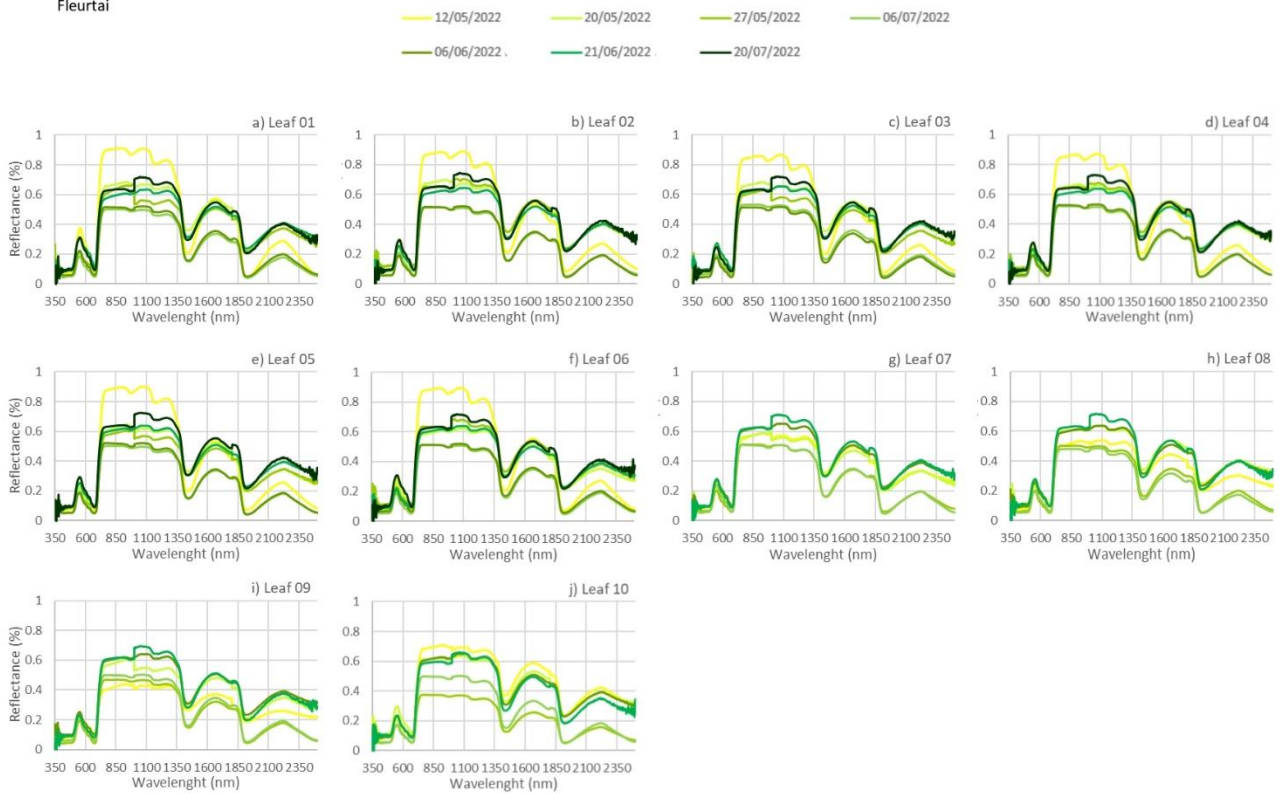


Figure 32: Fleurtaï spectral behavior

C. Cortis

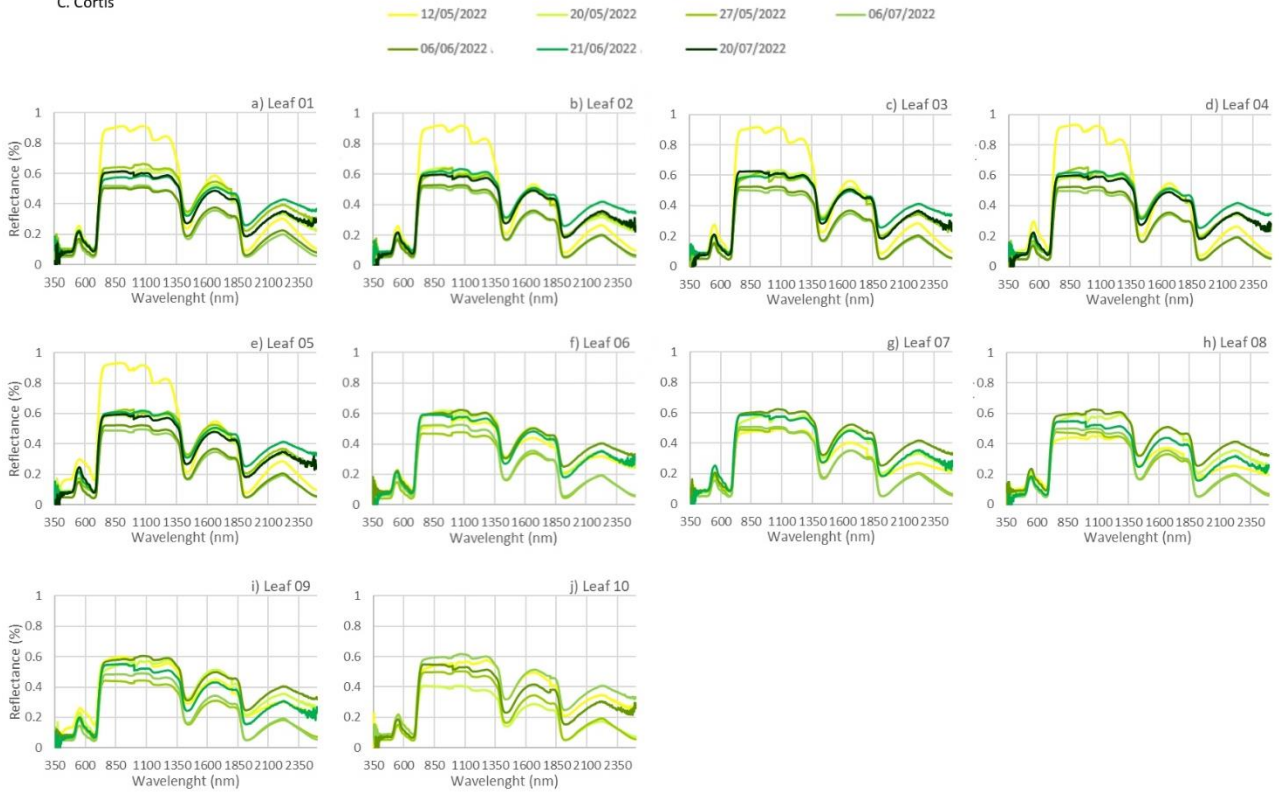


Figure 33: C. Cortis spectral behavior

Merlot Khantus

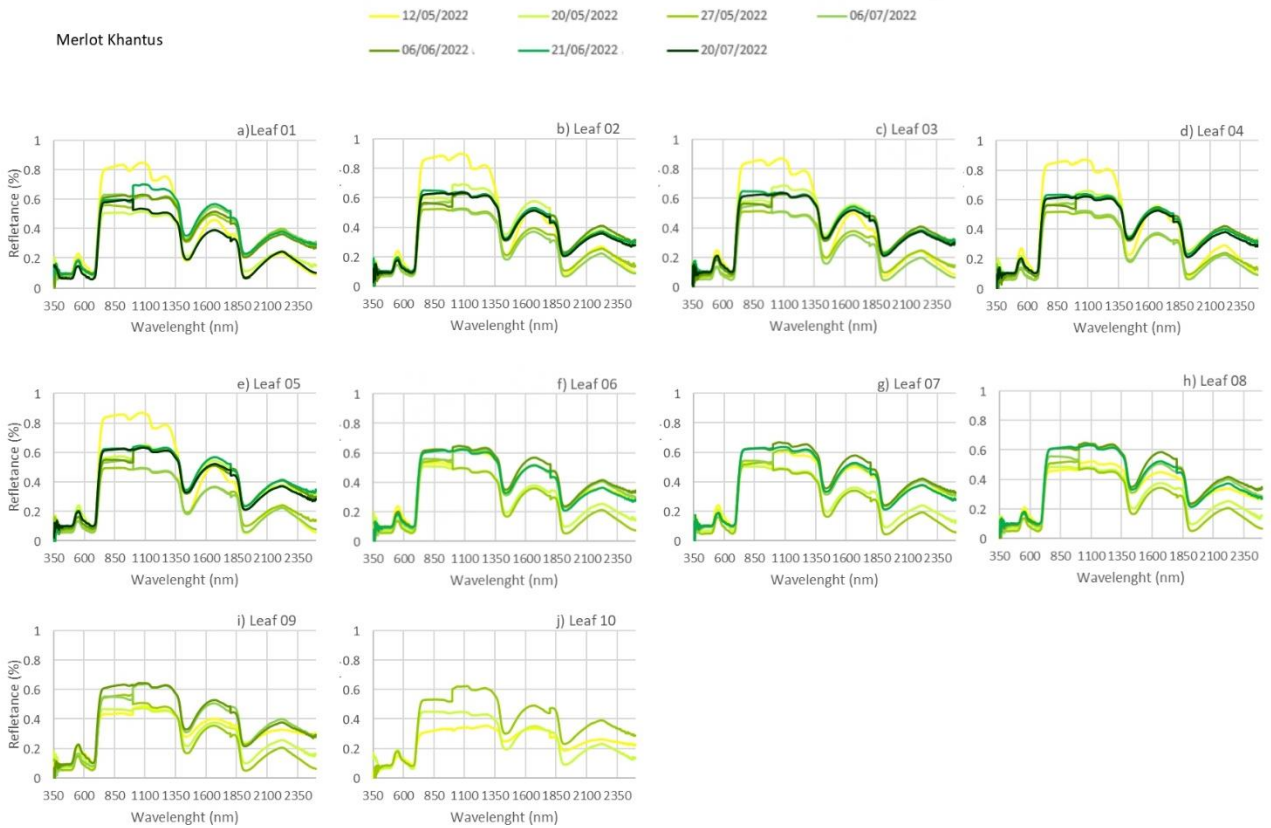


Figure 34: M. Khantus spectral behavior

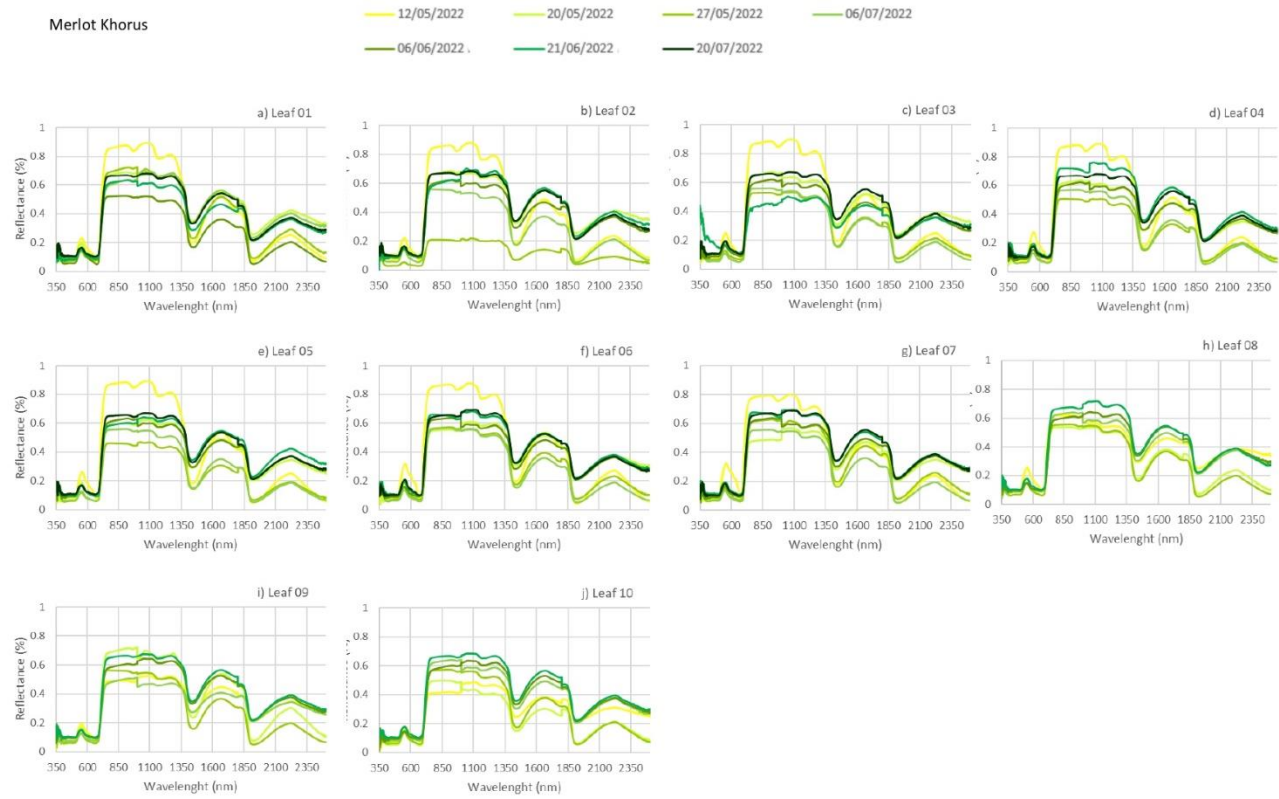


Figure 35: *M. Khorus* spectral behavior

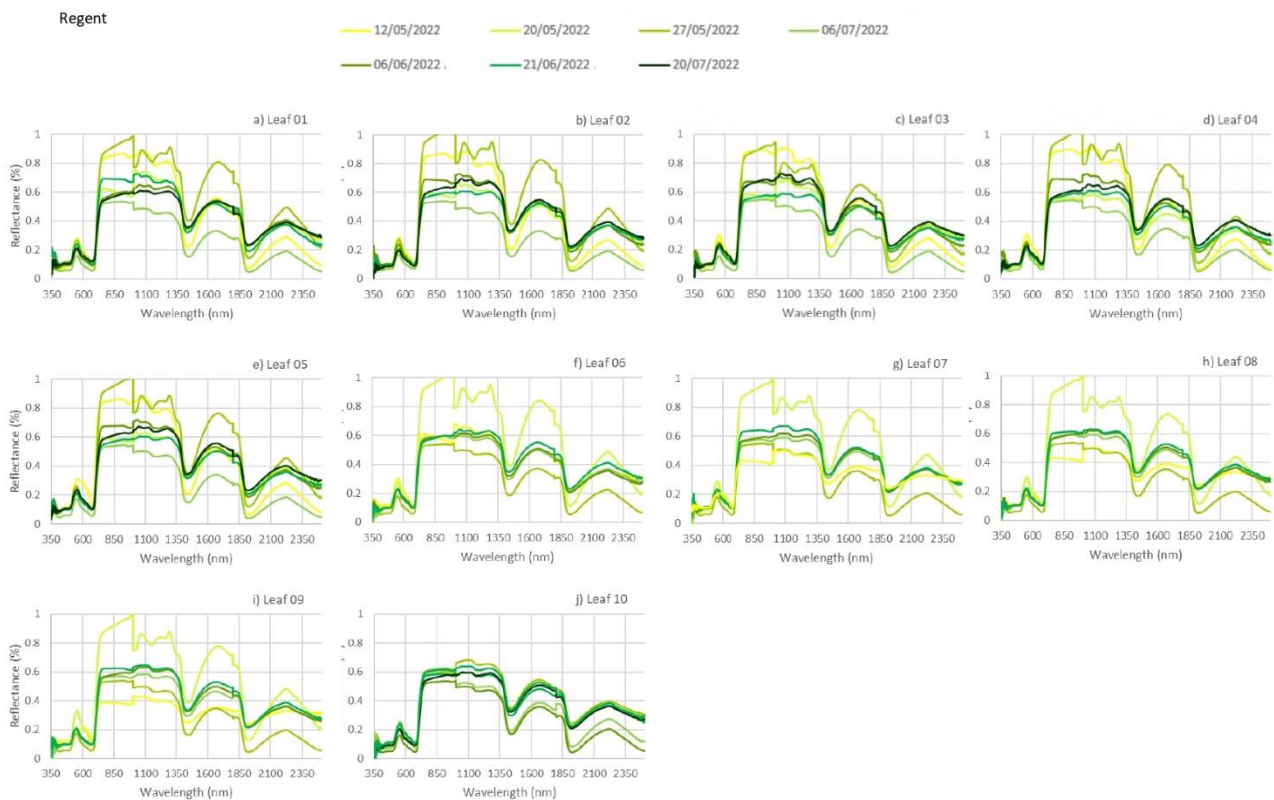


Figure 36: *Regent* spectral behavior

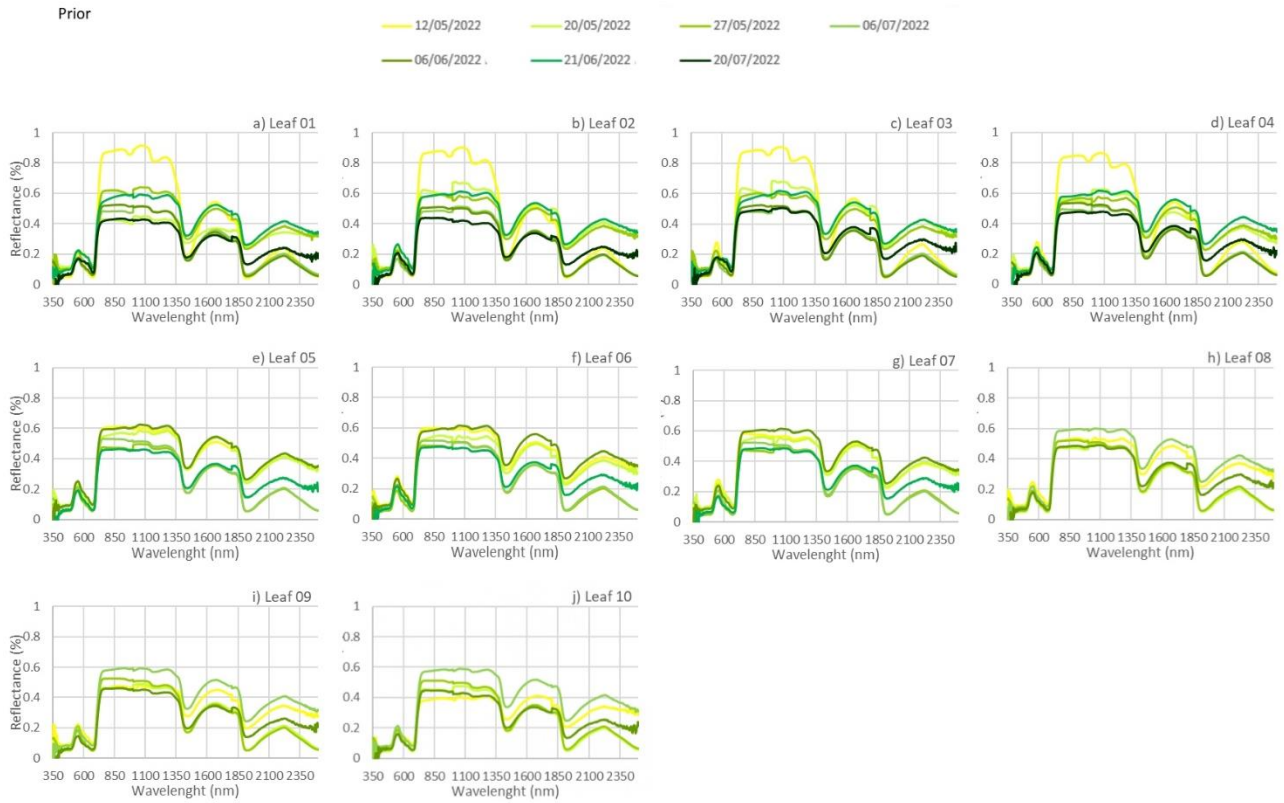


Figure 37: Prior spectral behavior

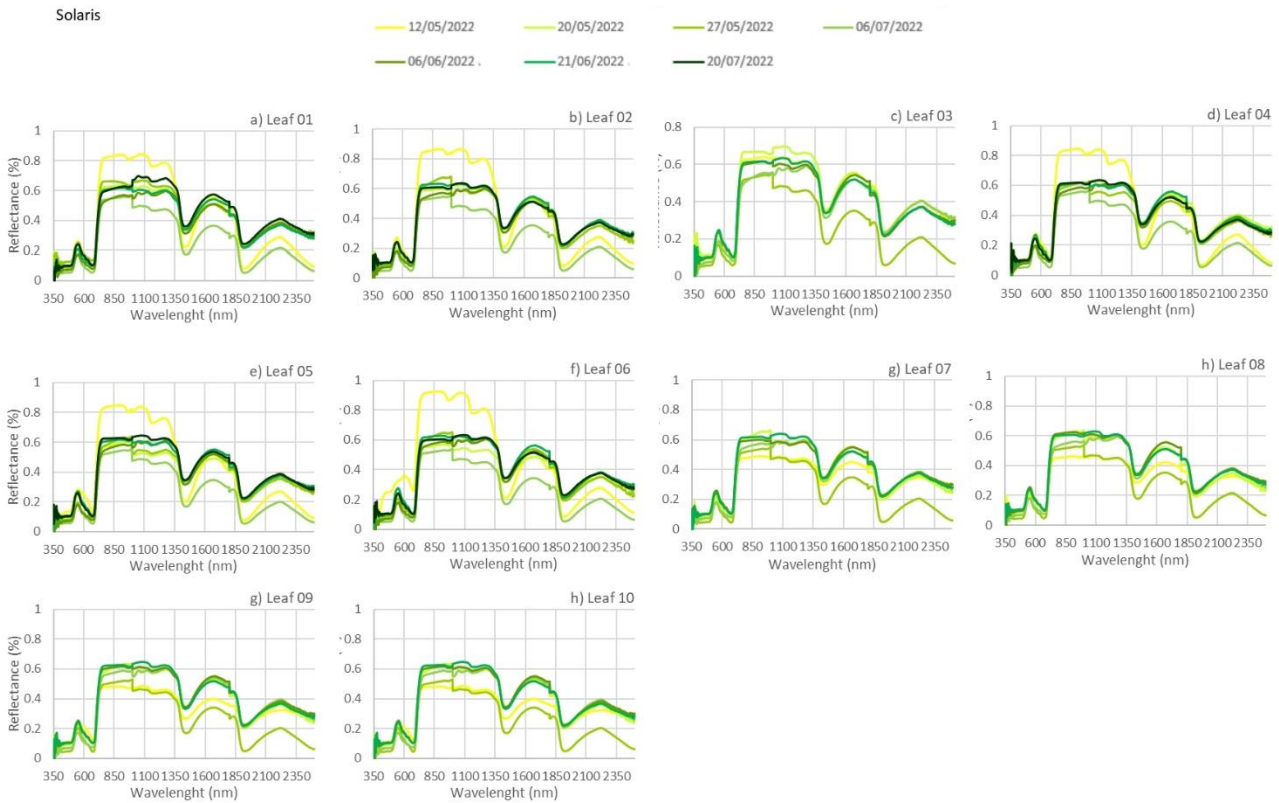


Figure 38: Solaris spectral behavior

S. Nepis

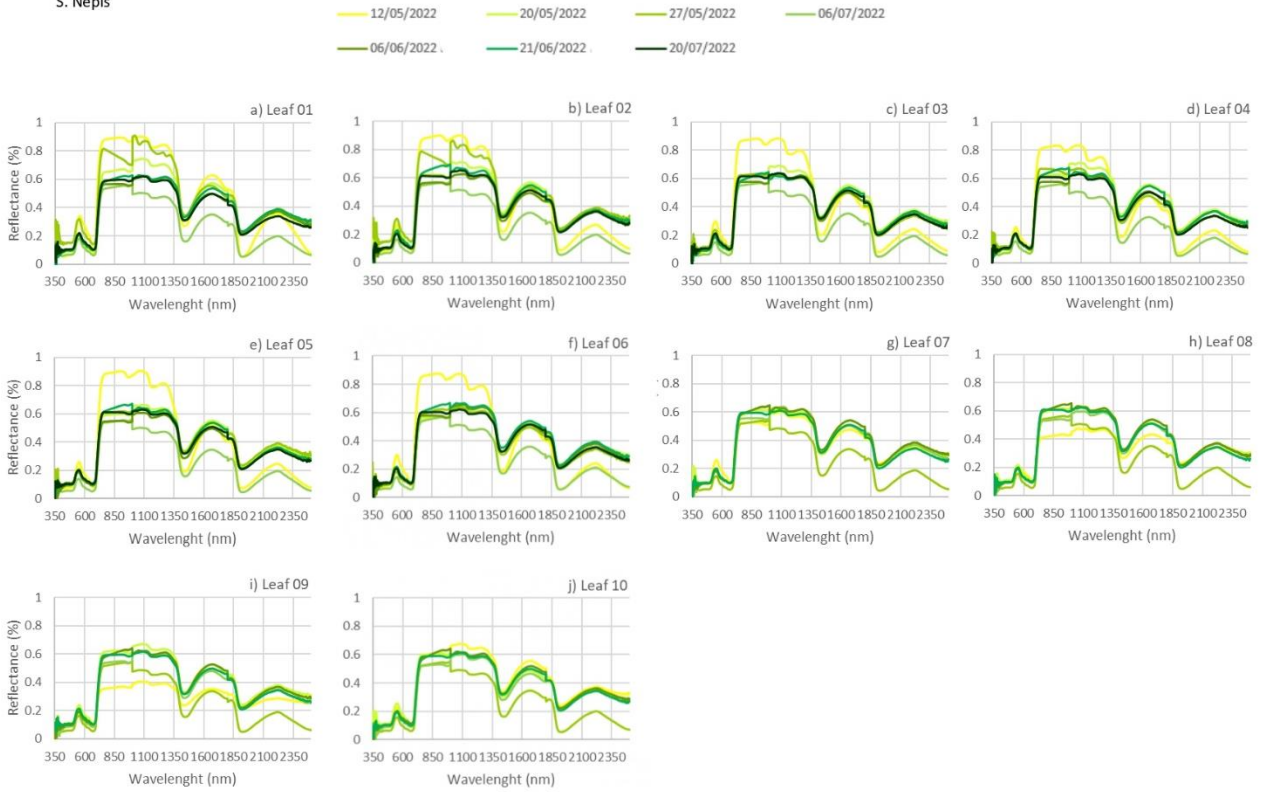


Figure 39: *S. Nepis* spectral behavior

S. Kretos

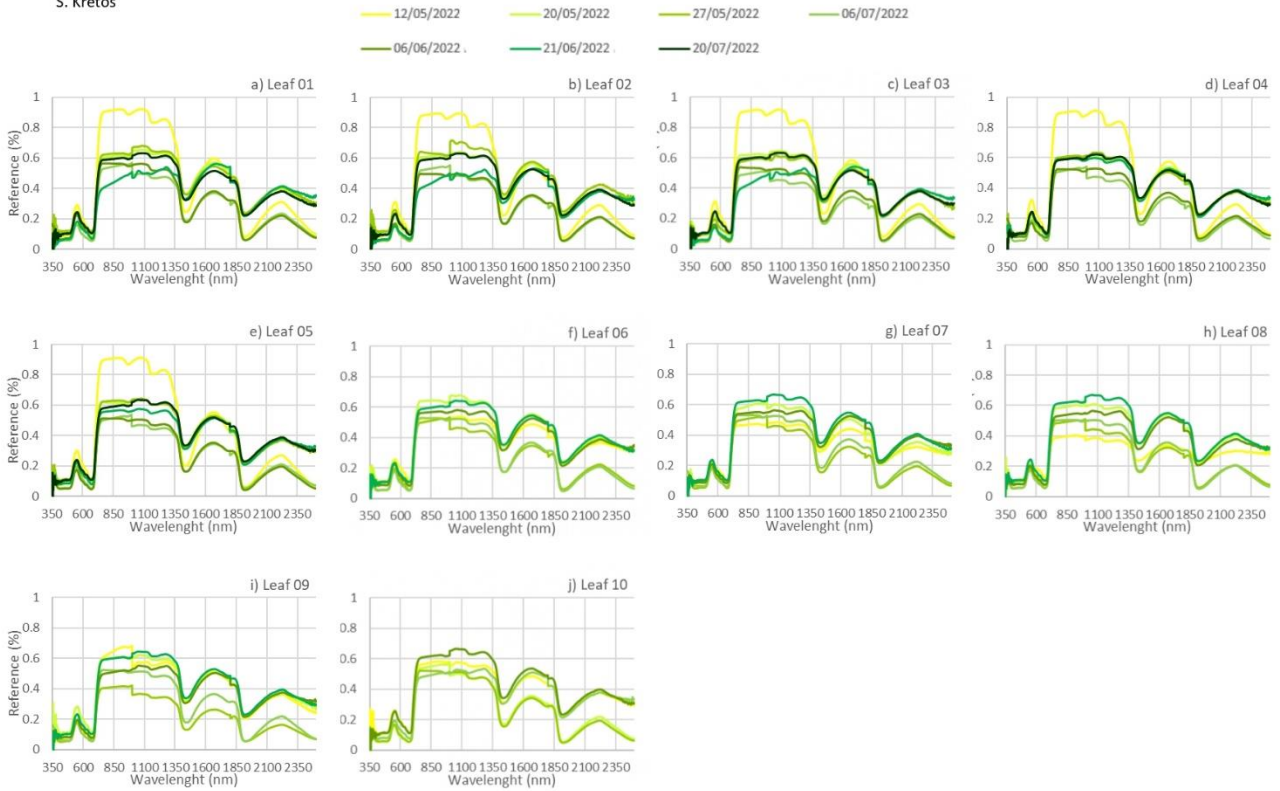


Figure 40: *S. Kretos* spectral behavior

## 5.2.1 Effect of leaf biological age (LPI/PI) on spectral indices

For each variety, the effect of the evolution of the biological age of the leaves on the leaves spectral responses was assessed using two indices: the Leaf Plastochron Index (LPI) and the Plastochron Index (PI). While LPI is specific of each single leaf, for each date and within each cultivar, a PI was computed for each shoot (replicate) to assess the impact of different leaf demographics on selected vegetation indices (VI). For this study, a total of 11 VIs were evaluated selected among those available in literature. In the following analysis, only those leading to interesting and significant correlations with LPI and PI were used.

The main objective of this analysis was to assess which VIs are impacted by changes in the leaf biological age and which, on the contrary, show similar responses even under contrasting leaf demographics.

The indices resulted as insensitive to foliar age variations, therefore, can be considered are less influenced by the heterogeneity of the leaf demography typically present in a vine canopy. As a result, where no differences were found in the index as a function of biological age, the specific bands used for the VI computation could be used do not consider the part of the spectrum that affects biomass.

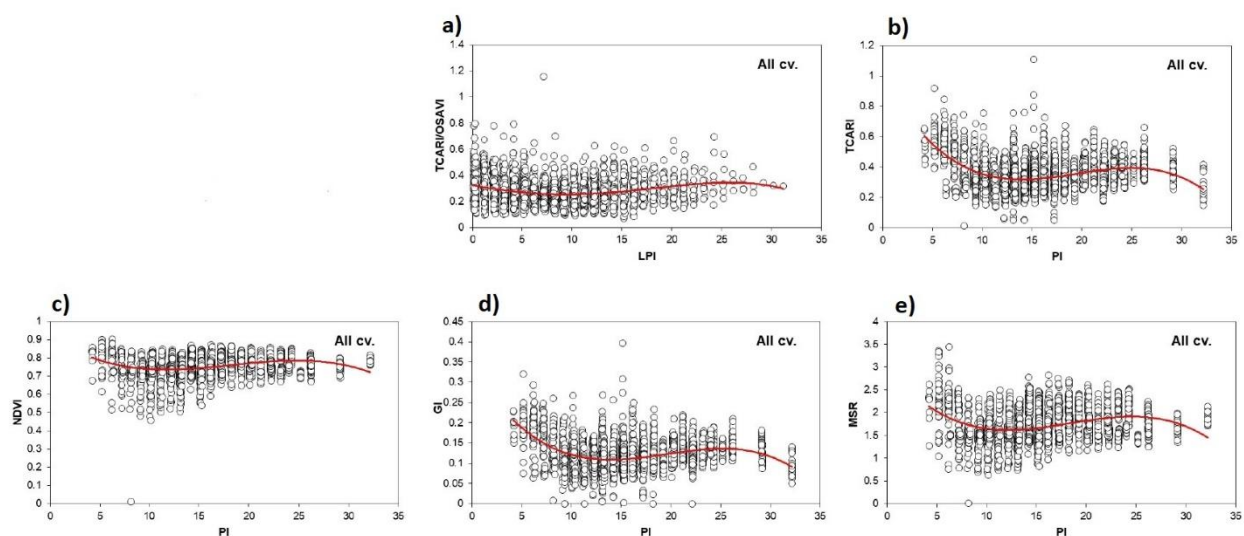


Figure 41: Effect of leaf biological age (LPI/PI) on spectral indices considering all the varieties. a) TCARI\_OSAVI/LPI b) TCARI/PI c) NDVI/PI d) GI/PI e) MSR/PI

Considering all cultivars together, most significant correlations were found with TCARI, NDVI, GI and MSR with PI and between TCARI/OSAVI and LPI (Figure 41). Considering, on the contrary, individual varieties it was not possible to underline a common behavior and results appeared heterogeneous (Figure 42, 43, 44, 45,46, 47).

The NDVI index appears to be less affected by the mean PI age; as the biological age of the shoots increases, in fact, the NDVI remains relatively constant and does not show large differences. Also for the TCARI/OSAVI in relation to LPI, no significant fluctuations are observed with increasing leaf age.

The relationships between MSR, IM, EVI and TCARI with the biological age index PI have in common a sinusoidal trend: with very low PI values there is a consequent increase in VI values, the curve stabilizes with PI ranging from 10 to 25, and then have a slight drop as the shoot age increases.

MSR, GI EVI and TCARI indices were developed to provide an improvement of the traditionally used NDVI by optimizing the vegetation signal and allowing to have more precise results even in high LAI conditions. They also make it possible to reduce background signals produced by the atmosphere and soil. This may explain why the NDVI does not show any specific difference as PI changes, while the use of more specific indices makes it possible to observe variations.

In particular, the TCARI focuses on the amount of chlorophyll going to analyze the bands of the spectrum affected by it. The sinusoidal trend therefore also describes the trend in the quantity of



chlorophyll, which in the initial stages is relatively high, and then decreases over time with the increase of PI.

As regards the relation between PRI and PI, on the other hand, the data shows a behavior that deviates from the previous ones. In the early stages, when the sprout is very young, there is a slight increase in PRI up to PI values of 10 from this point on PRI tends to remain almost constant with increasing leaf age. PRI is an index that is based on the cycle of xanthophylls, or carotenoids that have an important role in dissipating the excess energy received by the leaf.

The content of chlorophyll and carotenoids in the initial leaf development phases is inversely proportional, which explains why this index shows a trend opposed to TCARI in conjunction with low PI value.

While for some varieties such as Bronner, Cab. Cortis and Merlot Khantus there were no consistent results regarding the relationship between PI and VI. For Aromera and Prior, on the other hand, there was a tendency to have consistent relationships between VI/LPI and VI/PI.

The same ratio between indices maintains the same trend in the different varieties. For example, the relationship between VARI and PI has been evaluated on Aromera, Fleurtai and Cab. Carbon and all the varieties mentioned have been found to have the same behavior (Figure 41). Generally we do not notice particularly evident variations of VARI with the increase of PI, but the resulting curve has a sinusoidal trend. Higher VARI values increase with PI. One of the cases where this behavioral uniformity between varieties is not respected, namely when considering the relationship between SRI and LPI. The ratio between these indices is analyzed on 3 varieties: Sauvignon Nepis and Merlot

Khantus have no variation of SRI with the variation of LPI, while Merlot Khorus has a positive correlation between the two indices.

Similar behaviors were observed between the different VIs in the different varieties.

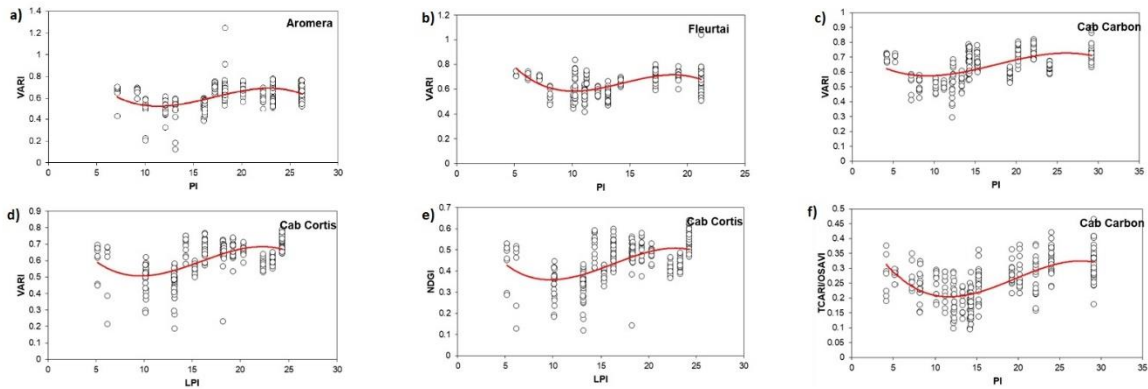


Figure 41: Effect of leaf biological age (LPI/PI) on spectral indices. a) Aromera: VARI/PI b) Fleurtaï: VARI/PI c) Cab. Carbon: VARI/PI d) Cab. Carbon: VARI/LPI e) Cab. Cortis: NDGI/LPI f) Cab. Carbon: TCARI-OSAVI/PI

NDGI/PI (Aromera), VARI/PI (Aromera, Fleurtaï, Cab Carbon), VARI/LPI (Cab. Cortis) TCARI/OSAVI/PI (Cab. Carbon, Solaris), NGDI/LPI (Cab. Cortis), NDVI/PI (Fleurtaï) can all be described by a sinusoidal curve that moves as PI increases along the Z axis (Figure 41, Figure 42, Figure 43). At low PI or LPI the VIs have relatively high values, follow a slight decrease and subsequent stabilization of the same.

Then there is a further increase in the index at high values of PI/LPI, until returning to the initial levels.

Other similarities between indices have been observed in GI/LPI (Bronner), GI/PI (S. Kretos, Prior, Mer. Khorus), EVI/LPI (Mer. Khorus) and SRI/PI (S. Nepis, Cab, Carbon).

A further description can be made considering the cases in which a similar behavior was observed at the same VI, but with different index of biological age:

- GNDVI/PI and GNDVI/LPI (Figure 42): in the different varieties, with both indices of biological age, similar behaviors were found. GNDVI does not appear to be affected by the variation of LPI or PI.
- GI/LPI and GI/PI: in both cases and on different varieties the curve remains consistent. Initial high GI values tend to stabilize as PI/LPI increases.

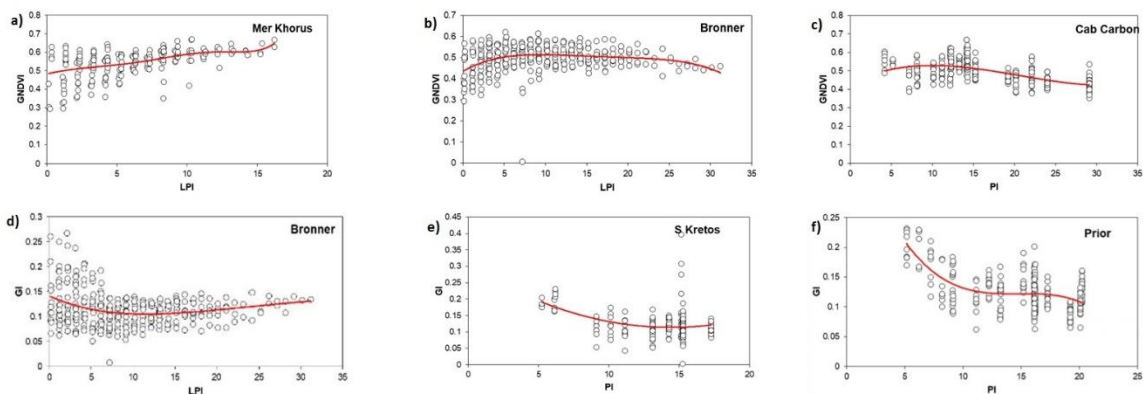


Figure 42: Effect of leaf biological age (LPI/PI) on spectral indices. a) Merlot Khorus: GNDVI/LPI b) Bronner: GNDVI/LPI c) Cabernet Carbon: GNDVI/PI d) Bronner: GI/LPI e) Sauvignon Kretos: GI/PI f) Prior: GI/PI

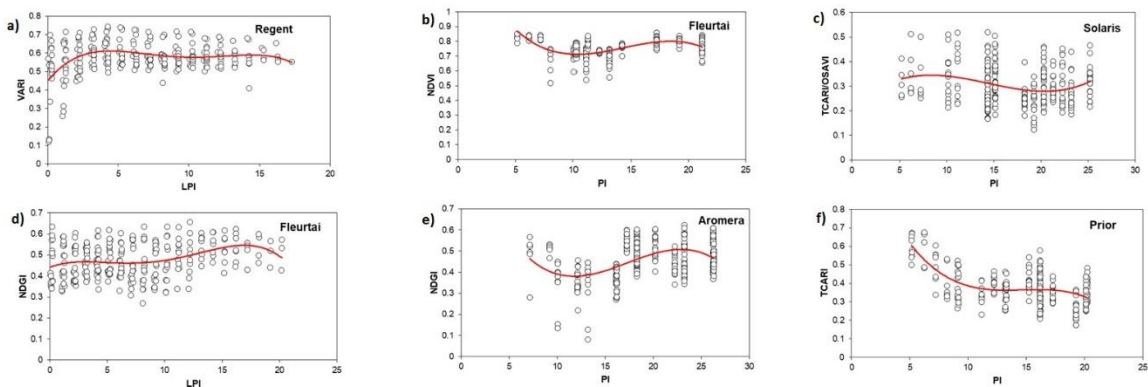


Figure 43: Effect of leaf biological age (LPI/PI) on spectral indices. a) Regent: VARI/LPI b) Fleurtaï: NDVI/PI c) Solaris: TCARI-OSAVI/PI d) Fleurtaï: NDGI/LPI e) Aromera: NDGI/PI f) Prior: TCARI/PI

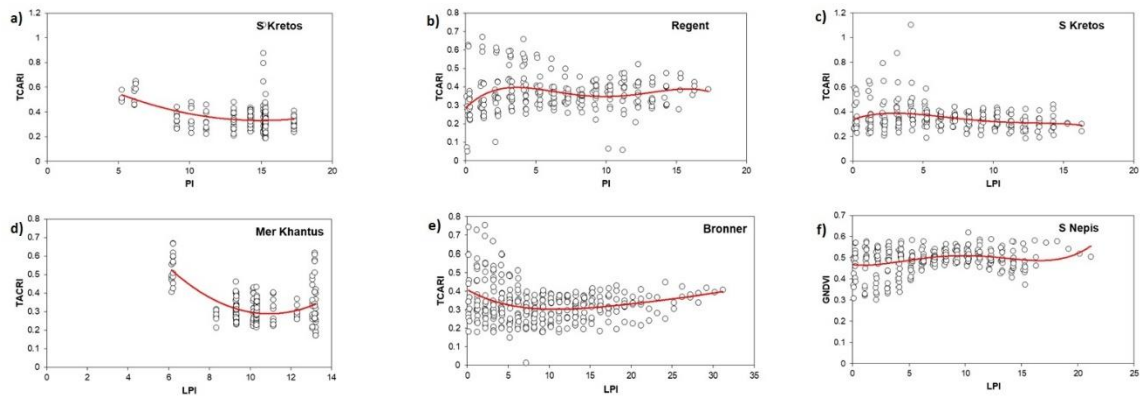


Figure 44: Effect of leaf biological age (LPI/PI) on spectral indices. a) Sauvignon Kretos: TCARI/PI b) Regent: TCARI/PI c) Sauvignon Kretos: TCARI/LPI d) Merlot Khantus: TCARI/LPI e) Bronner: TCARI/LPI f) Sauvignon Nepis: GNDVI/LPI

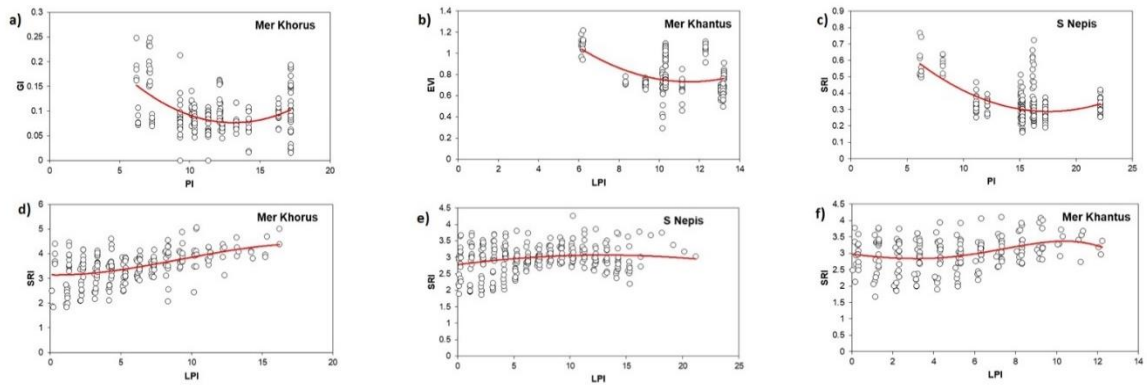


Figure 45: Effect of leaf biological age (LPI/PI) on spectral indices. a) Merlot Khorus: GI/PI b) Merlot Khantus: EVI/LPI c) Sauvignon Nepis: SRI/PI d) Merlot Khorus: SRI/LPI e) Sauvignon Nepis: SRI/LPI f) Merlot Khantus: SRI/LPI

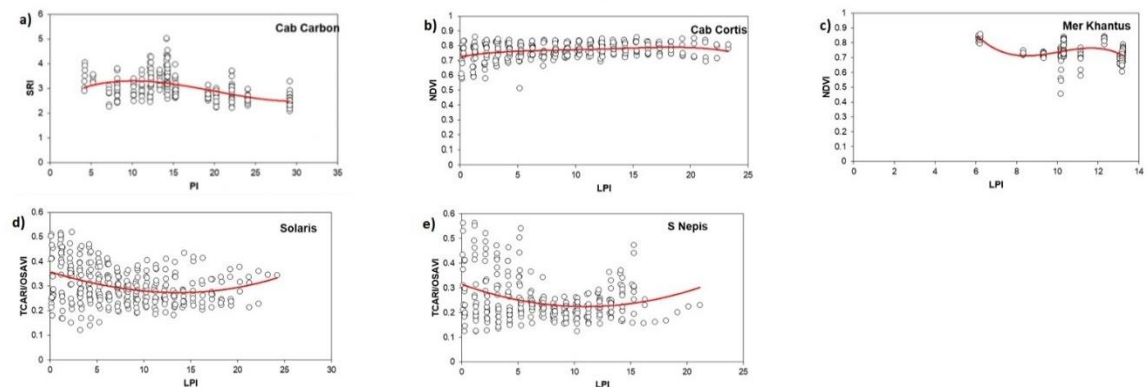


Figure 46: Effect of leaf biological age (LPI/PI) on spectral indices. a) Cabernet Carbon: SRI/LPI b) Cabernet Cortis: NDVI/LPI c) Merlot Khantus: NDVI/LPI d) Solaris: TCARI-OSAVI/LPI e) Sauvignon Nepis: TCARI-OSAVI/LPI

It is worth to notice how the same vegetational index related to a different index of biological age can perform different behaviors by changing the variety:

- VARI/LPI: In Cab. Cortis has the same behaviour as VARI/PI of Fleurtaï, Aromera and Cab. Carbon. In Regent, however, there is a behavior that differs from the previous ones.
- NDGI/LPI: In Fleurtaï there is a different trend from NDGI/PI Aromera. In Cab Cortis there is a similar trend to NDGI/PI Aromera
- TCARI\_OSAVI/LPI and TCARI\_OSAVI/PI
- SRI: the relationship between PI and SRI tends to be inversely proportional, while when related to LPI there are no substantial differences, SRI remains constant

TCARI/LPI and TCARI/PI in different varieties found different behaviours. The responses were very heterogeneous.

On the contrary, for PRI/LPI there was a consistency of behaviour in the different varieties analyzed. Both in Cab. Carbon and Bronner PRI does not seem to be affected by the biological age of the leaves.

In some cases, it has been noted that results were heterogeneous between the different varieties when maintaining the same VI and biological age index.

- VARI/LPI: In Cab. Cortis there is a sinusoidal pattern similar to other VI, while in Regent VARI increases slightly and then stabilizes.
- TACRI\_OSAVI/PI: In cab. Cortis there is a sinusoidal pattern comparable with other indices. In Solaris there is a sinusoidal trend but opposite to Cab. Cortis.
- TCARI/LPI: In Solaris and S. Nepis it was found a curve with a comparable trend, that is a curve that tends to decrease with intermediate values of LPI and that subsequently has the tendency to rise with the increase of LPI. In Med. Khorus and S. Kretos there is a similar trend in which there is a negative correlation between the indices. However, the values do not differ significantly with increasing LPI.

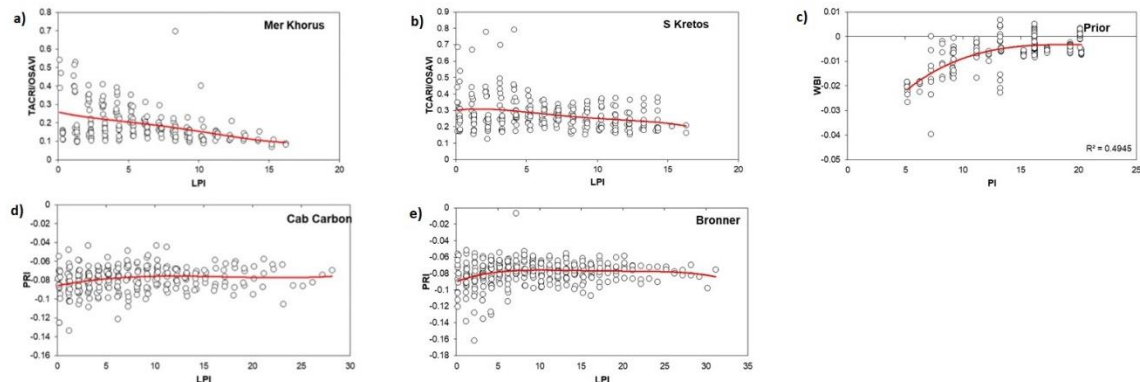


Figure 47: Effect of leaf biological age (LPI/PI) on spectral indices. a) Merlot Khorus: TCARI-OSAVI/LPI b) Sauvignon Kretos: TCARI-OSAVI/LPI c) Prior: WBI/PI d) Cabernet Carbon: PRI/LPI e) Bronner: PRI/LPI

## 5.2.2 Relation between gws and LPI

This analysis allows to determine at which leaf age occurs the maximum values of leaf gas exchanges, expressed here as stomatal conductance values. For traditional varieties, has been reported in literature that leaf photosynthetic capacity showed a maximum at LPI values between 12.5 and 17, approximately at 45-60 days after budburst, and a decline thereafter (Schultz et al., 1996; Zufferey et al., 2000; Petrie et al., 2000; Schultz, 2003).

The results of the present study were different by those reported in literature showing a peak in leaf gas exchanges around LPI values of 5-7. Nonetheless, a marked variability was observed among cultivars and even for each cultivar among dates and replicates. This may be caused by the fact that stomatal conductance was measured with a porometer (LI-600) that, while it allows a fast and reliable measurement of stomatal conductance and leaf transpiration, its measurements are affected by the environmental conditions that were experienced by the leaf under, radiative in particular.

All the varieties considered individually have a consistent behavior of  $g_{ws}$  in relation to LPI, which can be described with a Gaussian-like function. The peak of  $g_{ws}$  occurs at LPI values between 5 and 7. It has been observed that these values are reached at leaf maturity and then 30 days after leaf appearance.

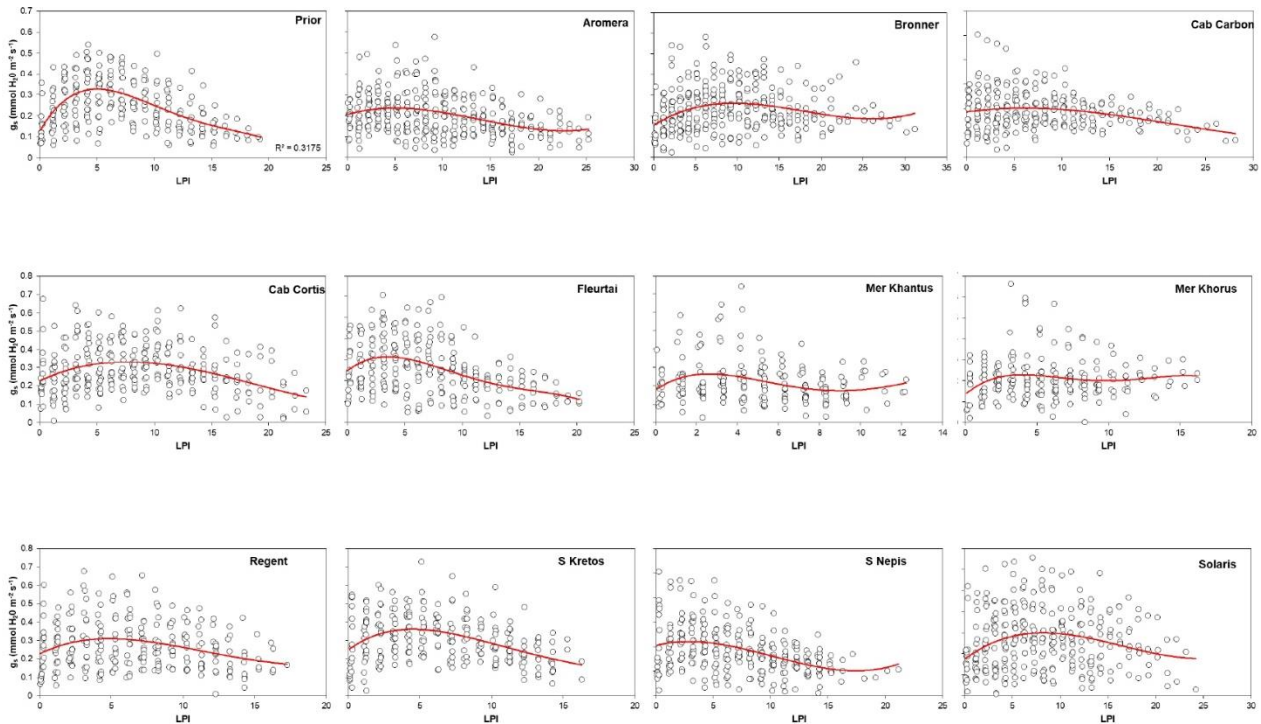


Figure 48: LPI and  $g_{ws}$  correlation in different resistant grapevine varieties

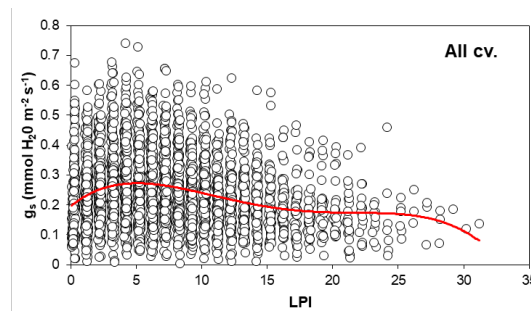


Figure 49: Relation between  $g_{ws}$  and LPI considering all the 12 varieties

### 5.2.3 Variation of $g_{ws}$ over time in different leaf ages

The stomatal conductance data revealed different results among cultivars that could be classified in two different groups. The first group consists of the varieties Bronner, Aromera, M. Khantus, M. Khorus, Regent, Solaris, Fleurtaï and in the second group are C. Carbon, C. Cortis, Sauvignon Kretos, SauvignonNepis, Prior.

For group 1, a peak in stomatal conductance levels was observed at the end of May/beginning of June and then stabilized in the subsequent period analyzed. This phenomenon is most evident for the first 9 leaves, in which the peak of conductance is particularly evident. The period in which the highest values of gsw occur coincides with the achievement of leaf maturity. It is observed that especially from leaf 10 onwards, the peak of stomatal conductance moves forward in time. To reach the maximum expansion and activity, the leaf needs on average 30-40 days, the leaves 10+ having appeared chronologically after the leaves 1-9, will move the peak of conductance later in time. For group 2, however, the levels of gsw remain relatively stable throughout the period considered. The data do not differ considerably both in time and for the different leaf ages. However, it is possible to observe the tendency to have higher values of conductance at two points corresponding to the sampling dates of 27/05 and 21/06. This time distance of about 30 days can be consistent with the time needed for the leaf to reach maturity.

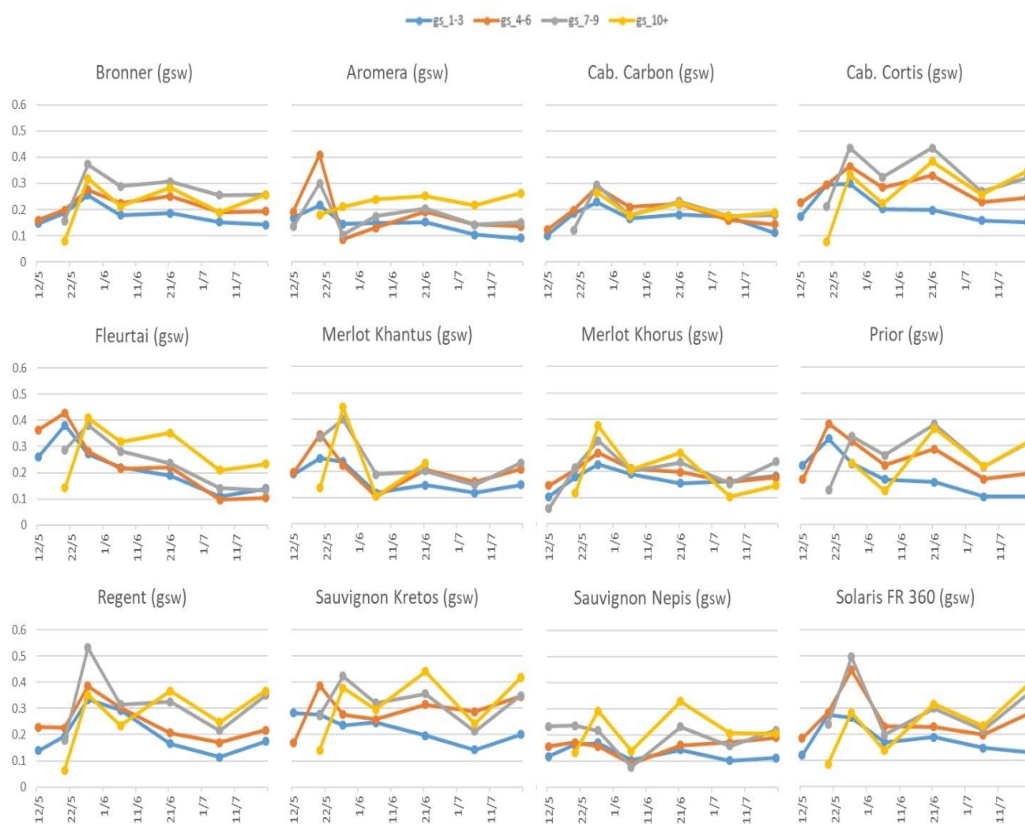


Figure 50: Variation of gws over time in different leaf ages



### 5.2.3 Plastochron Index

During the growing season of the varieties examined, canopy was trimmed at two different times, one before 06/06 and the other before the data collection of 21/06. These green prunings did not affect all varieties, but only those that had a particularly high vegetative development at that time. Shoot trimming has certainly affected the growth of the remaining leaves and consequently led to variations in the actual data, and in particular in the results of the Leaf Plastochron index.

Also environmental factors, such as a hail event registered on 03/07, are factors that may have affected the vegetative development of the plants under evaluation. The comparison between the pre- and post-trimming shows how much the canopy practice affected the final LPI of some varieties such as M. Khorus. By comparing PI data, it could be possible to hypothesize information about the earliness or lateness of the different varieties examined. This type of analysis, however, finds limits and needs further investigation.

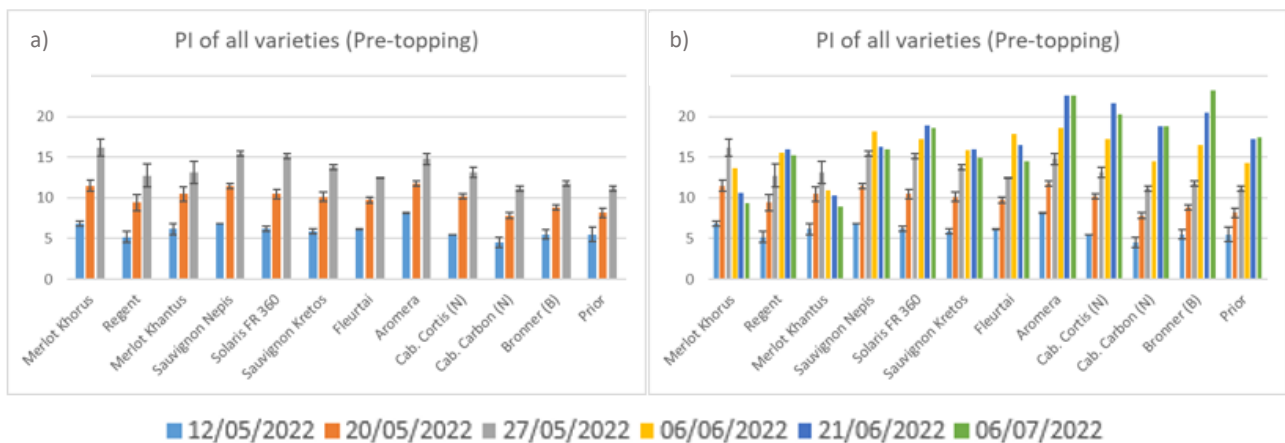


Figure 51: The effect of trimming on Plastochron Index. a) PI of the date before the trimming b) PI of all the sample dates.

## 5.3 Conclusion

The varieties examined in this study are relatively new and on the contrary of traditionally planted ones, both international and local varieties that were used for decades and their agronomical and physiological attributed allowed the definition of specific terroir, these resistant varieties were developed by crossing between traditional and resistant varieties and to date there is still a lot to known about them. In this thesis, the aim was to explore different methods of analysis with the aim of characterizing new resistant varieties from the biological, physiological and spectral basis.

The spectral results revealed a certain homogeneity in the behavior of the leaves over time over different ages. The varietal factor did not have a noticeable impact on the final results.

The study about the transpiration response highlighted similar behaviors among varieties. In fact, it was noticed that the peak of stomatal conductance always coincides with the maximum of foliar ripening but it falls earlier than traditionally grown varieties.

In the spectral analysis on the vegetation indices in relation to biological age indices, many heterogeneous data were found. Some indices were found to be independent on LPI and PI. This behavior can be exploited when measurements need to be made within a canopy with high demographic variability, without the leaf age affecting with the final spectral response results

# CHAPTER 6: Bibliography and sitography

## 6.1 Bibliography

- Alaa A., Naseer K. and Hazim H.. 2020. Performance Improvement of CIGS PV Solar Grid Tied System Using Planer Concentrators, Case Study: Baghdad
- Bhagooli R., Mattan-Moorgawa, S., Kaullysing, D., Louis, Y., Gopeechund, A., Ramah, S., et al. 2021. Chlorophyll fluorescence-A tool to assess photosynthetic performance and stress photophysiology in symbiotic marine invertebrates and seaplants. *Marine Pollution Bulletin* (165): 112059.
- Bryson, A. E., Wilson Brown, M., Mullins, J., Dong, W., Bahmani, K., Bornowski, N., Chiu, C., Engelgau P., Gettings B., Gomezcano F., Gregory L. M., Haber A. C., Hoh D., Jennings E. E., Ji Z., Kaur P., Kenchanmane Raju S. K., Long Y., Lotreck S. G., Mathieu D. T., Ranaweera T, Ritter E. J., Sadohara R., Shrote R. Z., Smith K. E., Teresi S. J., Venegas J., Wang H., Wilson M. L., Tarrant A. R., Frank M. H., Migicovsky Z., Kumar J., VanBuren R., Londo J. P., Chitwood D. H.. 2020. Composite modeling of leaf shape along shoots discriminates *Vitis* species better than individual leaves. *Applications in Plant Sciences* 8( 12): e11404.
- Bussotti F., Kalaji M. H., Desotgiu R., Pollastrini M., Łoboda T., Bosa K.. 2012. Misurare la vitalità delle piante per mezzo della fluorescenza della clorofilla. Firenze: Firenze University Press. Strumenti per la didattica.
- Carter G. A. and Knapp A. K..2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *American Journal of Botany* 88(4): 677–684
- Chen C., Chen H., 3 and Chen Y.. 2009. A new method to measure leaf age: leaf measuring-interval index. *American Journal of Botany* 96(7): 1313–1318
- Chessa F..2022. Impiego di sensori ottici di riflettanza per la diagnosi dello stato fisiologico delle colture agricole. Supervisor: Prof. Prosdocimi Gianquinto G.. Co-supervisor: Cerasola V. A.

- Cogato A., Wu L., Jewan S.Y.Y., Meggio F., Marinello F., Sozzi M., Pagay V.. 2021. Evaluating the Spectral and Physiological Responses of Grapevines (*Vitis vinifera* L.) to Heat and Water Stresses under Different Vineyard Cooling and Irrigation Strategies. *Agronomy* 2021(11). 1940
- Davis P.D., Parbrook G. D. and Kenny G. N. C..1995. Basic Physics and Measurement in Anaesthesia. Diffusion and Osmosis. (),89–102. Elsevier
- Dipartimento di Fisica e Astronomia. Faculty of Physics. Alma Mater Studiorum -Università di Bologna. Bologna
- Du L., Gong W. and Yang J..2018. Application of spectral indices and reflectance spectrum on leaf nitrogen content analysis derived from hyperspectral LiDAR data. *Optics and Laser Technology* (107): 372-379
- Feilhauer H., Gregory P. Asner G. P. and Martin R. E.. 2015. Multi-method ensemble selection of spectral bands related to leaf biochemistry. *Remote Sensing of Environment* (164): 57-65
- Ferreiral R. B.. Monteiro S. S.. Piçarra-Pereira M. A.. and Teixeira A. R.. 2004. Engineering grapevine for increased resistance to fungal pathogens without compromising wine stability. *TRENDS in Biotechnology*. Vol 22 (4): 168-173
- FieldSpec® 3 User Manual. 2010. ASD Inc. ASD Document 600540 Rev. J
- Ford H.. 1982. Leaf demography and the plastochron index. *Biological Journal of the Linnean Socup* (17): 361-373.The Linnean Society of London
- Garcia R. L , Norman J. M. and. McDermitt D. K..1990. Measurements of canopy gas exchange using an open chamber system. *Remote Sensing Reviews*. Vol 5(1): 141-162
- Guidi L.;Tattini M. and Landi M..2017. Chlorophyll. How Does Chloroplast Protect Chlorophyll Against Excessive Light?..22-33. Intech
- Hendrickson L. , Furbank R. T. and Chow W. S..2004. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. *Photosynthesis Research* 82: 73–81
- Hatfield J. L, Prueger J. H, Sauer T. J., Dold C., O’Brien P. and Wacha K.. 2019. Applications of Vegetative Indices from Remote Sensing to Agriculture: Past and Future. *Inventions* (4): 71

- Keller M..2010. Phenology and Growth Cycle. (1) 49-83. Burlington. California. London: Elsevier Science Publishers
- Knipling E. B.. 1970. Physical and Physiological Basis for the Reflectance of Visible and Near-Infrared Radiation from Vegetation. *Remote Sensing of Environment* (1): 55-1 59. American Elsevier Publishing Company. Inc.
- Kriedemann P.E.. Kliewer W.M. and Harris J.M.. 1970. Leaf age and photosynthesis in *Vitis vinifera* L. *Vitis* (9): 97-104
- Lucchin M..2020.Specie Autogame, Università degli Studi di Padova. DAFNAE: Department of Agronomy, Food, Natural resources, Animals and Environment
- Lüttge U..2008. Encyclopedia of Ecology. *Plant Physiology*: 2837-2845. Elsevier
- Maimaitiyiming M., J. Miller A. J., and Ghulam A.. 2016. Discriminating Spectral Signatures Among and Within Two Closely Related Grapevine Species. *Photogrammetric Engineering & Remote Sensing* Vol. 82 (2): 51–62
- Manes F., Fusaro L. and Salvadori E..2019. Misura degli scambi gassosi fogliari per il monitoraggio dello stato funzionale della vegetazione. Sapienza università di Roma. Department of Ambiental biology. Faculty of mathematics, physics and natural science. Progetto Nazionale Lauree Scientifiche
- Meer F. v. d..(2018). Near-infrared laboratory spectroscopy of mineral chemistry: A review. *International Journal of Applied Earth Observation and Geoinformation*.65():71–78.
- Meggio<sup>1</sup> F., 2021, Grapevine Structure and Function – part2, Università degli Studi di Padova, DAFNAE: Department of Agronomy, Food, Natural resources, Animals and Environment
- Meggio<sup>2</sup> F., 2021, Grapevine Growth – part02, Università degli Studi di Padova, DAFNAE: Department of Agronomy, Food, Natural resources, Animals and Environment
- Meicenheimer R. D..2014. The plastochron index: still useful after nearly six decades. *American Journal of Botany* 101 (11): 1821 – 1835
- Miner G. L.,. Bauerle W. L and Baldocchi D. D.. 2017. Estimating the sensitivity of stomatal conductance to photosynthesis: a review. *Plant, Cell and Environment* (40):1214–1238

- Montaigne E., Coelho A. and Khefifi L..(2016). Economic issues and perspectives on innovation in new resistant grapevine varieties in France. *Winw Economics and Policy*. Vol (5) 2: 73-77
- Nobel P. S..2020. *Physicochemical and Environmental Plant Physiology* (5):409-488. Elsevier
- Ollinger S. V..2010. Sources of variability in canopy reflectance and the convergent properties of plants. *New Phytologist* (189): 375–394. Tansley review
- Pedneault, K., Provost, C.. 2016. Fungus resistant grape varieties as a suitable alternative for organic wine production: Benefits, limits, and challenges. *Sci. Hortic*
- Pirotti F.. 2019. Corso di Telerilevamento e Sistemi Informativi Territoriali- parte 3. Università degli Studi di Padova. TESAF. Dipartimento Territorio e Sistemi Agro-Forestali
- Poni S., Palliotti A., Mattii G. and Di Lorenzo R.. 2007. Funzionalità fogliare ed efficienza della chioma in *Vitis vinifera* L.. *Italus Hortus* 14 (4): 29-46
- Prieto J. A. , Louarn G, Perez Peña J. , Ojeda H , Simonneau T. and Lebon E.. 2012. A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). *Plant, Cell and Environment* (35), 1313–1328
- Jacquemoud S.. Ustin S.L.. Verdebout J.. Schmuck G.. Andreoli G. and Hosgood B..1996. Estimating leaf biochemistry using the PROSPECT leaf optical properties model. *Remote Sensing of Environment* (56):194-202
- Schultz H. R.1992. An empirical model for the simulation of leaf appearance and leaf area development of primary shoots of several grapevine ( *Vitis vinifera* L. ) canopy-systems. *Scientia Horticulturae*, (52): 179-200. Elsevier Science Publishers B.V., Amsterdam
- Strever A.E.. 2012. Non-destructive assessment of leaf composition as related to growth of the grapevine (*Vitis vinifera* L. cv. Shiraz). Supervisor: Prof Hunter J.J..Co-supervisor: Dr Young P.R .. Department of Viticulture and Oenology. Faculty of AgriSciences. Stellenbosch University. Stellenbosch
- Using the LI-600 Porometer/Fluorometer Instruction Manual. 2022. LI-COR inc. 984-19037

## 6.2 Sitography

[www.alspergis.altervista.org](http://www.alspergis.altervista.org)

[www.atascientific.com.au](http://www.atascientific.com.au)

[www.cd-genomics.com](http://www.cd-genomics.com)

[www.ch.ic.ac.uk](http://www.ch.ic.ac.uk)

[www.chimica-online.it](http://www.chimica-online.it)

[www.compoundchem.com](http://www.compoundchem.com)

[www.directindustry.it](http://www.directindustry.it)

[www.en.wikipedia.org](http://www.en.wikipedia.org)

[www.galenotech.org](http://www.galenotech.org)

[www.gmrstrumenti.com](http://www.gmrstrumenti.com)

[www.licor.com](http://www.licor.com)

[www.piwi-international.org](http://www.piwi-international.org)

*[www.tesaf.unipd.it](http://www.tesaf.unipd.it)*

[www.weather.gov](http://www.weather.gov)

[www.winesvinesanalytics.com](http://www.winesvinesanalytics.com)