

# Potentials of Spectral Imaging for Stress Monitoring in Viticulture

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## **Abstract**

Remote sensing technologies are widely used to monitor quantity and quality of plants in agriculture and forestry. While conventional panchromatic and RGB cameras can provide spatial information equivalent to human vision, high-resolution spectroscopy can reveal information about the chemical and structural composition of plants and provide detailed insight about plant health. With the development of commercial high-resolution multi- and hyperspectral cameras in the past decade, it is now possible to combine spectral and spatial information to obtain high-throughput and accurate predictions of plant condition. In this article, the underlying optical phenomena in plants will be reviewed and projected to potential imaging applications for plant stress monitoring with a focus on viticulture.

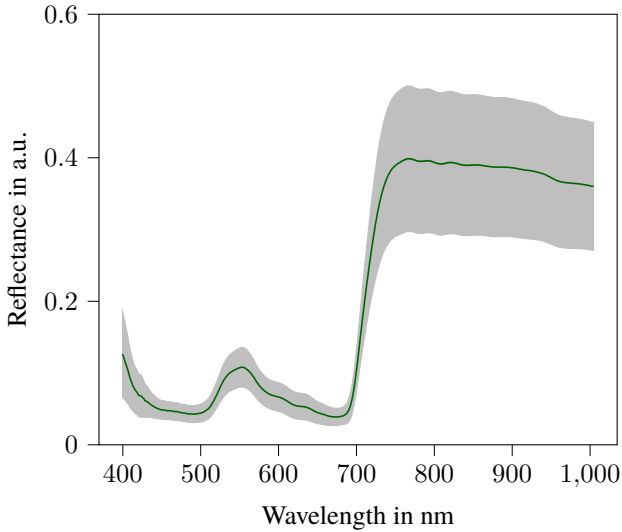
## **1 Introduction**

As world population continuously grows and agriculture is increasingly under pressure of climate change, precisely monitoring the state of crops is getting more important. Imaging technologies have become an invaluable tool for

plant phenotyping, i.e. the recording of all visible characteristics of a plant, which result from the interaction of the genetic information and environmental influences. RGB-cameras have been widely available for decades and have proven to be suitable for a large range of applications, including prediction of shoot biomass [19], assessment of disease severity [41] and monitoring of leaf injury [27]. However, the low spectral resolution of RGB-images does not allow detailed conclusions to be drawn about the chemical composition of the plants under observation and, in many cases, does not reveal the cause of specific symptoms. In grapevine, several conditions can result in similar symptoms that can be hard to distinguish by the human eye or RGB-imaging. To overcome this limitation, multi- and hyperspectral cameras have become increasingly powerful tools for plant phenotyping, providing high resolution spectra as well as spatial information. Typically, the investigated spectral range is extended into the near infrared (NIR)- or even short-wave infrared (SWIR) regime, allowing predictions about the chemical composition. A widely-known approach for plant state monitoring is given by the normalized difference vegetation index (NDVI), first introduced in 1973 [37]. It has been derived from satellite imaging bands and describes the ratio of the difference of the signal measured by the red and NIR band, given by  $g_{red}$  and  $g_{NIR}$ , over their sum

$$NDVI = \frac{g_{NIR} - g_{red}}{g_{NIR} + g_{red}}. \quad (1.1)$$

In the first publication, band 5 and 7 of Landsat 1 were utilized [37], corresponding to 600-700 and 800-1100 nm, respectively. Until today, it has remained one of the most widely used indices to predict general plant condition [18]. The broad validity of the NDVI, not only in agriculture, is based on the optical properties of the leaf pigment chlorophyll and the structure of leaves. The influence of the three main pigments in leaves, chlorophyll, carotenoids and anthocyanins as well as the influence of leaf cellular structure will be reviewed in more detail in the following. In Section 3, the influence of plant stress on the optical properties will be described. The technologies and approaches to exploit these properties for stress identification are introduced in Section 4. The article concludes with an outlook on open research questions and future work.



**Figure 2.1:** Average reflection spectrum measured on a leaf of *Vitis vinifera* L., cultivar 'Riesling' (green line). The shaded area illustrates the standard deviation of all pixels across the whole leaf.

## 2 Optical Properties of Leaves

As photosynthesis takes place in the leaves, their state is of vital importance for all green plants. The optical properties of leaves can provide valuable insights into potential stress factors affecting the plant, which shall be illustrated in the following on the example of grapevine leaves. An exemplary spectrum of a healthy grapevine leaf of cultivar 'Riesling' is shown in Figure 2.1.

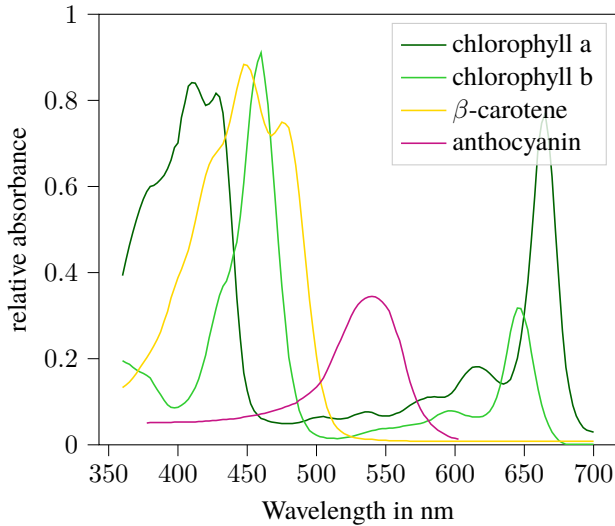
### 2.1 Optical Phenomena in Leaves

Interaction of light with leaves is governed by surface reflection, internal reflection, light absorption by pigments and transmission through the leaf tissue [43]. Light reflected from leaf surfaces is specularly reflected and typically polarized [43]. This portion of reflected light is not influenced by pigments or

the internal leaf structure. Depending on the surface structure, i.e. the amount and shape of surface wax as well as the number of hair, surface reflectivity varies and can in some species account for most of the reflected light [45, 20]. Light penetrating through the leaf surface is reflected from each cell wall-air interface of the epidermal cells along the optical path due to the higher refractive index of water-filled cells than air-filled spacings between cells [43, 8]. When leaving the leaf after multiple internal reflections, this portion of light is unpolarized. It could be demonstrated that soaking a leaf in water will fill enclosed air gaps and consequently reduce the difference in refractive indices and thus significantly reduce internal reflection in the NIR spectral range [8]. Light propagating inside the leaf tissue is selectively absorbed by pigments, resulting in strongly wavelength-dependent internal reflection depending on the concentration of individual pigments. The characteristic absorption properties of the most prominent pigments are evaluated in the following sections.

## **2.2 The Sieve Effect and Detour Effect**

It is well known from Lambert-Beer's law that absorbance linearly increase with increasing concentration of the absorbing species. However, this relation only holds for homogeneously distributed particles. This is not the case in plant leaves, where particles are not freely floating but are concentrated within cell organelles [25]. Thus, the absorbance of pigments comprised in organelles is smaller compared to the same concentration of pigments homogeneously suspended in the surrounding medium, which is known as the sieve effect and leads to underestimated pigment concentration from absorption spectra. A decrease of the absorption maximum as well as broadening of absorption bands at increased chlorophyll concentration due to the sieve effect has been observed by [8]. On the contrary, abundant air-cell interfaces result in light scattering and thus increase the optical path length within leaves, increasing the chance of light interaction with pigments. This is known as detour effect and, in return, increases absorption and overestimates pigment concentration [43]. In the near infrared regime where chlorophyll does not absorb, scattering results in very high leaf reflectivity. With increasing age of the plant, the intercellular air spaces become more abundant, resulting in increased scattering and thus increased



**Figure 2.2:** Schematic absorption spectra of chlorophyll a (dark green), chlorophyll b (light green),  $\beta$ -carotene (yellow) and anthocyanin (purple). Reproduced from [16].

reflectivity in the near infrared [8]. The relation of sieve effect and detour effect is strongly tissue-dependent and thus not generally describable [25].

## 2.3 Molecular Absorption of Leaf Pigments

### 2.3.1 Chlorophyll

The most prominent pigment in plants is chlorophyll, responsible for the green appearance of leaves. Different types of chlorophyll exist that can be distinguished by their side chains. In plants, chlorophyll a and b are present. Their absorption spectra are shown in Figure 2.2. Comparing the absorption spectra to the reflectance spectrum in Figure 2.1, the reflectance dips in the blue and red spectral regions and the green appearance of the leaf can be readily explained. Chlorophyll a strongly absorbs in the blue and red regime, while

chlorophyll b presents a prominent absorption peak in the blue-green and a smaller peak in the orange regime. While chlorophyll a is used as the primary light absorbing molecule in photosynthesis, chlorophyll b is supplemented to enlarge the absorption range and is thus relatively more abundant in shade plants [6]. In grapevine, the ratio of chlorophyll a/b typically ranges between 2 and 3 [28].

The combination of chlorophyll absorption and strong NIR-scattering results in the red edge, the sharp increase of reflectivity in the far red, which can be seen in Figure 2.1 around 700 nm. This effect is the result of two phenomena: firstly, chlorophyll absorption sharply decreases towards the red edge as can be seen in Figure 2.2, resulting in increased reflectivity. Secondly, it can be attributed to strong internal scattering at the leaf cell boundaries in the infrared regime causing strong reflectivity [24]. As shown above, the NDVI describes the relation between red and NIR reflectance and thus a normalized ratio of both sides of the red edge. As the red edge is invariant to background coverage [24], it has become a valuable tool for remote sensing with numerous applications.

### **2.3.2 Anthocyanins**

Anthocyanins are a subgroup of flavonoids, secondary plant metabolites that are responsible for pigmentation [17]. Flavonoids fulfill numerous important tasks for the protection of plants, thus their synthesis rate can be triggered by stress [35]. Anthocyanins encompass a large group of molecules responsible for characteristic red, blue, purple or black coloring of plants.

Malvidin and in particular malvidin-3-O-glucoside has been determined as the most abundant anthocyanin in grapevine [12]. It should be noted that anthocyanin absorption is strongly pH-dependent [46] and the representation in Figure 2.2 is thus only illustrative without general validity.

In red grapevine varieties, anthocyanins are responsible for the purple or red coloring of grapes, wine and sometimes leaves, whereby the concentration is strongly dependent on the cultivar [12]. While the genes responsible for anthocyanin generation are also present in white cultivars, their synthesis is inhibited by mutant regulatory genes [44].

### **2.3.3 Carotenoids**

Carotenoids appear yellow and are molecules involved in the photosynthetic process just like chlorophyll. They consist of liposoluble tetrapenes and can be subdivided into two main classes due to their chemical structure: carotenes, represented by a specific group of hydrocarbons; and xanthophylls, represented by oxygenated carotenes [40].  $\beta$ -carotene is the most abundant carotene and lutein the most abundant xanthophyll in grapevine [23]. Carotenes fulfill two main functions in plant photosynthesis: photoprotection by dissipating excess heat as well as a possible contribution to light harvesting by absorbing light in the green spectral range which is not efficiently absorbed by chlorophyll [15, 22]. Carotenoids are present in leaves all season long, but are typically masked by chlorophyll. With declining chlorophyll concentration in autumn, the influence of carotenoids becomes visible with leaves turning to orange and yellow hues [3].

## **3 Influence of Stress on Plant Spectra**

Pigments are subject to associated metabolic pathways and thus susceptible to environmental influence. Therefore, their concentration can change due to biotic or abiotic stress, resulting in altered reflectance and absorption spectra. Additionally, the cell structure may change due to external influence, which in turn influences leaf scattering properties. The individual effects and their impact on the reflectance spectra of grapevine leaves are explained in the following section.

### **3.1 Change in Pigment Concentration**

#### **3.1.1 Chlorophyll and Carotenoids**

As chlorophyll and carotenoids are both involved in photosynthesis, the rate of change of both pigments is often highly correlated. Upon stress exposure, leaf chlorophyll content as well as the concentration of most carotenoids typically

declines. This has been reported for several biotic conditions in grapevine, including plants infected with the bacterial infection Bois Noir [38] and the virus infection Grapevine Leafroll disease GLRaV-3 [21]. It was found that Esca, a fungal disease, results in strongly decreased chlorophyll content, whereas the carotenoid content did not change significantly [34].

The influence of different abiotic stress factors on the concentration of chlorophyll and carotenoid was investigated by [10] on two different red cultivars, 'Touriga Nacional' (TN) and 'Trincadeira' (TR). They observed very different reaction of cultivars upon abiotic stress: while water stress, light stress and heat stress individually resulted in an increase in total chlorophyll concentration in TN, it slightly decreased in TR when compared to a non-stressed control group. Combined light, heat and water stress, in return, resulted in the increase of chlorophyll content in both cultivars. Carotenoid content slightly decreased during exposure to water, light and heat stress for TN, but slightly increased in TR in response to water- and light stress. Combination of water, light and heat stress resulted in increased carotenoid content in TN but was not significantly affected in TR.

### **3.1.2 Anthocyanins**

The change of anthocyanin concentration has been observed as response to a wide range of biotic and abiotic stresses in grapevine, but is decoupled from chlorophyll and carotenoid concentration change. Several studies showed an increase of anthocyanin concentration in red cultivars as response to biotic stress. [21] could identify the presence of anthocyanins only in Grapevine Leafroll disease GLRaV-3 infected leaves, while the healthy control group did not contain any measurable amount of anthocyanin. The response of the two red cultivars 'Nebbiolo' and 'Barbera' to the bacterial infection *Flavescence dorée* (FD) were measured by [31]. 'Barbera', being highly susceptible to FD, showed an increase of anthocyanin by up to ten times compared to the healthy control group, whereas in the less susceptible cultivar 'Nebbiolo', anthocyanin concentration doubled at maximum. Upon recovery, both plants did not exhibit remarkable difference compared to the control group.



The influence of abiotic stress on the cultivars 'Touriga Nacional' (TN) and 'Trincadeira' (TR) on anthocyanin concentration was investigated by [10]. In their experiments, water, light and heat stress individually decreased anthocyanin concentration in TN, while TR showed a slight increase in anthocyanin when exposed to water stress and slight decrease as reaction to light- and heat stress. Combination of both three stresses yielded a strong increase in TN but left TR unaffected.

### **3.2 Change in Cell Structure**

Cell structure is subject to seasonal effects as well as environmental impact and can change significantly with leaf age [26, 11]. During senescence, the reflectance decreases, which could be attributed to cell wall breakdown and the resulting changes in optical properties [26]. Plant water status has been successfully correlated to water absorption bands [33, 13]. However, the water absorption bands with strong impact on NIR spectra are not in the range of inexpensive silicon sensors and thus water status has also been indirectly correlated to the visible spectral range [29, 36]. Decreasing NIR reflectance in grapevine infected with root rot disease have been observed in [9] and attributed to structural change due to wilting of infected leaves. [32] investigated the spectral response of three red cultivars to the fungal infection *Plasmopara viticola*. They observed a slight increase in NIR reflectance for the susceptible cultivar 'Mueller-Thurgau', but a decrease in the resistant cultivars 'Regent' and 'Solaris', indicating that no general rule for structural change of leaves can be derived from specific pathogens.

## **4 Discussion**

### **4.1 Spectral Imaging for Stress Identification in Viticulture**

As mentioned above, stress triggers biological processes that, in turn alter the optical properties of plants. Spectral imaging has thus been successfully implemented for numerous applications for stress monitoring in viticulture.

In-field monitoring of Grapevine Leafroll disease GLRaV-1 and -3 has been demonstrated by [4] using a hyperspectral camera mounted inside a grape harvesting machine, illuminated by halogen lamps. The obtained data was analyzed by Linear Discriminant analysis (LDA), Partial Least Squares (PLS), Multilayer Perceptron (MLP) and Radial-Basis Function Network with Relevance (rRBF), achieving classification accuracies of 67-94% in the NIR- and 74-96% in the SWIR spectral range. The same algorithms have been tested in [5] to identify grapevine yellows on whole shoot hyperspectral images taken in the lab. Here, classification accuracies between 89 and 97% could be achieved. Spectral features obtained by a point spectrometer were combined with textural features extracted from RGB images in [39] to monitor Grapevine yellows and Esca. The effect of nutrient deficiency was observed by [14]. They analyzed single leaves collected in the greenhouse in a laboratory hyperspectral imaging (HSI) setup and evaluated the extracted mean leaf spectra using a binary Support Vector Machine (SVM) classifier. The suitability of HSI for water stress monitoring has been demonstrated by [29]. They used a hyperspectral camera in the visible range mounted on a tripod in the field under sunlight illumination to identify stressed vines. Making use of Random Forest and Extreme Gradient Boosting, they could correctly identify between 77 and 83% of stressed plants.

For large-scale applications, high throughput is required. Thus, the use of unmanned aerial vehicles (UAV) has become increasingly popular and has already been used in several studies. A UAV-mounted multispectral camera was utilized by [1] to map *Flavescence dorée* and Grapevine Trunk Disease. Grapevine water status was monitored by [2] using a multisensor platform carried by a UAV, including multispectral and thermal imaging systems. Airborne RGB-multispectral- and hyperspectral imaging was employed by [42] to monitor the insect pest grape phylloxera. In all these studies, sunlight provided the necessary illumination which subject to variation.

As processing hyperspectral datacubes requires substantial computational resources, the analysis is oftentimes reduced to indices and ratios of only a limited number of wavebands [1, 2, 42]. As monitoring water stress as in [2] is clearly correlated to water absorption, the use of indices for water stress monitoring is widely used. Considering biotic stresses, the change in plant status can not as clearly be attributed to the presence or absence of single substances.

Although [39, 1] could achieve satisfying classification accuracy for biotic stresses using indices from the literature, custom chemometric models based on the full spectrum could possibly achieve higher accuracy by taking into account the individual changes in pigment concentration and leaf structure. Also, generalization could be difficult since the presented studies could only observe a limited number of cultivars. As described in Section 3, cultivars can substantially differ in their reaction to stress. Thus, more research is needed to find a suitable spectral configuration for specialized cameras that can record the relevant change in pigments, cellular structure and abiotic factors, but is at the same time cost-effective and computationally efficient by limiting the number of channels.

## 4.2 Future Prospects

Most studies introduced above have only focused on individual cultivars. As described in Section 3.1, different cultivars show substantially divergent behavior, varying tolerance to stress influence and exhibition of symptoms. To further promote the use of spectral imaging, further work with a focus on generalization to varying environmental and biological conditions is needed. It is therefore not sufficient to extract relevant spectral bands for a single cultivar only, but also those shared by a wide range of cultivars growing under varying conditions and ideally in different climates. However, data collection of such large datasets under stable measurement conditions would be time-consuming and financially demanding. One possible solution could be the exploitation of multispectral Very High Resolution satellite imagery. Although limited in spatial and spectral resolution and flexibility, this would allow mapping of virtually any vineyard on a regular basis with comparable equipment for extended time spans and several seasons. To date, few studies have focused on the seasonal fluctuations in plant appearance, which should also be considered for further generalization.

Another common problem is given by heavily imbalanced classes. Typically, significantly more data is available for healthy reference plants than for those with known and properly defined stress or combination of stresses. Balancing techniques as suggested in e.g. [7] should be used more intensively to counteract imbalance and consequently produce more reliable models. As was evident

from the results of [10], the combination of different stresses should also be investigated in detail. In this study, only the combination of abiotic stresses was considered. Thus, further research regarding the interplay different stresses is needed for generalization.

As described above, several stresses can result in similar symptoms and similar pigment- and structural changes. Since pigment change represents an easily detectable and obvious symptom to the human observer, more general understanding on the mechanisms triggering pigment change is required across cultivars. Spectral unmixing methods could be employed for reliable pigment concentration estimation that could then be attributed to specific stresses. Some stress conditions yield characteristic patterns on leaves, e.g. the prominent stripes resulting from Esca. Thus, important information is lost if only the average spectrum of the leaf is considered. therefore, further evaluation of spectral and spatial features combined is needed, which could improve differentiation between different stresses. Additionally, combining spectral imaging with other sensing techniques such as thermography and chlorophyll fluorescence could lead to increased accuracy in diagnosing stress symptoms. This approach has been tested for disease monitoring in wheat already [30]. In grapevine, combination of multispectral and thermal imaging has been tested to monitor water status [2], but could also potentially enhance disease monitoring.

## **5 Conclusion**

Spectral imaging is a promising technology for large-scale stress monitoring in viticulture. When exposed to stress, the pigment concentration of plants is changed, resulting in a change of coloration that is obvious on leaves. Genotypes react differently upon stress exposure, therefore spectral changes due to variation in pigment concentration cannot be generally described but must be determined for each individual cultivar. Thus, changes in pigment concentration may not be a sufficient general indicator, especially for abiotic stress. Additionally, different stress factors can influence each other and yield changes in pigment concentration that substantially differ from individual factors. Only considering spectra in the visible spectral range that is dominated by pigment absorption may

not provide sufficient evidence to differentiate between stress factors but can only give a general impression of the overall plant status and detect anomalous plants. Additional features are necessary for more selective diagnosis. These features could be represented by evaluating characteristic patterns of symptoms, e.g. discoloration in the shape of spots, stripes or whole leaf segments, which can only be obtained by imaging- but not point spectrometers. Consequently, evaluation of single pixels can be misleading since these changes oftentimes do not take place uniformly across the leaf and plant, but the symptom pattern gives more detailed insight into the type of stress. Combining spatial and spectral features, multi- and hyperspectral imaging have proven to be capable tools for stress monitoring. Further morphological information like leaf rolling or necrotic segments could provide additional evidence and could also be detected by imaging systems. Extending the spectral range under consideration to the NIR- and SWIR-spectral range can provide further information about leaf structure and water status, which could be important parameters for stress differentiation.

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