An experimental setup for the study of *Plasmopara viticola* on vine leaves by fluorescence

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***Abstract*— The early recognition of a specific disease on a plant permits of intervening quickly and efficiently, avoiding the spreading of the infection on all the plants, reducing the use of substances, and incrementing the quantity and quality of production.**

In Italy the precision agriculture applied to wine industry is particularly interesting: one of the major productive sectors is wine, with the production of quality grapes resulting in wine of great quality.

The aim of this study is to detect and investigate the presence of *Plasmopara viticola* on the surface of vine leaves using an experimental setup based on a spectrophotometer, operating in the UV-NIR spectral band.

The results were aimed at differentiating healthy leaves from those affected by *Plasmopara viticola*.

Keywords—precision agriculture, fluorescence, ultraviolet radiation

I. INTRODUCTION

The early recognition of plant pathologies is an issue of great interest and impact for the agricultural production worldwide.

Recognizing a disease early makes it to intervene quickly and efficiently, while at the same time using lower doses of pesticides or substances that can pollute the environment and preserving any production in terms of quantity and quality.

From a scientific point of view, the study of precision agriculture methodologies is widely widespread, given the important impact on agriculture, and many steps forward have been made to improve and preserve the quantity and quality of agricultural products.

In Italy the agricultural heritage is very important; one of the major productive sectors is wine, with the production of quality grapes resulting in fine wines.

Therefore, it is essential that the grape production is at its maximum yield, and that the quality of the grapes is such as to guarantee the highest standards. To this aim, in this study we analysed the fluorescence spectral response of vine leaves affected by downy mildew (*Plasmopara viticola*), a very common fungal disease in certain humidity and temperature conditions.

The aim of this study was to detect and investigate the presence of mould on the surface of vine leaves using an experimental setup based on a spectrophotometer, operating in the UV-NIR spectral band.

II. THE STATE OF THE ART OF FLUORESCENCE PROCESS

When light strikes the surface of leaves or other parts of the plant, the photons are absorbed by pigment molecules and chlorophyll. Part of the energy is absorbed by the plant for photosynthetic processes, part is reflected by the plant, and part is re-emitted in the form of fluorescence. The energy resulting from the fluorescence emission process is very low and corresponds to about 3-5% of the total energy.

In the fluorescence spectrum, the maximum emission wavelength is longer than that of absorption by chlorophyll. The peak of fluorescence emission in a chlorophyll solution is at 668 nm, while the maximum absorption is at 663 nm. The maximum peak of fluorescence emission in a leaf at room temperature is at 685 nm, and the fluorescence spectrum extends up to 800 nm.

When a leaf sample is adapted in the dark and subsequently illuminated with a saturating light source, an increase in fluorescence intensity to a maximum level is observed. Once the maximum fluorescence peak is reached, a slow decay can be observed over time, stabilising after about 3 minutes.

The literature presents many works on the study of chlorophyll fluorescence, to determine the state of health of the plant, following excitation by the UV component of sunlight [1,2].

The spectrum of sunlight is highly variable and cannot be easily controlled. Furthermore, the determination of the spectral response of the fluorescence from leaves or other parts of the plant is very difficult for re-absorption effects that plays a key role in the process [3]; moreover, it is necessary a variable time of dark adaption before the measurement [4].

Despite these challenges, a study of the spectral response in fluorescence is possible to provide for a screening of the status of health of the plants [5] considering that exposed leaves accumulate substances (as flavonoids) that presumably protect the leaf tissues from the UV radiation damages. [6]

As example the chlorophyll fluorescence imaging has been widely used to study some plant stress or disease as the leaf rust and powdery mildew of wheat [7,8], while many studies show the use of the visible fluorescence emitted from Ultraviolet (UV) excitation, for the identification of bacterial and fungal samples [9-14].

In [15] a survey of autofluorescence upon infection of downy mildew has been conducted showing a possible detection of the pre symptomatic infection, while in [16] has been studied the UV- fluorescence in case of infection by *Erysiphe necator*, showing the possibility of detecting the infection considering the fluorescence ratios.

[17] analyses the structure of carotenoids in various kind of fungal infection, evidencing the presence of carotenoids as antioxidant, against reactions generated by photosynthesis, while in [18] is evidenced the possibility of detecting the presence of carotenoids through the process of fluorescence.

By using the experimental setup described in the next section we show the first result acquired, highlighting a possible spectral response to UV fluorescence due to the presence of carotenoids on the vine leaf infected by downy mildew (*Plasmopara viticola*).

III. THE EXPERIMENTAL SETUP

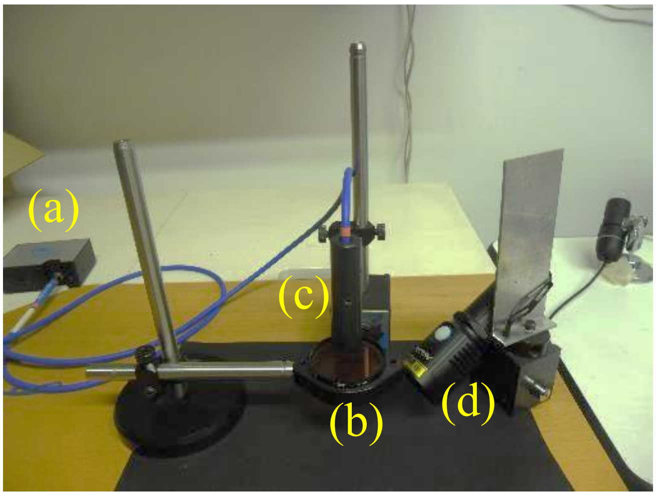

Fig. 1. The experimental setup. (a) is the USB4000 spectrometer, (b) is the filter, (c) is the CCD sensor, (d) is the UV LED flashlight

The experimental setup in Fig. 1 is based on the Ocean Optics (now Ocean Insight) USB4000 with principal features:

- Sensor based on the Toshiba TCD1304AP linear CCD array, with 3648 pixels of size 8µm wide by 200 µm tall.
- Responsive from 200 nm - 1100 nm, specific range and resolution depends on your grating and entrance slit choices.
- Sensitivity of up to 130 photons/count at 400 nm; 60 photons/count at 600 nm
- An optical resolution of ~0.3 – 10.0 nm (FWHM)
- Integration times from 10 µs to 65 seconds
- Connection to other devices through USB, RS232 ports and SPI/I2C
- Low power consumption of only 250 mA @ 5 VDC
- CE Certification

The UV source is a very light, simple, and compact 365nm UV LED flashlight, with a power output of 10W, USB-C port for charging and a pocket clip.

To filter out the spectral components related to the UV source, a 52mm bandpass filter was used.

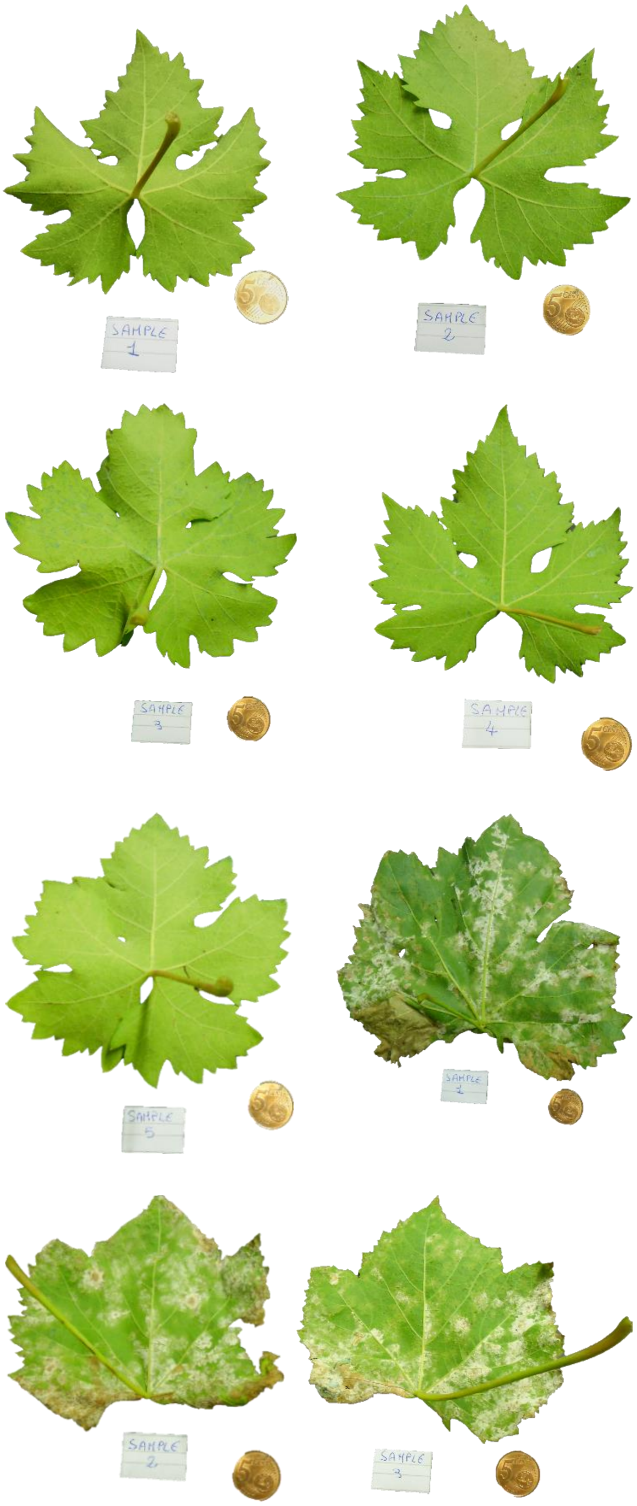

Fig. 2. Ten leaves samples. Five healthy and five with mould.

IV. SAMPLES AND FIRST RESULTS

The purpose of this first test was to analyse the fluorescence emitted by a healthy vine leaf and one affected by downy mildew (*Plasmopara viticola*).

Downy mildew or Peronospora is induced by a fungus, the *Plasmopara viticola*. It is a fungal disease that can infect various types of plants. It mainly affects plants that live in a humid and rainy climate: heavy and frequent rains favour this type o infection. This fungus causes yellowing of seedling leaves, with patches and spots that disrupt the regularity of the tissue. As the disease progresses, the spots become darker; if the *Peronospora* attacks the stem, the crop is seriously damaged.

The samples were taken directly from the vineyard, chosen according to have a several leaves variety, varying the shape of leaf, their distribution and the amount of dry leaf.

The first step was to prepare 5 samples of healthy leaves and five samples of naturally infected leaves; samples were examined 3 hours after harvesting and stored in a cardboard box to preserve their natural moisture content. Healthy leaves and those with mould are shown in Fig. 2.

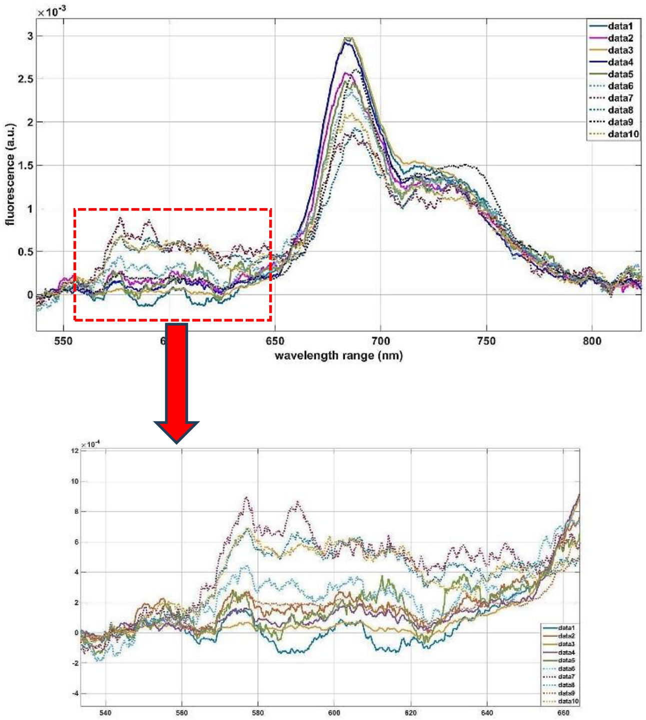

Fig. 3. The normalized spectra for the leaves healthy (data1 - data 4) and with mold. The detail shows a higher value of fluorescence for the leave with mold (data 5 - data 10)

The second step has been to detect the fluorescence emitted by healthy and mouldy leaves at the beginning of the process and after 3 minutes that corresponds to the stabilization of the fluorescence phenomenon.

Prior to excitation of the sample with ultraviolet light, the sample was placed in a no-light situation to give it time to adapt.

In addition, analysed data have been normalized with respect to background noise and darkness, being the measuring instrument very sensitive.

Fig.3 shows the normalized spectra of whole the 10 samples acquired by using our experimental setup; it evidences both the main peak of fluorescence of the chlorophyll placed at 668 nm, and the fluorescence emitted by moulds fixed in the wavelength range of 540 nm and 650 nm.

This pattern is presumably due to the fluorescence from carotenoids pigments present in the mould.

The carotenoids are natural organic compounds that range from yellow to red-orange and can protect against photooxidation, being able to inactivate oxygen radicals and quench oxygen. There are various types of carotenoids, the most common and present in the most of fungi is β-carotene [17], but each fungus has its characteristic in carotenoids. However, they have other important biological functions in fungi [19].

V. CONCLUSIONS

This study present an experimental setup, based on a spectrophotometer, operating in the UV-NIR spectral band, to detect the presence of moulds on the surface of vine leaves.

Acquisition was done under dark conditions, with a spectrophotometer with an optical resolution of ~0.3 to 10.0 nm, using software provided by the parent company, Ocean Optics. A total of 10 leaves were analysed, 5 healthy leaves and 5 leaves affected by a specific fungus, *Plasmopara viticola*, whose intensity of infection was variable from leaf to leaf.

The results have been graphed, showing a significant spectral response for or the diseased leaves, in a wavelength range of 540 nm and 650 nm, that has not been found in the healthy leaves. This result, presumably, can be attributed to the presence of carotenoids synthesized by the fungus to reduce the oxidative stress of photosynthesis.

This study was limited to highlight the possibility of detecting the presence of moulds, through fluorescence study, also in agreement with the literature; however, further testing is needed to confirm the spectral response of carotenoids in the indicated spectral band.

REFERENCES

[1] Murchie, E. H., & Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of experimental botany, 64(13), 3983-3998.

[2] Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence - a practical guide. Journal of experimental botany, 51(345), 659-668.

[3] R. Pedrós, I. Moya, Y. Goulas, S. Jacquemoud, “Chlorophyll fluorescence emission spectrum inside a leaf “Photochemical and Photobiological Sciences”, 2008, 7 (4), pp. 498 - 502 DOI: 10.1039/b719506k

[4] L. Chaerle, R. Valcke, D. Van Der Straeten, “Imaging techniques in plant physiology and agronomy: From simple to multispectral approaches”, Plant Physiology and Plant Molecular Biology in the New

Millennium, Hemantaranjan, A., Jodhpur, India, 2003; Volume 5, pp. 135–155

[5] S. Lenk, L. Chaerle, E.E. Pfündel, G. Langsdorf, D. Hagenbeek, H.K. Lichtenthaler, D. Van Der Straeten, C. Buschmann, "Multispectral fluorescence and reflectance imaging at the leaf level and its possible applications", J Exp Bot. 2007;58(4):807-14. doi: 10.1093/jxb/erl207

[6] MLF. Ferreyra, P. Serra, P. Casati, "Recent advances on the roles of flavonoids as plant protective molecules after UV and high light exposure", Physiol Plant. 2021 Nov;173(3):736-749. doi: 10.1111/ppl.13543

[7] K. Bürling, "Potential of Fluorescence Techniques with Special Reference to Fluorescence Lifetime Determination for Sensing and Differentiating Biotic and Abiotic Stresses in Triticum aestivum L." Ph.D. Thesis, University of Bonn, Bonn, Germany, 2011.

[8] J. Kuckenberg, I. Tartachnyk, G. Noga, "Temporal and spatial changes of chlorophyll fluorescence as a basis for early and precise detection of leaf rust and powdery mildew infections in wheat leaves", Precision Agric 10, 34–44 (2009). https://doi.org/10.1007/s11119-008-9082-0

[9] M.S. Ammor, "Recent Advances in the Use of Intrinsic Fluorescence for Bacterial Identification and Characterization". J Fluoresc 17, 455–459 (2007). https://doi.org/10.1007/s10895-007-0180-6.

[10] J.M. Herzog, , V. Sick, "Quantitative Spectroscopic Characterization of Near-UV/visible E. coli (pYAC4), B. subtilis (PY79), and Green Bread Mold Fungus Fluorescence for Diagnostic Applications", J Fluoresc (2023). https://doi.org/10.1007/s10895-023-03183-6

[11] L. Leblanc, E. Dufour, "Monitoring the identity of bacteria using their intrinsic fluorescence", FEMS microbiology letters. 211. 147-53. 10.1016/S0378-1097(02)00636-5.

[12] M. Sohn, D..S Himmelsbach, F.E. Barton, P.J. Fedorka-Cray (2009) "Fluorescence spectroscopy for rapid detection and classification of bacterial pathogen", Applied Spectroscopy. 2009;63(11):1251-1255. doi:10.1366/000370209789806993

[13] F. Awad, C. Ramprasath, N. Mathivanan, P.R. Aruna, S. Ganesan, "Steady-state and fluorescence lifetime spectroscopy for identification and classification of bacterial pathogens". Biomedical Spectroscopy and Imaging. 3. 381-391. 10.3233/BSI-140095

[14] L. Smeesters, T. Kuntzel, H. Thienpont, L. Guilbert, "Handheld Fluorescence Spectrometer Enabling Sensitive Aflatoxin Detection in Maize". Toxins 2023, 15, 361. https://doi.org/10.3390/toxins15060361

[15] S. Bellow, G. Latouche, S.C. Brown, A. Poutaraud, Z.G. Cerovic, "Optical detection of downy mildew in grapevine leaves: daily kinetics of autofluorescence upon infection". J Exp Bot. 2013 Jan;64(1):333-41. doi: 10.1093/jxb/ers338

[16] M.C. Bélanger, J.M. Roger, P. Cartolaro, A. Viau, V. Bellon Maurel, "Detection of powdery midiou in grapevine using remotely-sensed UV-induced fluorescence", International Journal of Remote Sensing - INT J REMOTE SENS. 29. 1707-1724. 10.1080/01431160701395245

[17] G. Sandmann, "Carotenoids and their biosynthesis in fungi, Molecules (Basel,Switzerland),27(4),1431,https://doi.org/10.3390/molecules27041431

[18] T. Gillbro, R. J. Cogdell, "Carotenoid fluorescence", Chemical Physics Letters,Volume 158, Issues 3–4,1989,Pages 312-316,ISSN 0009-2614, https://doi.org/10.1016/0009-2614(89)873

[19] J. Avalos, M.C. Limón, "Biological roles of fungal carotenoids", Current genetics, 61(3), 309–324. https://doi.org/10.1007/s00294-014-0454-x