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Application of fluorimetric indices in Vermentino plants affected by GPGV

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Abstract. Grapevine Pinot Gris virus (GPGV), a single-stranded RNA virus classified under the Trichovirus, infects grapevines, manifesting symptoms such as growth arrest, chlorotic mottling, and leaf deformation. Typically, when the viral infection is active, symptoms manifest in late spring, facilitating the visual identification of symptomatic and asymptomatic vines, even if there may also be latent forms. This visual classification is often detected through laboratory tests, such as the polymerase chain reaction prevented by reverse transcriptase as RNA virus (RT-PCR). This work focused on field monitoring, using an alternative method for detect GPGV in a Vermentino vineyard in Olmedo (Italy). A proximal fluorescence sensor was employed as a non-destructive tool to assess leaf physiological activities. In the summer of 2021, 50 vines were sampled and classified in two groups N (Negative) or P (Positive) after laboratory assays RT-PCR: 20 vines were classified as N, and 30 as P. Before collecting the samples, the plants were analyzed with a proximal fluorimetric sensor, which utilized indices related to leaf physiological characteristics. The statistical analysis, performed by ANOVA, revealed a significant difference between N and P vines, particularly in the anthocyanin FER_RG index. From the analysis, the FER_RG index was superior in P plants, indicating a higher anthocyanin density than in asymptomatic plants, and thus estimating a lower chlorophyll content. This suggests the potential of proximal fluorescence sensing as a valuable tool for early GPGV detection in grapevines, offering a non-destructive and efficient tool of assessing plant health.

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1 Introduction

In viticulture, the acquisition of virus-affected vegetative material for new vineyard implantations has generated new management problems in Italian and European vineyards [1]. Among several viruses reported, the Grapevine Pinot Gris Virus (GPGV) represents one of the most important due to the damage it causes to vines when present[2].

Specifically, GPGV manifests during the stage that follows vegetative reawakening, causing phenomena of reduction in sprout size, diffused chlorosis on the leaf's upper page and typical morphological deformations on leaves[3]. The virus presence alters the plant's metabolism, compromising the development phases and leading to a potential collapse in productivity. One of the causes of GPGV diffusion is geographically associated with an eriophyid mite, Colomerusvitis[4], which has also been shown to be a vector of the virus on vine but not for other types of plants, such as Chenopodium albus, Rosa sp., Rubus sp. and Silene lotiflora on which the virus is supposed to arrive through a winged vector of the pathogen in wine-growing areas [5].
Traditionally, the primary technique for pathogen recognition is associated with the leaf's destructive sampling method and its laboratory molecular method of Polymerase chain reaction after Retro-transcriptase[6].Precision agriculture (PA) could play a crucial role in developing field identification of the infestation, seeking among the technologies and practices of the tools used in PA for monitoring and estimating the pathogen in the field[7]. Among the various instruments employed, the proximal field fluorimetry [8], capable of obtaining real-time general information about the physiological activities of the plant, is particularly relevant.

The primary purpose of the present research was to investigate a non-invasive method for detecting GPGV in grapevines. In association with RT-PCR, this study proposes a preliminary investigation into the feasibility of using a proximal fluorescence sensor to assess leaf physiological activities as a potentialalternative to traditional monitoring methods in GPGV detection.

2 Materials and Methods

2.1 Site and experimental design

The study took place in a 15 years-old commercial Vermentino vineyard in Olmedo, Italy, during 2021 summer. Plant sampling was conducted in a random design within the field during the plant growing phase on June 24th. The monitoring and sampling procedures involved a total of 50 vines, including vinesmanifesting the characteristic symptoms of GPGV and others that appeared asymptomatic, to create a heterogeneous dataset of symptomatic and asymptomatic vines.

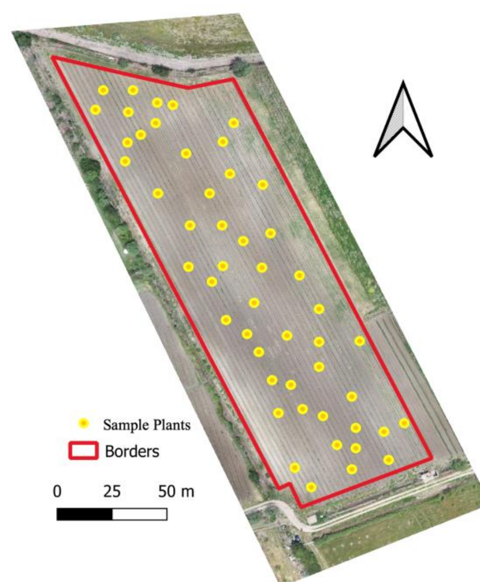

Fig. 1. Field borders of the vineyard analysed during the survey.

2.2 Fluorimetric survey

During the leaf sampling process, proximal monitoring was conducted in the field using the Force-A Multiplex 3.6 (MFA, Orsay, France) sensor for a fluorimetric survey. The sensor array of the MFA includes three RGB excitation LED channels (blue, green, and red at 625 nm, 516 nm and 470 nm, respectively) and six LEDs (375 nm) for ultraviolet radiation.

The fluorimetric outputs provide information on the metabolic and physiological activity of the plant during its vegetative and production stages [11].The sensors detect fluorescence in red, near-infrared, and blue-yellow, and the captured fluorescence is correlated with energy excitation. The MFA fluorimetric indices are employed to assess different physiological and biochemical aspects of vines, capable to analyse both vegetative and productive elements. For instance, the Simple Fluorescence emission Ratio (SFR) can evaluate photosynthetic activity by measuring chlorophyll fluorescence. Nitrogen Balance Index (NBI) supports detecting variations in plant health, particularly related to nitrogen nutrition [12]. Concerning biotic and abiotic stress analysis, the Blue-Red emission Ratio to Far-Red Fluorescence (BRR_FRF) serves as an indicator of plant reaction to external factors such as viruses, water stress and heat phenomena. Similarly, Fluorescence Excitation Ratios in the Red and Blue (FER_RB) and Fluorescence Excitation Ratios in the Red and Green (FER_RG) are used to detect physiological stress and changes in anthocyanin content, which play a role in berry pigmentation and stress adaptation. These indices collectively provide valuable estimation of crop health, aiding in precision agriculture and sustainable crop management [9].

2.3 Laboratory analysis

Leaf sampling was conducted with 5-6 leaves and petiole per plant, depositing the samples in a cooler to maintain tissue integrity. The leaves were then transported to the laboratory for molecular analysis to detect and confirm the presence of GPGV. RT-PCR was chosen to obtain etiological confirmatory analysis and to identify the virus-positive plants in the laboratory. According to the specific protocol, the first step was to extract the total RNA from the samples. The RNA was retrotranscripted for amplification and then analysed by PCR. The results were verified through an electrophoretic run to confirm the RT-PCR analysis. The protocol used involved three different primer pairs. The first pair, detf/detr, amplifies the gene regions that include MP and CP [13]. The second pair partially amplifies the gene regions that include MP and CP. Once the positive samples were identified, they were subjected to a more specific PCR to identify the individual MP and CP sequences using 5637f/3939r and 6609F/7020R. These sequences were then sequenced to recreate the phylogenetic trees.

2.4 Statistical analysis

The results from the laboratory analysis were combined with the data from MFA fluorimetric monitoring. This allowed linking the positivity or negativity to the various fluorimetric indices at each sample point.The dataset was then statistically examined using the *open-source*Rstudio platform and the RCommander package for graph construction and statistical analysis[14]. The t-test analysis was choose to elaborate the dataset, where the MFA indices presented in Table 1 were associated with the laboratory results to define the Positive (P) and Negative (N) groups. During the statistical analysis, a boxplot graph was created for each MFA index to visualize the data distribution within the groups.

3 Result and Discussion

The laboratory analysis by RT-PCR allowed the identification of positives vines, dividing the plant sampled in two categories in 20 N and 30 P. The t-test analysiswas performed with the chlorophyll, stress,flavonolsand anthocyanins MFA indices SFR_G, SFR_R, BRR_FRF, FLAV, FER_RG and FER_RB, associating the boxplot data distribution to observe the two groups behaviour.

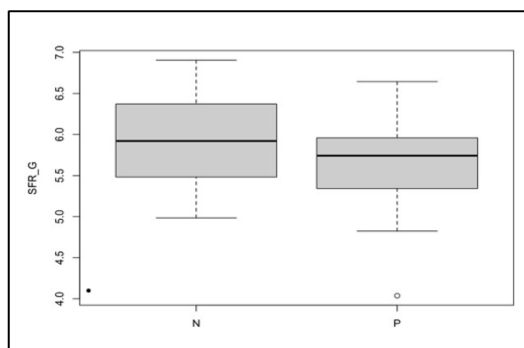

Fig. 2. Boxplot of SFR_G chlorophyll index comparing the Negatives (N) and Positives (P) vines.

Table 1. Principals fluorimetric indices elaborated by MFA and related formulas.

Index	Target	Formula	
SFR_R	Chlorophyll activity	FRF/RF	(1)
SFR_G		FRF/GF	(2)
BRR_FRF	Stress Index	FRF/UV	(3)
FLAV	Flavonoids	LogFER_RUV	(4)
FER_RG FER_RB	Anthocyans	FRF_R/FRF_G FRF_R/FRF_BY	(5)

Due to the delay in monitoring (24 June), many leaves had lost the chlorotic appearance that characterises the manifestation of the disease. The statistical analysis of SFR_G and SFR_R revealed a non-significative response due to the high p-value (0.095 and 0.165, respectively). The boxplot graphical analysis reports a similar result for both indices, where the chlorophyll values for the P vines remained lower than those for the N vines. Figure 2 shows the SFR_G index boxplot graph.

Theflavonols index (FLAV, Fig. 3) did not assess a difference between P and N groups, as the high p-value significance (0.211).
Furthermore, the BRR_FRF index boxplot graph shows higher values in the P vines despite the N ones, suggesting higher stress levels in the positive plants.

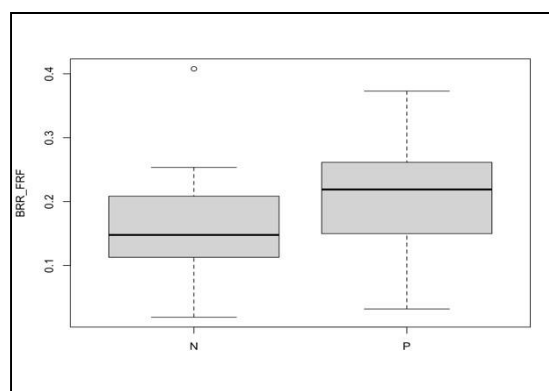

Fig. 3. The BRR_FRFstress index boxplotfor N and P groups.

However, t-test analysis reported a non-significative result between the two groups (0.107).

Due to the non-significant ANOVA analyses for the primary indices, the research focused on secondaries indices application, which are not directly correlated to chlorophyll and stress phenomena but may provide an estimation of the trend of values between the N and P groups observed for the SFRs and stress indices. Since GPGV is a plant disease that affects, among other, vascular tissues causing browning[15], the approach adopted was to consider an index that could analyse these discolorations.

Vermentino is a cultivar characterised, among other things, by an absence of visible anthocyanins on the leaf[16], so in a healthy leaf, anthocyanin estimation

values should tend to be low. Since chlorosis phenomena mainly affected the perinerval area of the leaf, the anthocyanin estimation index was chosen to observe the detection capability of this anomaly.

The index analysed was the FER, with the green and red excitation channel, which estimates the anthocyanin content in the leaf epidermis [17]. The use of its inverse under green wavelengths, FER_RG^{-1}, is a valid estimator of chlorophyll [9]in substitution to the more applied indices SFRs.

The FER_RG, FER_RB and FER^{-1} indices measured in P and N groups were compared using t-test analysis at 0.05 significance level. Table 3reports the statistical analysis result for the two group comparisons.

Table 2. t-test significativityfor anthocyans content (FER_RB and FER_RG) and chlorophyll activity (FER_RG^{-1}).

Index	*p-value*
FER_RB	0.035
FER_RG	0.002
FER_RG^{-1}	0.002

The boxplots displayed in Figures 5 and 6 illustrate epidermal anthocyanin content, revealing a significant difference compared to the previous indices.

P vines display a higher overall anthocyanin index than Negative ones, as confirmed by the data from both green and red radiation channels. This trend suggests that the N leaves have a significantly lower total anthocyanin content compared to the P group, likely due to the absence of chlorosis and, consequently, a higher level of photosynthetic activity in the P group. The overall range of values for P plants is higher, suggesting a higher exposure of nerve and perinerval anthocyanins, probably accompanied by a reduction in chlorophyll content.

This assumption is associated with the observation in Fig. 2, where the index used to estimate photosynthetic activity identified higher values in N plants despite the absence of statistical significance (Table 2).

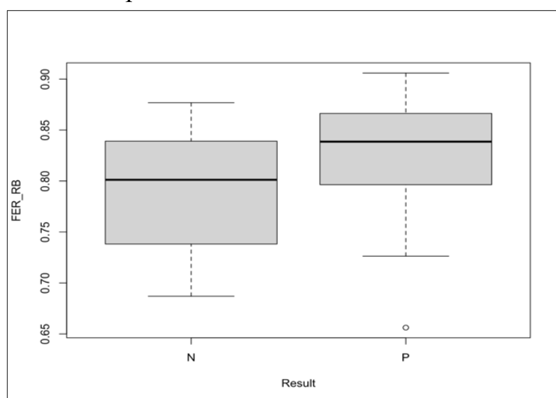

Fig. 4. FER_RB boxplot. The difference observed from N and P groups exhibits a higher value on P vines,suggesting a critical situation instead of N vines.

The inverse index of FER_RG (FER_RG^{-1}) shows how the instrument identifies a significant difference between the plants analysed by the “Result” variable.
Despite the delayed monitoring operation, the MFA instrument reached the identification of physiological variations between symptomatic and asymptomatic Vermentino plants.As expected, the graphical result (Fig.6) shows lower values in the P plants compared with the N group.

These results, in association with the observations in the graphs for SFR, BRR_FRF and FLAV, suggest that the fluorimetric approach could represent a rapid detection and analysis system for the symptomaticity of Vermentino plants to GPGV pathology.

4 Conclusion

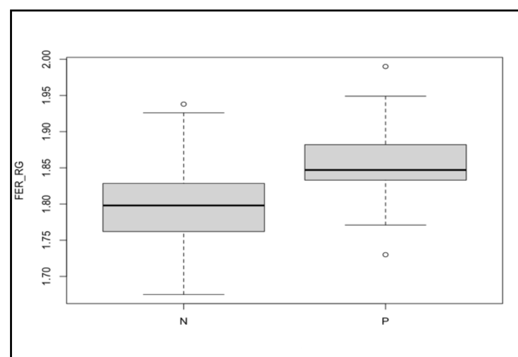

Fig. 5. FER_RG index boxplot for N and P vines.

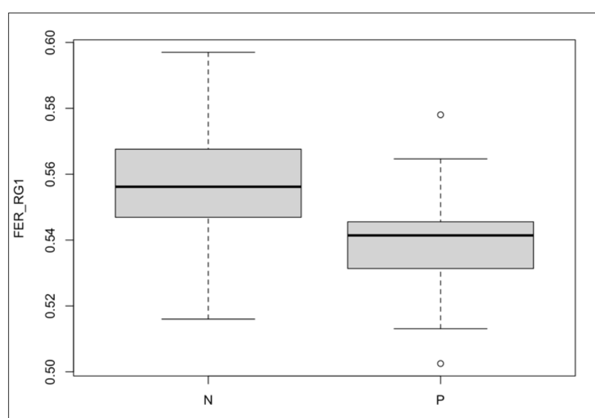

Fig. 6. The derived chlorophyll activity index shows, as expected, higher values on N group then lower activity on P group.

The MFA's preliminary analysis of GPGV revealed some helpful observations in the domain of proximal sensing in viticulture. The fluorimetry application allowed the statistical identification and graphical division of the N and P groups, despite the late survey on the crop and, therefore, in a phase of regression of the symptoms characterising the infestation. The statistical differences illustrated by the fluorimetric indices FER_RG, FER_RB, and FER_RG-1 in Vermentino plants affected by GPGV indicate that these indices may also play a significant role in symptom detection. The non-destructive use of proximal fluorescence shows promise as a valuable tool for monitoring and potentially identifying viral entities like GPGV in viticulture. However, this detection process did not account for external contaminants, such as other viruses or infections of different origins. To optimise the detection capabilities, further investigations are planned to assess additional contaminations in vines and develop a calibration curve for GPGV identification.

Further studies will involve a system for classifying healthy and diseased plants by clustering analysis, using both the indices and channels of the MFA to capture the GPGV contamination.

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Foundings
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Conflicts of Interest
The authors declare no conflict of interest.

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