Optical Devices Evaluation for Diagnosis of *Plasmopara viticola* on Vine

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ABSTRACT

Remote sensing (RS) is the most widely adopted technique for crop monitoring in precision viticulture (PV). Recent research looks at the development of proximal sensing technologies alternative to RS. The present work considers the possible use of proximal sensing optical devices for diagnosis in vineyard; in particular, we evaluated the GreenSeeker RT100 and the Crop Circle (two commercial optical sensors) in detecting different levels of grapevine downy mildew symptoms. The analysis was conducted on vine leaves that had been picked from plants of cv. Cabernet Franc infected by *Plasmopara viticola*. Leaves were divided into homogeneous infection classes and then analyzed through the optical devices and a portable Vis/NIR (visible/near infrared) spectrophotometer used as tester. Data showed a linear relation between the percentage of symptomatic leaf area and normalized difference vegetation index NDVI calculated through the two optical sensors (R^2 = 0.708 for GreenSeeker; R^2 = 0.599 for Crop Circle; R^2 = 0.950 for the spectrophotometer). The regression obtained for GreenSeeker is more significant than the regression obtained for Crop Circle. This fact suggests a greater capability of GreenSeeker than Crop Circle in detecting different disease levels and its possible use in diagnosis application in the vineyard.

Keywords: Precision viticulture, diagnosis, NDVI, proximal sensing, optical devices.

1. INTRODUCTION

Precision viticulture (PV) may be defined as the methodologies allowing site-specific monitoring and management in vineyard. PV includes the monitoring and the management of productivity spatial variability within the single vineyard, both in quantity and quality (Lamb and Bramley, 2001). Therefore, crop monitoring is the most studied application in PV systems and it considers data and information coming from observation carried out on the crop directly, pertaining to details such as phenological, nutritional and phytosanitary status, production expectations, etc. Early diagnosis of vegetation stress, which includes a variety of production limiting factors, is of growing importance in the framework of precision agriculture (PA) and PV applications, especially with regards to integrated pest management. Early information on crop health and disease detection can: a) facilitate the control of disease through proper management strategies, b) allow for a more efficient application of agrochemicals and c) improve productivity (Sankaran et al., 2010). This is especially important for capital-intensive perennial crops, such as various fruit species and vine.

Currently, scouting is the most widely used mechanism for monitoring stress in tree crops, though it is an expensive, labor-intensive, and time consuming process. Spectroscopic and imaging techniques are the unique disease monitoring methods that have been used to detect diseases and stress due to different factors in plants and trees.

Studies were conducted with various crops to distinguish diseased leaves from healthy leaves (Blanchfield et al., 2006; Moshou et al., 2006). Many diseases are known to cause changes in leaf pigments, biochemical components and metabolic alterations in infected leaves (Lehrer et al., 2007). These pathological conditions influence spectral characteristics of leaf tissue that can be detected in the visible and/or the NIR (Near Infrared) regions of the electromagnetic spectrum. Thus, the different spectral reflectance between healthy and infected leaves can be used to identify the health status of a plant. Delalieux et al. (2007) investigated the capability of hyperspectral analysis for early white apple scab (*Venturia inaequalis*) detection; Naidu et al. (2009) investigated the potential of leaf spectral reflectance changes between virus-infected and uninfected grapevines (*Vitis vinifera* L.) in developing non-invasive techniques for field-based "real-time" diagnosis of grapevine leaf roll disease (GDL). Cséfalvay et al. (2009) conducted a study on the pre-symptomatic detection of *Plasmopara viticola* (Berk. et Curt.) Berl. and De Toni infection in grapevine leaves using chlorophyll fluorescence imaging at high resolution.

The oomycete *P. viticola* is an important pathogen of grapevines. It is the etiological agent of grapevine downy mildew, which is one of the most serious diseases of *Vitis vinifera* L. in cool climates (optimum 20°–25°C; extremes 10°–29°C) with abundant rains in late spring, as are most of the European vineyards that fall short of the Mediterranean regions. This pathogen impairs leaf physiology soon after the onset of infection (Polesani et al., 2008). In the 5–15 days following infection, depending on environmental conditions, *P. viticola* grows within the leaf tissue and causes significant economic loss if no chemicals are applied. It induces the appearance of pale yellow oilspots on leaves and extended necrosis on clusters. Eventually, the spots become necrotic and result in a premature defoliation of vines, mostly in severely affected vineyards. The macroscopic symptoms of the grapevine downy mildew are particularly evident and can allow a significant evaluation of optical device performance in detecting different disease levels in vineyards.

Many spectroscopy-based studies use different vegetative indices for evaluating the change in spectral reflectance for diseased or healthy plant (Bravo et al., 2004). One of the most diffused indices is the NDVI (Normalized Difference Vegetation Index), that is calculated according to the following formula (Rouse et al., 1974):

$$\mathbf{NDVI = (NIR - red) / (NIR + red)} \quad (1)$$

where **NIR** is the fraction of emitted NIR radiation returned from the sensed area (reflectance) and **red** is the fraction of emitted visible red radiation returned from the sensed area (reflectance). The present work aims to evaluate the efficiency of two optical commercial devices (computing NDVI in real time) in detecting symptoms of grapevine downy mildew caused by *P. viticola*: the GreenSeeker RT100 (NTech Ind.; Ukiah, CA, USA) and the Crop Circle (Holland Scientific Inc.; Lincoln, NE), normally used to monitor cover crops in order to manage nitrogen fertilization according to the logic of variable rate application (Govaerts et al., 2007; Sripada et al., 2008). These devices were proposed for use in monitoring crop vigor variations as an alternative to the acquisition of multispectral images (Lamb et al., 2009); in particular, Drissi et al. (2009) and Mazzetto et al. (2009) proposed the application of the GreenSeeker to monitor vigor variations of *Vitis vinifera* L. in vineyards. In this experimentation, data captured from the two devices were compared to data collected by a portable Vis/NIR spectrophotometer used as reference because of its optimal characteristics and its high spectral resolution - this in order to investigate the possible use of optical devices in diagnosis application in vineyard (Mazzetto et al., 2010).

2. MATERIALS AND METHODS

The evaluation of the two commercial sensors described above was conducted on leaves characterized by increasing infection levels. Leaves were picked from plants of *Vitis vinifera* L. cv. Cabernet Franc previously inoculated with the pathogen *P. viticola*. Leaves were divided into 8 homogeneous groups based on a disease scale which is normally used for disease visual assessment in vineyard. In fact, the monitoring of grapevine downy mildew is based on the method described by Townsend and Heuberger (1943). This method consists in counting the number of infected leaves and classifying them according the extension of the symptoms on the leaf surface. The disease scale has 8 classes (tab. 1) ranging from 0 – healthy - to 7 - 75 to 100% of symptomatic surface (fig. 1). When used for the monitoring in vineyard, the results coming from the classification are normally used to calculate the percentage infection index (I%I) of *P. viticola* according to the equation:

$$\mathbf{I\%I = [\Sigma\ (n \cdot v)] / [(n° \text{ of classes} - 1) \cdot N]} \quad (2)$$

Where **n** is the frequency of leaves in each class, **v** is the identifying number of each class, and **N** is the sample size.

Table 1: Infection classes used to classify leaves infected with *P. viticola*.

Class	Symptomatic leaf area (%)
0	0
1	0-2.5
2	2.5-5
3	5-10
4	10-25
5	25-50
6	50-75
7	75-100

In the present work an expertise carried out the visual classification of the leaves on each inoculated plant and then 9 leaves of each class were picked up. Leaves of about the same age were used for the analysis in order to reduce the variation of the measurements due to different factors that influence the spectral response (age, thickness, chlorophyll concentration). Since the tests are destructive, it was no possible to include more leaves into each group because of the difficulty of selecting a bigger number of leaves characterized by the same disease level and vegetative stage. Leaves belonging to the same class were then fixed on a black plastic panel to form a single layer and investigated through the optical devices. The black panel was used as a background to reduce interferences in vegetation canopy reflectance measurements. In fact, it is known that dark elements absorb almost the total incident radiation, and consequently has a low reflectance. This assure that the radiation captured by the optical devices comes mostly from the vegetation.

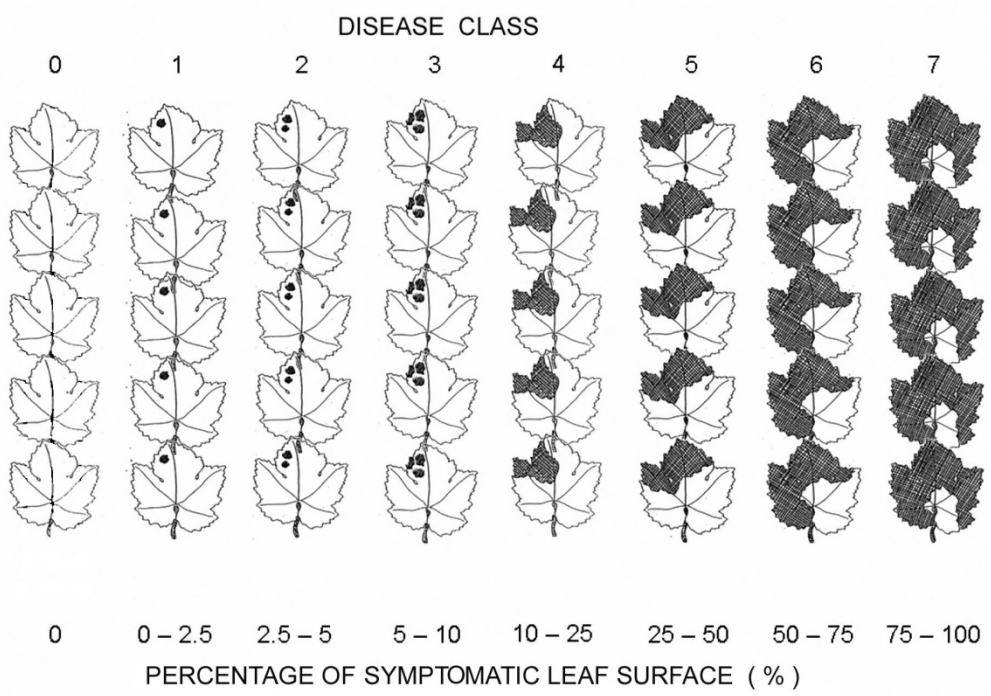

Figure 1. Schematic layout of the leaves arranged on the panel.

Leaves were analyzed in the Vis/NIR range (400–1000 nm) using a portable spectrophotometer that can do a punctual measurement where the fiber optic get in contact with the sample. The system consists of five elements (Guidetti et al., 2010).

1. Lighting system: The light source is a 50 W halogen spotlight with a color temperature of 4500°K and maximum emission at 500 nm. The light source is embedded in a metal holder, enabling the lamp to face the optical fiber steadily.
2. Fiber optic probe: Light radiation is carried over leaves through a fiber optic probe ("step index," mod. FCR-19IR200-2-ME-S1 by Avantes©). Spectral acquisitions are performed using a diffusive reflectance technique. The probe consists of 19 fibers with 200 μm diameter: 17 of these fibers carry light over the samples and 2 carry back radiation from the leaves to the spectrophotometer. The probe is characterized by a total field of view of about 0.8 mm^2.
3. Portable spectrophotometer: A fiber optic probe is connected to the AvaSpec-2048 portable spectrophotometer by Avantes©. The spectrophotometer is equipped with a diffractive grating for acquisition in the spectral range of 450–980 nm and a CCD sensor with a 2048-pixel matrix to record each wavelength's signal intensity with a resolution of 0.3 nm.
4. PC for data acquisition control: The system is controlled by a portable PC with dedicated software for data processing and DAC for automatic control of the spotlight.
5. Battery: The system is powered by a 12 V battery.

During the tests carried out through the spectrophotometer, twenty points were investigated in each group of leaves at different infection levels - this in order to include reflectance data coming from healthy and diseased leaf tissue. Leaves were lit by the radiation coming from a lighting system and the reflected component was measured by a spectrophotometer and registered by acquisition software. During the acquisition, one spectrum was recorded without lightening the lamp. The registered signal, obtained only with environmental light was used as a baseline and subtracted form the spectra of leaves.

Regarding the two commercial devices described above, it is important to highlight that the sensor units are designed to be mounted on mobile vehicles and measurements are taken as the sensors are passed over the crop surface. In particular, GreenSeeker senses a 0.6 × 0.01 m spot when held at a distance of approximately 0.6 to 1 m from the illuminated surface. The active optical sensor is made up of electroluminescent diodes (LEDs) emitting high intensity light in red (650 ± 10 nm full-width half-magnitude - FWHM) and NIR (770 ± 15 nm FWHM). The LEDs are pulsed at 100 Hz with an average reading of 10 Hz. The reflected light is captured by silicon photodiode positioned in front of the device.

On the other hand, Crop Circle uses a polychromatic LED with peak emission wavelengths at 650 nm (red) and 880 nm (NIR). The LED-lens configuration provides an approximately collimated beam with a source-ground footprint divergence angle of about 32° × 6°. The photodiode array comprises a couple of photodiodes: one conditioned to detect the visible radiation (<700 nm) and the other the NIR radiation (>800 nm). The sensor outputs NDVI at a programmable rate of from 1 sample per second to 20 samples per second.

Both the two tools does not need any calibration before their use, because they contain an autocalibration system.

Three measurements were conducted respectively with the GreenSeeker and the Crop Circle for each group of leaves. During these tests, static readings of reflectance were carried out by the two devices. Each sensor was made to stop over every group of leaves for an acquisition time of 5 seconds. Tools were alternately mounted on a metallic support, at an optimal distance of 0.60 m from the leaves. At this distance, GreenSeeker projects a beam of light of about 0.7 and 0.01 m in length and width respectively (fig. 2 A), whereas Crop Circle projects 15 light spots that cover a total surface of about 0.4 and 0.15 m in length and width respectively (fig. 2 B).

Data collected by the spectrophotometer were used in order to check whether NDVI is suitable for recognizing the symptomatic level of leaf tissue according the eight infection classes described above. Moreover, it is possible to extrapolate the same red and NIR wavebands investigated by the GreenSeeker and the Crop Circle from the reflectance measured by the spectrophotometer. In this way, we verified which NDVI - and consequently which of the two commercial devices - is more sensitive to infection classes. The wavelengths used to calculate the different NDVI are summarized in tab. 2. Then, the two optical sensors were tested on the same plant material in order to verify their real capability in detecting different disease levels. However, it should be stressed that the spectrophotometer measured the reflectance at precise points of the leaf surface, whereas NDVI values coming from GreenSeeker and Crop Circle referred to a larger portion of the vegetation. Data analysis was carried out through the software SPSS Statistics ver. 17.0.

Figure 2. A) The metallic bar used as support for GreenSeeker and Crop Circle during the measurements. The red line represents the light emitted by GreenSeeker. B) The field of view of Crop Circle consists of 15 spotlights.

Table 2: Specific wavelength used for calculating NDVI using the three devices.

Device	Vegetative Index	Wavelengths
GreenSeeker	$NDVI_G$	Red: 650±10 nm NIR_G: 770±15 nm
Crop Circle	$NDVI_C$	Red: 650±10 nm NIR_C: 880±15 nm
Spectrophotometer	$NDVI_{SG}$	Red: 650±10 nm NIR_G: 770±15 nm
	$NDVI_{SC}$	Red: 650±10 nm NIR_C: 880±10 nm

3. RESULTS AND DISCUSSION

Data collected by the spectrophotometer were processed through the Unscrambler software package (Version 9.6, CAMO ASA; Norway), normally used in chemometric analysis. In this way, the spectra of each infection class can be visualized on a graph. Figure 3 shows reflectance spectra obtained for each infection class. Each spectrum (plotted with a different color) corresponds to each infection class and is obtained as the average of twenty spectra collected for each class. Dotted lines highlight portions of spectra corresponding to wavelength used to compute NDVI in the red (A) and NIR (B = NIR_G, C = NIR_C) wavebands. These wavebands are the same used by GreenSeeker (A and B) and by Crop Circle (A and C) to compute NDVI indices.

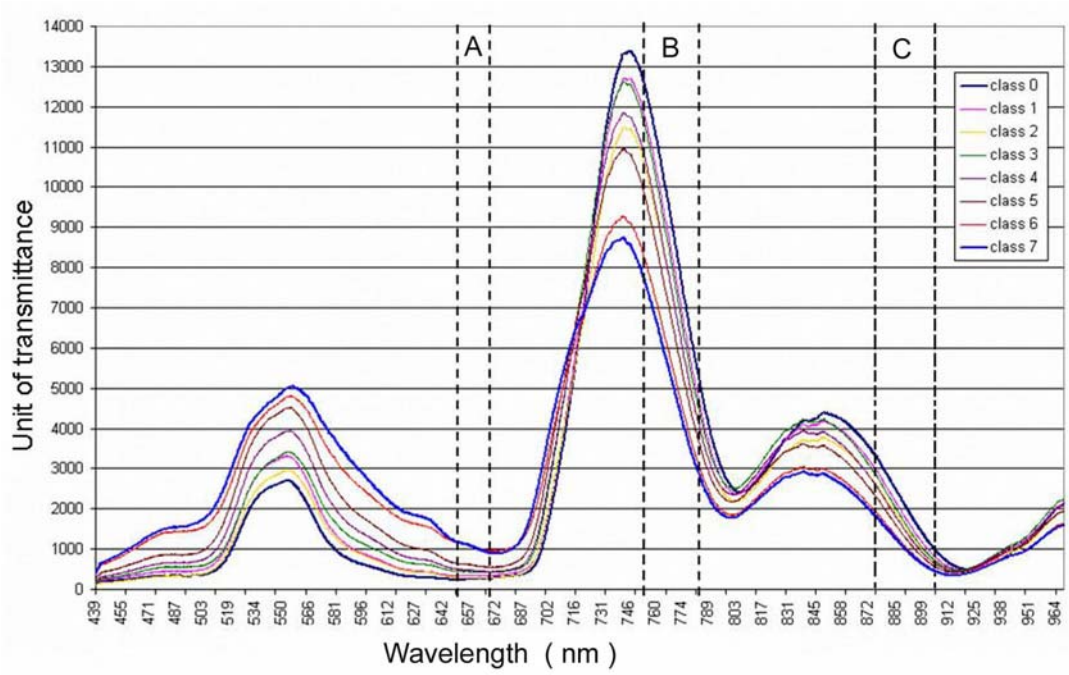

Figure 3. Reflectance spectra obtained from the analysis of homogeneous groups of leaves based on the percentage of symptomatic leaf area. Dotted lines highlight wavelengths used by GreenSeeker (A and B) and by Crop Circle (A and C) to compute NDVI.

From the graph presented in figure 3, it can be observed that all spectra show the typical trend of leaf tissue. In fact, as it is known, it is characterized by a greater reflectance in the NIR band than in the red band; moreover, it shows a peak reflectance in green (at about 550 nm) and NIR (about 740 nm) and minimum reflectance in red band. Spectra of different classes are arranged according to increasing symptomatic surface. From class 0 to 7 they show increasing reflectance in the Vis band, along with decreasing reflectance in the NIR band. For example, in the green band reflectance ranges from about 2598 for class 0 to 4869 for class 7. The increase of the reflectance can be observed also in the red band, even if its absolute value is lower than the reflectance in green (class 0: 257; class 7: 1030). In the NIR band, as expected, the reflectance decreases from class 0 (NIR_G: 8602; NIR_C: 2864) to class 7 (NIR_G: 4942; NIR_C: 1535). This fact confirms the general loss of functionality of leaf tissue due to the reduced photosynthetic activity and the senescence of vegetation caused by grapevine downy mildew. This first result is particularly interesting because it confirms multispectral analysis potential in diagnosis and detecting different levels of grapevine downy mildew manifestation and symptoms. Moreover, reflectance spectra in the red and NIR bands are arranged in order, according to infection classes. In particular, class 2 shows lower reflectance than class 1, even when it includes leaves with higher percentage of symptomatic surface than class 1. In NIR wavebands, it can be observed the overlap of spectra referred to class 2 and 4.

Afterwards, NDVI was calculated using reflectance data acquired with the spectrophotometer in the selected wavebands. For each sampling point, reflectance values were averaged, respectively in the red and NIR bands, in order to obtain a single reflectance value. Then, the mean value obtained in NIR and red was combined according to equation 1, obtaining NDVI values that corresponded to each investigated point. Then, NDVI values corresponding to sampling points that belonged to the same infection class were averaged again. In this way, a single NDVI value

was obtained for each class of infection (these processing steps allow calculating NDVI according to a procedure similar to that used by both GreenSeeker and Crop Circle; in fact, these devices return only one value of the considered vegetation index in correspondence to each class of leaves). Then, a linear regression analysis was conducted in order to investigate the importance of the infection level (independent variable) in NDVI variability (dependent variable) and to evaluate which instrument has a better performance in distinguishing the different percentages of symptoms. The scale employed for the disease visual assessment uses classes based on a range of the percentage of symptomatic leaf area, but in this work the mean value of each class was considered. The regressions obtained were compared through the coefficient of determination (R^2) which takes into account errors with the data, or outliers, and the regression sum of squares. In fact, R^2 is an adimentional coefficient and it is completely unrelated to the size of the sample. For this reason, it can be considered a standard indicator for the comparison among different models. Results are summarized in figure 4. The regressions are highly significant (P<***) for both NIR wavebands, as confirmed by the determination coefficient (R^2 = 0.955 and R^2 = 0.946) and root mean standard error (RMSE = 0.026 and RMSE = 0.058). The significance of the result is confirmed also by the verification of the intervals confidence of the regression angular coefficient, β ($NDVI_{SG}$: β = -0.034; t = -11.324; SE = 0.0003; $NDVI_{SC}$: β = -0.0075; t = -11.073; SE = 0.00068). In particular, it can be observed that $NDVI_{SC}$ calculated using the reflectance at 880 nm (fig. 4, graph B) covers a wider range ($NDVI_{SC}$ between 0.20 and 0.84) than $NDVI_{SG}$ calculated at 770 nm (fig. 4, graph A; $NDVI_{SG}$ between 0.65 and 0.95). This is particularly evident for disease classes with high percentages of symptomatic leaf surface. Moreover, regression highlight a certain variability of the vegetation index beginning with class 4, which indicates the diffusion of grapevine downy mildew symptoms on leaf surface within a range of 10–20%.

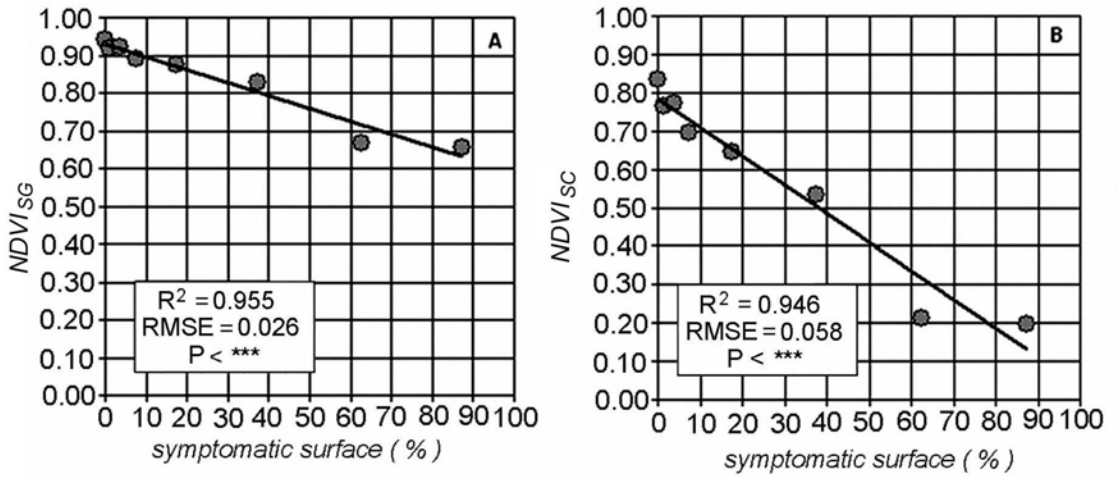

Figure 4. Linear regression between percentage of symptomatic leaf area and NDVI values obtained from data collected by the spectrophotometer, using Red and NIR_G (graph A) and Red and NIR_C (graph B) wavebands.

Concerning the two commercial optical tools, in order to evaluate GreenSeeker and Crop Circle reliability, the eventual relation between the mean percentage of symptomatic leaf area (of each infection class) and NDVI values from the two tools was investigated. To this aim, a linear regression analysis was conducted.

Figure 5 shows the graph with the regression line obtained considering the average of the three measures for GreenSeeker and Crop Circle, respectively. Graphs presented in figure 4 represent the average of the three NDVI measures. Regression is more significant for data collected by GreenSeeker (fig. 5, graph A) than for data collected by Crop Circle (fig. 5, graph B), as confirmed by R^2 (R^2 = 0.708 and R^2 = 0.599 for GreenSeeker and Crop Circle, respectively) and RMSE (RMSE = 0.028 and RMSE = 0.058 for GreenSeeker and Crop Circle, respectively). The regression angular coefficient is correctly estimated for both the two instruments, but it is possible to observe that the SE is higher in the case of data collected by the Crop Circle, probably because of data are more spread out ($NDVI_G$: β = -0.0014; t = -5.827; SE = 0.00023; $NDVI_C$: β =0.0022; t = -4.575; SE = 0.00048). The Crop Circle returned lower NDVI values than GreenSeeker, even though the devices investigated the same leaves. This is probably due to the different surface area of investigation for the two devices. In fact, the Crop Circle field of view is larger than that of GreenSeeker, a fact that ensures greater uniformity of sampling. During the acquisition, some spotlights of the Crop Circle did not light only leaf tissue, but also part of the black panel; as a consequence, the correspondent NDVI values were more influenced by the reflectance of the background (fig. 2 B). In the present case study, the analysis seems to highlight a greater aptitude of GreenSeeker for identification of symptoms of grapevine downy mildew. In addition, experimental data collected by GreenSeeker show a significantly decreasing trend of NDVI in correspondence to symptomatic leaf area within a range of 10–20% (class 4).

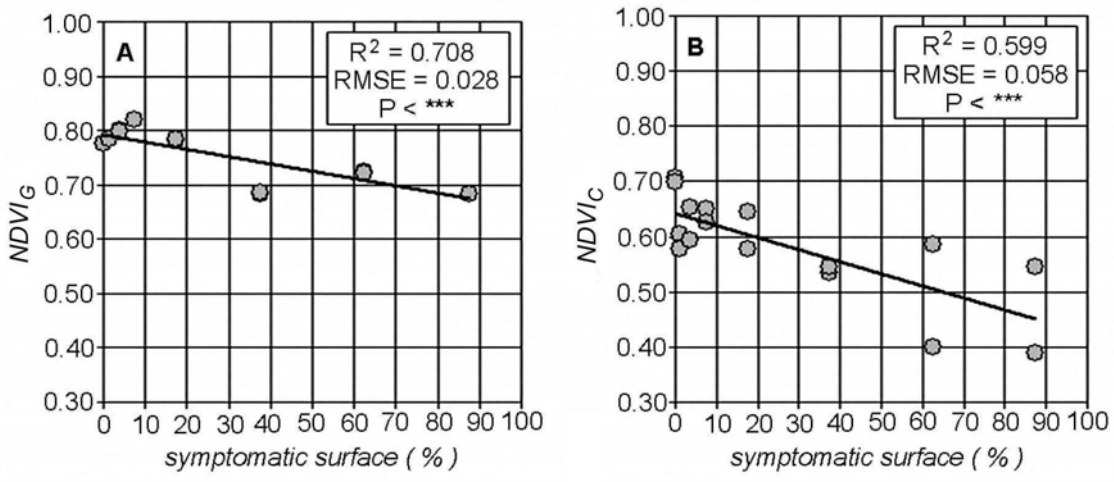

Figure 5. Linear regression between NDVI and percentage of symptomatic leaf area for GreenSeeker (graph A) and Crop Circle (graph B). Number of data points is the same in both the two graphs. The apparent difference is due to the overlapping of multiple points measured by the GreenSeeker (graph A) caused by the high repeatability of the measurements.

The comparison between results obtained by the spectrophotometer and the two commercial devices confirms the aptitude of GreenSeeker for diagnosis application. In fact, NDVI calculated by the spectrophotometer - considering the same wavelengths investigated by the GreenSeeker - decreases beginning with class 4. Nevertheless, the regression corresponding to NDVI values derived from reflectance data collected through the spectrophotometer (R^2 = 0.955) is more significant than the regression for GreenSeeker (R^2 = 0.708). The difference is due to the higher quality of spectrophotometer measurements. In fact, this instrument is able to do a hyperspectral

analysis of leaf tissue, thus ensuring a superior accuracy and precision of reflectance data and, consequently, of NDVI values. As for Crop Circle, it gave back NDVI values that different significantly from results obtained using reflectance data coming from the spectrophotometer. These results seem to suggest the greater capability of GreenSeeker than Crop Circle in diagnosis application in the open field.

4. CONCLUSIONS

The present work considers the possible use of two commercial optical devices - GreenSeeker and Crop Circle - and of a portable Vis/NIR spectrophotometer used as reference in detecting different levels of symptoms of grapevine downy mildew.

Regression analysis carried out on NDVI values corresponding to leaves with increasing percentages of symptomatic areas showed a decreasing relationship between the two variables for all the optical tools tested in the present work. This fact confirms the effect of physiological alterations of leaf tissue on NDVI variability values collected by the devices.

In particular, the regression corresponding to the spectrophotometer is more significant than the regression corresponding to either GreenSeeker or Crop Circle. This is due to the optimal characteristics and to the high spectral resolution of the spectrophotometer. As for the two pulsed-light devices, regression is more significant for GreenSeeker than for Crop Circle. Moreover, experimental data show a significant NDVI decrease in correspondence with percentage of symptomatic area within a range of 10–20%, both for GreenSeeker and the spectrophotometer. This seems to indicate a greater capability of the GreenSeeker in detecting different disease levels.

Therefore, results seem to confirm the possibility of using GreenSeeker for evaluation of the temporal evolution of crop vegetative growth and diagnosis application, in particular when used in conjunction with devices for the identification of vegetation presence along rows. In this way, timely operations can be carried out according to a site-specific management approach, in order to reduce stress incidence, which negatively affects crop production.

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