

## Evaluation of “ground sensing” optical sensors for diagnosis of *Plasmopara viticola* on vines

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### Abstract

The present work considers the possible use of two commercial optical devices, the GreenSeeker RT100 and the Crop Circle, 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 eight homogeneous infection classes and then analyzed (on the leaves' adaxial surfaces) through the optical devices and a portable visible/near infrared (Vis/NIR) spectrophotometer used as tester. Data showed a linear relation between the percentage of symptomatic leaf area and the Normalized Difference Vegetation Index (NDVI) calculated through the 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 one 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. Finally, the NDVI measurements carried out through the two commercial sensors, showed lower values on abaxial surfaces than on adaxial surfaces, and a reduced range of values. Moreover, the identification of different infection classes was more difficult on the abaxial surface. This is due to both the different structure of the leaf tissue and the different symptoms of *P. viticola* on the abaxial and adaxial surfaces. The present work will allow, in the future, applying these optical devices to diagnosis directly in vineyards.

**Additional key words:** Crop Circle; diagnosis of phytopathogenic fungi; GreenSeeker; NDVI; plant health status; proximal sensing.

### Resumen

#### Evaluación de sensores ópticos para el diagnóstico de *Plasmopara viticola* en vides

El presente estudio describe la posibilidad de utilizar dos sensores ópticos comerciales, el GreenSeeker RT100 y el Crop Circle, para identificar diferentes niveles de síntomas de mildiu en vid. La experimentación ha sido realizada en hojas de vid cv. Cabernet Franc infectadas por *Plasmopara viticola*. Las hojas se dividieron en ocho clases homogéneas por nivel de infección y analizadas (en las caras superiores) con instrumentos ópticos y con un espectrofotómetro Vis/NIR (visible/infrarrojo), utilizado como testigo. Los resultados muestran la presencia de una regresión lineal entre el porcentaje de superficie foliar con síntomas y el índice NDVI calculado por medio de los sensores ópticos ( $R^2 = 0,708$  para GreenSeeker;  $R^2 = 0,599$  para Crop Circle;  $R^2 = 0,950$  para el espectrofotómetro). La regresión obtenida con el GreenSeeker es más significativa que la obtenida con el Crop Circle. Esto sugiere una mayor capacidad del GreenSeeker para detectar diferentes niveles de infección. Por último, las mediciones realizadas por medio de los dos sensores comerciales en el envés de las hojas mostraron valores de NDVI menores que los obtenidos en la cara superior, así como un menor rango de valores. Por lo tanto, la identificación de las diferentes clases de infección fue más difícil de realizar en el envés de las hojas. El presente estudio permitirá, en el futuro, aplicar estos sensores ópticos al diagnóstico directamente en los viñedos.

**Palabras clave adicionales:** Crop Circle; diagnóstico de hongos fitopatógenos; estado sanitario de la planta; GreenSeeker; NDVI; sensores locales.

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Abbreviations used: CCD (charge-coupled device); DAC (digital analogue converter); FWHM (full-width half-magnitude); GLD (grapevine leaf roll disease); LED (light emitting diode); NDVI (normalized difference vegetation index); NIR (near infrared); PA (Precision Agriculture); PV (Precision Viticulture); RMSEC (root mean square error of calibration); SE (standard error); Vis (visible).

## Introduction

Crop monitoring is one of the most studied applications in Precision Viticulture (PV) systems. It considers data and information from observations carried out directly on the crop, such as phenological, nutritional, and phytosanitary status. Crop monitoring is done to maximise the production in quantity and quality (Lamb & Bramley, 2001; Arnó *et al.*, 2009). 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. In fact, health monitoring in plants and trees is critical for sustainable agriculture. 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 vines.

Currently, scouting is the most widely used mechanism for monitoring stress in tree crops, though it is an expensive, labour-intensive, and time consuming process. Recent developments in agricultural technology have led to a growing demand for a new era of automated non-destructive methods of plant disease detection. Spectroscopic and imaging techniques are unique disease monitoring methods that have been used to detect diseases and stress caused by different factors in plants and trees.

Various studies have been conducted regarding the identification of biotic and abiotic plant stresses such as herbicide application (Smith *et al.*, 2005), fungal infection (Zhang *et al.*, 2005), water stress (Kriston-Vizi *et al.*, 2008), and nutrient deficiency (Zhao *et al.*, 2005). Studies were conducted with various crops to distinguish diseased leaves from healthy leaves (Blanchfield *et al.*, 2006; Moshou *et al.*, 2006; Huang *et al.*, 2007; Lins *et al.*, 2009). 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 of plants can influence spectral characteristics of leaf tissue that can be detected in the visible and/or the NIR (Near Infrared) regions of the electromagnetic spectrum. In fact, the visible and infrared regions are known to provide the maximum information on the physiological stress levels in plants (Xu *et al.*, 2007). Thus, the

different spectral reflectance between healthy and infected leaves can be used to identify a plant's health status. Such changes in reflectance characteristics have been used to diagnose disease symptoms in plants. Delalieux *et al.* (2007) investigated the capability of hyperspectral analysis for early white apple scab (*Venturia inaequalis*) detection and to develop an approach to explore this potential. 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. 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 (GLD).

In addition to statistical models for classification, many spectroscopy-based studies use different vegetative indices for evaluating the change in spectral reflectance for different plant conditions (diseased or healthy) (Bravo *et al.*, 2004). As it known, one of the most diffused indices is the NDVI (Normalized Difference Vegetation Index; Lamb *et al.*, 2001). This index is closely related to vegetal vigour (biomass and leaf area) and to photosynthetic activity.

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 *Plasmopara viticola*: the GreenSeeker RT100 (NTech Ind.; Ukiah, CA, USA) and the Crop Circle (Holland Scientific Inc.; Lincoln, NE, USA). Both proximal sensors are normally used to monitor cover crops to manage nitrogen fertilization according to the logic of variable rate application (Govaerts *et al.*, 2007; Sripada *et al.*, 2008). These devices were proposed to monitor crop vigour 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) suggested the application of the GreenSeeker to monitor vigour variations of *Vitis vinifera* plants in vineyards. Previous experiences were carried out by the authors in field using a mobile lab equipped with GreenSeeker sensor, in order to evaluate its efficiency in detecting the dynamic diffusion of disease incidence directly in vineyard (Mazzetto *et al.*, 2010, 2011). The analysis of the obtained NDVI geo-referred maps demonstrates correspondence between GreenSeeker observations and the real vine phytosanitary status, even if at a qualitative level. In the present

work, in particular, the goal of the experimentation was to evaluate the possibility to identify plant disease (*P. viticola*, even if at leaf level) using the GreenSeeker and the Crop Circle. To this aim NDVI collected by these two devices are 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, in future, the possible use of proximal sensors in diagnosis application directly in vineyards

## Material and methods

### The *Plasmopara viticola* oomycete

The oomycete *P. viticola* 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°C to 25°C; extremes 10°C to 29°C) with abundant rains in late spring, as are most of the European vineyards close to 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 it causes significant economic loss if no chemicals are applied. *P. viticola* infects all green parts presenting stomata of the host plant. On the adaxial surface of the leaves, translucent pale yellow spots appear, the so called “oil spots”. Sporulation only occurs on the abaxial leaf surface, where the sporangia appear on sporangiophores as a white mould. On old leaves, the sporulation primarily occurs on the margins of the spot. During time, oil spots become dry and necrotic, at first in the centre and then throughout the entire spot, causing the reduction of the photosynthetic area of the leaf. In mostly severely affected vineyards, grapevine downy mildew can result in a premature defoliation of vines. 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.

### The commercial optical sensors

The GreenSeeker RT100 (NTech Ind., Inc., Ukiah, CA, USA) and the Crop Circle (Holland Scientific Inc., Lincoln, NE, USA) are two commercial optical de-

vices normally used to monitor cover crops in order to manage nitrogen fertilization according to the logic of variable rate application (Govaerts *et al.*, 2007; Sri-pada *et al.*, 2008). Both the GreenSeeker and the Crop Circle use patented techniques to measure vegetation spectral reflectance and to calculate NDVI in real time and they are designed to be mounted on a mobile vehicle and to realize dynamic surveys. The two tools feature self-contained illumination in both the red and NIR bands. The devices measure the fraction of the emitted light in the sensed area that is returned to the sensor (reflectance). These fractions are used within the sensor to compute NDVI according to the following formula (Rouse *et al.*, 1974):

$$\text{NDVI} = \frac{\text{NIR} - \text{red}}{\text{NIR} + \text{red}} \quad [1]$$

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). In particular, GreenSeeker senses a  $0.6 \times 0.01$  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 ( $650 \pm 10$  nm full-width half-magnitude FWHM) and NIR ( $770 \pm 15$  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.

The 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  $32^\circ \times 6^\circ$ . 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 from 1 sample  $\text{s}^{-1}$  to 20 samples  $\text{s}^{-1}$ . Both tools do not need any calibration before their use because they each contain an autocalibration system.

### The experimental plan

The evaluation of the two commercial sensors 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 *Plasmopara viticola*. Leaves were picked from 12 vine plants grown in pots and cultivate in greenhouse. Vine plants had the same age (3 years) and the same rootstock (Kober 5BB). The sampling was carried out in a three day period (26-28 of July 2010) and all tests were conducted in laboratory; this in order to investigate, at the same time, fresh leaves at different disease level. Leaves were divided into eight homogeneous groups based on a disease scale that is normally used for disease visual assessment in vineyards according to the method described by Townsend & Heuberger (1943). This method involves counting the number of infected leaves and classifying them according to the extension of the leaf surface symptoms. The disease scale has eight classes ranging from 0 (healthy) to 7 (75 to 100% of symptomatic surface). When used for in-vineyard monitoring, the results coming from the classification are normally used to calculate the percentage infection index (I%I) of *Plasmopara viticola* according to the Eq. [2]:

$$I\%I = \frac{\sum (n \cdot v)}{(n^{\circ} \text{ of classes} - 1) \cdot N} \quad [2]$$

where n = frequency of leaves in each class, v = identifying number of each class, and N = sample size.

In the present work, we carried out, in collaboration with an expert in plant pathology, the visual classification of the leaves on each inoculated plant and picked up 9 leaves of each class. Visual inspection, carried out directly in vineyard, is the method usually employed for disease assessment. Leaves of about the same age were used for the analysis in order to reduce the variation of the measurements caused by different factors that influence the spectral response (age, thickness, chlorophyll concentration, etc.). Since the tests were destructive, it was not possible to include more leaves in each group because of the difficulty of selecting more leaves characterized by the same disease level and vegetative stage. Leaves belonging to the same class (9 leaves class<sup>-1</sup>) were then fixed on a black plastic panel to form a single layer and were investigated through the optical devices. For each class, leaves were arranged to allow measurement of the same portion of leaf surface with the two sensors (GreenSeeker and Crop Circle) and, at the same time, to reduce the portion of black panel that can be intercepted by the devices. The black panel was used as 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, have low reflectance. In this way, optical devices captured radiation mostly derived from the vegetation.

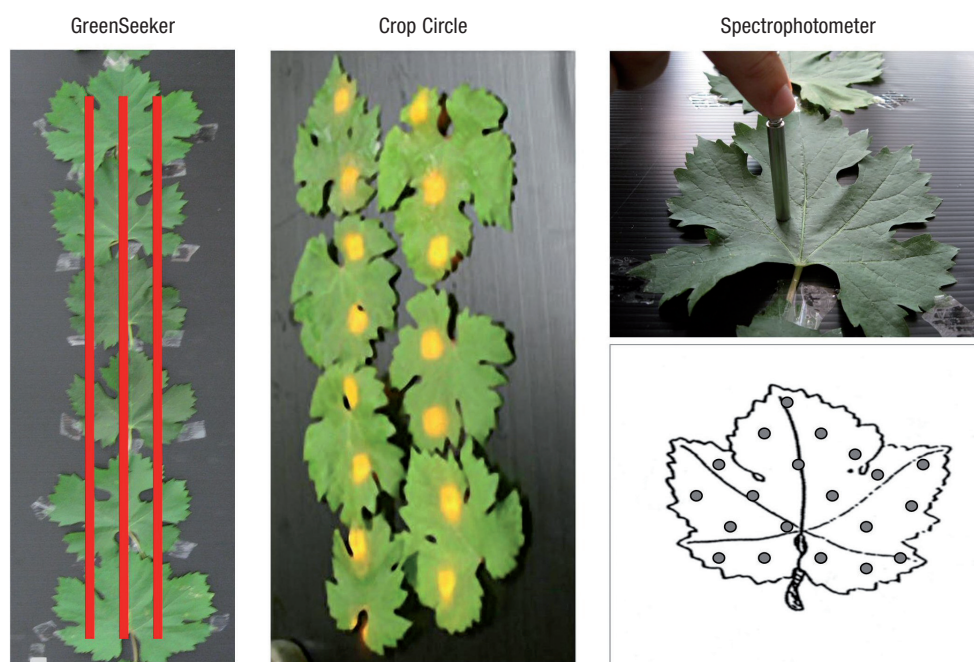
Regarding the commercial devices, a total of six measurements were conducted in each group of leaves: 3 with the GreenSeeker and 3 with the Crop Circle (in total 24 measurements for each sensor). Static readings of reflectance were carried out by the two devices. Each sensor was made to stop over each group of leaves for an acquisition time of 5 s. Tools were alternately mounted on a metallic support at an optimal distance of 0.60 m from the leaves. At this distance, the GreenSeeker projects a beam of light of about 0.7 and 0.01 m in length and width, respectively, whereas Crop Circle projects 15 light spots covering a total surface of about 0.4 and 0.15 m in length and width, respectively. In order to investigate one leaf surface as similar as possible for both sensors (about 25-30% of the total leaf surface), for GreenSeeker we considered the NDVI average value related to three measurements on different contiguous leaf portions for each class.

Afterwards, leaves were analyzed using a portable spectrophotometer that can take a punctual measurement where the fibre optics contact the sample. The spectrophotometer allows realizing measurements in the Vis/NIR range (400-1,000 nm). From this range the same Red and NIR wavelengths investigated by GreenSeeker and Crop Circle were extracted. This because the two devices calculate the NDVI index using the same values in Red band (650 ± 10 nm) but different values in NIR band (770 ± 15 nm for GreenSeeker, 880 ± 10 nm for Crop Circle); so it is necessary to investigate which NDVI is more sensible in plant disease detection. As consequence, if for the comparison between the two sensors is necessary to investigate the same portion of leaf area, for the spectrophotometer is enough to carry out a regular sampling on every single leaf belonging to each infection class. Fig. 1 shows the GreenSeeker and Crop Circle fields of view on the leaves and the sampling method used for the spectrophotometer measurements.

The spectrophotometer consists of the following elements:

- Lighting system: the light source is a 50-W halogen spotlight with a colour temperature of 4,500°K and maximum emission at 500 nm. The light source is embedded in a metal holder, enabling the lamp to face the optical fibre steadily.

- Fibre optic probe: light radiation is carried over leaves through a fibre optic probe (“step index”, mod.



**Figure 1.** The fields of view of the two tested sensors and example of sampling carried out with the spectrophotometer (20 point investigated for each leaf). The red lines represent lights emitted by GreenSeeker during three successive measurements (the average of them corresponds to the NDVI value for each class) while the field of view of Crop Circle consists of 15 yellow spotlights.

FCR-19IR200-2-ME-S1 by Avantes©). Spectral acquisitions are performed using a diffusive reflectance technique. The probe consists of 19 fibres 200  $\mu\text{m}$  in diameter: 17 fibres carry light over the samples and two fibres carry back radiation from the leaves to the spectrophotometer. The probe is characterized by a total field of view of about 0.8  $\text{mm}^2$ .

— Portable spectrophotometer: a fibre 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 2,048-pixel matrix to record each wavelength's signal intensity with a resolution of 0.3 nm.

— 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.

For each leaf belonging to each class, twenty points were investigated by the spectrophotometer in each group of leaves at different infection levels (180 point for each class in total). This was done 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 com-

ponent was measured by the spectrophotometer and registered by the acquisition software. During the acquisition, one spectrum was recorded without lighting the lamp. The registered signal, obtained only with environmental light, was used as a baseline and was subtracted from the leaves' spectra. Data collected by the spectrophotometer were used to check NDVI suitability in recognizing the symptomatic level of leaf tissue. In fact, 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, it has been verified that NDVI is more sensitive to infection classes and, consequently, which of the two commercial devices is more suitable for symptoms identification. The wavelengths used to calculate the different NDVI are summarized in Table 1. The two optical sensors were tested on the same leaves used for the spectrophotometer analysis in order to verify their real capability in detecting different disease levels. However, it should be underlined that the spectrophotometer measures the reflectance in precise points of the leaf surface, whereas NDVI values coming from GreenSeeker and Crop Circle referred to a larger portion of the vegetation.

**Table 1.** Specific wavelength used for calculating NDVI using the three devices

Device	Vegetative index	Wavelengths
GreenSeeker	NDVI <sub>G</sub>	Red: 650±10 nm NIR <sub>G</sub> : 770±15 nm
Crop Circle	NDVI <sub>C</sub>	Red: 650±10 nm NIR <sub>C</sub> : 880±15 nm
Spectrophotometer	NDVI <sub>SG</sub>	Red: 650±10 nm NIR <sub>G</sub> : 770±15 nm
	NDVI <sub>SC</sub>	Red: 650±10 nm NIR <sub>C</sub> : 880±15 nm

NDVI<sub>G,C</sub>: NDVI calculated through GreenSeeker and Crop Circle sensors respectively. NDVI<sub>SG,SC</sub>: NDVI calculated through the spectrophotometer using GreenSeeker and Crop Circle wavelengths, respectively.

Then, to verify the possibility to use the NDVI index measured by the two optical sensors to identify different level of *P. viticola* on vine leaves, we realized a prediction model, even if limited to the considered vine cultivar (Cabernet Franc) that links NDVI with the symptomatic surface of the leaves (%). The model was validated through the cross validation leave-one-out technique (the choice of this method is due to the reduced sample size).

Finally, GreenSeeker and Crop Circle were used to measure NDVI on the abaxial surface of the investigated leaves. In this way, it is possible to evaluate the difference NDVI values measured on the adaxial and abaxial leaf surfaces and the eventual discrimination of the infection classes linked to the presence of white mould.

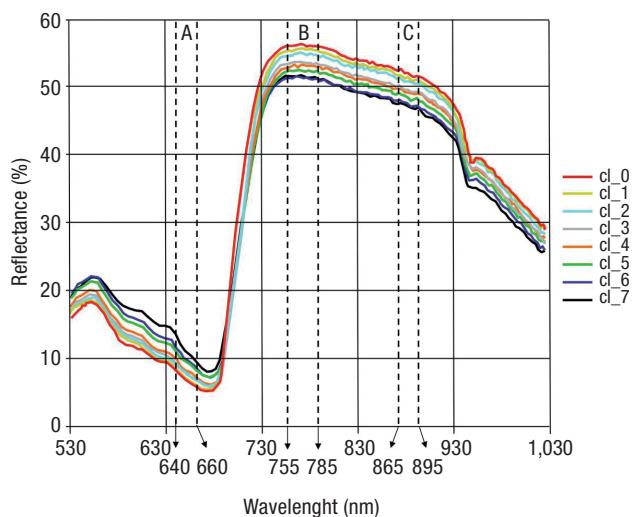
All measurements on the leaves were conducted within few minutes from their drawing from vine plants, in order to minimize the dehydration of the leaves. Data analysis was carried out through the software SPSS Statistics ver. 17.0 and Unscrambler software package (Version 9.6, CAMO ASA; Norway), which is normally used in chemiometric analysis. In this way, the spectra of each infection class can be displayed on a graph.

## Results and discussion

Fig. 2 shows reflectance spectra obtained for each infection class. Each line (plotted with a different colour) corresponds to each infection class, and it is obtained as the average of 20 spectra collected for each class. Dotted lines highlight portions of spectra cor-

responding to wavelength used to compute NDVI in the red (A) and NIR (B = NIR<sub>G</sub>, C = NIR<sub>C</sub>) wavebands. These wavebands are the same as those used by GreenSeeker (A and B) and by Crop Circle (A and C) to compute NDVI indices.

From the graph presented in Fig. 2, it can be observed that all spectra showed the typical trend demonstrated by leaf tissue. In fact, it is characterized by a greater reflectance in the NIR band than in the red band; moreover, it shows a peak of reflectance in green (about 550 nm) and NIR (about 740 nm) and minimum reflectance in the 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 18.3% for class 0 to 22.5% 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: 6.4%; class 7: 10.7%). As expected, in the NIR band, the reflectance decreases from class 0 (NIR<sub>G</sub>: 55.9%; NIR<sub>C</sub>: 51.7%) to class 7 (NIR<sub>G</sub>: 50.7%; NIR<sub>C</sub>: 46.9%). This fact confirms the general loss of functionality of leaf tissue because of reduced photosynthetic activity and vegetation senescence caused by grapevine downy mildew. This first result is particularly interesting because it confirms



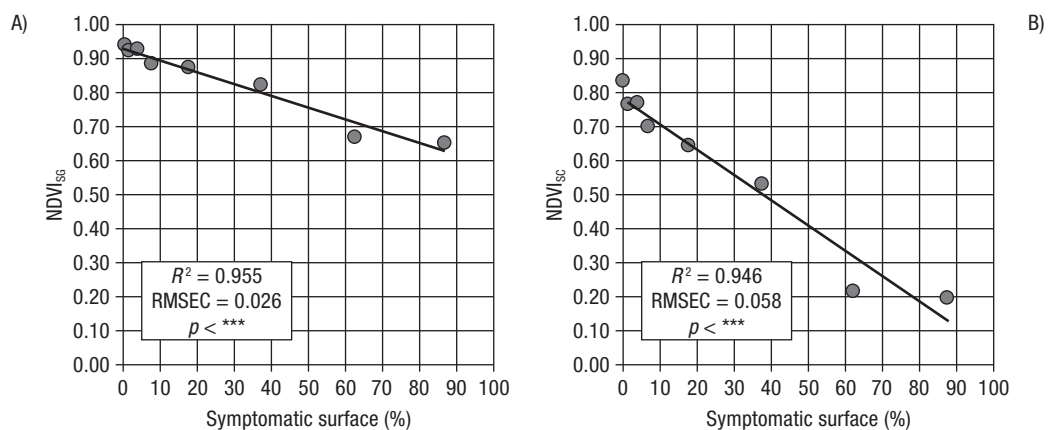
**Figure 2.** Reflectance spectra obtained from the analysis of homogeneous groups of leaves based on the percentage of symptomatic leaf area through the spectrophotometer. Dotted lines highlight wavelengths used by GreenSeeker (A and B, respectively 650±10 nm and 770±15 nm) and by Crop Circle (A and C, respectively) to compute NDVI.

multispectral analysis potential in diagnosis and detecting different levels of grapevine downy mildew symptoms. Moreover, reflectance spectra in the red and NIR bands are arranged in order, according to infection classes. In particular, in NIR wavebands, a partial overlap of spectra referred to class 6 and 7 can be observed.

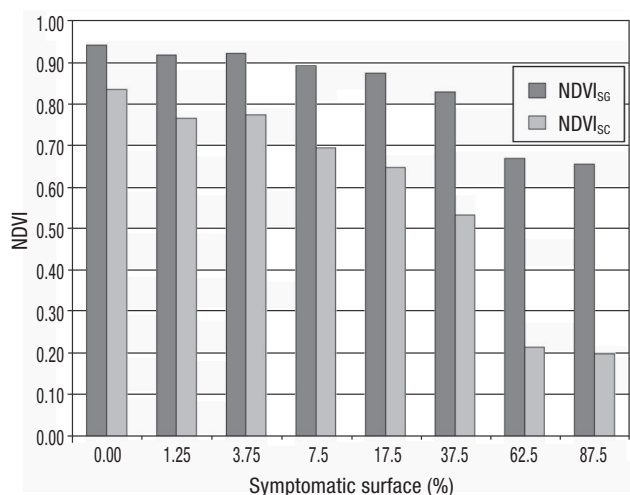
Afterwards, NDVI was calculated using reflectance data acquired by the spectrophotometer in the selected wavebands. For each sampling point, reflectance values were averaged in order to obtain a single reflectance value, respectively in the red and NIR bands. Then, the mean value obtained in NIR and red was combined according to Eq. [1], obtaining NDVI values corresponding to each investigated point. Then, NDVI values corresponding to sampling points belonging to the same infection class were averaged again. In this way, a single NDVI value was obtained for each class of infection. These steps allow calculation of NDVI according to a procedure similar to the one 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. A linear regression analysis was conducted 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 different percentages of symptoms. The scale employed for the disease visual assessment was based on classes including a range of the percentage of symptomatic leaf area, but in this work the mean value of each class was used. The regressions obtained were compared through the determination coefficient

( $R^2$ ), which considers errors in the collection of data, or outliers, and the regression sum of squares. In fact,  $R^2$  is a dimensionless 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 Fig. 3. 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 the root mean square error of calibration expressed as NDVI unit (RMSEC = 0.026 and RMSEC = 0.058). The significance of the result is also confirmed by the verification of the intervals of confidence of the regression angular coefficient,  $\beta$  (NDVI<sub>SG</sub>:  $\beta = -0.034$ ;  $t = -11.324$ ; SE = 0.0003; NDVI<sub>SC</sub>:  $\beta = -0.0075$ ;  $t = -11.073$ ; SE = 0.00068). In particular, it can be observed that NDVI<sub>SC</sub> calculated using the reflectance at 880 nm (Fig. 3B) covers a wider range (NDVI<sub>SC</sub> between 0.20 and 0.84) than NDVI<sub>SG</sub> calculated at 770 nm (Fig. 3A; NDVI<sub>SG</sub> between 0.65 and 0.95). This is particularly evident for disease classes with high percentages of symptomatic leaf surface. Moreover, regression highlights a certain variability of the vegetation index starting from class 4, which indicates the diffusion of grapevine downy mildew symptoms on leaf surface within a range of 10-20%. Fig. 4 shows the histograms of NDVI values calculated starting from wavelengths used by GreenSeeker (NDVI<sub>SG</sub>) and Crop Circle (NDVI<sub>SC</sub>).

Regarding the two commercial optical tools, in order to evaluate GreenSeeker's and Crop Circle's reliability, the eventual relation between the mean percentage



**Figure 3.** Linear regression between percentage of symptomatic leaf area and NDVI values obtained from data collected by the spectrophotometer, using Red and NIR<sub>G</sub> (A) and Red and NIR<sub>C</sub> (B) wavebands.



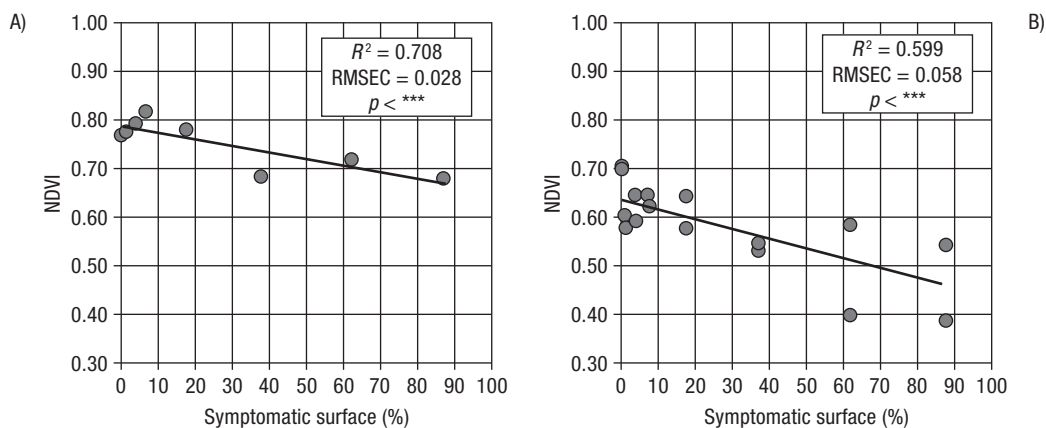
**Figure 4.** Histograms of NDVI values calculated starting from wavelengths used by GreenSeeker ( $NDVI_{SG}$ ) and Crop Circle ( $NDVI_{SC}$ ).

of symptomatic leaf area (of each infection class) and NDVI values was investigated. To this aim, a linear regression analysis was conducted.

Fig. 5 shows the graph with the regression line obtained considering the average of the three performed measurements with GreenSeeker and Crop Circle, respectively. The number of data points is the same in both the two graphs (three points for each class). The apparent difference is caused to the overlapping of multiple points measured, especially by the GreenSeeker (graph A) due to the high repeatability of the measurements. The regression is more significant for data collected by GreenSeeker (Fig. 5A) than for data

collected by Crop Circle (Fig. 5B), as confirmed by  $R^2$  (0.708 and 0.599 for GreenSeeker and Crop Circle, respectively) and RMSEC (0.028 and 0.058 for GreenSeeker and Crop Circle, respectively). The regression angular coefficient is correctly estimated for both instruments, but it is possible to observe that the SE is higher in the case of data measured by the Crop Circle, probably because data are more spread out ( $NDVI_G$ :  $\beta = -0.0014$ ;  $t = -5.827$ ;  $SE = 0.00023$ ;  $NDVI_C$ :  $\beta = 0.0022$ ;  $t = -4.575$ ;  $SE = 0.00048$ ). In the investigation of the same leaves, the Crop Circle detected lower NDVI values than GreenSeeker did. This is probably due to the different technology that sensors use for calculating NDVI index. In fact, Crop Circle has a larger field of view than GreenSeeker, so it ensures a more uniform sampling. On the contrary, GreenSeeker project a thin beam of light on the leaf surface: this makes GreenSeeker more suitable to identify different health status of the leaves. During the acquisition, some of the Crop Circle spotlights lit only leaf tissue and part of the black panel; as a consequence, the correspondent NDVI values were influenced by the reflectance of the little portion of background. In the present case study, the analysis seems to highlight a greater aptitude of GreenSeeker in the identification of grapevine downy mildew symptoms. Moreover, 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).

The comparison between results obtained by the spectrophotometer and the two commercial devices confirms the aptitude of GreenSeeker for diagnosis



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



application. In fact, considering the same wavelengths investigated by the GreenSeeker, NDVI calculated with the data collected by spectrophotometer decreases starting from class 4. The regression referred to NDVI values derived from reflectance data collected through the spectrophotometer ( $R^2 = 0.955$ ) is more significant than the regression referred to GreenSeeker ( $R^2 = 0.708$ ). This difference is due to the higher quality of spectrophotometer measurements. In fact, this instrument performs a hyperspectral analysis of leaf tissue, ensuring a superior accuracy and precision of reflectance data and of NDVI values. As for Crop Circle, NDVI values are significantly different from results obtained using reflectance data coming from the spectrophotometer. Results seem to suggest that GreenSeeker has greater capability than Crop Circle in open-field diagnosis applications.

Table 2 shows the results of models developed to predict the percentage of symptomatic surface using different type of NDVI (Table 1). An univariate data analysis on experimental data sets, with cross validation leave-one-out, has been carried out.  $R^2$  values of calibration presented in Figs. 3 and 5 are the same, because the same data have been used in both analyses. Good results were obtained when the spectrophotometer is used; in particular in calibration, where the RMSEC value is of 6.434% ( $NDVI_{SG}$ ) and 6.584% ( $NDVI_{SC}$ ). Whereas in prediction, RMSECV increased (10.812%, for  $NDVI_{SG}$ ; 11.192 for  $NDVI_{SC}$ ). The regression models obtained using NDVI collected by the GreenSeeker and the Crop Circle are worse, with high RMSEC (16.298% for  $NDVI_G$ ; 19.298 for  $NDVI_C$ ). In prediction, correlation fell slightly with  $R^2 = 0.587$  (for  $NDVI_G$ ) and  $R^2 = 0.513$  and RMSECV increased, reaching 19.587% (for  $NDVI_G$ ) and 21.264% (for  $NDVI_C$ ).

Finally, the results of measurements carried out with GreenSeeker and Crop Circle sensors on the abaxial surfaces of the leaves are shown in Figs. 6 and 7. Measurements

were conducted according to the same experimental plan used to investigate the adaxial surface. In both the two cases, we considered the average value of NDVI related to each class of infection correlated to the symptomatic surface (%). It is possible to observe that both sensors measured lower NDVI values on the abaxial surface than on the adaxial surface.

Student's two-tailed t-test for the mean of two coupled population showed significant difference between NDVI values measured on the two leaf surfaces (GreenSeeker:  $\text{stat } t = -8.609$ ,  $t \text{ crit} = 2.364$ ; Crop Circle:  $t = -4.206$ ,  $\text{critical } t = 2.364$ ,  $\text{significance } \alpha = 0.05$ ). This is due to the different physiology of the leaf tissue. In fact, under the upper epidermis there is the palisade parenchyma (or chlorophyllose parenchyma), whose cells contain most of the leaf's chloroplasts. The spongy parenchyma (under the lower epidermis) is characterized by large intercellular air spaces. Stomata are connected to the intracellular air spaces and they allow the air exchange between the leaf and the environment.

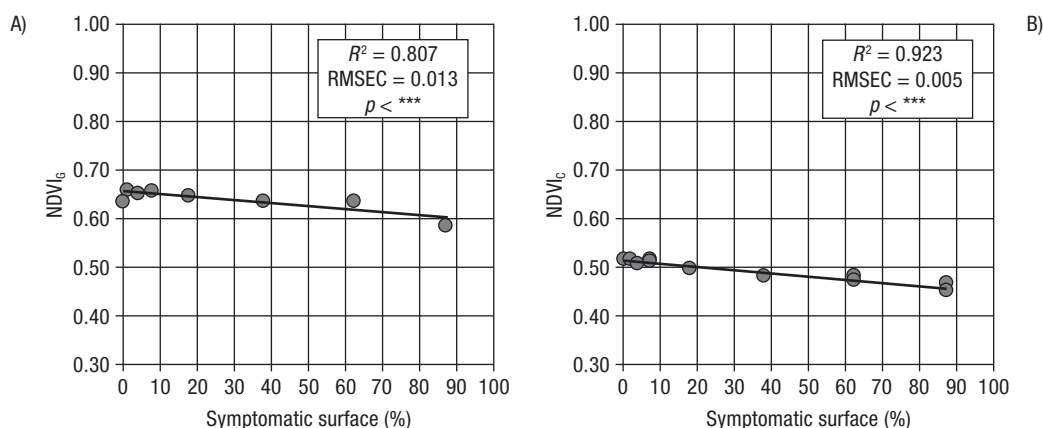
Since the NDVI index is correlated with the chlorophyll content into the leaf tissue, higher NDVI values occur on the adaxial surface where chlorophyll is more concentrated.

Regarding the infection classes identification, the linear regression obtained is more significant for Crop Circle than for GreenSeeker ( $R^2 = 0.923$  vs.  $R^2 = 0.807$ ,  $\text{RMSEC} = 0.005$  and  $0.013$  respectively, Fig. 6). This suggests a better performance of Crop Circle when NDVI is measured on the abaxial surface. Nevertheless, the eight infection classes are included in a very small range of NDVI (0.077 GreenSeeker, 0.057 Crop Circle). So the identification of healthy and unhealthy leaf tissue is very difficult. The two sensors appear less suitable in the detection of the disease when used on the abaxial surface of the leaves. Therefore, it is important to consider the influence of leaf exposure on

**Table 2.** Models to predict the percentage of symptomatic leaf surface of Cabernet franc. Models have been computed using NDVI collected by the different tools described in the text

Vegetative index	Estimated parameter	Number of samples	Calibration		Cross-validation	
			$R^2$	RMSEC	$R^2$	RMSECV
$NDVI_{SG}$	Symptomatic leaf surface (%)	8	0.955	6.434	0.874	10.812
$NDVI_{SC}$	Symptomatic leaf surface (%)	8	0.953	6.584	0.865	11.192
$NDVI_G$	Symptomatic leaf surface (%)	24	0.708	16.298	0.587	19.587
$NDVI_C$	Symptomatic leaf surface (%)	24	0.599	19.298	0.513	21.264

$R^2$ : coefficient of determination; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross validation.



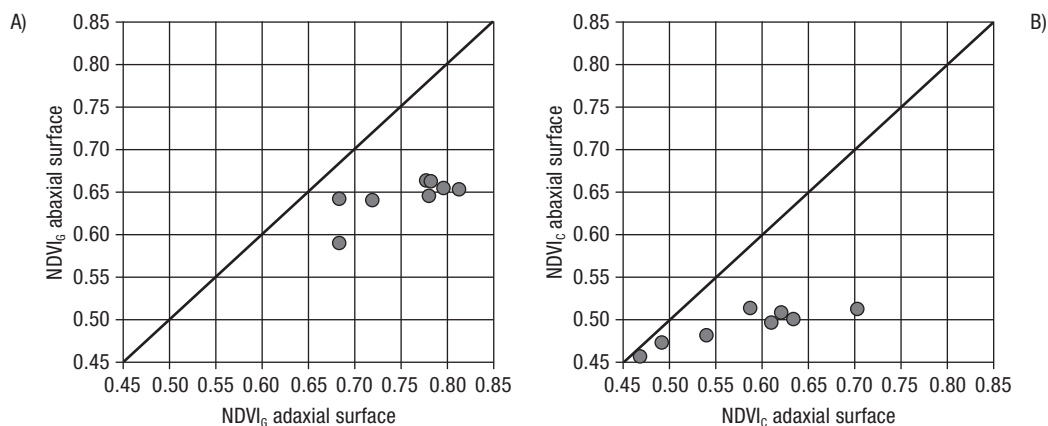
**Figure 6.** Linear regression between NDVI measured on the abaxial leaf surface and the percentage of symptomatic leaf area for GreenSeeker (A) and Crop Circle (B). The apparent difference in the sample set is caused by the overlapping of multiple points measured by sensors due to the high repeatability of the measurements.

NDVI when the sensors are used in vineyard, because it is probable not get reliable results in case of surveys carried out in windy conditions.

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 grapevine downy mildew symptoms.

Regression analysis was carried out on NDVI values referring to leaves with increasing percentages of symptomatic areas. It 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 when the percentage of symptomatic area is within a range of 10-20%, both for GreenSeeker and the spectrophotometer. It seems to indicate a greater capability of the GreenSeeker in detecting different disease levels. This capability seems to be confirmed also by models developed to predict the percentage of symptomatic surface using different type of NDVI. This



**Figure 7.** Comparison between NDVI values measured on the adaxial and abaxial surfaces of the leaves for GreenSeeker (A) and Crop Circle (B). In this analysis the mean values of NDVI for each class of infection were considered.

analysis, conducted on a reduced sample size, demonstrates that considered optical sensors can identify grapevine downy mildew symptoms only at a clear infection phase. In any case, this aspect needs further investigation. Finally, NDVI showed lower values on the abaxial surface of the leaves than on the adaxial surface. NDVI is correlated with infection classes also in this case, but this aspect is important for vineyard monitoring. In fact, vineyard surveys must not be conducted under windy condition because wind can modify the orientation of the leaves. Factors that can negatively affect field observations should be minimized. 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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