

HYPERSPETRAL REMOTE SENSING IN ENVIRONMENTAL MONITORING: STRESS DETECTION IN A PLANT ECOSYSTEM

Kalinka Velichkova¹, Dora Krezhova²

¹University of Mining and Geology “St. Ivan Rilski”, 1700 Sofia; E-mail: k.velichkova@mgu.bg

²Space Research and Technology Institute, Bulgarian academy of sciences, 1113 Sofia; E-mail: dkrezhova@stil.bas.bg

ABSTRACT. Hyperspectral remote sensing offers unique opportunities in the environmental monitoring and sustainable use of natural resources. In this study, a non-invasive remote sensing technique based on hyperspectral reflectance measurements was applied for the detection of a viral infection (sharka) in an open-field plum orchard. Changes in the leaf spectral reflectance are a sensitive indicator for the impact of a variety of adverse environmental factors on the plant ecosystems such as stress, diseases, drought, etc. Spectral data were collected by means of a portable fiber-optics spectrometer in the spectral range of 350-1100 nm. The differences between the leaf reflectance spectra of healthy and infected with Plum Pox Virus (PPV) trees were appreciated by hyperspectral analyses of the first derivative spectra for extraction of the red edge position (REP) and red edge symmetry, and of five narrowband vegetation indices (VIs) - Normalized Difference (NDVI), Modified Red Edge Normalized Difference (MRENDVI), Photochemical Reflectance Index (PRI), Red Green Ratio Index (RGR), and Structure Insensitive Pigment Index (SIP), as indicators of stress and disease symptoms. Statistical analyses (Student's t-test and Fisher's LSD test) were used to assess the significance of differences between spectral data of healthy and infected plum leaves. All VIs gave statistically significant differences except for NDVI. Most sensitive to the changes in the physiological status of plum trees turned out to be MRENDVI.

Key words: hyperspectral remote sensing, spectral reflectance, vegetation indices, stress detection, red edge position.

МОНИТОРИНГ НА ОКОЛНАТА СРЕДА ЧРЕЗ ХИПЕРСПЕКТРАЛНИ ДИСТАНЦИОННИ ИЗСЛЕДВАНИЯ: ОТКРИВАНЕ НА СТРЕС В РАСТИТЕЛНА ЕКОСИСТЕМА

Калинка Величкова¹, Дора Крежова²

¹Минно-геоложки университет „Св. Иван Рилски“, 1700 София

²Институт за космически изследвания и технологии – Българска академия на науките, 1113 София

РЕЗЮМЕ. Хиперспектралните дистанционни изследвания предлагат уникални възможности за мониториране на околната среда и за устойчиво управление на природните ресурси. В това изследване е приложена неинвазивна техника за дистанционни изследвания, основаща се на хиперспектрални измервания на отразена радиация, за да се оцени наличието на вирусна инфекция в сливова овощна градина. Промените в отразената от листата радиация са чувствителен индикатор за въздействието на различни фактори на околната среда като стрес, болести, суша и др. върху растителните екосистеми. Спектралните данни са регистрирани с портативен спектрометър в спектралния диапазон 350-1100 нм. Разликите между спектрите на отражение на листата на здрави и заразени с Plum Pox Virus (PPV) дървета бяха оценени чрез хиперспектрални анализи на спектрите на първите производни за извличане на положението и симетрията на червения ръб и на пет теснолентови вегетационни индекса – индекс на нормираната разлика (NDVI), индекс на модифицирана нормирана разлика на червения ръб (MRENDVI), фотохимичен индекс на отразената радиация (PRI), индекс на отношението червено/зелено (RGR) и нечувствителен към структурата пигментен индекс (SIP). Приложени са статистически анализи (Т-тест на Стюдънт и тест на Фишер за най-малка значима разлика), за да се оцени значимостта на разликите между спектралните данни на здрави и заразени сливови листа. С изключение на NDVI, всички вегетационни индекси дават статистически значими разлики. Най-чувствителен към промените във физиологичното състояние на сливовите дървета се оказва MRENDVI.

Ключови думи: хиперспектрални дистанционни изследвания, отразена радиация, вегетационни индекси, стрес, положение на червения ръб.

Introduction

Environmental sustainability is one of the biggest issues faced by mankind at present. The increasing human population, increment in per capita consumption, as well as the rapid growth of industrialisation, lead not only to overexploitation of natural resources but also to the risk of contamination with toxic chemicals leading to the degradation of the environment becoming greater and greater (Li et al., 2017).

Plant growth and health have a considerable effect on the environment and climate. Vegetation, which covers 70% of the global land area, is an essential indicator of the change of the

land ecological environment. It is also the major object of earth observation with remote sensing techniques. The ecological processes related to plant material energy exchange, e.g. photosynthesis, transpiration, respiration, and primary productivity, are in close connection with the biophysical and biochemical parameters of the vegetation (Zhou et al., 2020).

Forest ecosystems fulfill a whole host of ecosystem functions that are essential for life on our planet. However, an unprecedented level of anthropogenic influences is reducing the resilience and stability of our forests and orchards, as well as their ecosystem functions. That is why environmental monitoring is of great significance for natural resource protection and

management. Today, an increasing amount of forest and orchard health data is available from monitoring programs (McRoberts et al., 2012; Traub et al., 2017), from experimental studies of forest ecosystems (Henttonen et al., 2017), as well as from remote sensing data.

In recent years, sensors and remote sensing (RS) techniques have improved significantly the capability to gather information about natural resources and the environment. Many types of sensors including photographs, airborne multi-spectral scanners, satellite imagery, and ground-based spectrometer measurements collect electromagnetic information. RS technologies are capable of providing detailed spectral (tone, colour, and spectral signature), spatial (size, shape, and orientation), and temporal information on terrestrial ecosystems.

The development of hyperspectral sensors, collecting data in hundreds of narrow spectral bands simultaneously, allows accurately studying terrestrial vegetation on regional and global scales. The RS technique based on hyperspectral measurements of leaf reflectance in the optical portion of the electromagnetic spectrum makes it possible to monitor the amount and condition of photosynthetic activity of green vegetation. Changes in the leaf spectral reflectance are a sensitive indicator for the impact of a variety of adverse environmental factors (natural and human-induced) on the plant ecosystems causing stress, diseases, drought, etc. (Krezhova et al., 2017; Gold et al., 2020). Changes in reflectance properties of plants are a consequence of changes in their biophysical and biochemical properties (Mutanga and Skidmore, 2007).

There has been a significant increase in the scientific literature in recent years focusing on detecting stress in plants using hyperspectral data analysis (Ray et al., 2010; Velichkova and Krezhova, 2017). Plant disease detection is a major activity in the management of crop plants in both agriculture and horticulture. In particular, early detection of stress and diseases is of great benefit to farmers and growers as it enables earlier interventions to help mitigate crop loss and its quality. There are various techniques available to analyse the data to detect biotic and abiotic stress in plants.

Over time and through many scientific studies, RS experts have come to understand how combinations of the measured reflectance at two or more wavelengths, known as Vegetation Indices (VIs), reveal specific characteristics of the vegetation. Significant wavelengths combined together can indicate the chlorophyll (Chl) content, health, or disease status occurring within a specific species. VIs are quite simple and effective algorithms for quantitative and qualitative evaluations of vegetation cover, vigour, growth dynamics, and senescence. The quantity of VIs is increasing every year. Some of them are more general in nature but others are used to reveal the general health of the vegetation.

The aim of this study is to assess the efficiency and sensitivity of two different approaches for hyperspectral data analysis of leaf reflectance data for the detection of a viral infection (Plum pox, also known as sharka) in a plum orchard. Analyses have been conducted on the first derivatives of the reflectance spectra for the extraction of the red edge position (REP) and red edge symmetry (RES), as well as on five narrowband VIs, selected as indicators of stress and disease symptoms. Statistical analyses (Student's t-test and Fisher's LSD test) were used to assess the significance of differences between spectral data of healthy (control) and infected plum leaves.

Materials and Methods

Plant material

Sharka caused by the Plum pox virus (PPV) is one of the most harmful diseases affecting stone (*Prunus*) fruit crops. Based on the scientific and economic importance, PPV is considered as one of the top ten plant viruses (Scholthof et al., 2011). The efficient transmission of PPV by many aphid species in a non-persistent manner, the wide range of isolates differing in their biological, serological, and molecular properties, as well as the rare presence of resistance genes within *Prunus* genes make it very difficult to implement control measures (Petrov, 2014).

The plum trees, cultivar Mirabelle, were grown in an open-field plum orchard in the Black Sea region near the town of Burgas. This cultivar is widespread in Bulgaria. Up to 10 branches from the different parts of the top of the crowns of six plum trees were subjected to analysis. Leaves without visual symptoms of sharka and damage (normal turgor and without signs of chlorosis) were collected from the branches. After the check with serological analyses, the leaves were divided into two groups (healthy, without of presence of PPV, and infected with PPV). Leaf samples were stored in plastic bags and kept cool for the analysis.

Spectral reflectance measurements

The reflected radiation from healthy leaves and leaves infected with PVY plum was collected by means of a portable fiber-optics spectrometer (Ocean Optics, USA) in the VIS and NIR spectral ranges (350-1100 nm) in 2048 narrow spectral bands with a step of 0.3 nm and at a spectral resolution of 1.5 nm (product/ocean-optics). The data was analysed in the spectral range 450-850 nm at 1170 spectral bands where more significant differences between the reflectance spectra of healthy and infected plants have appeared. Fresh detached leaves (about 30) from each leaf group were measured on the same day in a laboratory on an experimental setup. The light source was a halogen lamp providing homogeneous illumination of the leaf surfaces. The reflectance spectra (spectral reflectance characteristics) were determined as a ratio between the radiation reflected from the leaves and that reflected from the diffuse reflectance standard (BaSO_4). Specialised software was used for data acquisition and data processing.

Hyperspectral data analysis

Leaf spectral reflectance. Green vegetation species all have unique spectral features, mainly because of the chlorophyll and carotenoid, and other pigments and water content. Chl content is one of the most important biochemical materials, closely related to the photosynthetic process and with protective activity against a variety of degenerative diseases (Korus, 2013). Chl concentration is the main parameter that characterises plant growth conditions and health. Chl generally decreases under stress and changes in its content may thus indicate effects of disease and nutritional and environmental stresses in plants (Sonobe and Wang, 2017).

It is known that the reflectance from green vegetation is characterised by the low reflectance over the blue (400–500 nm) and red (620–700 nm) spectral ranges due to strong absorption by Chl and carotenoids in the blue wavelengths and Chl a in the red wavelengths. The abrupt change in reflectance between 680 and 750 nm, called the red edge, is caused by the combined

effects of increasing Chl absorption at wavelengths beyond 700 nm and internal cell structure. The leaf internal structure with large numbers of refractive discontinuities between cell walls and intercellular air spaces scatters incident radiation and allows a large proportion to pass back through the upper epidermis to be observed as reflected radiation (Blackburn, 2006).

Red edge position and first derivative analyses. The inflection point of the slope (red edge) on the reflectance spectrum (680–760 nm) is known as the red edge position (REP) and can be used for studying the Chl content or plant growth status (Horler et al., 1983; Boochs et al. 1990). The increase in Chl concentration results in a shift of REP towards longer wavelengths (Gates et al., 1965). Shifts of the REP to longer or shorter wavelengths have already been related to changes in the chemical and morphological plant status (Peng et al., 2011).

A first derivative (FD) spectrum displays the variations with wavelengths in the original reflectance spectrum. It has been suggested that spectral derivatives have important advantages over spectral reflectance, such as their ability to reduce variability due to changes in illumination or soil/litter reflectance (Elvidge and Chen, 1995).

The FD of reflectance spectrum has been used to detect specific points such as the green peak and the REP. The main method for defining the REP has been to identify the maximum amplitude of the peak of the FD in the region of the red edge. Horler et al. (1983) have applied derivative analysis techniques on the detection of the red edge shift and have identified two peaks in the derivatives. They attributed the first peak at around 700 nm to the Chl content in plant leaves, while the second one at around 725 nm was attributed rather to the leaf scattering properties than to the Chl content. A shift of the peaks to longer wavelengths was shown to be due to an increase in Chl concentration and leaf stacking, respectively.

Several other studies have revealed the existence of this double-peak feature in the first derivative of continuous spectra with different plants. Boochs et al. (1990) found the peaks at 703 and 735 nm; Smith et al. (2004) identified two peaks in the canopy spectra of grass near 702 and 725 nm.

Development of vegetation indices. The narrowband greenness VIs are designed to provide a measure of the overall amount and quality of photosynthetic material in vegetation, which is essential for understanding the physiological status of vegetation. Reflectance in particular wavelengths is selected for the calculation of ratios or VIs. Most

hyperspectral VIs for assessing Chl content generally use the wavelength domain ranging from 400 to 860 nm on either original reflectance, or derivative spectra.

The light use efficiency VIs provide a measure of the efficiency with which vegetation can use incident light for photosynthesis. Light use efficiency is highly related to carbon uptake efficiency and vegetative growth rates, and the physiological condition of plants.

The narrowband VIs used in this study for the detection of PPV infection on plum trees and details of these indices are presented in Table 1. The first two VIs are greenness and the next three are light use efficiency VIs.

The Normalised Difference Vegetation Index (NDVI) is a simple, but effective VI for quantifying green vegetation. The NDVI normalises green leaf scattering in the NIR wavelengths and Chl absorption in the red wavelengths. The value range of an NDVI is -1 to 1, whereas healthy vegetation generally falls between values of 0.2 to 0.8.

MRENDVI is a modification of the Red Edge NDVI705 that corrects for leaf specular reflection. Applications include precision agriculture, forest monitoring, and vegetation stress detection. The value of this index ranges from -1 to 1. The common range for green vegetation is 0.2 to 0.7.

The Photochemical Reflectance Index (PRI) is sensitive to changes in carotenoid pigments. Applications include vegetation health in evergreen shrublands, forests, and agricultural crops prior to senescence. The value of this index ranges from -1 to 1. The common range for green vegetation is -0.2 to 0.2.

Structure Insensitive Pigment Index (SIPI) applications include vegetation health monitoring, plant physiological stress detection, and crop production and yield analysis. An increase in SIPI is thought to indicate increased canopy stress (carotenoid pigment). The value of this VI ranges from 0 to 2. The common range for green vegetation is 0.8 to 1.8.

The Red Green Ratio Index (RGRI) is an indicator of leaf production and stress. Applications include plant growth cycle (phenology) studies, canopy stress detection, and crop yield prediction. The value of this index ranges from 0.1 to more than 8. The common range for green vegetation is 0.7 to 3.

A new red edge parameter, defined as red edge symmetry (RES), was calculated and analysed. Compared to the commonly used red edge parameters (REP, red edge amplitude, and red edge area), RES was a better predictor of the leaf Chl content. Furthermore, RES was easily calculated using the reflectance of red edge boundary wavebands at 675 and 755 nm (R_{675} and R_{755}) and reflectance of red edge centre

Table 1. Calculated narrowband vegetation indices for detection of PPV infection on plum trees

Vegetation Index (VI)	Computation	Reference
NDVI (Normalized Difference)	$(R_{NIR} - R_{red}) / (R_{NIR} + R_{red})$	Rouse et al., 1973
MPENDVI (Modified Red Edge Normalized Difference)	$(R_{750} - R_{705}) / (R_{750} + R_{705} - 2R_{450})$	Datt, 1999, Sims and Gamon, 2002
PRI (Photochemical Reflectance Index)	$(R_{531} - R_{570}) / (R_{531} + R_{570})$	Peñuelas et al., 1995; Gamon et al., 1997
SIPI (Structure Insensitive Pigment Index)	$(R_{800} - R_{445}) / (R_{800} + R_{445})$	Peñuelas et al., 1995
RGRI (Red Green Ratio Index)	$\frac{\sum_{i=600}^{699} R_i}{\sum_{i=500}^{599} R_i}$	Gamon and Surfus, 1999
RES (Red Edge Symmetry)	$(R_{718} - R_{675}) / (R_{755} - R_{675})$	Chang-Hua et al., 2010

wavelength at 718 nm (R_{718}), with the equation (Chang-Hua et al., 2010) given in Table 1.

Data processing. All data were processed using the Microsoft Excel and the Origin Pro software. The spectra collected from the healthy and infected plum leaves were first cropped in the

spectral range of 450 to 850 nm because this range is most informative for green vegetation. Secondly, the raw spectra were converted to reflectance spectra using the ViewSpec Pro version 6.2.0 software (ASD Inc., Boulder, CO, USA). These reflectance spectra were then subjected to a Savitzky–Golay filtering method (Savitzky and Golay, 1964) to reduce instrumental noise. The filtered reflectance spectra were used to compute the first derivative of the mean reflectance spectra that represents the signal change between two adjacent wavelengths.

Statistical analyses were carried out by means of the Statistica 8 software. Fisher's F-test, Student's t-test and Fisher's least significant difference test (Fisher's LSD test) were applied. Fisher's F-test was performed to determine the ratio between the variances of the compared data sets (30 reflectance spectra of healthy, control leaves and 28 from infected leaves) at a level of significance 0.05. The Student's t-test was applied to assess the statistical significance of the differences (p_{t-stat}) between values of calculated VIs for healthy and infected leaves at a level of significance 0.05 using equal or unequal variances (σ), determined from the F-test. The least significant difference for each VI was compared with the differences between its mean values of control (x_c) and infected (x_{PPV}) plants. If the difference ($x_c - x_{PPV}$) > LSD, then it could be accepted that the two mean values compared were different at significance level p_{t-stat} and it could be concluded that this VI could be used for the detection of a PPV infection.

Results and discussion

The red edge shift of the reflectance spectra and the shape and maximum of the red peak (REP) of the FD curves have been analysed. Because the data analyses are focused on the red, red edge, and NIR ranges of the electromagnetic spectrum, both the reflectance and first-order derivative spectra were cropped to the 680 to 780 nm. The averaged spectral reflectance characteristics from all 30 measurements of control and 28 from infected plum leaves are shown in Fig. 1. Differences between the two curves have appeared in the spectral range of 690 to 780 nm and it is seen that this part of the PPV spectrum is shifted toward the shorter wavelengths.

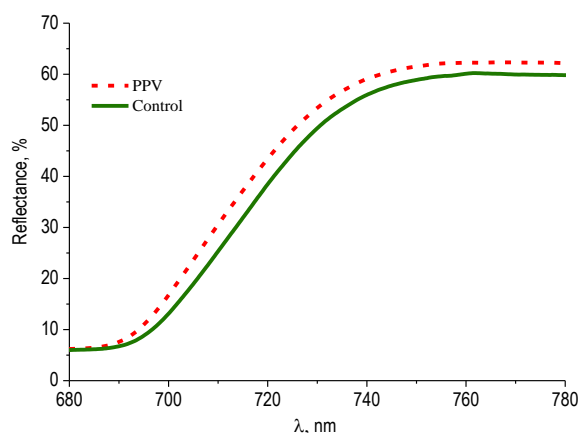


Fig. 1. Spectral reflectance of healthy (control) leaves and leaves infected with PPV in the red and red edge region

A high-order curve fitting technique, such as third-order polynomials, according to Savitzky and Golay (1964), was applied for smoothing the FDs. The FDs of the reflectance spectra in the spectral range 660–780 nm are shown in Fig. 2. It is observed that the shapes of the derivatives have a bi-modal structure. The maximum FD values of infected leaves have increased and the red peak is shifted toward the shorter wavelengths. The maximum FD values of control leaves are around 705–720 nm, whereas for the infected leaves, they are around 690 to 705 nm. Their amplitudes are 1.18 and 1.27, respectively.

For more detailed analyses, deconvolution of the first derivative spectra on their two components was applied using the Multiple Peak Fit tool, available in the OriginPro software. The deconvolution of the FDs was performed with two Gaussian functions positioned in the regions around 690–705 nm for infected leaves and 705–720 nm for control leaves. The fitting operations end with Fit converged. The obtained coefficients of determination are: $R^2 = 0.996$ for the control leaves and $R^2 = 0.993$ for the infected leaves. The deconvolution of the FD spectra for control and infected leaves in the spectral range of 670–770 nm is demonstrated in Fig. 3 and fig. 4, respectively. The first peaks around 700 nm were attributed to the Chl content in leaves, the second peaks around 720 nm were attributed to the effect on leaf scattering properties and cell structures.

The maximum values of first and second peaks for control and infected leaves are 0.486 and 1.108, and 0.676 and 1.071 respectively. The corresponding wavelengths are 702.4 nm and 718.9 nm for the control group and 700.6 nm and 717.3 nm for the infected group. The amplitude of the first peak of FD for the infected group increases because of a decrease in the Chl content in the leaves and an increase in the reflectance in the red range. The amplitude of the second peak is slightly reduced owing to a disturbance of leaf structure, which contributes to the stronger reflection in the NIR due to light scattering in the internal structures of the leaves. A shift toward the shorter wavelengths for the two peaks of FD of infected leaves in comparison with control is observed. That is an indicator of the presence of changes in the physiological condition of the leaves.

Narrowband VIs were calculated using the mean values of the reflectance spectra of control and PPV infected leaves in the spectral range of 450 to 850 nm.

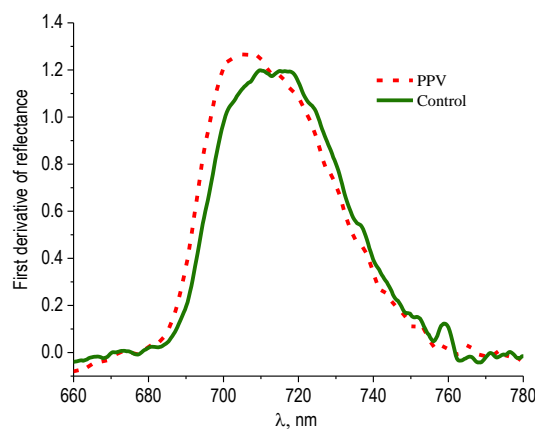


Fig. 2. First derivative spectra of control leaves and leaves infected with PPV: $REP_c = 709.94$ nm, $REP_{PPV} = 705.58$ nm

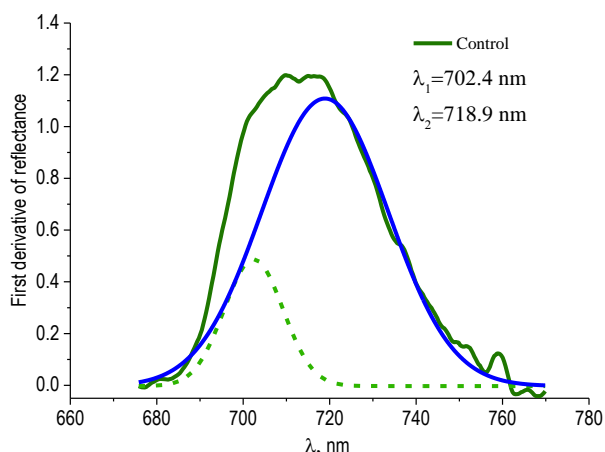


Fig. 3. Deconvolution of the first derivative spectrum of healthy (control) plum leaves

The calculated values of VIs and the results from the applied statistical analyses, Student's t-test, and Fisher's LSD test are shown in Table 2. The difference between the mean values of VIs for both types of leaves is statistically significant for all VIs according to Student's t-test with the exception of NDVI. The values of NDVI are very close (0.815 and 0.812) and this result correlates with the early degree of the PPV infection, i.e. the leaves are with similar "greenness". The MRENDVI and light use efficiency VIs (PRI, SIPI, and RGRI) are sensitive to the

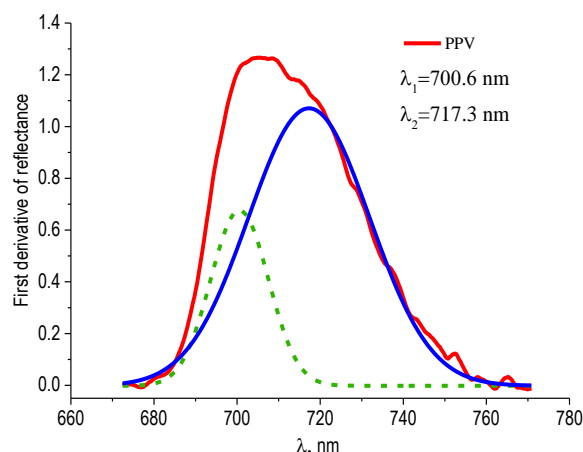


Fig. 4. Deconvolution of first derivative spectrum of infected plum leaves

infection. Also, statistically significant are the differences of red edge symmetry (RES) for healthy and infected leaves that indicate the changes in the size of the red peak of FD.

The Fisher's LSD test shows that the indices MRENDVI, RES, and SIPI are sensitive to the infection. This result is a consequence of their purpose and main applications (vegetation health monitoring and plant physiological stress detection). The index MRENDVI yields the best results.

Table 2. Statistical results: mean values of control and PPV infected plants, F- values, level of statistical significance (p_{F-stat}) and conclusion obtained by F-test ($F_{crit} = 4.073$), t-values, level of statistical significance (p_{t-stat}), critical values of t (t_{crit}), least significant difference (LSD), and difference between mean values of both type of plants

VI	Control	PPV	F	p_{F-stat}	σ_1 / σ_2	t	p_{t-stat}	t_{crit}	LSD	$ X_C - X_{PPV} $
NDVI	0.815	0.812	1.111	0.807, ns	$\sigma_1 = \sigma_2$	-0.312	0.756, ns	2.018	0.0206	0.0032
PRI	0.028	0.020	2.056	0.110, ns	$\sigma_1 = \sigma_2$	-3.301	0.0020, ***	2.018	0.0052	0.0085
SIPI	1.017	1.024	3.189	0.012, *	$\sigma_1 = \sigma_2$	5.474	2.3×10^{-6} , ***	2.018	0.0026	0.0069
MRENDVI	0.542	0.474	17.080	2.5×10^{-8} , ***	$\sigma_1 \neq \sigma_2$	-7.743	1.3×10^{-9} , ***	2.060	0.0175	0.0682
RGRI	0.823	0.769	1.933	0.136, ns	$\sigma_1 \neq \sigma_2$	-4.905	2.5×10^{-5} , ***	2.028	0.0234	0.0543
RES	0.619	0.680	13.028	2.8×10^{-7} , ***	$\sigma_1 \neq \sigma_2$	8.747	1.6×10^{-9} , ***	2.057	0.0138	0.0607

ns – no statistical significance; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.005$.

Conclusions

Hyperspectral leaf reflectance measurements were used for biotic stress detection (PPV viral infection in an early stage) in a plum orchard. This is a non-invasive process where the plum leaves are scanned to collect high-resolution data. First derivatives and mathematical transformations (VIs) of the original spectral reflectance were used for interpreting the health and physiological status of the plum trees. Many factors should be taken into consideration for the efficient application of the vegetation indices in the environmental research. Some of the VIs are more general in nature (NDVI, MRENDVI), whereas several others combining significant wavelengths together can

indicate the health or disease status occurring within a specific species (PRI, SIPI, and RGRI). FD analyses and REP extraction procedure were performed through deconvolution of the red peak of FDs. A shift toward the lower wavelengths (about 5 nm) was established for the FD of infected leaves, which is an indicator for the presence of an infection in plum leaves. MRENDVI has turned out to be most sensitive to the changes in the physiological status of plum trees. This study has demonstrated the potential of hyperspectral reflectance data for stress detection in plants.

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