APPLICATION OF HYPERSPECTRAL VEGETATION INDICES FOR DISEASE DETECTION IN YOUNG APPLE TREES

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ABSTRACT: Recent advances in hyperspectral remote sensing make it possible to develope new ways for monitoring of plant ecosystems and environment changes as well as for detection of plant diseases under field conditions. Hyperspectral (narrow-band) vegetation indices (Vis) have been shown to provide additional information being decisive in characterizing the physiological state, biochemical composition, physical structure,, and water content of the plants. The present study aims to determine narrow spectral bands that are best suited for characterizing the influence of a viral infection (at an early stage) on young apple trees, cultivar Florina, infected with Apple Stem Grooving Virus (ASGV). An empirical-statistical approach was developed and applied on hyperspectral reflectance data collected by means of a portable fiber-optics spectrometer USB2000 in the visible and near infrared spectral ranges (450-1000 nm) with a spectral resolution of 1.5 nm. Several narrow-band VIs - normalized difference vegetation index (NDVI), modified NDVI (mNDVI), simple ratio (SR), photochemical reflectance index (PRI), chlorophyll/pigment related indices ($Chl_{red\ edge}$, Chl_{green}), pigment index (PI), chlorophyll absorption ratio index (CARI), modified CARI (MCARI), and disease index f_d were selected and calculated for estimation of the applicability of the indices to detect changes that occured in the physiological state of the trees infected with ASGV. Statistical analyses (Students' t-test and F-test) were applied to assess the sensitivity of the VIs. Indices CARI, $Chl_{red\ edge}$, and PRI gave the best results.

Keywords: Remote sensing, hyperspectral leaf reflectance, vegetation indices, apple stem grooving virus (ASGV)

ПРИЛОЖЕНИЕ НА ХИПЕРСПЕКТРАЛНИ ВЕГЕТАЦИОННИ ИНДЕКСИ ЗА УСТАНОВЯВАНЕ НА ЗАБОЛЯВАНЕ НА МЛАДИ ЯБЪЛКОВИ ДЪРВЕТА

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РЕЗЮМЕ: Последните постижения в хиперспектралните дистанционни изследвания дават възможност за развитие на нови начини за мониториране на растителните екосистеми, на промените в околната средаи за откриване на болести по растенията. Хиперспектралните (теснолентови) вегетационни индекси (ВИ) предоставят допълнителна информация, която се оказа от решаващо значение при характеризиране на физиологичното състояние, биохимичния състав, физическата структура и водно съдържание на растенията. Настоящото изследване има за цел да определи теснолентови спектрални ленти, които са най-подходящи за характеризиране на влиянието на вирусна инфекция върху млади ябълкови дървета, сорт Флорина, заразени с вируса на ябълковото стъблено набраздяване (ASGV). Спектралните данни за отразена от листата радиация са регистрирани с портативенспектрометър USB2000 във видимата и близката инфрачервена области (450-1000 nm) със спектрална разделителна способност 1,5 nm. Разработен и приложен е емпирично-статистически подход върху тези данни и са изчислени и оценени няколко теснолентови ВИ: нормирана разлика (NDVI), модифициран NDVI (mNDVI), просто съотношение (SR), фотохимичен (PRI), два хлорофилни индекса (Chl red edge, Chl green), пигментен (PI), абсорбция на хлорофил (CARI) и негова модификация (MCARI), и индекс на болестта fd. За оценка на чувствителността на ВИ към промените във физиологичното състояние на заразените с ASGV дървета са приложени статистически анализи чрез t-тест на Стюдънт и F-тест). Най-добри резултати дадоха индексите CARI, $Chl_{red\ edge}$, and PRI.

Ключови думи: Дистанционни изследвания, хиперспектрално отражение от листа на растения, вегетационни индекси, вирус на ябълковото стъблено набраздяване

Introduction

Precise estimates of the plant diseases and their effect on the quality and quantity of crop production are important for horticulture, precision agriculture, as well as for basic and applied plant researches. Reliable and timely assessments of plant disease occurrence and spread are the basis for planning plant protection activities in field or greenhouse production.

Remote sensing data and techniques have already proven to be relevant to many requirements of agricultural applications. Different studies and experiments demonstrated their usefulness and feasibility to address various agricultural issues, such as crop classification and mapping, predicting crop yield, soil survey, irrigation planning, and damage assessment by disaster, pest or diseases (Wang et al., 2010; Usha and Bhupinder, 2013; Krezhova et al., 2017). Remote sensing methods are widely used in managing abiotic stresses, such as nitrogen and water deficiency, salinity and herbicide stress, in order to improve crop yield. When it comes to biotic stress, remote sensing is only able to assess the damage from diseases; and yet, it is not useful for preventing the losses. Therefore, further research is needed to investigate the early detection of biotic stress in plants before the occurrence of visible symptoms.

Recent hyperspectral remote sensing (HRS) techniques based on leaf reflectance measurements are successfully used to derive meaningful biophysical variables related to plant physiological state, like the concentration of foliar pigments (Panigada et al., 2010), nitrogen concentration (Fava et al., 2009), water content (Colombo et al., 2008), leaf structure (Monteiro et al., 2012), etc.

Spectral reflectance of plants in the visible (VIS) and near infrared (NIR) regions of the electromagnetic spectrum is primarily affected by plant pigments, mainly chlorophylls (Chls) and carotenoids, and cellular structure of the leaves. Chls absorb light energy and transfer it into the photosynthetic apparatus. Carotenoids (yellow pigments) can also contribute energy to the photosynthetic system. Chls tend to decline more rapidly than carotenoids when plants are under stress or during leaf senescence (Gitelson and Merzlyak, 1996). From the optical point of view, these pigments have different spectral behaviour, with specific absorption features at different wavelengths, which allows remote sensing techniques to discriminate their respective effects on vegetation reflectance spectra.Thus, the variations in leaf pigment content provide useful information concerning the state of the plants.

The increasing importance of hyperspectral reflectance data motivated researches for defining optimal wavebands to estimate changes in plant physiological state (Stellacci et al., 2016). The complexity of a rich hyperspectral dataset requires techniques for reduction of such large volumes of data, characterized by redundancy of information due to the high degree of correlation of neighbouring wavebands (Thenkabail et al., 2012; Rinaldi et al., 2014). Finding efficient solutions is essential for exploiting the full potential of hyperspectral data. Most of the approaches proposed are based on optical vegetation indices (VIs) that summarize the information contained in the reflectance spectrum through mathematical combinations of reflectance at different wavelengths. Large number of narrowband VIs, derived from hyperspectral measurements, was developed allowing several combinations for each biophysical variable (Wang et al., 2012). The use of VIs may improve the sensitivity to vegetation parameters investigated minimizing the influence of extraneous factors.

In recent years, researchers have studied various spectral vegetation indices to detect different vegetation diseases (Delalieux et al., 2009; Ranjan et al., 2012; Velichkova et al., 2016). Efficient use of spectral data in detecting plant disease depends on the application. The spectral regions from 400 to 700 nm and 700 to 1100 nm are mainly influenced by leaf composition of pigments, structure, and water content (Mahlein et al., 2013). The effects of a disease on the pigments and structure of a plant and the change in their spectral responses enable spectroradiometry and remote sensing techniques to detect plant disease effectively (Oerke et al., 2016).

The aim of this study is to detect a biotic stress (latent viral infection) on young apple trees caused by apple stem grooving virus (ASGV) in an early stage without visual symptoms. An empirical-statistical approach was applied on hyperspectral leaf reflectance data on the basis of calculation of several narrowband vegetation indices and evaluation of their sensitivity to the changes in the physiological state of the infected trees.

Materials and methods

Plant material

Young (one-year-old) apple trees, cultivar Florina, grown in a small non-commercial orchard were used for investigations. The trees were without disease symptoms on the aerial parts and organs. In the summer, a few trees (about 25) were checked trough Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) for the presence of viruses. Some of them were infected with ASGV. For data analysis we chose five trees – four infected to different degrees with ASGV and one non-infected that was adopted as control tree.

Spectral measurements

Leaf reflectance spectra of the five apple trees were collected using a portable fiber-optic spectrometer USB2000 (Ocean Optics, 2017) in the spectral range 450-1000 nm at a spectral resolution of 1.5 nm (bandwidth at half maximum). The measurements were carried out on an experimental setup in a laboratory. The light signal from the freshly detached leaves is guided to the entrance lens of the spectrometer by a-meter-long fiber-optic cable directed perpendicular to the measured surface. As a source of light, a halogen lamp providing homogeneous illumination of measured leaf areas was used. Leaf reflectance measurements were made at about 10 cm above the illuminated sides of 25 to 30 leaves on the healthy and infected leaves from each tree. At the beginning of each measurement, the emitted spectrum of the light source was registered from a diffuse reflectance standard. Spectral analyses were carried out in spectral range 450-850 nm at 1130 narrow spectral bands (0.3 nm). The spectral reflectance characteristics (SRC) of the investigated leaves were determined as the ratio between the reflected from leaves radiation and this one reflected from the standard.

Narrowband vegetation indices used in this study

VIs were commonly calculated from combinations of reflectance at two or three spectral bands (most common in red and NIR spectral ranges) in order to obtain a single value (index) that is related to the vegetation growth.

For assesment of the changes in the physiological state of the apple trees infected with ASVG, we selected ten narrowband VIs, given in Table 1. VIs included were applied at the leaf level and were expected to be related to photosynthetic activity, biomass, Chl content and plant stress.

Normalized Difference Vegetation Index (NDVI) was first proposed by Rouse et al. (1974) and is one of the most known and widely used VIs. It is based on the contrast between reflectance in the red region due to maximum absorption of foliar pigments (Chls and carotenoids) and reflectance in NIR where the maximum of the reflection caused by leaf cellular structure and biomass has appeared (Davenport and Nicholson, 1993). NDVI is affected by plant photosynthetic activity, total plant cover, plant and soil moisture and is commonly used for estimation of plant ''greenness''. In most of the researches NDVI shows non-linear relationship with biophysical parameters such as green leaf area index (LAI) and biomass (Baret and Guyot, 1991).

Table 1
Calculated narrowband vegetation indices for detection of ASGV virus infection on apples trees

Index	Equation	Full name	Reference
NDVI	$(R_{NIR}– R_{red})/(R_{NIR}+R_{red})$, NIR=845 nm, red=665 nm	Normalized Difference Vegetation Index	Rouse et al. (1974)
mNDVI	$(R_{750}– R_{705})/(R_{750}+ R_{705})$	Modified Normalized Difference Vegetation Index	Jurgens (2010)
SR	R_{NIR} /R_{red} NIR = 760 nm, red=695 nm	Simple Ratio	Tucker (1979)
CARI	$(R_{700} – R_{670})$-0.2$(R_{700} – R_{550})$	Chlorophyll Absorption Ratio Index	Kim (1994)
MCARI	$[(R_{700} – R_{670})-0.2(R_{700} – R_{550})](R_{700}/R_{670})$	Modified Chlorophyll Absorption Ratio Index	Daughtry (2000)
$CI_{red\ edge}$	$(R_{NIR} /R_{red\ edge}) – 1$ red edge=714 nm, NIR=760 nm	Chlorophyll Index at green range	Gitelson et al. (2005)
CI_{green}	$(R_{NIR} /R_{green}) – 1$ green=550 nm, NIR=760 nm	Chlorophyll Index at red edge	Gitelson et al. (2005)
PI	R_{NIR} /R_{red} NIR=677 nm and red=554 nm	Pigment index	Tilley et al. (2003)
PRI	$(R_{531}– R_{570})/(R_{531} + R_{570})$	Photochemical Reflectance Index	Gamon et al. (1992)
f_D	$R_{500} / (R_{500}+ R_{570})$	Index of disease	

Modified NDVI (mNDVI) with wavelength of 705 nm was an improved version of NDVI (Sims and Gamon, 2002). It was developed to eliminate the effects of surface reflectance by incorporating the blue band. This VI is more strongly correlated with total Chl content and eliminates the effect of surface reflectance. Li et al. (2015) found that mNDVI is very sensitive to minor changes in the vegetation canopy, gap fraction, and senescence, and has been used for precision agriculture, forest monitoring,, and vegetation stress detection. The value of this index is between -1 and 1, as the values in range 0.2 to 0.7 are an indicator for green vegetation (Snirer, 2013).

The simple ratio index (SR) (Jordan, 1969) is probably the first index and is the most commonly used to derive LAI for a forest canopy. When it is calculated at wavelengths 760 and 695 nm (SR in this case was called also Carter index), it is specialized narrow band index for the monitoring of stress (Tucker, 1979). Its value is in the interval 0 – 30 (Snirer, 2013).

Chlorophyll Absorption Ratio Index (CARI) was first developed by Kim et al. (1994) and measures the magnitude (depth) of Chl absorption at the red region (670 nm) where the maximum of Chl absorption is, to the green (550 nm) and red-edge (700 nm) regions of the spectrum, where absorption of the photosynthetic pigments is minimum. By CARI, a reduction of the variability of the photosynthetically active radiation due to the presence of diverse non-photosynthetic materials could be achieved (Wu et al., 2009).

Modified Chlorophyll Absorption Ratio Index (MCARI) was proposed by Daughtry et al. (2000).It was designed to measure photosynthetically active radiation related to Chl absorption in red and red-edge regions. Thus, it is mostly affected by Chl variability, showing high sensitivity even at high chlorophyll levels (Haboudane et al., 2004). Authors found that MCARI has a great potential for LAI predictions, because 60% of MCARI variation is due to the LAI, although they did not consider NIR band in its formula.

Photochemical Reflectance Index (PRI) was developed by Gamon et al. (1992) to estimate rapid changes in the relative levels of carotenoid pigments (particularly xanthophylls). Carotenoid pigments indicate if photosynthetic light was used efficiently. Thus PRI determines directly light use efficiency by remote sensing (Raddi et al., 2005). PRI is used in studying vegetation productivity and stress. Its value ranges from -1 to 1. The common range for green vegetation is from - 0.2 to 0.2 (Snirer, 2013).

Chl Index at green range (Chl_{green}) and Chl Index at red edge ($Chl_{red\ edge}$) belong to three-band model for non-invasive estimation of Chl and carotenoid contents. Both were proposed and studied by Gitelson et al. (2003, 2005). Because of the strong linear correlation with Chl content, the Chl_{green} could be applied for estimation of canopy Chlcontent at any leafscale, under a wide range of canopy conditions and seasonal changes and variation in photosynthesis patterns (Thanyapraneedkul et al., 2012). $Chl_{red\ edge}$ did not depend on the crop type and exhibited low sensitivity to soil background effects. It was a suitable surrogate of Green LAI as it objectively responded to changes in both leaf area and foliar chlorophyll content (Wu et al., 2009).

The leaf pigment vegetation indices (PIs) were designed to provide a measure of stress-related pigments present in vegetation, such as carotenoids and anthocyanins, which tend to be present in higher concentrations when vegetation is in a weakened state. PIs do not quantity Chl, which is measured using the greenness indices. Applications of PIs include crop monitoring, ecosystem studies, analyses of canopy stress, and precision agriculture (Sims and Gamon, 2002).

Disease indices f_D are specific for singular study. As f_D increased, the reflectance decreased significantly in NIR regions.

Data analyses

For the assessment of the sensitivity of considered 10 VIs (Table 1) to changes in the physiological state of apple trees infected with ASGV, statistical analyses (extended Student t-test and Fisher-test) were performed by applying a two-step procedure. To produce reliable conclusions, these tests require normally distributed data; therefore, the VIs datasets were preliminary tested for normality with the Shapiro-Wilk test at a significant level of 0.05.

As a first step, the Student two-sample t-test was carried out to determine the statistical significance of the differences between the values of the calculated 10 VIs for infected trees against the control ones. The differences are affirmed as statistically significant at level $p < 0.05$.

Then, we applied the Fisher's Least Significant Difference (LSD) test. Accordingly, the difference between two mean values is declared statistically significant at a given level of significance if found to exceed the value of LSD. In our case the value $LSD_{0.05}$ is calculated from the expression

$$LSD_{0.05} = t_{0,05} . s_d \ ,$$

where: $t_{0,05}$ is tabulated t-value at the level of significance 0,05 with degrees of freedom n_1+n_2-2, (n_1 and n_2 are the numbers of the control sample and the infected sample, respectively), and s_d is the pool standard deviation of the difference between the means.

Results and discussion

The averaged (over 30 measured areas) SRCs of the control apple trees and four trees infected by ASRG are shown in Figure 1. SRCs of the infected trees differ against the control trees in several spectral ranges: green (520–580 nm, maximum reflectivity of green vegetation), red (640–680 nm, maximum chlorophyll absorption), red edge (680–720 nm,

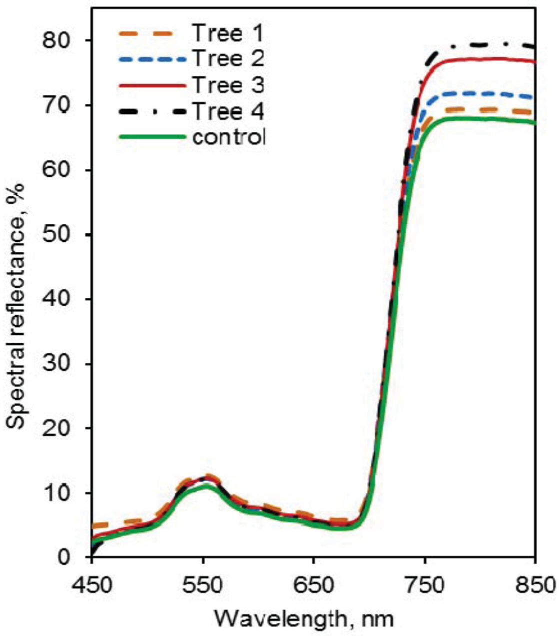

Fig. 1. Averaged SRC of leaves of five studied apple trees

maximum slope of the reflectance spectra), and NIR (720–770 nm, plateau of the characteristics). In all spectral ranges, the SRCs values are higher than the control. The differences at green and NIR regions are most significant.

We checked the VIs datasets for normality by means of Shapiro-Wilk test investigating the values of skewness and kurtosis. The standard error (SE) and the value of the ratios skewness/SE_{skew} and kurtosis/SE_{kurt} were calculated and they are within the interval (-1.96; +1.96), so that data are normally distributed. In Figure 2, we exemplify results from the Shapiro-Wilk test at a significance level 0.05 applied to the pigment index (PI) dataset for apple tree 4.

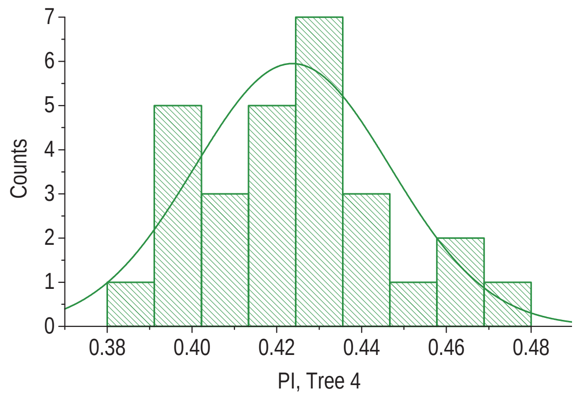

Fig. 2. The distribution of pigment VI dataset for tree 4 infected by ASGV

Figure 3 shows box plots summarizing the results of assessment of the normality of PI datasets derived from hyperspectral reflectance data of control and four infected trees. The results show that VIs datasets satisfy the normal distribution reasonably well.

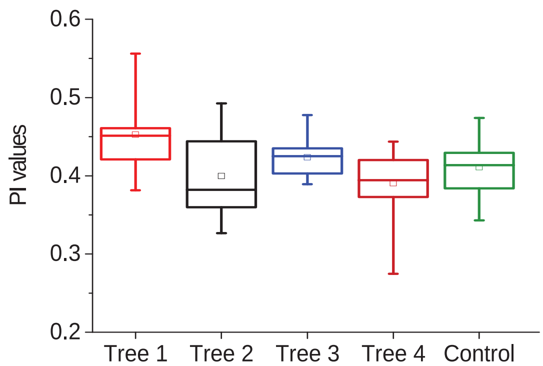

Fig. 3. Box plots ofPI datasets of all investigated trees: median (line across box), mean value (small empty square box), minimum and maximum values (lower and upper ends of the whisker, respectively), interquartile range containing 50% of values (box)

Student t-test was performed at a level of statistical significance $p<0.05$ for assessment of the significance of the differences between mean of sets of the calculated VI values of control and infected trees. Mean values, p-values, F-values, and LSD for the sets of hyperspectral VIs used in the study are shown in Table 2. F-values are calculated as a ratio of variances (squares of standard deviations) of two compared groups (healthy and infected).

Table 2.
Mean values, p-values, F-values, and LSD for the sets of hyperspectral VIs used in the study

tree	1	2	3	4	1	2	3	4	1	2	3	4
NDVI					mNDVI				SR			
Mean healthy	0.870	0,870	0.870	0.870	0.644	0.644	0.644	0.644	10.325	10.325	10.325	10.325
Mean infected	0.842	0,871	0.869	0.863	0.609	0,625	0.643	0.636	8.588	10,328	10.336	11.256
F-ratio	1.747	2,390	1.486	2.969	2.177	9,081	1.676	2.601	1.625	5,700	1.064	5.730
p	ns	*	ns	ns	ns	***	ns	ns	ns	***	ns	***
LSD	0.006	0,005	0.004	0.006	0.011	0,016	0.009	0.010	0.315	0,484	0.290	0.516
difference	0.028	0,001	0.001	0.013	0.034	0,019	0.001	0.012	1.738	0,003	0.011	0.931
CARI					MCARI				Chl_{green}			
Mean healthy	5.176	5,176	5.176	5.176	18.910	18.910	18.910	18.910	5.176	5.176	5.176	5.176
Mean infected	5.753	5,980	5.712	5.921	19.616	23,401	20.739	22.824	4.509	5,080	5.298	5.613
F-ratio	2.318	7,388	1.002	2.506	1.941	6,528	1.075	2.532	1.057	5,816	1.008	3.619
p	*	***	ns	*	ns	***	*	*	ns	***	ns	**
LSD	0.396	0,493	0.261	0.346	1.920	2,400	1.320	1.779	0.239	0,342	0.200	0.302
difference	0.576	0,804	0.536	0.744	0.707	4,492	1.830	3.915	0.667	0,095	0.123	0.437
$Chl_{red\ edge}$					PI				PRI			
Mean healthy	1.482	1.482	1.482	1.482	0.411	0.411	0.411	0.411	0.027	0,027	0.027	0.027
Mean infected	1.328	1,362	1.479	1.561	0.453	0,400	0.424	0.390	0.028	0,326	0.026	0.022
F-ratio	2.128	8,074	1.792	2.692	1.684	1,823	2.544	1.093	9.090	2,679	2.427	13.772
p	ns	***	ns	*	ns	ns	*	ns	***	*	*	***
LSD	0.074	0,098	0.058	0.068	0.025	0,020	0.015	0.019	0.005	0,009	0.002	0.005
difference	0.154	0,120	0.003	0.080	0.042	0,011	0.013	0.021	0.001	0,005	0.001	0.005

*ns – no statistical significance; * - p<0.05; ** - p<0.01; *** - p<0.001*

The results show that NDVI is insensitive to ASGV viral infection at an early stage. The mean NDVI values of control and infected trees are close (about 0.85). In healthy leaves, NDVI values are positive and have a maximum value of 1. The higher values are an indicator for more amount of biomass and more Chl content. In our case, mean NDVI values of infected trees weakly decrease against the control and this VI can be used as a measure of the greenness and vigour of the vegetation.

Index mNDVI is also insensitive to the infection. Its values decrease when the vegetation is subject to a state of stress. The values in range from 0.2 to 0.7 are an indicator for green vegetation. In our case, the values are between 0.64 and 0.61 (for infected trees) and the decrease is not significant. This is due to the fact that no visual symptoms occured in the infected leaves but still mNDVI indicates slight changes in its physiological state.

Pigment VI (PI) appears less sensitive since it indicates that no changes occured in the ratio Chl/carotenoids. This is the reason why VIs SR, Chl_{green} and $Chl_{red\ edge}$ also do not show good results for detection of viral infection at an early stage.The changes in SRCs (Fig. 1) of infected trees in green spectral range around 550 nm (maximum of the reflectivity of green vegetation due to Chl content) are not statistically significant for trees 1, 3, and 4 (with lower ASGV content). The results of serological test DAS-ELISA applied on ifected trees for the presence of ASGV are displayed in Figure 4. Disease index f_d calculated for reflectance in the choosen wavelengths proved to be not suitable for this investigation and the results are not shown in Table 2.

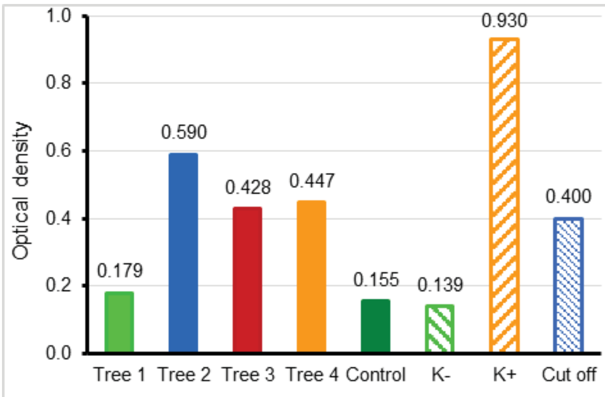

Fig. 4. Results of DAS-ELISA test on leaf samples from infected with ASGV young apple trees

Indices PRI, CARI, and MCARI show the best results. PRI directly determined light use efficiency by remote sensing data - leaf reflectance at 531nm, where changes in xcantophyll cycle are manifested as narrow absorbtion feature and are an indicator for the changes in the physiological state of the plants. CARI and MCARI were designed to measure the Chl influence in the green, red and red-edge regions. In our case, average SRCs of infected trees differ more significant against the control (Fig. 1) in these regions. The mean values of both VIs increase for all infected trees.

The normalised differences of mean values of 10 VIs calculated from spectral data of infected trees against the control tree are presented in Figure 5. It is seen that differences are highest for tree 2 and tree 4 in correspondence with the ASGV concentration.

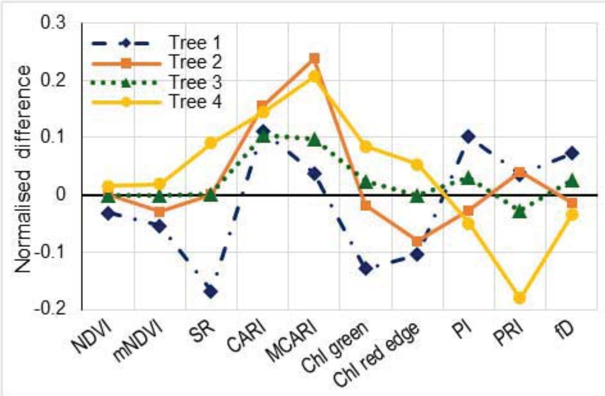

Fig. 5. Normalized differences of vegetation indices of the infected apple trees against the control tree

Conclusions

Ten hyperspectral vegetation indices were tested to explore their potential in assessment of influence of viral infection, caused by ASGV, at an early stage. In principle, all considered vegetation indices should be suitable to detect differences in the reflection between healthy and diseased plants. Different narrow band combinations were applied to derive from the indices better sensitivity to changes in physiological state (biophysical variables) such as Chl and pigment contents, cell structure, vegetation vigour, etc. Statistical methods were implemented to assess their sensitivity. Thus, for the investigation of the ASGV infection, two of the indices (NDVI and mNDVI) were found inapplicable. The sensitivity of other indices, such as SR, Chl_{green} and $Chl_{red\ edge}$, was not very high. PRI, CARI, and MCARI showed the best results as the differences of SRCs of infected trees in the selected wavebands were more significant.

This paper has shown that the selection of optimal narrow spectral bands that were better adjusted to study of a given application, allows to reduce the amount of hyperspectral data and computer time used for their proccesing, sometimes making the data interpretation easier.

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