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SPECTRAL VEGETATION INDEXES AS INDICATORS OF LEAF PIGMENT CONTENT IN APPLE (*Malus domestica* Borkh.)

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Abstract

Methods of operational remote (satellite and unmanned) agricultural monitoring are currently based on the use of spectral vegetation indices as some integral indicators of plant condition. Since the first of them (Normalized Difference Vegetation Index — NDVI) appeared in the early 1970's, rich experience has been accumulated in their use to detect various properties of agricultural plants and agrophytocenoses as a whole. About a hundred different indices have been proposed to detect different properties, e.g., moisture, leaf structure, architecture of plants in crops, the content of various substances, including pigments regulating photosynthesis and plant productivity. In many cases, the proposed indices function reliably for specific plants or for the vegetation as a whole. For fruit crops and, in particular, for apple-tree, there are practically no such indices. In this paper, it is shown for the first time that the spectral vegetation indices proposed for the detection of pigments in agricultural plants need to be refined when they are used for similar detection of pigments in the leaves of an apple tree of a particular variety. Our goal was to analyze the relationship between the spectral vegetation indices calculated for the leaves of the Imrus apple tree (*Malus domestica* Borkh.) with the leaf content of chlorophyll and carotenoids. We evaluated the applicability of several dozen vegetation indices proposed for determining the content of chlorophylls and carotenoids in the leaves of various plants to the non-contact determination of these pigments in the leaves of the Imrus apple tree. On October 19, 2021, leaves were collected at noon randomly from 2-5-year old branches of the middle part of the crown of model Imrus trees grown from 2011 at the test plot (Stupino District, Moscow Province, Russia). In total, 26 mixed leaf samples were collected for pigment content analysis. The content of chlorophylls a + b was determined in the laboratory by the Wintermans-De Mots method, carotenoids by the von Wetshtein method. For the same leaves, spectral reflectance was measured under field conditions using a SR-6500 field spectroradiometer (Spectral Evolution, USA), which operates in the 350-2500 nm range with a resolution of 1 nm. Spectral reflectivity curves were plotted in 5 replicates for the upper surface of the leaves, averaged for each leaf, and then for each of the 26 mixed groups of leaves. Based on the averaged spectral reflectance curves, the most common spectral vegetation indices were calculated, followed by an analysis of the relationship between the values of the spectral vegetation indices and the content of pigments in the leaves. It has been established that the previously proposed numerous vegetation indices cannot be used for non-contact detection of the content of chlorophyll and carotenoids in the leaves of the Imrus apple tree. There is practically no connection between the index value and pigment content. It is also not possible to group the analyzed leaves according to the content of pigments based on the construction of a dendrogram of the similarity between the spectral reflectance curves of leaves in the range of 350-2500 nm. Based on the correction of the indices that showed the most accurate dependence, new vegetation indices were proposed for non-contact detection of the content of carotenoids and chlorophyll in apple leaves, which make it possible to obtain regression models with R^2 above 0.65. Before widespread use, they must be tested for leaves of apple trees of other varieties, as well as for leaves at different stages of development.

Keywords: spectral reflectance, *Malus domestica*, apple leaves, chlorophyll content, carotenoids content, vegetation indexes

Remote sensing data (mainly satellite data) is now widely used as the main source of information for quickly and cost-effectively obtaining information about the condition of agricultural plants over large areas. According to scientific publications, satellite agricultural monitoring allows for assessment of sown areas [1, 2], operational monitoring of crop state [3-5], yields [6-8], and monitoring of agronomically important soil properties [9].

Methods of satellite agricultural monitoring during their development since the mid-1960s have evolved from visual analysis of paper photographs to interactive interpretation on a computer monitor [10] and further to the construction of fully automated analysis algorithms [11] due to the transition from analogue images to satellite data as a set of digital (pixel) scenes. As a result, it became possible to perform automated computer pixel-by-pixel analysis of satellite data using a combination of several imaging channels in the form of derivative images obtained by arithmetic operations on individual channels. This significantly expanded the list of potential satellite predictors of the vegetation or soil properties as objects of remote monitoring. It turned out that in many cases the use of derivatives of satellite images rather than original satellite images is more effective for detecting and monitoring the properties of soils and vegetation.

In 1972, the first spectral vegetation index NDVI (normalized difference vegetation index) [12] was proposed for remote monitoring of vegetation, which was calculated as NDVI = (R – NIR)/(R + NIR), where R is the image brightness in the red shooting channel, NIR is the image brightness in the near-infrared shooting channel.

Numerous studies using the example of different plant associations have shown that this index well reflects the state of vegetation and correlates with many of its properties (leaf color, aboveground phytomass, leaf surface, etc.) [13]. Until now, NDVI is widely used in agricultural remote monitoring systems around the world [2, 4, 14].

However, a search was carried out for other spectral vegetation indices that would be more sensitive to the specific properties of vegetation and soils. Currently, there are more than a hundred of them proposed and the number is constantly growing [15]. Of significant practical interest are vegetation indices developed for non-contact (remote) detection of the amount of pigments in plant leaves (mainly chlorophyll and carotenoids), since the efficiency and productivity of photosynthesis depends on their content [16, 17].

As a rule, authors test and validate their models and their proposed indices using the example of specific plants (in agriculture, these are mainly annual plants) [18], and the convenience of their use for other plants remains unexplored.

The possibilities of using vegetation indices to monitor perennial fruit plantations have been least studied. Moreover, many publications focus on the development of new methods for extracting information about the content of pigments in plants. Thus, for apple leaves in China [19, 20], approaches based on machine learning methods and neural networks have been proposed. C. Li et al. [21] assessed the capabilities of remote (satellite) detection of chlorophyll content for individual apple trees. However, there are few such publications and non-contact methods for assessing the content of pigments in apple leaves are still not sufficiently developed.

The presented article shows for the first time that spectral vegetation indices proposed for detecting pigments in agricultural plants need to be clarified when used for similar detection of pigments in the leaves of a particular apple tree variety.

Our goal was to analyze the relationship between the spectral vegetation indices calculated for the leaves of the Imrus apple tree with the content of chlorophyll and carotenoids in them.

Materials and methods. An analysis of the leaf spectral reflectance of apple tree (Malus domestica Borkh.) Imrus variety planted in 2011 was carried out on the territory of the experimental garden of the Federal Scientific Center for Horticulture (Mikhnevo village, Moscow Province, Stupinsky District) on October 19, 2021. At this time, the leaves of the trees are in different states (from completely green to already yellowed or reddened), which ensured the most complete coverage of possible options for pigment content. Imrus is a winter scab-immune (*Vf*) variety (Antonovka vulgaris × OR18T13) bred at the All-Russian Research Institute for Breeding Fruit Crops (Oryol Province)

Mixed leaf samples were taken from two adjacent rows in plots with 15-20 trees in each row, which were located opposite each other. Leaves were selected randomly at midday and from branches 2-5 years old in the middle part of the crown. A total of 26 samples, each from 30-40 trees, were mixed to analyze the pigment content. Chlorophylls a + b were measured in lab test by the Wintermans-De Mots method [22], carotenoids by the von Wettstein method [23].

Spectral reflectance was assessed using a field spectroradiometer SR-6500 (Spectral Evolution, USA) that operates in the range of 350-2500 nm with a resolution of 1 nm. Spectral reflectance curves were obtained in 5 replicates for the upper leaf surfaces and averaged for each leaf and then for each of the 26 mixed leaf groups.

Based on the averaged spectral reflectance curves, the most common spectral vegetation indices were calculated. After this, an analysis was carried out of the relationship between the values of spectral vegetation indices and the content of pigments in the leaves.

At the first stage, a simple correlation analysis was carried out. Then clustering of the spectral reflection curves was performed and an analysis of the grouping of the chlorophyll and carotenoids contents in different groups of curves, identified by the similarity dendrogram of the reflection curves, was carried out. At the last stage, an attempt was made to correct the most suitable indices in order to adapt them to determine the pigment content in apple leaves based on linear regression analysis.

Statistical processing of data. i.e., the calculation of average values, confidence intervals, assessment of the statistical significance of differences ($t_{0.05}$), preliminary processing of spectral reflection curves (their smoothing and removal of outliers) was carried out using the stats and prospectr packages in the R environment (https://www .r-project.org/). The similarity dendrogram was constructed using the Statistica 6.0 package (StatSoft, Inc., USA). Regression analysis and calculation of p-value using the *F*-test were performed in Microsoft Excel.

Results. Table 1 presents formulas for calculation of vegetation indices for non-contact determination of pigment contents in leaves.

Regression analysis between the content of pigments in leaves and the value of various vegetation indices showed an almost complete absence of reliable regression dependencies. For carotenoid content, the highest R^2 value was found for the ARI index (ARI = 0.36), for chlorophyll content for the G index (G = 0.36). All other R^2 values turned out to be lower than 0.2 (Table 2). Only two models were statistically significant (at p = 0.01).

By the dendrogram of similarity of spectral reflection curves of apple tree leaves in the analysis of 26 mixed samples, all curves were quite reliably divided into two large groups and one curve (19av) was not included in any of these groups (Fig. 1).

1. Spectral vegetation indices for non-contact determination of the chlorophyll and carotenoids contents in plant leaves

Formula for calculation	Pigment	Reference
ARI = $1/R_{550} - 1/R_{700}$	Carotenoids	[24]
CRI = $1/R_{510} - 1/R_{550}$	Carotenoids	[24]
CRI2 = $1/R_{510} - 1/R_{700}$	Carotenoids	[24]
PSSRc = R_{800}/R_{500}	Carotenoids	[25]
SIPI = $(R_{445} - R_{800})/(R_{670} - R_{800})$	Carotenoids	[26]
CSI1 = R_{695}/R_{420}	Chlorophyll	[27]
CSI2 = R_{695}/R_{760}	Chlorophyll	[27]
G = R_{554}/R_{677}	Chlorophyll	[28]
GM1 = R_{750}/R_{550}	Chlorophyll	[29]
GM2 = R_{750}/R_{700}	Chlorophyll	[29]
gNDVI = $(R_{750} - R_{550})/(R_{750} + R_{550})$	Chlorophyll	[30]
MCARI = $[(R_{700} - R_{670}) - 0{,}2\cdot(R_{700} - R_{550})]\cdot(R_{700}/R_{670})$	Chlorophyll	[31]
NPQI = $(R_{415} - R_{435})/(R_{415} + R_{435})$	Chlorophyll	[32]
PRI = $(R_{528} - R_{567})/(R_{528} + R_{567})$	Chlorophyll	[33]
SR705 = $SR_{705} = R_{750}/R_{705}$	Chlorophyll	[34]
TCARI = $3\cdot[(R_{700} - R_{670}) - 0{,}2\cdot(R_{700} - R_{550})]\cdot(R_{700}/R_{670})/$ $/(1 + 0{,}16)\cdot(R_{800} - R_{670})/(R_{800} + R_{670} + 0{,}16)$	Chlorophyll	[35]
TVI = $0{,}5\cdot[120\cdot(R_{750} - R_{550}) - 200\cdot(R_{670} - R_{550})]$	Chlorophyll	[36]
VOG1 = R_{740}/R_{720}	Chlorophyll	[37]
VOG2 = $(R_{734} - R_{747})/(R_{715} - R_{720})$	Chlorophyll	[37]
ZTM = R_{750}/R_{710}	Chlorophyll	[38]
SR (Chl a) = R_{675}/R_{700}	Chlorophyll	[30]
SR (Chl b) = $R_{675}/R_{650}\cdot R_{700}$	Chlorophyll	[30]
SR (Chl b2) = R_{672}/R_{708}	Chlorophyll	[30]
SR (Chl tot) = R_{760}/R_{500}	Chlorophyll	[30]
PSSRa = R_{800}/R_{675}	Chlorophyll	[25]
PSSRb = R_{800}/R_{650}	Chlorophyll	[25]
LCI = $(R_{850} - R_{710})/(R_{850} + R_{680})$	Chlorophyll	[39]

N o t e. R_{xxx} in formulas means reflection at the specified wavelength (xxx, nm).

2. The effectiveness of vegetation indices for regression modeling of pigment content in the leaves of the apple tree (*Malus domestica* Borkh.) variety Imrus (Mikhnevo village, Moscow Province, Stupinsky District, 2021)

Index	Linear Regression R^2	p-value	Pigment
ARI	0.36	8.84184E–05	Carotenoids
CRI	0.08	0.11982	Carotenoids
CRI2	0.03	0.41109	Carotenoids
PSSRc	0.12	0.66258	Carotenoids
SIPI	0.17	0.11075	Carotenoids
CSI1	0.02	0.10390	Chlorophyll
CSI2	0.04	0.06195	Chlorophyll
G	0.36	8.89972E–06	Chlorophyll
GM1	0.11	0.46123	Chlorophyll
GM2	0.07	0.13564	Chlorophyll
gNDVI	0.03	0.48826	Chlorophyll
MCARI	0.16	0.37879	Chlorophyll
NPQI	0.19	0.37090	Chlorophyll
PRI	0.08	0.65917	Chlorophyll
SR705	0.03	0.24438	Chlorophyll
TCARI	0.18	0.37874	Chlorophyll
TVI	0.03	0.33811	Chlorophyll
VOG1	0.02	0.11980	Chlorophyll
VOG2	0.04	0.78741	Chlorophyll
ZTM	0.09	0.14587	Chlorophyll
SR (Chl a)	0.11	0.02127	Chlorophyll
SR (Chl b)	0.17	0.08067	Chlorophyll
SR (Chl b2)	0.08	0.13811	Chlorophyll
SR (Chl tot)	0.12	0.02885	Chlorophyll
PSSRa	0.02	0.16915	Chlorophyll
PSSRb	0.06	0.14184	Chlorophyll
LCI	0.16	0.01012	Chlorophyll

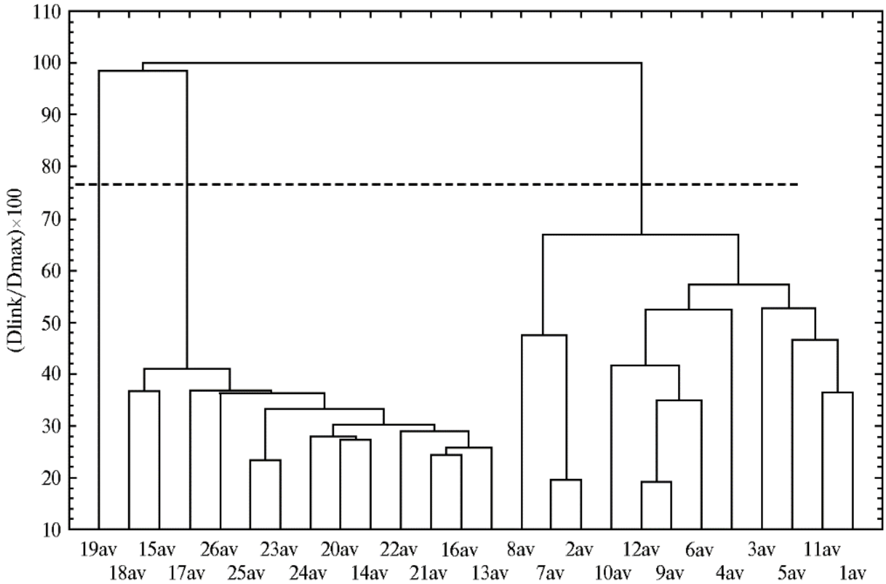

Fig. 1. Dendrogram of similarity of spectral reflection curves of apple tree leaves (*Malus domestica* Borkh.) variety Imrus: 1av-26av — each of 26 mixed leaf samples (Mikhnevo village, Moscow Province, Stupinsky District, 2021).

An attempt to establish connections between the content of pigments in leaves with the indicated groups was also not successful. In particular, the content of carotenoids in one of the groups was 0.57±0.06 mg/g, in the other 0.56±0.06 mg/g, and the content of chlorophylls a + b was 2.27±0.27 and 2.17±0.26 mg/g (at $p = 0.05$), respectively.

Thus, the spectral vegetation indices proposed by other researchers for the non-contact detection of pigments in plant leaves, in our case, did not provide satisfactory results. This is most likely due to the fact that most of the indices (see Table 1) were proposed and tested for vegetation at the level of phytocenosis, rather than individual leaves, without division into species (24, 26, 32) or for specific agricultural plants (28, 30, 31). The structure of the leaves of an apple tree has its own specifics and differs significantly from that of other plants, which determines the characteristics of light reflection.

Having analyzed the relationship between the previously proposed vegetation indices and the content of pigments in apple leaves, we tried to select more reliable indices. Since the general patterns of constructing indices should be preserved, the indices that showed the best results in regression analysis were selected as the base ones, and then we refined them for apple tree leaves by changing the wavelengths involved in the calculation.

The G index was chosen to detect chlorophyll content [28]. When specifying the wavelength for which the reflection value is taken when calculating using the formula, the quality of the regression model (as per R^2) increased almost 2 times. As a result, a new vegetation index was obtained for non-contact detection of chlorophyll content in apple tree leaves:

$$G\_apple = R_{580}/R_{685}.$$

The regression dependence ($R^2 = 0.66$) with this index is presented in Figure 2, A, the parameters of the regression model are in Table 3.

For carotenoids, we used the ARI vegetation index [24] as the base one:

$$ARI\_apple = (1/R_{560}) - (1/R_{690}).$$

The R^2 value of the regression model with this vegetation index reached 0.65 (see Fig. 2, B), table 3 shows the parameters of the regression model.

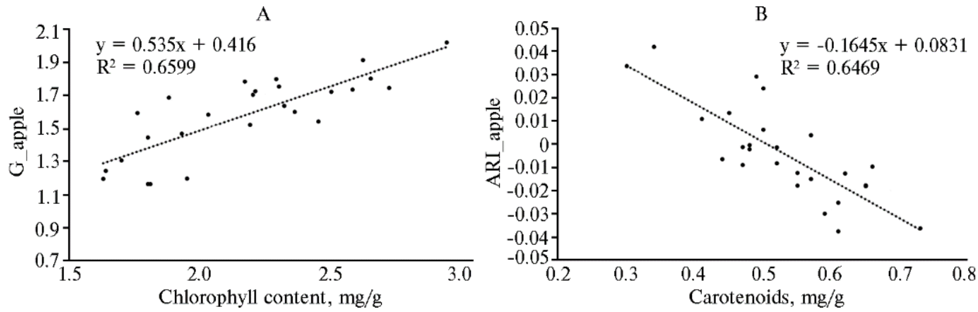

Fig. 2. Regression dependence of the spectral vegetation indices ARI_apple (A) **and G_apple** (B) **on the content of chlorophylls a + b** (A) **and carotenoids** (B) **in the leaves of the apple tree (*Malus domestica* Borkh.) variety Imrus** (Mikhnevo village, Moscow Province, Stupinsky District, 2021).

3. Parameters of regression models characterizing the dependence of the spectral vegetation indices on the content of pigments in the leaves of the apple tree (*Malus domestica* Borkh.) variety Imrus (Mikhnevo village, Moscow Province, Stupinsky District, 2021)

Index ARI_apple—chlorophylls a + b (Fig. 2, A)					
Regression statistics					
Plural R	0.804310212				
R-square	0.646914918				
Normalized R-squared	0.632203039				
Standard error	0.060379302				
Observations	26				
Analysis of variance					
	df	*SS*	*MS*	*F*	*F* significance
Regression	1	0.160308005	0.160308005	43.97228542	7.3744E-07
Remainder	24	0.087495841	0.003645660		
Total	25	0.247803846			
	coefficient	standard error	*t*-statistics	p-value	
Y-intersection	0.51319766	0.012052075	42.5816848	4.00502E-24	
Variable X1	–3.933057307	0.593117523	–6.631160187	7.3744E-07	
Index G_apple—carotenoid (Fig. 2, B)					
Regression statistics					
Plural R	0.812355896				
R-square	0.659922102				
Normalized R-squared	0.645752189				
Standard error	0.217909933				
Observations	26				
Analysis of variance					
	df	*SS*	*MS*	*F*	*F* significance
Regression	1	2.211462417	2.211462417	46.57206635	4.65864E-07
Remainder	24	1.139633737	0.047484739		
Total	25	3.351096154			
	coefficient	standard error	*t*-statistics	p-value	
Y-intersection	0.225012363	0.288248014	0.780620689	0.442657485	
Variable X1	1.23344918	0.18074176	6.824372964	4.65864E-07	

Thereof, our findings show that simple vegetation indices can be applicable to non-contactly determine the pigment content in apple leaves, but they must be adjusted for a specific variety. The quality of the results is quite comparable to that obtained using machine learning methods [19] or methods based on the use of neural networks [20]. Moreover, unlike complex methods, the approaches we propose are easier to use. Our studies confirm the results of C. Li et al. [21], although they were obtained for individual trees.

Therefore, the previously proposed numerous vegetation indices cannot be used for non-contact detection of the chlorophyll and carotenoid contents in the leaves of the Imrus apple tree. There is practically no connection between the index value and pigment content. It is also not possible to group the analyzed leaves according to pigment content based on constructing a dendrogram of similarity between the leaf spectral reflectance curves in the range of 350-2500 nm.

By the correction of the indices that showed the most accurate dependence, we proposed new vegetation indices for non-contact detection of the content of carotenoids and chlorophyll in apple leaves. Based these indices, we suggest regression models with R^2 above 0.65. Before widespread use, such models need to be tested for leaves of other apple varieties, as well as for leaves at different stages of development.

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