Possibilities of cucumber powdery mildew detection by visible and near-infrared spectroscopy

I. Alsiņa[1,*], G. Bimšteine[1], L. Dubova[1], J. Kaņeps[1], K. Kviesis[2], B. Bankina[1], M. Dūma[3] and A. Avotiņš[2]

[1]Latvia University of Life Sciences and Technology, Faculty of Agriculture, Institute of Plant and Soil Science, Liela street 2, LV-3001 Jelgava, Latvia
[2]Riga Technical University, Faculty of Power and Electrical Engineering, Kaļķu street 1, LV-1658 Riga, Latvia
[3]Latvia University of Life Sciences and Technology, Faculty of Food Technology, Department of Chemistry, Liela street 2, LV-3001 Jelgava, Latvia
*Correspondence: Ina.Alsina@llu.lv

Abstract. Cucumbers are one of the most demanded and widely grown greenhouse vegetables. Important factors that influence quality and quantity of yield are diseases. Powdery mildew (caused by *Podosphaera xanthii* and/or *Golovinomyces cichoracearum),* is one of the most harmful cucumber diseases. Early detection of mildew via non-destructive methods can optimize schemes of fungicide application. The study aimed to find regularities in the reflected light spectra, indices described in the literature, and severity of mildew. Plants were grown in the polycarbonate greenhouse under artificial lighting in a 16 h photoperiod with PAR at the tips of plants 200 ± 30 µmol m^{-2} s^{-1}. Leaf reflection spectra were obtained using spectroradiometer RS-3500 (Ltd. Spectral Evolution). Spectral range 350–2,500 nm, bandwidth 1 nm. The severity of cucumber mildew was evaluated using 10 point scale (0- no symptoms, … 9 - the plant is dead). The vegetation indices found in the literature have been calculated. The obtained results show that the calculated indices have different sensitivities. The strongest correlation between the degree of cucumbers infection with powdery mildew and the light reflectance spectrum was found in the green range of visible light around 550 nm. Disease-Water Stress Index-2 (DSWI-2), Structure Intensive Pigment Index (SIPI), and Normalized Difference Vegetation Index (NDVI) are the most suitable indices for determining powdery mildew in cucumbers. New indices for detection of powdery mildew have been created. None of the studied indices allows determining the powdery mildew at the early stages of disease development when powdery mildew severity is below 10%.

Key words: *Cucumis sativus*, NDVI, SIPI, DSWI, *Podosphaera xanthii, Golovinomyces cichoracearum.*

INTRODUCTION

Cucumber (*Cucumis sativus L.*) is one of the most demanded and widely produced greenhouse-grown vegetables. In 2010, a total area of greenhouses in Latvia was 84.0 ha with an average cucumber harvest of 76.5 t ha^{-1}. Throughout the following years, a steady decline in the total greenhouse area was observed. In 2019, the total greenhouse area decreased by 35.5% to 54.2 ha compared to 2010. At the same time, greenhouse productivity increased by 48.4% up to 113.5 t ha^{-1} (Central Statistical Bureau of Latvia, 2019).

Cucumber is a thermophilic crop, temperature above 20 °C is optimal for growing. Greenhouse-grown cucumbers have high requirements for irradiance, air humidity, soil moisture, temperature, and fertilizers (Singh et al., 2017).

Diseases are an important factor that influences the quality and quantity of yield. Powdery mildew (caused by *Podosphaera xanthii* and/or *Golovinomyces cichoracearum*), is one of the most harmful cucumber diseases in greenhouses. The powdery mildew can decrease yield potential and reduce fruit quality if it is not controlled during the early infection phases. Visual diagnostic of diseases in early infection stages is problematic, due to the presence of symptoms on lower, more matured leaves, which often are covered by other leaves. Early disease detection is beneficial for optimal powdery mildew management strategy (i.e fungicide application) (Abdulridha et al., 2020).

Conventional scouting for foliar diseases relies primarily on the visual inspection of leaves (color, pattern, crown structures). Laboratory test approaches, such as polymerase chain reactions, an enzyme like immunosorbent assays, etc. are highly specific and sensitive to identify diseases, but these methods are destructive and labor-consuming and require specialized skills (Lu et al., 2018). Hyperspectral imaging is one of the most efficient and fast-developing non-destructive techniques for obtaining detailed information about plants (Golhani et al., 2018). Recent studies have attempted to explain the role of spectral bands in differentiation between healthy and diseased plants. Any disease that affect chlorophyll and/or water content in the plant leaves, that damage plant cells would affect leaf spectral reflectance (Semeraro et al., 2019). For accurate disease detection it is crucial to identify the most significant wavelengths and find or create vegetation indices for early diagnosis of diseases (Abdulridha et al., 2020). Despite the strong focus on powdery mildew research, further research is needed to diagnose the disease before the symptoms become apparent (Fernández et al., 2021).

The study aimed to find regularities in the reflected light spectra, indices described in the literature, and severity of mildew.

MATERIALS AND METHODS

The experiments were carried out in a polycarbonate greenhouse of Latvia University of Life Sciences and Technologies during the autumn season of 2020. Plants were grown under artificial lighting with a 16 h photoperiod with PAR at the tips 200 ± 30 µmol m^{-2} s^{-1}.

Cucumber variety 'Atos F1' was chosen for the experiments. Plants were grown in plastic pots; the volume of pots was adapted to plant size throughout cucumber vegetation. During the production of cucumbers, the pot volume was 12 L. Peat substratum KKS-S from Laflora (pH_{KCl} 5.8–6.6, EC 0.25 mS cm^{-1}, PG Mix (NPK 15-10-20) 1 kg m^{-3}, Ca 1.78%, Mg 0.21%). Plants were fertilized once a week with 1% solution of Kristalon Green (NPK 18-18-18) with Mg, S and microelements during the vegetative phase of plant growth and with Kristalon Red (NPK 12-12-36) with microelements during the reproductive phase; in proportion v:v_{pot} = 1:50.

Cucumbers were analyzed between 27 October and 22 December 2020, totally 4 times. Number of analyzed plants 15. Three leaves were selected for each plant, where the severity of infection was assessed for each leaf, then the leaf was torn off and the reflection spectrum of the leaf was determined immediately. Totally 180 leaves were visually evaluated.

Powdery mildew (caused by *Podosphaera xanthii* and/or *Golovinomyces cichoracearum*), was observed at a three week interval. The severity was assessed for individual leaves according to the same 10-point scale:

0 point – leaf without powdery mildew symptoms (Fig. 1, a);
1 point – 1–5% of leaf with powdery mildew symptoms;
2 points – 6–10% of leaf with powdery mildew symptoms;
3 points – 11–15% leaf with powdery mildew symptoms;
4 points – 16–25% of leaf with powdery mildew symptoms;
5 points – 26–40% of leaf with powdery mildew symptoms (Fig. 1, b);
6 points – 41–55% of leaf with powdery mildew symptoms;
7 points – 56–70% of leaf with powdery mildew symptoms;
8 points – 71–85% of leaf with powdery mildew symptoms;
9 points – more than 86% of leaf with powdery mildew symptoms (Fig. 1, c).
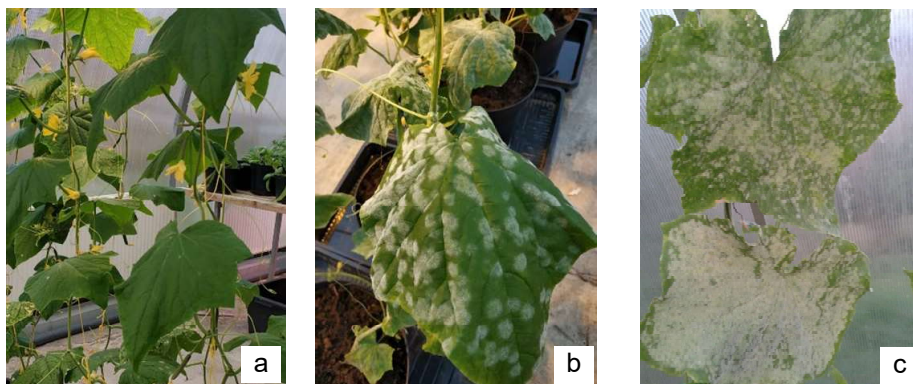
Figure 1. The severity of powdery mildew: a – healthy leaves; b – rated 5 points; c – rated 9 points.

Each leaf reflectance was measured with spectroradiometer RS-3500 (Ltd. Spectral evolution) in 10 replicates. Spectral range 350–2,500 nm, sampling bandwidth was 1 nm.

Indices used for the evaluation of cucumbers vitality and diseases detection are shown in Table 1.

Table 1. Vegetation indices (VI) calculated from leaf reflectance

Vegetation index	Abbreviation	Equation	Reference
Carotenoids	CRI1	$\dfrac{1}{W510} - \dfrac{1}{W550}$	Gitelson et al., 2001
Greenness Index	GI	$\dfrac{W554}{W677}$	Zarco-Tejada et al., 2001
Lichtenthaler index 1	LIC1 (NDVI)	$\dfrac{W800 - W680}{W800 + W680}$	Lichtenthaler, 1996
Lichtenthaler index2	LIC2	$\dfrac{W440}{W690}$	Lichtenthaler, 1996
Normalized Difference Vegetation Index	NDVI (1)	$\dfrac{W760 - W670}{W760 + W670}$	Padilla, 2017
Plant Senescence Reflectance Index	PSRI	$\dfrac{W678 - W500}{W750}$	Merzlyak et al., 1999
Structure Intensive Pigment Index	SIPI	$\dfrac{W800 - W445}{W800 - W680}$	Peñuelas & Filella, 1998, Zarco-Tejada et al., 2001
Simple Ratio Pigment Index	SRPI	$\dfrac{W430}{W680}$	Peñuelas et al., 1994
Water use efficiency	WBI3	$\dfrac{W950}{W900}$	Peñuelas et al., 1993
Water Index	WI	$\dfrac{W900}{W970}$	Peñuelas et al., 1997
Flavonoid reflectance Index	FRI	$\left(\dfrac{1}{W410} - \dfrac{1}{W460}\right)W800$	Skoczowski et al., 2021
Normalized Difference Index	CNDVI	$\dfrac{W750 - W705}{W750 + W705}$	Lu et al., 2018
Red Edge Index	REI	$\dfrac{W760}{W730}$	Padilla, 2017
Healthy index	HI	$\dfrac{W534 - W698}{W534 + W698} - 0.5W704$	Mahlein et al., 2013
Powdery Mildew Index	PMI	$\dfrac{W520 - W584}{W520 + W584} + W724$	Mahlein et al., 2013
Disease-Water Stress Index 1	DSWI-1	$\dfrac{W800}{W1660}$	Apan et al., 2004
Disease-Water Stress Index 2	DSWI-2	$\dfrac{W1660}{W550}$	Apan et al., 2004
Disease-Water Stress Index 3	DSWI-3	$\dfrac{W1600}{W680}$	Apan et al., 2004
Disease-Water Stress Index 4	DSWI-4	$\dfrac{W550}{W680}$	Apan et al., 2004
Disease-Water Stress Index 5	DSWI-5	$\dfrac{W800 - W550}{W1660 + W680}$	Apan et al., 2004

Wn – reflectance at a given wavelength.

To evaluate the difference between values of vegetation indices of healthy and infected plants the M value is used (Abdulridha et al., 2020). It is considered as a significant discriminant between different vegetation indices. As the M value increases better reparability is observed.

$$M = \frac{Mean_{healthy} - Mean_{infected(1\ point)}}{\sigma_{healthy} + \sigma_{infected\ (1\ point)}} \qquad \text{where } \sigma\text{- standard deviation} \qquad (1)$$

The critical value of vegetative index (VI_{crit}) was calculated by equation

$$VI_{crit} = Mean_{healthy} \cdot \sigma_{healthy} - \sigma_{infected\ (1\ point)} \qquad (2)$$

Using the obtained critical value of the vegetative index (VI_crit) and the corresponding line equation (Fig. 3), the identifiable powdery mildew threshold (PMT) was calculated.

RESULTS AND DISCUSSION

During the cucumber growing season, only symptoms of powdery mildew (caused by *Podosphaera xanthii* and/or *Golovinomyces cichoracearum*) were observed. The development of the disease was relatively intensive. The severity varied from 1 to 8 points during the observations.

Early detection of powdery mildew by using non-destructive methods can minimize direct human intervention and optimize schemes of fungicide application. Fig. 2, A shows the differences between the reflectance spectra of cucumber leaves. Different parts of the spectrum show different sensitivity. The largest differences between the leaves of different infection severity were found in the visible light part of the spectrum with a reflection maximum at 552–553 nm. (Fig. 2, B). Wavelengths around 550 nm also appear in other authors' works as a sensitive indicator of plant physiological and biochemical status (Alsiņa et al., 2016; Sytar et al., 2020; Skoczowski, et al., 2021) and health (Mahlein et al., 2012; Mahlein et al., 2013; Berdugo et al., 2014; Kuska et al., 2015; Lu et al., 2018).

The reflectance grows rapidly at the 'red edge' zone. Correlation between the severity of infection with powdery mildew and reflectance derivate is observed (Fig. 2, C). In the range from 699 nm to 712 nm, the correlation of the first derivative of reflectance spectrum with the degree of infection is less than -0.9, reaching a minimum at 703 nm ($R = -0.953$).

Wavelengths 720–960 nm indicate the peculiarities of the plant leaf surface cell structure (Roman & Ursu, 2016), the reflection spectra are relatively stable. In this part of the spectrum, the highest reflection is observed for leaves with low and medium infection degrees. The reflection spectra of severely infected leaves and asymptomatic leaves are not significantly different.

At the wavelength corresponding to the water absorption maximum (1,460 nm), it was found that there were no significant differences between the leaves with various levels of disease severity. That is in contradiction to the work of Lu and co-authors, where at this wavelength the affected leaves had lower reflection than healthy leaves (Lu et al., 2018).

To determine the severity of powdery mildew, the 21 vegetation indices shown in Table 2 were used. Correlation analysis was performed between the calculated vegetation indices and the severity of powdery mildew. A very strong correlation ($R > 0.8$) was found in only one case, with the calculated second Disease-Water stress index DSWI-2, coefficient of correlation $R = -0.84$. High correlation coefficients were also found for disease severity and SIPI and DSWI-5, in both cases $R = -0.797$. A correlation coefficient larger than 0.7 was also observed for the most widely used indices

characterizing plant physiological conditions NDVI and LIC-1 (Table 2). Analyzing the wavelengths used to obtain these vegetation indices, it was found that the most sensitive regions are 445, 550, 670, 680, 760, 800 and 1,600 nm. Similar wavelengths for the determination of plant health status are mentioned also by other authors. 540 and 660 nm are mentioned by Atanassova et al., 2019, spectrum ranges from 500–700 nm and 450 and 720 nm especially for powdery mildew by Mahlein et al., 2013; 650 and 700 nm by Berdugo et al., 2014; 500–730 nm by Abdulridha et al., 2020. Correlation coefficients for health index HI and DSWI-3 were between |0.6–0.7|: -0.674 and -0.603, respectively. Correlation coefficients above |0.5| were for the two vegetation indices used in the red edges (REI and CNDVI) and for indices that can be used to determine carotenoid and flavonoid content in plant leaves. Several authors (Lu et al., 2018; Atanassova et al., 2019; Abdulridha et al., 2020) mention changes in the water content of diseased plant leaves. In our experiment, the correlation between the indices for determining the water content and the prevalence of the disease was low ($R = \pm 0.054$).
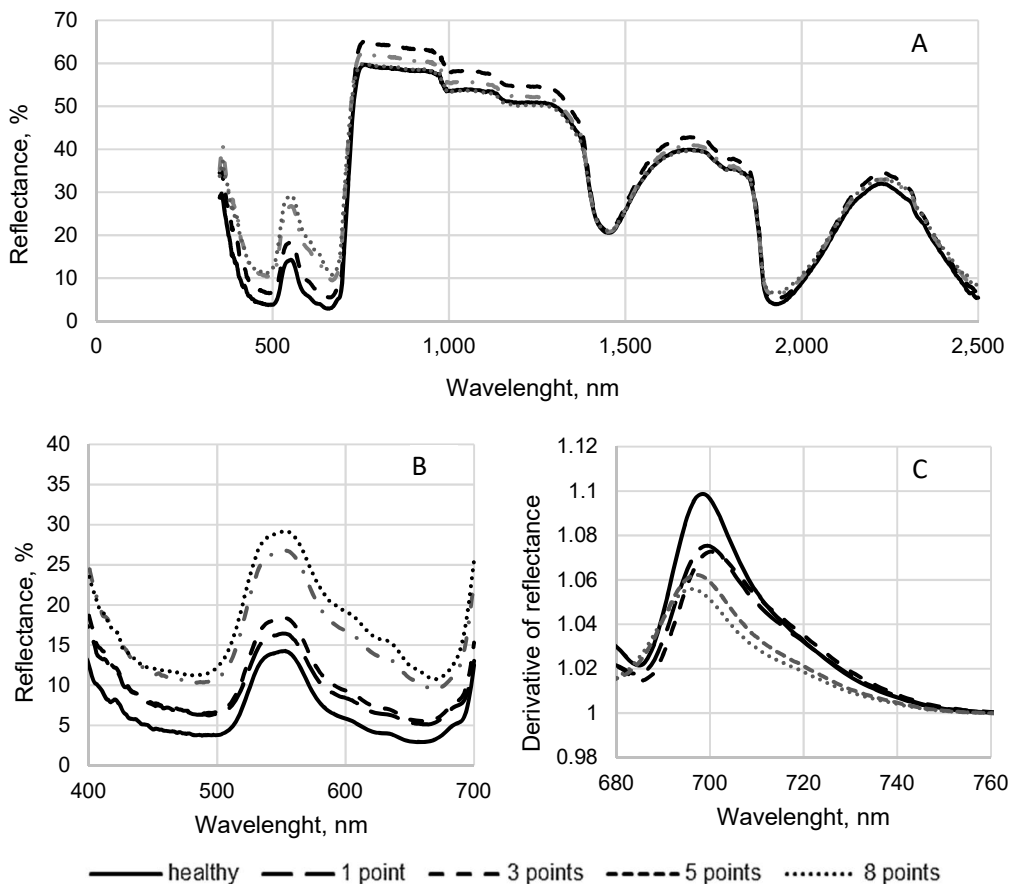
Figure 2. Average cucumber leaf reflectance spectra depending on the severity of mildew: A – spectral range within interval 350–2,500 nm; B – zoomed-in spectral range of 400–700 nm and C – first-order derivative zoomed in a wavelength of 680–760 nm.

The maxima (553 nm; 759 nm; 1,188 nm; 1,684 nm and 2,226 nm) and minima (1,452 nm and 1,925 nm) of the obtained reflectance spectra were used to find out whether it is possible to create indices with which would more accurately reflect the degree of mildew infection. Mentioned reflectance values were used to develop normalized difference and modified normalized difference indices. In total, 13 different indices were created, which were tested. The three best of the created indices are included at the end of the Table 2. The highest correlation coefficients (> 0.75) were obtained using the reflectance in 553 nm and 759 nm.

Table 2. Coefficients of correlation (R) between powdery mildew severity and calculated indices

Parameter	Vegetation index	Abbreviation	R
Plant structure and pigment content	Carotenoids	CRI1	-0.564
	Greenness	GI	-0.172
	Simple Ratio Pigment	SRPI	-0.070
	Structure Intensive Pigment	SIPI	**-0.797**
	Normalized Difference Vegetation	NDVI	**-0.729**
	Lichtenthaler 1	LIC-1	**-0.701**
	Lichtenthaler 2	LIC -2	-0.033
Biochemical content	Flavonoid reflectance	FRI	0.553
Plant Senescence	Plant Senescence	PSRI	-0.406
Water Use efficiency	Water use efficiency	WBI	0.054
	Water Index	WBI-2	-0.054
Red Edge	Red Edge Index	REI	-0.512
	Normalized Difference	CNDVI	-0.562
Plant Health	Healthy	HI	-0.674
	Powdery Mildew	PMI	0.483
	Disease-Water Stress	DSWI-1	-0.018
		DSWI-2	**-0.840**
		DSWI-3	-0.603
		DSWI-4	-0.118
		DSWI-5	**-0.797**
New generated Powdery Mildew Indices	$\dfrac{W759 - W553}{W759 + W553}$	PMI-1	**-0.859**
	$\dfrac{W759 - W553}{W1188}$	PMI-2	**-0.796**
	$\dfrac{W759 - W553}{W1684 - W1925}$	PMI-3	**-0.774**

$R_{0.05;\,36} = 0.332$.

The best results for the identification of powdery mildew were shown by the vegetation index DSWI-2, which has the highest correlation and determination coefficient (Fig. 3, Tables 2 & 3). Wavelengths of 550 and 1,660 nm were used to calculate the index value. Unfortunately, the M value for this index is lower than for SIPI and NDVI. That suggests that DSWI-2 is less sensitive to the early detection of powdery mildew. Calculations show that infected cucumber can be distinguished from a healthy plant when the visual assessment of the degree of mildew development is 2.7 points.
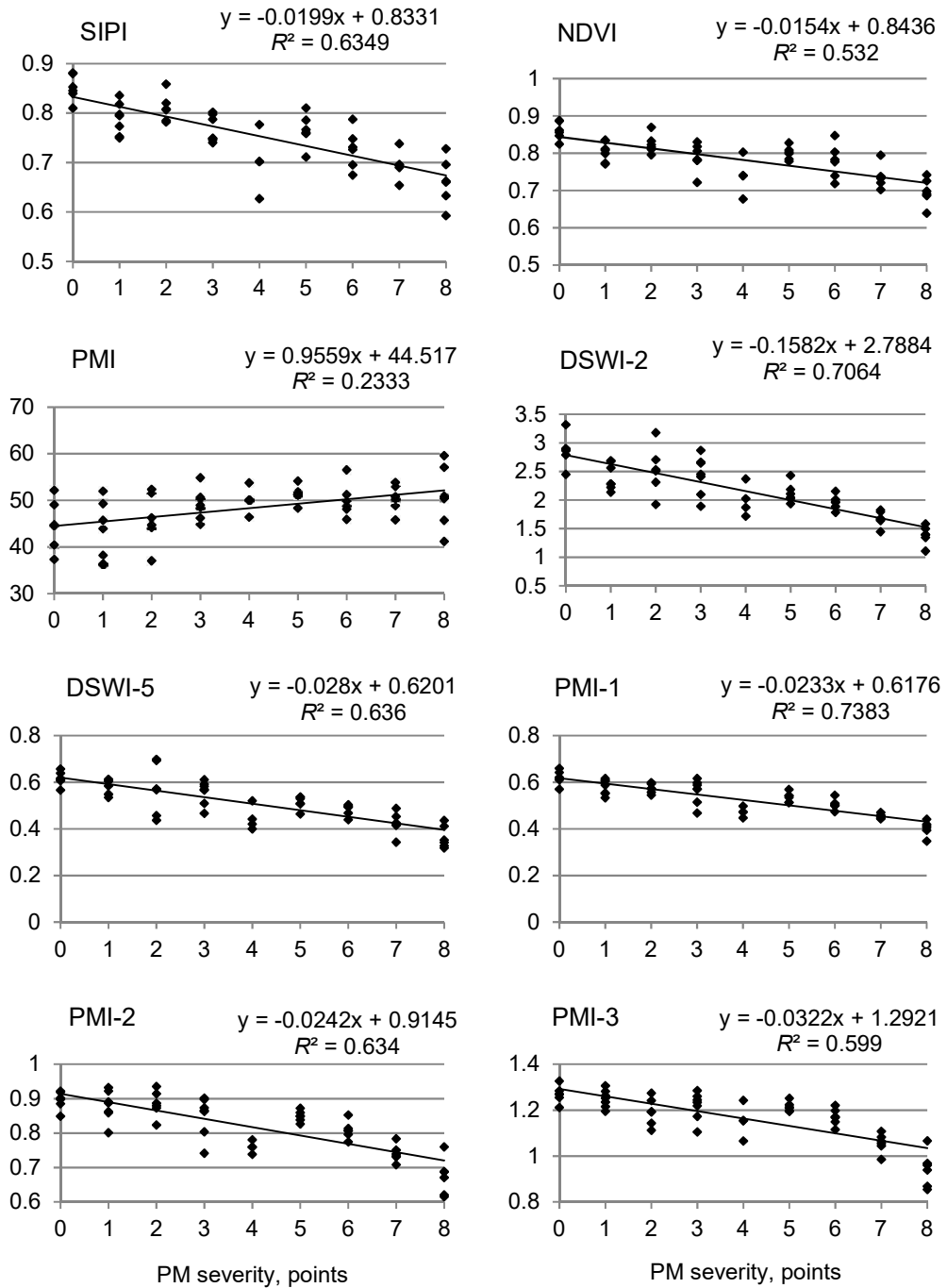
Figure 3. Relationship between powdery mildew (PM) severity and calculated vegetative indices (SIPI, NDVI, PMI, DSWI-2, DSWI-5), and created indices (PMI-1, PMI-2, PMI-3).

The most sensitive indices are SIPI (Structure Intensive Pigment Index) and the widely used NDVI (Normalized Difference Vegetation). The disease can be identified according to the visual assessments 2.0 and 2.2. The calculated critical values of SIPI and NDVI are 0.79 and 0.81, respectively (Table 3). Unfortunately, these indices do not have the necessary selectivity, as they are widely used (especially NDVI) to assess plant vitality, chlorophyll content, N supply (Haboudane et al., 2002; Mahlein et al., 2013; Alsiņa et al. 2016; Padilla et al., 2017; Atanassova et al., 2019; Alsiņa et al., 2020). The vegetation index PMI, specially developed for the determination of powdery mildew, in our experiments, showed low sensitivity. The critical value for identification of powdery mildew was 32.9, which corresponds to a visual assessment value of 5.5 points, making it less sensitive/effective compared to visual disease assessment. At this point, the disease has damaged nearly 50% of the plant photosynthetic area, which is too late for effective disease control.

Table 3. The fittingness of vegetative indices for the determination of powdery mildew on the leaves of the cucumber

Parameter	Abbrevation of vegetative index								
	SIPI	NDVI	PMI	HI	DSWI-2	DSWI-5	PMI-1	PMI-2	PMI-3
M	1.06	1.12	0.14	0.07	0.91	0.61	0.71	0.37	0.25
VI_{crit}	0.79	0.81	32.9	-12.4	2.36	0.55	0.56	0.83	1.19
PMT	2.0	2.2	5.5	5.4	2.7	2.4	2.7	3.6	3.2

The use of reflectance peaks can improve the correlation between the visual and calculated by reflectance spectrums severity of powdery mildew. The correlation coefficient for the index mentioned in literature DSWI-2 was -0.840, but the already established PMI-1 is -0.859. Unfortunately, the lowest detected powdery mildew threshold (PMT) lags behind the SIPI and NDVI mentioned in the literature.

The study demonstrates the potential of using a spectroradiometer for the identification of powdery mildew, but further research is needed to find more appropriate indices for the identification of powdery mildew at the early stages of the disease.

CONCLUSIONS

The strongest correlation between the degree of cucumbers infection with powdery mildew and the light reflectance spectrum was found in the green range of visible light around 550 nm.

Disease-Water Stress index-2 (DSWI-2), Structure Intensive Pigment Index (SIPI), Normalized Difference Vegetation Index (NDVI) and created index for powdery mildew detection (PMI-1) are the most suitable indices for determining powdery mildew in cucumbers. None of the studied indices allows determining the powdery mildew at the early stages of disease development (powdery mildew severity is below 2 points or less than 10% of cucumber leaves are infected).

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