

# POTENTIAL OF CHLOROPHYLL FLUORESCENCE AND VIS/NIR SPECTROSCOPY MEASUREMENT USE FOR THE DETECTION OF NITROGEN CONTENT AND DISEASE INFECTION OF APPLE LEAVES

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## Abstract

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A possibility of using spectral methods for determining a nutritional status and detecting pathogens in apple-tree cvs. 'Jonagold' and 'Idared' was verified in an orchard and pot experiments in 2007–2010. Treatments differed in the fertilizer or fungicide dose. Leaf samples were collected from the experimental variants to determine nitrogen content and to measure spectral reflectance (spectrophotometer Avantes USB 2000) and chlorophyll fluorescence imaging (FluorCam). Results of the measurements were correlated to leaf analyses for nitrogen content in dry matter. At the same time, a health status (the occurrence of fungal pathogens *Venturia inaequalis* and *Podosphaera leucotricha*) was assessed and changes of photochemical efficiency of PSII of infected leaves were evaluated. The parameters providing the best description of differences in the photosynthetic activity of leaves depending on treatments (parameter Fv/Fm and parameter GENTY, known as  $\Phi_{\text{PSII}}$  – effective quantum yield of PSII) were selected. The values of correlation coefficients of Fv/Fm and  $\Phi_{\text{PSII}}$  depending on fertilization treatments were as follows: Fv/Fm:  $r = -0.4735$ ,  $p < 0.000089$ ,  $\alpha = 0.05$ ;  $\Phi_{\text{PSII}}$ :  $r = 0.755$ ;  $p < 0.00038$ ,  $\alpha = 0.05$ . Data obtained from measuring with a spectrophotometer was used for the calculation to normalized difference vegetation indices NDVI; a significant relationship was found for the index GNDVI ( $r = 0.4691$ ,  $p < 0.0002$ ,  $\alpha = 0.05$ ). The significant difference between healthy leaves and leaves infected by the pathogens *V. inaequalis* and *P. leucotricha* was confirmed using the spectrophotometer, and the largest differences in reflectances were found in wavelengths around 400 nm. The values of indices GNDVI, RNDVI and NDVI 450 obtained from measuring reflectance of leaves with symptoms of *V. inaequalis* and *P. leucotricha* infections were significantly lower compared to the indices of healthy leaves. The values of indices NDVI were as follows: GNDVI 0.930; RNDVI 0.912; NDVI 450 0.917 for healthy leaves and GNDVI 0.519/0.623; RNDVI 0.428/0.540; NDVI 450 0.432/0.499 for leaves infected by pathogens *V. inaequalis*/*P. leucotricha*, respectively. There was found significant difference between infected and healthy leaves for all indices ( $\alpha = 0.05$ ). Also, the  $\Phi_{\text{PSII}}$  exhibited significant responses to the presence of *V. inaequalis* and *P. leucotricha* ( $\Phi_{\text{PSII}}$ : healthy leaves 0.182; *V. inaequalis*/*P. leucotricha* presence 0.232/0.222;  $\alpha = 0.05$ ).

apple tree, spectral reflectance, chlorophyll fluorescence, *Venturia inaequalis*, *Podosphaera leucotricha*, nitrogen content

Light energy absorbed by chlorophyll molecules in photosynthesis, for thermal energy, and it in chloroplasts of green plant leaves is used in is released to the environment or emitted in the three ways: for synthesis of simple sugars the form of red fluorescence radiation. These

processes are competitive among each other and stress conditions (high temperatures, drought, frost, deficient or abundant nutrients, infection by pathogens, herbicide effects) induce changes in the activity of the photosynthetic process. Use of photochemical energy decreases resulting in increased release of thermal energy, and especially fluorescence radiation. There is an inversely proportional relationship between the efficiency of photosynthesis and chlorophyll fluorescence. Upon UV-A excitation, the fluorescence spectrum of green leaves presents not only the red and far-red emission bands respectively centred near to 690 and 740 nm due to the emission of the protein-bound chlorophyll *a* in the chloroplasts of the subepidermal mesophyll cells, but also a maximum (430–450 nm) in the blue region and visible near to 520 nm (CHAPELLE *et al.*, 1984). A complex description of the photosynthetic apparatus activity, including distribution of loss processes of photosynthesis into photochemical and thermal is provided by quenching analysis, the most widespread technique of measuring chlorophyll fluorescence. The quenching analysis is based on highly-intensive flashes of a xenon lamp, so-called saturation light pulses. The measurement is usually carried out on leaves that were adapted to dark (for at least 15 min). Analysis of quenching kinetics for small regions can be used to diagnose distribution of the photosynthetic activity in leaves.

During the growing season, apple trees are exposed to different stress factors. In plant physiology, two basic groups of stress are identified: abiotic stress (chemical factors or mechanical damage) and biotic stress (it is caused by living organisms). Chlorophyll fluorescence imaging can determine changes in leaf photosynthesis induced by both types of stresses - abiotic and biotic.

Biotic factors influencing the plant metabolism and decreasing yield of crops are various infections (fungal, viral or bacterial). Pathogen infections induce changes in the primary metabolism of the affected plant tissue (CSÉFALVAY *et al.*, 2010). A lot of plant pathogens damage photosynthetic tissues (necrotic lesions, chloroses, yield reduction) (BEDBROOK & MATTHEWS, 1973). Tobacco mosaic virus probably acts on chloroplasts as a parasite damaging the photosynthetic apparatus indirectly through depleting nitrogen supplies which are used for the synthesis of ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco). A similar principle of influence can be found in various virus species (REINERO & BEACHY, 1989). CHAERLE *et al.* (2007) tested the use of chlorophyll fluorescence imaging for screening resistance to *Cercospora beticola* in sugar beet. The method is useful for automatic quantification of the stress induced by damage during diagnosis using fluorescence, i.e. before visual symptoms of infection, and for detection of pre-visual expressions of the pathogen presence in leaves of infected plants of sugar beet.

Abiotic factors causing stress can be added heat, drought or nutrient deficiency. They negatively influence the plant metabolism but as well as leaf photosynthesis. SCHAPENDONK *et al.* (1992) verified a possibility of using chlorophyll fluorescence for fast assessment of a photosynthetic status in plants. His experiments with maize plants exposed to water and cold stress confirmed a possibility to employ this method and demonstrated that it is a non-destructive technique detecting damage of the photosynthetic apparatus of plants. Cold stress was determined by a decrease in maximum quantum yield of PSII – Fv/Fm, water stress was determined by increased  $q_n$ . ZULINI *et al.* (2007) conducted experiments aimed at detecting drought influence on chlorophyll fluorescence and photosynthetic pigments in grapevine (cv. White Riesling). He compared leaves of grapevine grown under optimum conditions and those exposed to water stress. Leaf pigment concentrations (chlorophyll *a*, chlorophyll *b* and carotenoids) were not influenced by water stress, but in stressed plants, the correlation between Fv/Fm and chlorophyll concentrations was significant. They concluded that photosynthetic efficiency was only affected at a high intensity of drought. Fv/Fm could therefore be a good indicator to define threshold between moderate and excessive drought stress in the field.

HEISEL *et al.* (1999) investigated detecting of other stress factors which were caused especially by a lack (N, K, Mg) or excess (Ca) of nutrients in wheat, grapevine and apple trees. The effect of nitrogen supply on chlorophyll emission intensity in apple cv. 'Jonagold' was studied. Under 355 nm excitation, the intensities at 690 and 740 nm of the chlorophyll emission, as well as the blue/red and blue/far-red ratios, were modified by the nitrogen fertilization in an opposite way for rosette leaves (much lower intensities and slightly higher ratios when nitrogen was supplied) than for the 1-year short leaves (weak intensities increase and important decrease of the ratios). For 532 nm excitation, the fertilization decreased the red emission and increased the far-red intensities for both leaves, markedly for rosette leaves.

BREDEMIER & SCHMIDHALTER (2001) assumed a close relationship between nitrogen content in plants and chlorophyll synthesis, and a possibility to detect its content basing on changes in the fluorescence spectrum of plants. An important component of tetrapyrrole chlorophyll core is nitrogen and the chlorophyll component in leaves is correlated with nitrogen concentration. The author not only confirmed a relationship between fluorescence intensity and chlorophyll content in leaves, especially at 680 nm, but also a negative relationship between values of the fluorescence ratio 680/740 nm and chlorophyll values in winter wheat leaves. DALEY (1994) exposed tobacco plants to slight stress of nitrogen deficiency. Due to this stress, fluorescence quenching considerably changed, particularly during stomata movement.

LIMA *et al.* (1999) tested the effect of nitrogen and phosphorus deficiency on chlorophyll fluorescence in beans. He demonstrated that the phosphorus and nitrogen deficiency affected the growth and parameters of photosynthesis, and decreased maximum fluorescence yield by up to 25%.

Detection of physiological defects of apples using chlorophyll fluorescence during storage was studied by CISCATO *et al.* (2001). In apple cvs. 'Golden Delicious Smoothe' and 'Jonagold' he proved a possibility to use chlorophyll fluorescence for assessing physiological damage of fruits depending on storage potential prior to emerging symptoms. He found a relationship between total chlorophyll content and chlorophyll fluorescence intensity. In cv. 'Jonagold' internal damage could be visualized before emerging symptoms, especially in the range of fluorescence radiation of 690–740 nm.

DELALIEUX *et al.* (2009) tested a possibility of using fluorescence imaging to detect the stress induced at early development stages in the apple scab pathogen on leaves of cv. 'Breaburn'. Evaluation of fluorescence imaging showed that shortly after inoculation the leaves exhibited relatively low quantum efficiency of PSII. The  $\Phi_{\text{PSII}}$  values differed in control and scab inoculated leaves. The values of  $\Phi_{\text{PSII}}$  were around 0.92–0.97 for control leaves, while they ranged from 0.76–0.85 for scab inoculated leaves.

DELALIEUX *et al.* (2009) tested potential use of SWIR spectral domain (1,300–2,500 nm) to early detect apple scab infections. Leaves inoculated with scab spores showed a larger SWIR absorption area compared to the control leaves. An appropriate index 1480/2135 (calculated from the ratio of values measured in wavelengths of 1,480 and 2,135 nm) for detecting apple scab at early stages of its development was found.

The objectives of the work were to assess relationships between parameters of chlorophyll fluorescence and health status of apple trees, and to verify the effect of fungal pathogens *Venturia inaequalis* and *Podosphaera leucotricha* on the photosynthetic activity of plant tissues. The effect of nutrition on parameters of chlorophyll fluorescence, N content in leaves, yield and storage life of fruits (the presence of storage diseases) and other quality parameters (fruit size, content of refractometric dry matter) was verified too. The work was aimed at possible use of VIS/NIR spectral

reflectance for nitrogen content estimation as well as health status determination in apple leaves.

## MATERIALS AND METHODS

Experiments were conducted in 2007–2010 in a production apple (*Malus domestica* Borkh.) orchard with cvs. Jonagold and Idared, rootstock M9, 10 years old, trained in a slender spindle support system. Greenhouse experiments involved the identical cultivars, 3 years old, planted in pots of the 12 l volume. Variants differed in fertilizer type and dose (Tab. I); control variant was not fertilized. During the growing season, the experimental variants were treated by leaf spraying (an engine backpack sprayer Solo 444 with a hollow cone nozzle). Fertilized variants were treated with fungicides.

Leaf samples were collected from individual variants to determine nitrogen content according to Dumas (LEKO). To estimate N content in leaves, chlorophyll fluorescence imaging (FluorCam) and reflectance (Avantes USB 2000) were measured. In addition, chlorophyll fluorescence was imaged using a FluorCam (PSI s.r.o., Brno, Czech Republic) and reflectance was measured using a spectrophotometer Avantes USB 2000. The FluorCam is an instrument consisting of a CCD camera with the resolution of 512 × 512 pixels and sources of actinic light composed of a set of orange LED diodes. Uniform lightening of the 20 × 20 cm area enabled to measure a larger number of leaf samples at the same time without a risk of information loss. A time-consuming protocol of quenching analysis of modulated fluorescence by the saturation pulse method was used providing complex information about the efficiency of photosynthesis, the proportion of photochemical and non-photochemical losses. The measurement using FluorCam takes a large number of parameters, e.g.:  $F_0$ ,  $F_m$  at dark adapted state and e.g.:  $\Phi_{\text{PSII}}$  at light adapted state, after illumination with respectively actinic ( $I = 400 \mu\text{mol (photons).m}^{-2}.\text{s}^{-1}$ ) and saturating light ( $I = 1,800 \mu\text{mol (photons).m}^{-2}.\text{s}^{-1}$ ) intensities. Software for the recording and processing of the images has been developed by PSI s.r.o., Brno, Czech Republic. The measurement with a spectrophotometer Avantes USB 2000 (Avantes BV, Eerbeek, Netherlands) was performed in a reflectance probe of a circular shape of 10 mm diameter with artificial light source (a halogen lamp). The light was brought to the measuring

I: Crop treatment according to variants

Treatment	Commercial name of fertilizer	Active ingredient content	Dose (l, kg.ha <sup>-1</sup> )
1	untreated control		
2	SK SOL	K 26%, S 17%	0.3
3	SK SOL	K 26%, S 17%	0.6
4	Fruton Kombi	N 13.3%, CaO 21%, B 0.3%, Mn 0.55%, Zn 0.02%, MgO 2.2%	3
5	Fruton Kombi	N 13.3%, CaO 21%, B 0.3%, Mn 0.55%, Zn 0.02%, MgO 2.2%	6

area through a circular slit at 90°. At the angle of reflection (45°) an entrance lens was placed which brought the reflected beam by optic fibre to the spectrophotometer.

Chlorophyll fluorescence of apple-tree leaves was measured separately in each variant in four replications. Ten leaves of each replication were measured. Changes in the efficiency of photosynthesis were analysed at phenological stage BBCH 76 (the fruit size typical for the cultivar reached 60 %). Differences between treatments and N content and disease infection of leaves were tested with balanced ANOVA using software Statistica, version 8 (Statsoft Inc.). Due to a large number of parameters obtained from measuring the chlorophyll fluorescence, a multifactor analysis (analysis of main components) was used to identify usable parameters for N content in leaves estimation and plant pathogen presence detection. Indices NDVI were analysed by one-way balanced ANOVA for pathogen presence detection. The correlation between parameters of chlorophyll fluorescence and N content in leaves was determined using linear regression. To determine correlation between indices NDVI obtained from spectrophotometry Avantes 2000 and N content in leaves, linear regression was also used.

The parameters  $F_m/F_v$  and  $\Phi_{PSII}$  represent the evaluation of quenching analysis:

- $F_m$  is a maximum yield of fluorescence of dark adapted samples.
- $F_v$  is a total amount of variable fluorescence of dark adapted samples,

Upon the application of saturating flash ( $I = 1,800 \mu\text{mol (photons). m}^{-2}\text{s}^{-1}$ ) fluorescence raised from state value  $F_0$  to maximum value  $F_m$ . This measurement allowed the determination of the maximum quantum efficiency of photosystem II (PSII) primary photochemistry, given  $F_v/F_m$ . The parameter  $F_v/F_m$  is very useful to extend photoinhibition of photosynthesis.

- Effective quantum yield of PSII –  $\Phi_{PSII}$  describes the proportion between part of fluorescence

radiation  $F_q'$  and maximum fluorescence  $F_m'$  of light adapted state. It is calculated as:

$$F_q'/F_m' = (F_m' - F')/F_m'$$

$F_q'/F_m'$  has also been termed GENTY, Delta  $F/F_m'$ ,  $(F_m' - F')/F_m'$  and  $(F_m' - F_s)/F_m'$ . To calculate this parameter, only  $F_m'$  and  $F'$  are needed to be determined; both parameters can be measured in the light, i.e. no dark-adapted leaf material is needed (LEIPNER, 2007).

The parameter was correlated to leaf analyses for nitrogen content in dry matter.

The measurement with a spectrophotometer Avantes USB 2000 was performed on leaves of each variant in four replications. Individual leaves were placed under a light source and their reflectance was measured.

Based on data obtained from measuring with a spectrophotometer, reflectance was calculated by converting the measured values to the spectralon reflectance standards, and the calculation to normalized difference vegetation indices (NDVI) was used. NDVI indices can be calculated using the reflectances determined:

Green normalized difference vegetation index	$GNDVI = (R_{780} - R_{550}) / (R_{780} + R_{550})$ , APARICIO <i>et al.</i> (2000)
Red normalized difference vegetation index	$RNDVI = (R_{780} - R_{670}) / (R_{780} + R_{670})$ , RAUN <i>et al.</i> (2001)
Normalized 450 difference vegetation index	$NDVI_{450} = (R_{780} - R_{450}) / (R_{780} + R_{450})$ , BRONSON <i>et al.</i> (2005)

note:  $R_x$  describes indicated light reflectance in specific wavelength (nm).

To estimate nitrogen content in leaves, chlorophyll fluorescence imaging (FluorCam) and reflectance (spectrophotometer Avantes USB 2000) were used. At the same time, a health status of evaluated trees, the occurrence of the fungal pathogens (*V. inaequalis* and *P. leucotricha*) and changes in the photosynthetic

## II: Abbreviations

$F_m$	maximum chlorophyll fluorescence yield in dark-adapted state
$F_v$	variable chlorophyll fluorescence yield in dark-adapted state
$F_v/F_m$	maximum quantum yield of photosystem II photochemistry
$\Phi_{PSII}$	effective quantum yield of PSII
GNDVI	green normalized difference vegetation index
NDVI	normalized difference vegetation index
$NDVI_{450}$	normalized difference vegetation index (450 nm)
NIR	near infrared spectroscopy
PSII	photosystem II
RNDVI	red normalized difference vegetation index
SWIR	short wave infrared
VIS	visible spectroscopy

activity and reflectance of infected leaves were evaluated.

List of abbreviations is shown in Table II.

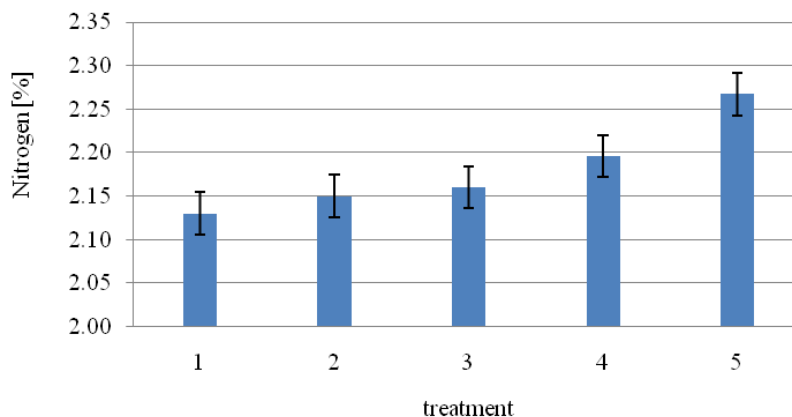
## RESULTS AND DISCUSSION

Measuring the photosynthetic activity of leaves demonstrated differences related to the treatment variant. The biggest statistical significant difference in nitrogen content was found between the untreated control and the variants treated with leaf fertilizer Fruton Kombi (variants No. 4, 5) (Fig. 1) only (confidence level  $\alpha = 0.05$ ). No increase in the nitrogen content in dry matter of leaves was confirmed for variant No. 2 (SK SOL 0.3 l.ha<sup>-1</sup>) in comparison with the untreated control.

Chlorophyll fluorescence parameter Fv/Fm and effective quantum yield of PSII –  $\Phi_{PSII}$  provided the best description of differences in the photosynthetic activity of leaves depending on N fertilization treatments. The highest values of Fm/Fv parameters were found for the untreated control. Their values decreased at rising nitrogen content in apple-tree leaves (Fig. 2).

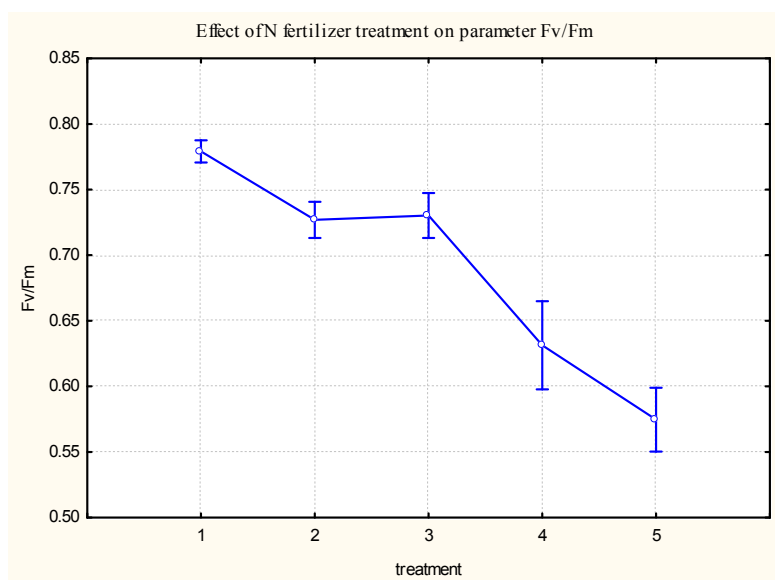
Having verified the relationship between nitrogen content in leaf dry matter and the parameters of chlorophyll fluorescence, correlation analysis was carried out confirming negative significant relationships between the nitrogen content and the parameters Fv/Fm (Fig. 3).

The variants with higher nitrogen content (compared with untreated control) increased the yield of fluorescence  $\Phi_{PSII}$ , expressed as GENTY



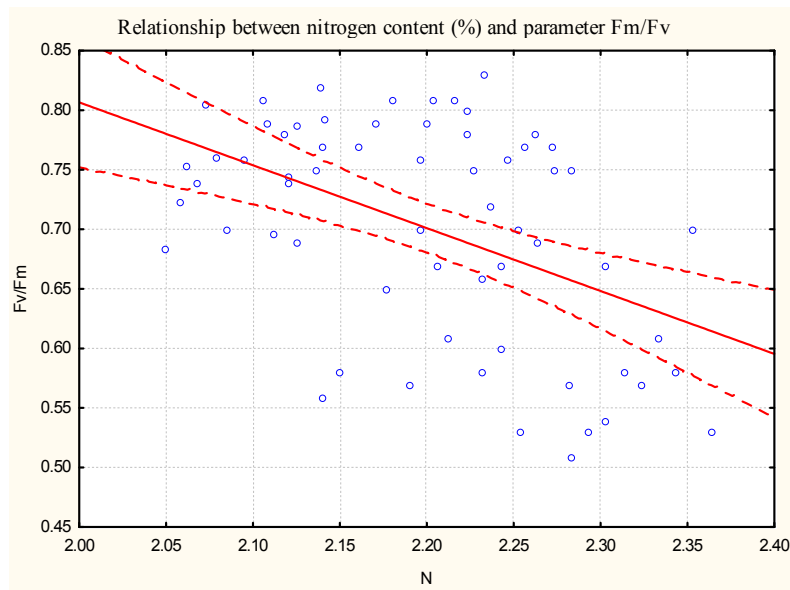
1: Nitrogen content in apple-tree leaves, cv. Idared, 2009

Note: Confidence level  $\alpha = 0.05$

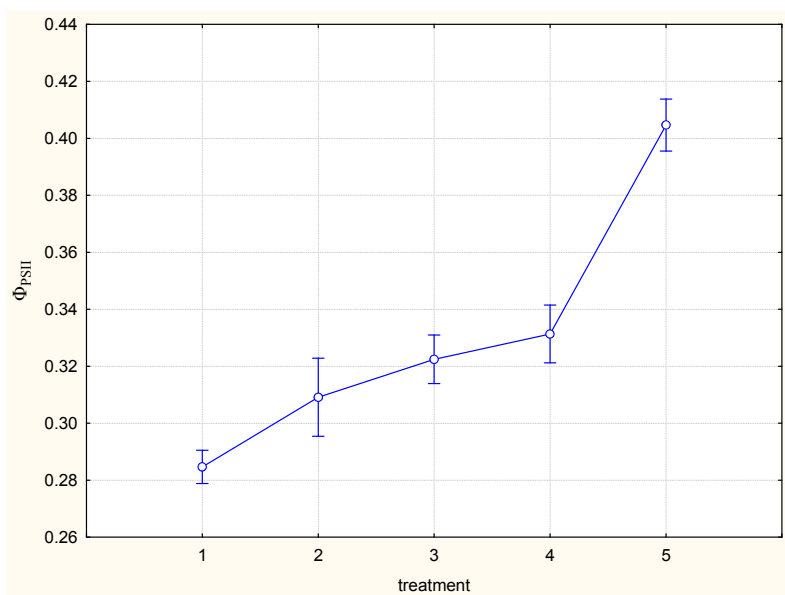


2: Effect of N fertilizer treatment on parameter Fv/Fm in cv. Idared, 2009

Note: Confidence level  $\alpha = 0.05$ ,  $p < 0.0000$



3: Relationship between nitrogen content (%) and parameter  $F_v/F_m$  in cv. Idared, 2009  
Note:  $r = -0.4735$ ,  $p < 0.000089$ , confidence level  $\alpha = 0.05$



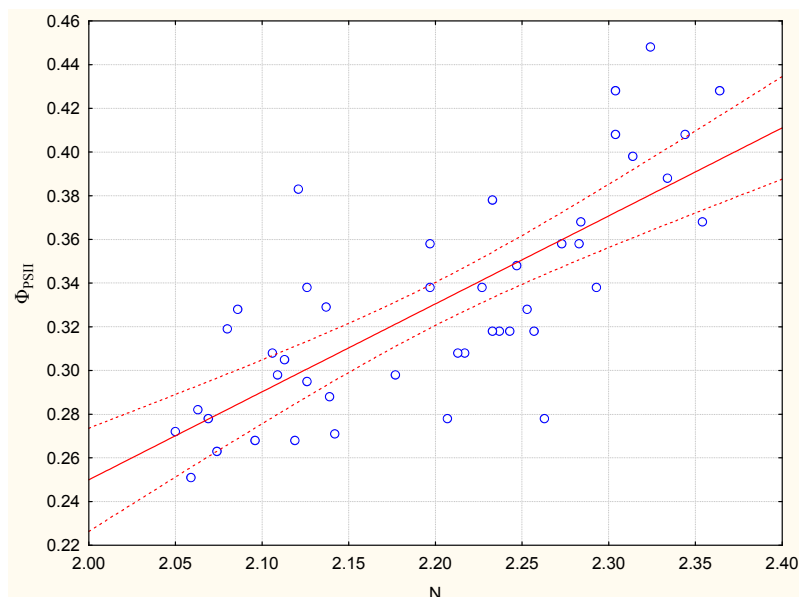
4: Effect of treatment on  $\Phi_{PSII}$  in cv. Idared, 2009  
Note: Confidence level:  $\alpha = 0.05$ ,  $p < 0.0000$

(Fig. 4). The highest value of this parameter was observed in variant No. 5, and a significant difference was found between the control and this variant. Correlation analysis confirmed a significant positive relationship between the nitrogen content and the effective quantum yield of PSII  $\Phi_{PSII}$  (Fig. 5). Parameters obtained from measuring with FluorCam were visualized to pre-determine the differences between treatments. Visualization allows to evaluate the spatial distribution at leaf. Visualization process was very rapid with relatively high estimation accuracy (Figs. 6, 7).

To verify the relationship between nitrogen content in leaves and NDVI indices, linear

regression analysis was performed. The values of correlation coefficients of NDVI indices depending on fertilization treatments were as follows: index GNDVI  $r = 0.469^*$ ; index RNDVI  $r = 0.107$ ; index NDVI 450:  $r = 0.0311$  (\*statistically significant correlation). When checking the relationship between nitrogen content in leaves and NDVI indices, the index GNDVI was chosen as the best for spectrophotometer.

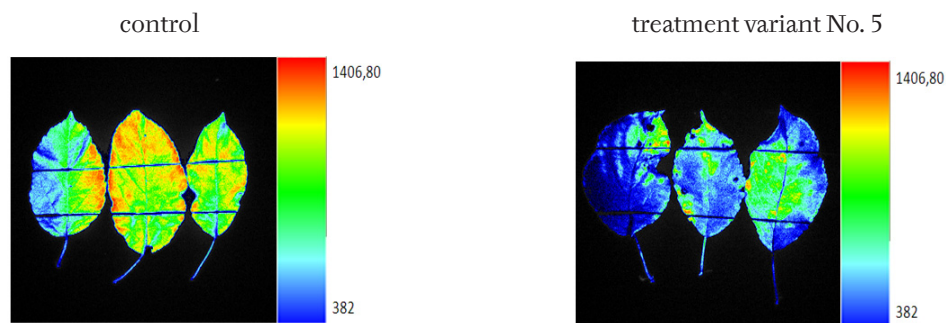
Differences in treatment variants are well described by the above-mentioned NDVI indices (Fig. 8). In the index GNDVI (Fig. 9) a significant relationship with nitrogen content was found only.



5: Relationship between nitrogen content and  $\Phi_{PSII}$  (cv. Idared)

Note: Correlation coefficient:  $r = 0.755$ ,  $p < 0.00038$ , confidence level:  $\alpha = 0.05$

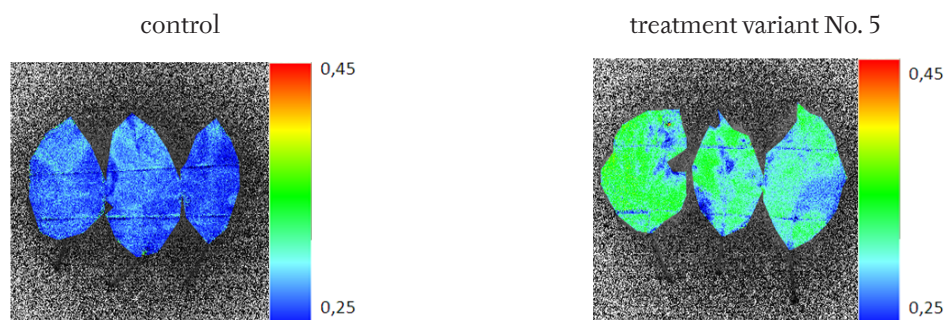
#### Parameter Fm



6: Visualization of the parameter Fm depending on a nutrition variant, cv. Idared, 2009

Note: The colours show low values in blue and high values in red. The rainbow bar on the right of each image row shows the scaling.

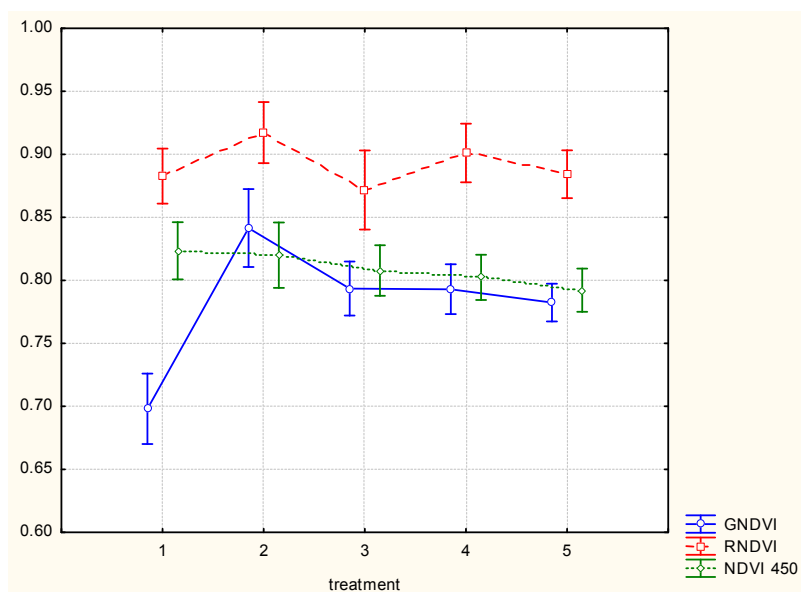
#### Parameter yield of fluorescence $\Phi_{PSII}$



7: Visualization of the  $\Phi_{PSII}$  depending on a nutrition variant, cv. Idared, 2009

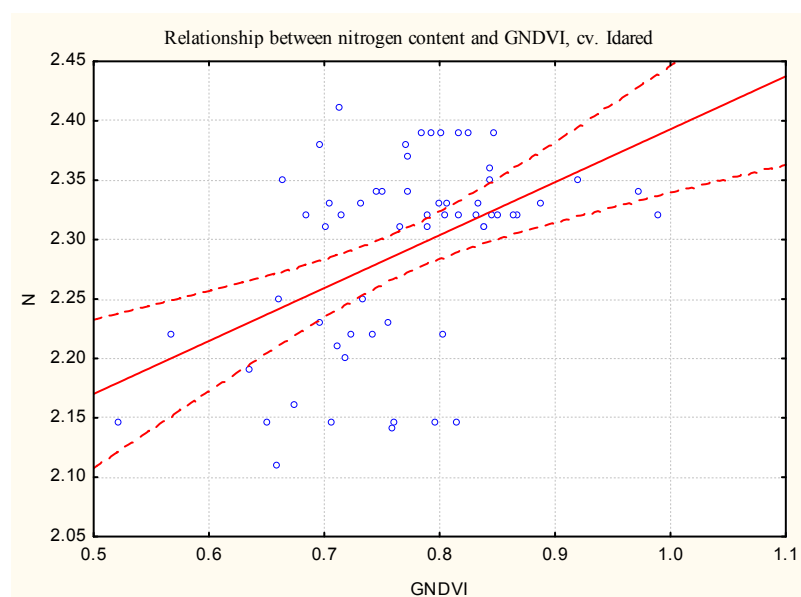
Good results were obtained at testing possibilities of using spectral methods for detecting diseases of apple-tree leaves caused by the fungal pathogens *V. inaequalis* and *P. leucotricha*. Statistical analysis of values obtained from measuring with a spectrometer

Avantes USB 2000 confirmed a significant difference between infected and healthy leaves. The biggest differences in reflectances between infected leaf tissues and the healthy control were found in wavelengths around 400 nm.



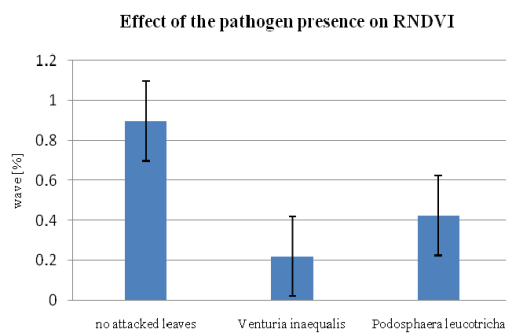
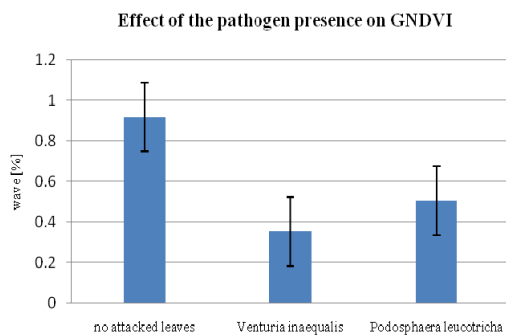
8: Effect of treatment on NDVI in cv. Idared, 2008

Note: Confidence level:  $\alpha = 0.05$ ,  $p < 0.0000$



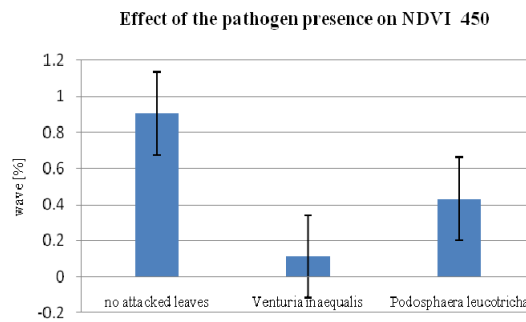
9: Relationship between nitrogen content and GNDVI (cv. Idared), 2008

Note: Correlation coefficient: 0.4691,  $p < 0.0002$ , confidence level:  $\alpha = 0.05$



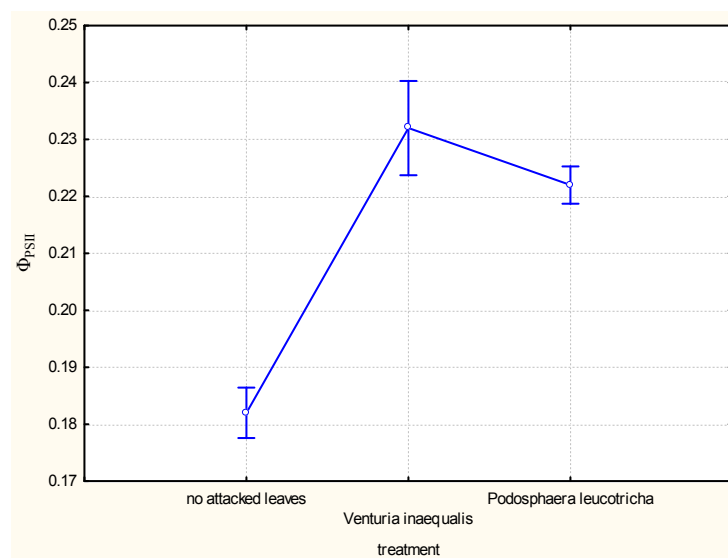
10, 11: Effect of the pathogen presence on apple-tree leaves, cv. Idared, on NDVI indices – GNDVI – RNDVI

Note: confidence level:  $\alpha = 0.05$



12: Effect of the pathogen presence on apple-tree leaves, cv. Idared, on NDVI 450

Note: confidence level:  $\alpha = 0.05$



13: Reaction of  $\Phi_{PSII}$  on pathogen presence

Note: confidence level:  $\alpha = 0.05$

The effect of the pathogen presence on apple-tree leaves on NDVI indices is demonstrated. The indices GNDVI, RNDVI and NDVI 450 were significantly affected by pathogen presence. The reflectance of leaves with symptoms of *V. inaequalis* and *P. leucotricha* infections was significantly lower for these indices (Figs. 10, 11, 12).

The  $\Phi_{PSII}$  was selected from parameters of chlorophyll fluorescence because it provided the best description of differences in photosynthetic activity of healthy and infected leaves. Increased effective quantum yield  $\Phi_{PSII}$  was found in infected leaves. A stronger rise of  $\Phi_{PSII}$  was found in leaves with *V. inaequalis* presence whereas the effect of *P. leucotricha* infection on the parameter was lower. It was confirmed that this parameter exhibited statistically significant reactions to the presence of both pathogens (Fig. 16). In agreement with DELALIEUX *et al.* (2009), effective quantum yield  $\Phi_{PSII}$  was useful in detecting diseases in leaf with its characteristic decrease during infestation of leaves. The infected leaves expressed typical  $\Phi_{PSII}$  pattern

that was already reported by DELALIEUX *et al.* (2009).

## CONCLUSION

During the study of the photosynthetic activity, differences related to the treatment variant were confirmed.

Verifying the relationship between nitrogen content in apple-tree leaves and measured chlorophyll fluorescence parameters, a significant relationship was confirmed for the quantum yield  $\Phi_{PSII}$  and a relationship for the parameters Fv/Fm.

A significant difference was confirmed between infected and healthy leaves at testing possibilities of using spectral methods for detecting diseases of apple-tree leaves caused by the fungal pathogens *V. inaequalis* and *P. leucotricha* with the spectrometer Avantes USB 2000 using normalized indices NDVI, especially in wavelengths around 400 nm.

Statistical assessment of the parameters of chlorophyll fluorescence  $\Phi_{PSII}$  confirmed significant responses to the presence of the pathogens *V.*

*inaequalis* and *P. leucotricha*. There was no significant difference in quantum yield  $\Phi_{\text{PSII}}$  between individual diseases.

Our results document that spectral reflectance (spectrophotometer Avantes USB 2000) and

chlorophyll fluorescence imaging (FluorCam) methods are suitable for detecting a health and nutritional status of apple trees.

## SUMMARY

This work deals with assessing the possibility of using spectral methods – chlorophyll fluorescence and spectroscopy – for determining a nutritional status (nitrogen content) and detecting pathogens (*Venturia inaequalis* and *Podosphaera leucotricha*) in apple tree. The results from seasons 2007–2010 showed that measuring photochemical efficiency of PSII of leaves using chlorophyll fluorescence or measuring reflectance using spectroscopy can demonstrate differences related to the presence of pathogens and nitrogen content in leaves. The effective quantum yield of PSII –  $\Phi_{\text{PSII}}$  provided the best description of differences in the photosynthetic activity of healthy and infected leaves ( $\Phi_{\text{PSII}}$ : healthy leaves 0.182; *V. inaequalis*/*P. leucotricha* presence 0.232/0.222;  $\alpha = 0.05$ ). The significant difference between healthy leaves and leaves infected by the pathogens *V. inaequalis* and *P. leucotricha* was confirmed using the spectrophotometer, and the largest differences in reflectances were found in wavelengths around 400 nm. The values of indices NDVI were as follows: GNDVI 0.930; RNDVI 0.912; NDVI 450 0.917 for healthy leaves and GNDVI 0.519/0.623; RNDVI 0.428/0.540; NDVI 450 0.432/0.499 for leaves infected by pathogens *V. inaequalis*/*P. leucotricha*, respectively. There was found significant difference between infected and healthy leaves for all indices ( $\alpha = 0.05$ ). The relationship between nitrogen content in leaf dry matter and the parameters of chlorophyll fluorescence  $\Phi_{\text{PSII}}$ , Fv/Fm was significant. The values of correlation coefficients of Fv/Fm and  $\Phi_{\text{PSII}}$  depending on fertilization treatments were as follows: Fv/Fm:  $r = -0.4735$ ,  $p < 0.000089$ ,  $\alpha = 0.05$ ;  $\Phi_{\text{PSII}}$ :  $r = 0.755$ ;  $p < 0.00038$ ,  $\alpha = 0.05$ . A significant relationship was found for spectrophotometer index: GNDVI ( $r = 0.4691$ ,  $p < 0.0002$ ,  $\alpha = 0.05$ ). The results document that imaging methods are suitable for detecting a health and nutritional status in apple trees.

## Acknowledgements

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