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INFLUENCE OF LATENT INFECTION BY APPLE STEM GROOVING VIRUS (ASGV) ON THE DEVELOPMENT OF YOUNG APPLE TREES CV. FLORINA – PRELIMINARY RESEARCH INVESTIGATION

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Abstract
Article presents the results of a survey of some morphological and physiological indicators of young apple trees infected with Apple Stem Grooving Virus, (ASGV). Trees were symptomless for viral infection during the period of apple orchard monitoring (2013 – 2014). Samples of young leaves collected from 23 apple trees cv. Florina were tested using enzyme linked immunosorbent assay (ELISA). Trees with extinction values at least three times greater than the negative control were defined as infected. Trunk diameter of infected trees increased statistically significant compared to control. Spectral reflectance characteristics obtained by remote sensing method of leaf reflectance confirmed the presence of two groups of infected trees against the group of healthy (control) trees. With calculated vegetation indices were corroborated the changes in some indicators characterizing the evolution and habitus of trees. Good correlation between vegetation indices and extinction values obtained by ELISA was found. Results of spectral analyses indicate that the leaf spectral reflectance is a reliable tool for early detection of stress in young apple trees.
Key words: young apple trees cv. Florina, ASGV infection, trunk diameter, remote sensing, vegetation index

Apple stem grooving virus (ASGV) is the type member of genus *Capillovirus* in the family *Betaflexiviridae* (King et al., 2012). According to Welsh and van der Meer (1989) ASGV is symptomless in most commercial cultivars and many authors reported that infection produced no disease symptoms (Yanase et al., 1990; Chen et al., 2014). Scions grafted onto the tolerant rootstocks *Malling* (M series) and *Malling-Merton* (MM series) display no symptoms of the disease.

Host plant species of the ASGV are *Aronia melanocarpa* (black chokeberry), *Malus domestica* (apple), *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus persica* (peach) (Internet link 1). The virus would cause symptoms in indicator plants such as *M. sylvestris* cv. Virginia Crab (Nemeth, 1986; Yanase et al., 1990), and in *M. micromalus*, *M. tschonoskii* and *M. yunnanensis* (Howell et al., 1996).

There are several methods to detect the ASGV infection. One of the methods is the using of biological indexing on woody indicator plants (Minoiu et al., 1983; Howell et al., 1996). In literature have been reported several serological (ELISA – Wu et al., 1998; Cagayan et al., 2006; Milusheva, 2014; and molecular (RT-PCR – Kundu, 2002; Marinho et al., 2003; Caglayan et al., 2006; Ji et al., 2013) methods and their modifications for detecting of ASGV infection in apple trees. In last decade the remote sensing (RS) techniques are increasingly valued as useful tool for investigation of plant stress. The spectral reflectance measurements, acquired in a high number of contiguous spectral bands in the visible (VIS) and near infra-red (NIR) spectral ranges, increases the potential to detect anomalies in the normal plant growth and production processes at an early stage, before the damage is occurred (Delalieux et al., 2007; Krezhova et al., 2012).

According Cembali et al. (2003) an ASGV infection could reduce to 27% the crop of an infected apple garden. ASGV infects many commercial apple cultivars with an infection rate up to 80 – 100% and cause yield loss up to 40% (Rana et al., 2011).

The aim of the present work is, using some of methods for virus detection to analyse the effect of ASGV on the development of young apple threes cv. Florina in a period when there are still not symptoms of infection.

MATERIAL AND METHODS

Plant material. The observed private non-comercial orchard in the vicinity of the town of Kostinbrod was planted in the end of 2012 year. The trees of apple cultivar Florina, propagated onto MM 106 stock, were planted in two rows 3 m x 3 m. Basic technological requirements were followed by the owner (Prodanov, 2005). The observations were carried out during 2013 and 2014 years. In the beginning of the first vegetation leaf samples were collected from 23 trees. All leaves were symptomless for virus infection. Trunk diameter of the same trees has been measured 0.5 m up to soil line for both years.

Virus detection. Double antibody sandwich-enzyme linked immune-sorbent assay (DAS-ELISA) was used for the virus detection. The test was performed on the leaves collected in the beginning of first vegetation (in 2013 year) and was realized with diagnostic reagents of LOWWE Ltd for Apple trunk grooving virus (ASGV). Threes with extension values at least three times greater than that of the negative control were defined as infected.

Leaf spectral reflectance. The remote sensing (RS) method of spectral reflectance (SR) was applied to assess the presence of stress in 10 of investigated apple trees during first vegetation. Hyperspectral reflectance data were collected from fresh detached leaves in VIS and NIR ranges (450 – 850 nm) of the electromagnetic spectrum by means of a portable fibre-optics spectrometer USB 2000 (Ocean Optics). In the investigated spectral range is concentrated the main part of the reflected from leaves radiation. Data were obtained at 1170 spectral bands with a step of 0.3 nm and a spectral resolution of 1.5 nm (Krezhova et al., 2014).

Mathematical and statistical methods. The data for trunk diameter of the trees were separated in three groups depending on their leaves extinction values (EV) – uninfected (EV < 0.400), plants with 0.417 < EV < 0.500 and plant with EV > 0.500. These groups formed a set of 3 variants as the first group was the control. The data, separately for every year, were processed by analysis of variance using F for test estimation and LSD (at level of significance P < 0.05, P < 0.01 or P < 0.001, depending of variation within the groups) for estimation of obtained differences between variant's means. The significance of reflectance sensitivities for infected and uninfected leaves of 10 of the observed trees was determined by t-student test. Reflectance in particular wavelengths is selected for calculation of some vegetation indices cite in literature. Statistical package of programs was used for analysis and calculations (Maneva, 2007).

RESULTS AND DISCUTION

Apple stem grooving virus (ASGV) was detected at different level of extinction values (EV) obtained by DAS_ELISA in the end of first vegetation (Figure 1). Trees with EV lower than the cut of extinction value (EV = 0.417) were 52.34% of all trees, 17.23% had EV near to the cut off value (T6, T21, T22 and T23) and 30.43% with EV > 0.417 (T1, T2, T4, T9, T12, T14, T16 and T18). After first vegetation T1 (EV = 0.551) died.

Data set, obtained from the measurement of the trunk diameter for both years of the investigation, showed that the infected trees (EV > 0.417) had larger diameter compared to control. The trunk diameter increases with increase of the EV of the respective trees (Figure 2). On the same figure is presented the linear dependence of the trunk thickness on the tree's EV. The coefficients of the linear regression were statistically significant – R = 0.752 (P < 0.05) and R = 0.793 (P < 0.05) for respective year.

Results of analysis of variance showed that diameter of the trunks for both groups infected plants (0.417 < EV < 0.500 and EV > 0.500) was significantly greater compared to control trees (Table 1 – **A**). There was not statistical significant difference between average diameter of trees with 0.417 < EV < 0.500 and those with EV > 0.500 (P > 0.500). This was the reason to assume these two groups as one – infected trees. United group was statistically significant different than the control for both years of the monitoring (P < 0.05), (Table 1 – **B**). There was a positive correlation between EV and the diameter of the trunks – the increase in extinction values leads to an increase in the diameter (**r** 2013 = 0.657 and **r** 2014 = 0.692 at P < 0.05).

Spectral reflectance. Averaged leaf spectral reflectance characteristics (SRC) from 7 of 10 investigated trees which were infected with ASGV (EV > 0.417) formed two groups, the first – SRC of trees with 0.417 < EV < 0.500 and the second – with EV > 0.500. Figure 3 shows the averaged SRC of each group. The values of

Table1. Average trunk diameter of young apple trees – statistical significance of the differences between mans
Таблица 1. Среден диаметър на стеблото на млади ябълкови дървета – статистическа достоверност на разликите между средните

Variant	**A**: Trunk thickness – cm		**B**: Trunk thickness – cm	
	2013	2014	2013	2014
Control – EV < 0.390	3.50 C	4.05 C	3,50 C	4.05 C
Infected – EV < 0.500	3.93 + C	4.97 + C	Infected – EV > 0.500* Infected – EV > 0.390	
Infected – EV > 0.500	4.07 + ns	5.10 + ns	4.00 +	5.04 +
F $LSD_{0.05}$	*16.94* *0.23*	*17.97* *0.53*	*12.94* *0.23*	*13.97* *0.53*

*EV – extinction values; ns - ; + (P < 0.05); ++ (P < 0.01); +++ (P < 0.001).

Table 2. Spectral vegetation indices, their formulas and coefficients of correlation (r) between indices and respective extinction values
Таблица 2. Вегетативни индекси и техните формули и коефициенти на корелация (r) между индексите и съответните екстинционни стойности

Index	Control	Infected	r	Formula	References
Physiological Reflectance Index – PRI	0.032	0.030 **	-0,171	$\frac{R531 - R570}{R531 + R570}$	Gamon et al., 1992
Normalized Difference Vegetation Index – NDVI	0.857	0.710 ***	-0.543 *	$\frac{R765 - R650}{R765 + R650}$	Rouse et al., 1974
Chlorophyll Index – ChI	1.745	1.670 ***	-0.694 **	R770/R710	Gitelson et al., 2005
Chlorophyl Absorption Reflectance Index – CARI	40.73	34.26 ***	-0.723**	1*	Kim et al., 1994
Moified CARI – MCARI	15.04	17.08 ++	0.689 *	2*	Daughtry et al., 2000
Vegetation Fraction – VF	0.441	0.404 **	-0.608 **	$\frac{R550 - R670}{R559 + R670}$	Gitelson et al., 2002a
Greenness Index – GI	2.565	2.213	-0.637 *	R554/R677	Zarco-Tejada, 2005

Ri = reflectance at I nm specific wavelength (e.g., R 550 nm);
1* CARI = p700|α670 + p670 + b|/ [p670(α2 + 1)0.5, where α = (p700 – p550)/150, b = p550 – 550 α;
2* MCARI = [(P700−P670) − 0.2(P700−P550)](P700/P670)
Statistical significance of mean differences and correlation coefficients at level:
(*) P < 0.05, (**) P < 0.01, (***) P < 0.001.

SRC of the second group are higher in the blue, green, red and NIR regions of the spectrum (450 – 850 nm). The most sensitive spectral reflectance range as determined from the data analyses was located in NIR region (730 – 850 nm). This was followed by selected ranges in the red region (640 – 680 nm) which shows maximum chlorophyll absorption and then in green (520 – 580 nm) near 550 nm. All differences between SRC in those regions were statistically significant (P < 0.01).

Vegetation indices. Numerous vegetation indices have been developed and many are reported to be correlated with plant condition, stress, vegetation fraction, or chlorophyll content (Gamon et al., 1992; Rouse et al., 1974; Vidal et al., 1994; Kim et al., 1994; Daughtry et al., 2000; Gitelson et al., 2002; 2005; Zarco-Tejada, 2005). In Table 2 are presented vegetation indices calculated on the base of respective spectral characteristics of ASGV infected and uninfected leaves of apple trees. Depending on changes of leaves attributes caused by viral infection, indices significantly increased (MCARI with 11.56%) or decreased (ChI<PRI<VF<GI<CARI< NDVI with 4.3, 6.2, 8.4, 13.79, 16.0 and 17.0% respectively) compare to control. All indices (except PRI) correlated well (significant r at P < 0.005 or P < 0.001) whit respective EV (Table 2).

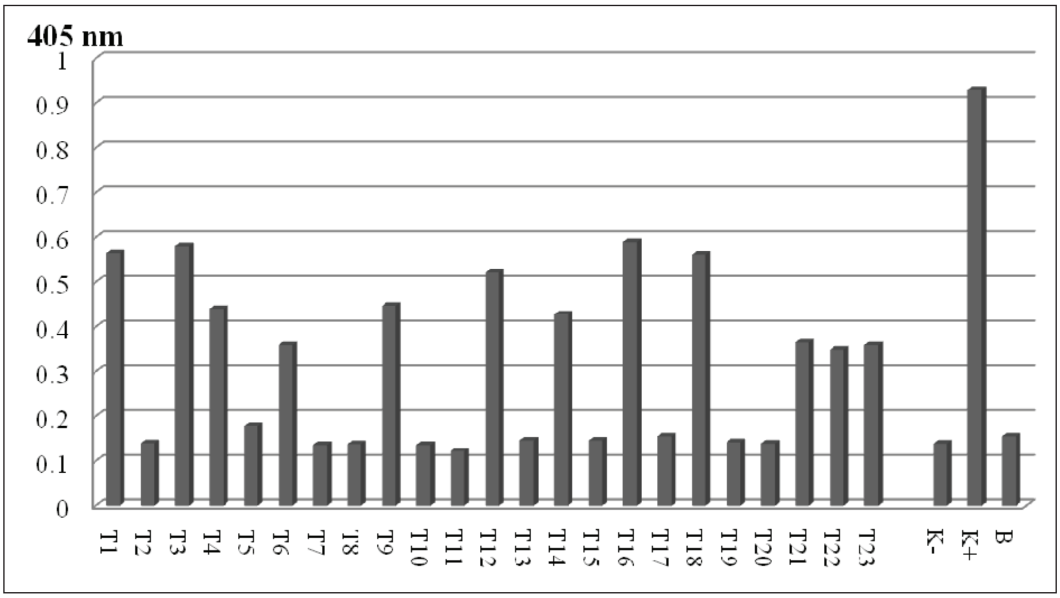

Fig. 1. Results from ELISA for ASGV in leaf samples collected form apple trees cv. Florina
Фиг. 1. Резултати от ELISA за ASGV в листни проби от ябълкови дървета на сорт Флорина

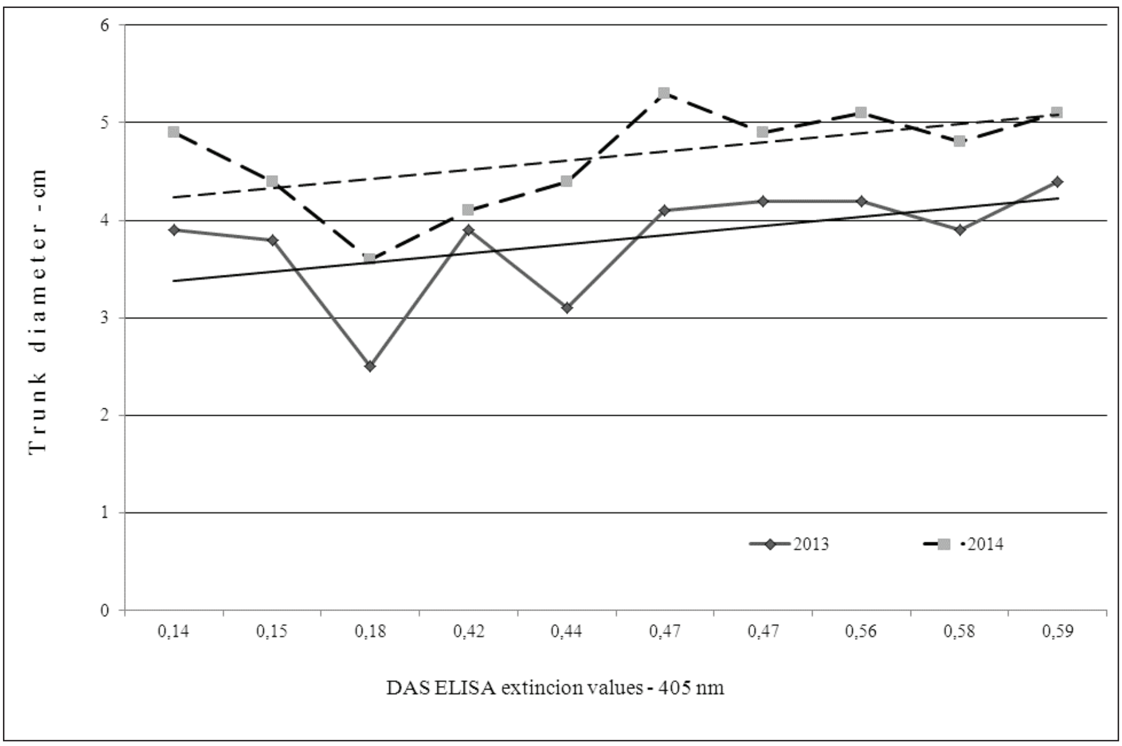

Fig. 2. Diameter of the apple tree trunks (cm) plotted against the respective tree EV
The straight lines are the linear trend of trunk diameter depending on EV.
Фиг. 2. Диаметър на стъблото на млади ябълкови дървета (cm) в зависимост от ЕС
Правата линия показва тенденцията на развитие на стъблото в зависимост от ЕС.

ASGV is often latent, causing no obvious symptoms in most commercial apple and pear cultivars, but will cause symptoms in indicator plants (Nemeth, 1986; Yanase et al., 1990, Howell et al., 1996). Yanase et al. (1990) reported that ASGV-infected scions of apple trees grown on the rootstocks *Malus prunifolia* var. ringo, *M. toringo* and *M. toringo* var. *arborescens* show symptoms of decline about 1-2 years after top-grafting. On sensitive species are identified symptoms on leaves (abnormal colours, abnormal leaf fall, yellowing or dead), fruits (reduced size) and stems

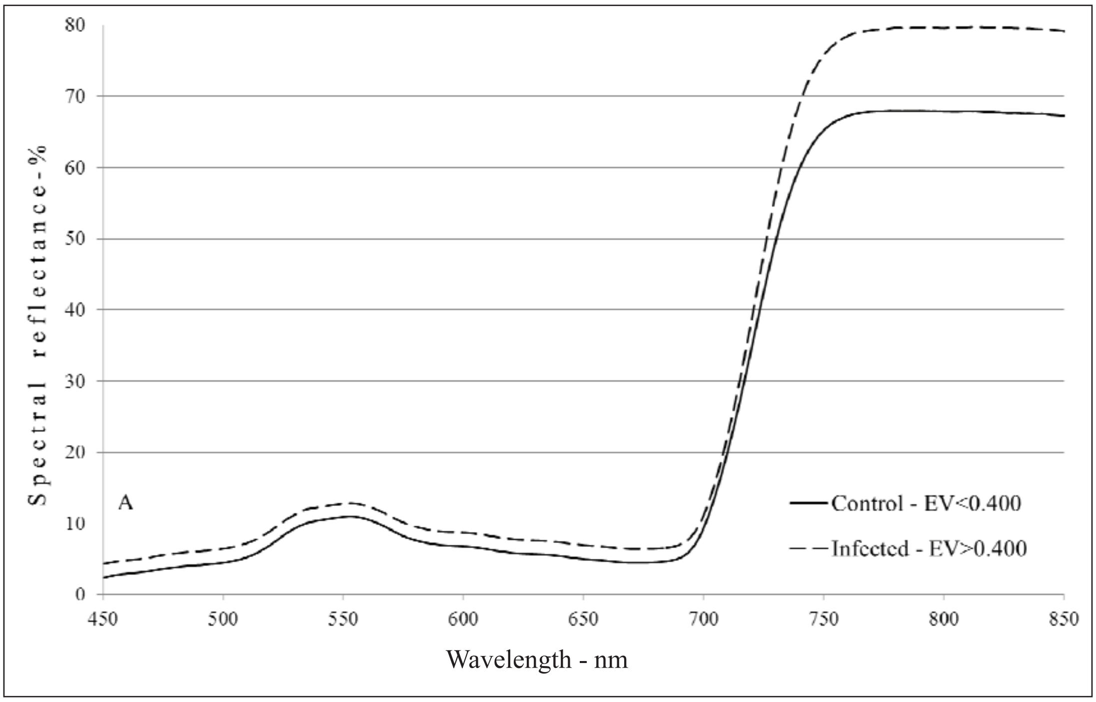

Fig. 3. Averaged spectral reflectance characteristics of control and ASGV infected apple trees
Фиг. 3. Спектрални характеристики на контролата и ASGV инфектираните дървета

(distortion) (Link 1). A brown line is often observed in tissue of Virginia crab immediately above the union, hence the disease has been called 'brown-line virus disease' (Nemeth, 1986). In the end of second vegetation ASGV infection detected by ELISA in leaves of apple trees cv. Florina remains latent. Data are reported that the trunk diameter of apple trees increases in the case of ASGV infection (Maxim et al., 2004; Bujdoso and Hrotko, 2006). There is not information that ASGV infection decreases trunk diameter. Kudela et al. (2009) reported for burrknots localised on the rootstock part of the trunk between the soil line and the graft junction as above them there was mild swelling, but the burrknots was not related to trunk diameter. According Lister (1970) ASGV produces stem grooving, swelling of graft unions and graft union necrosis symptoms upon apple seedling rootstock. ASGV infected trees of monitored orchard in the vicinity of the town of Kostinbrod had greater diameter compared to control for the both years of investigation.

One of the main applications of RS is monitoring of plants status and functioning to help in making timely management decisions. SR is a function describing the wavelength dependence of the ratios of the intensity of reflected light to the illuminated light. Changes in the absorption of incident light allow the identification of plant stress and disease. Reflectance in the visible portion of the electromagnetic spectrum (450 – 700 nm) is controlled by leaf pigments located in the chloroplasts. Strong absorption of energy required for photosynthesis causes low reflectance and transmittance in this region (Kumar et al., 2001). Live green plants absorb solar radiation in the photosynthetically active radiation spectral region, which they use as a source of energy in the process of photosynthesis. Potential of the viral infection to inhibit the production of chlorophyll reflect to a higher light reflectance of infected leaf and can be detected as an increase in the values of the reflected light at all spectrum. The results of our investigation are with agreement with this and confirm the presence of stress in observed trees.

The PRI is sensitive to changes in carotenoid pigments (e.g. xanthophyll pigments) in live foliage (Gamon et al., 1992). The NDVI is widely applied to recognize vegetated areas; it is very sensitive to the presence of biotic symptomatology (Rouse et al., 1974; Fletcher et al., 2004). NDVI provides a crude estimate of vegetation health and it remains the most well-known and used index to detect live green plant canopies in multispectral remote sensing data (Internet link 2). Negative values of NDVI (values approaching - 1) correspond to deep water. CARI and MCARI indicate the relative abundance of chlorophyll (Daughtry et al., 2000). On the basis of information that provide us calculated vegetation indices, a thorough analysis of all the changes occurring in infected trees could be done. It is not the purpose of this study. All calculated indices showed a significant change compared to same indices of the uninfected trees which is a good indicator of existing stress in the observed young apple trees, which was detected in an early stage of garden development, much before the viral symptoms to appear.

CONCLUSIONS

After two vegetations ASGV infected apples trees cv. Florina remain symptomless. On this stage of trees development trunk diameter of the ASGV infected trees is greater than that of the control. It was found statistical significant dependence between trunk diameter and extinction values (EV) Obtained by ELIS. The remote sensing (RS) technique based on spectral reflectance measurements, acquired in a high number of contiguous spectral bands in the visible (VIS) and near infra-red (NIR) spectral ranges, increases the potential to detect anomalies in the normal plant growth and production processes at an early stage, before the damage is occurred. RS detects stress even in early stage of plant development and gives additional information contributing phytosanitary monitoring. Vegetation indices (VI) give information concerning plant physiology that could be influenced by viral pathogen. There is good relation between VI and extinction values obtained by ELISA.

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Link 2: http://www47.homepage.villanova.edu/guillaume.turcotte/studentprojects/ arboretum/NDVI.ht

Влияние на латентна инфекция от ASGV върху развитието на млади ябълкови дървета сорт Флорина – предварително проучване

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Резюме

Статията представя резултати от изследване на някои морфологични и физиологични показатели при новозасадени ябълкови дървета, инфектирани с вируса на ябълковото стъблено набраздяване (apple stem grooving virus, ASGV). В периода 2013 – 2014 г. инфекцията на младите дървета е латентна. Вирусната инфекция е установена чрез имуноензимен тест (ELISA) на проби от млади листа, събрани от дървета на ябълковия сорт Флорина. Дърветата с екстинционни стойности, поне три пъти по-големи от отрицателната контрола, бяха приети за инфектирани. Сравнени с контролата, инфектираните дървета имаха статистически доказано нарастване на диаметъра на ствола ($P < 0,05$). Спектралните характеристики, получени чрез дистанционния метод от отразена от листата радиация, потвърдиха разделянето на изследваните дървета на две групи инфектирани дървета спрямо неинфектираните (контролни). Чрез изчислените вегетационни индекси бяха установени различия в някои показатели, характеризиращи развитието и хабитуса на дърветата.

Установена е значима взаимовръзка между индексите и екстинкционните стойности, измерени чрез теста ELISA. Резултатите от спектралните анализи показват, че отразената от листата радиация е надежден метод за ранно откриване на стрес в млади ябълкови дървета.