Pestic. Phytomed. (Belgrade), 29(1), 2014, 21–34
DOI: 10.2298/PIF1401021P

UDC 581.132:632
Review paper

Chlorophyll as a measure of plant health: Agroecological aspects

Danijela Pavlović[1], Bogdan Nikolić[1*], Sanja Đurović[1], Hadi Waisi[2], Ana Anđelković[1] and Dragana Marisavljević[1]

[1] Institute for Plant Protection and Environment, T. Drajzera 9, Belgrade, Serbia
[2] Institute for the Development of Water Resources "Jaroslav Černi", J. Černog 80, Belgrade, Serbia
**(bogdannik@mail2world.com)*

Received: December 13, 2013
Accepted: April 8, 2014

SUMMARY

As photosynthesis is the basic process during which light energy is absorbed and converted into organic matter, the importance of the plant pigment chlorophyll (*a* and *b* forms) as an intermediary in transformation of the absorbed solar energy and its activity in the process of photosynthesis and synthesis of organic substances in plants are crucial. Therefore, this paper provides an overview of methods for monitoring the optical activity of chlorophyll molecules and methods (non-destructive and destructive) for quantification of chlorophyll in plants. These methods are used to estimate the effects of different stress factors (abiotic, biotic and xenobiotic) on the efficiency of photosynthesis and bioproductivity, aiming to assess the impact that these limiting factors have on the yield of various cultivars. Also, those methods for analysis of chlorophyll optical activity and/or content are appropriate for assessing the reaction of weed species to different agricultural practices (mineral nutrition, treatment by herbicides, etc.) and studies of different aspects of weed ecophysiology and their influence on crop harvest.

Keywords: Chlorophylls; Photosynthesis; Plant health

Abbreviations: *$LHCP_2$, $LHCP_1$: antenna complexes in photosystems II and I with the main function of absorbing (sun)light quantum and converting it into excitation energy of chlorophyll molecules (excitons); PS II and PS I: photosystems II and I; RC: reaction centers of PS II and PS I with the function of transforming the energy of exciton into primary photochemical reaction and reducing the molecules of RC chlorophyll, which starts the photosynthetic electron transport; $NADPH_2$: the main reducing equivalent, i.e. the product of photosynthesis with a role in synthesis reactions in the „dark phase of photosynthesis"; NADP: the oxidized form of $NADPH_2$; ATP: the main energy equivalent, i.e. the product of photosynthesis with a function in synthesis reactions in the „dark phase of photosynthesis"; Qa and Qb: (plasto)quinones in PS II; D_1: herbicide- (photosynthesis inhibitors) and PS II (plasto)quinones-binding protein in the pigment-protein complex of PS II; Fd: a protein with a cofactor in PS I with the function of reducing NADP, as well as some other compounds important for the metabolism of nitrogen and sulfur; Fm, F_0, Fv: maximum, minimum level of fluorescence and variable fluorescence ($Fm-F_0$); ψ (=Fv/Fm): maximum quantum efficiency of photosynthesis in PS II (a measure of the potential photochemical efficiency of photosynthesis)*

INTRODUCTION

In the era of modern technologies and numerous natural disasters, there is a need to increase the production of plant organic matter in order to meet a growing global food demand. From the aspect of natural processes, this seems a simple task, there is sunlight, and a plant, and here comes the food. However, up-to-date biotechnology (i.e. plants tolerant to pesticides, drought, diseases, etc.), extensive environmental pollution (greenhouse gases, wastes in waters, etc.), reduction in arable land (urbanization and industrialization), deforestation, and other similar factors, lead to a destruction of natural food resources. The use of pesticides and organic food production stand in huge contrast. Farmers approach the issue from a standpoint of profit and crop yield, where the use of pesticides provides them with a feeling of security; while environmentalists believe that pesticides-free production protects the environment and human health; scientists transfer genes from one species to another and create genetically modified food that is supposed to combine both of these aspirations, although objections have been voiced to that approach as well.

1. CHLOROPHYLL: THE ESSENTIAL ELEMENT OF PHOTOSYNTHESIS

Photosynthesis is the basic process of substance and energy circulation in nature. In the light-dependent phase of photosynthesis, the energy of sunlight is taken from a chlorophyll-protein complex, the so-called antenna ($LHCP_2$, $LHCP_1$), and then transmitted as excitation energy to the reaction centers of both photosystems (PS II and PS I). In RC PS II excitation energy is converted into chemical energy by releasing of an electron, which is accepted by pheophytin as the primary electron acceptor in PS II. The process of molecule excitation and transfer of electrons goes on up to the NADP in PS I, which is the final electron acceptor in the photosynthetic electron transport chain (Malkin and Niyogi, 2000). Electron transport takes place in the chloroplast stroma and stromal lamellae via four protein complexes (PS_2, cytochrome b6/f complex, PS I and the complex of ATP- synthetase; Fuerst and Norman, 1991). The electrons from RC PS II are transmitted to Qa and Qb, the quinone of PS II. These electrons leave the D1 protein (it connects Qa and Qb quinones of PS_2) in the form of hydroquinone, PQH_2, and are transferred to cytochrome b6/f complex through the Q cycle, whence they undergo a noncyclic pathway from PS_2 to PS_1. In RC PS I (chlorophyll P_{700}), the received electron is further transferred to an iron-sulfur protein complex (Fx and Fa/Fb) in PS I. Electrons in PS I are then transferred to ferredoxin (Fd), which enables the reduction of $NADP^+$ into $NADPH_2$. In addition, an electron may be transferred back through the cyclic pathway to the cytochrome b6/f complex, which enables a conversion of quinone into hydroquinone and transmission of electrons through the thylakoid membrane, which leads to ATP synthesis (Moreland, 1980; Kastori, 1995; Nešković et al., 2003). The dark phase of photosynthesis takes place in the Calvin cycle. The cycle is dependent on energy (ATP) and reduction ($NADPH_2$, reduced Fd) equivalents created in the light reactions phase and the available CO_2. Carbon dioxide is the primary substrate of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). The assimilatory segment of the Calvin cycle consists of five enzymes: phosphoribulokinase, Rubisco, 3-phosphoglycerate kinase, NADP-glyceraldehyde-3-phosphate dehydrogenase, and triose-phosphate isomerase (Geiger and Servaites, 1994). Metabolic regulation of this cycle enables plants to respond to a variety of endogenous and exogenous factors. The level of CO_2 uptake is an important component of crop productivity and it is almost proportional to nitrogen content (Jinwen et al. 2009). The whole process is based on chlorophyll and therefore by monitoring its content in plants we can determine the productivity of photosynthesis. Chlorophyll molecules are esters of a dicarboxylic acid (chlorophyllins), and four five-membered pyrrole rings linked together by methyl groups (-CH=) (protoporfirin ring) (Kitchen et al., 1981). A change in chlorophyll content is one of the most obvious symptoms of plant stress (Lichtenhaler, 1996; Nikolić, 1997; Lichtenhaler and Babani, 2004; Pavlović, 2005). Numerous genetic (genotype), morphological (age and position of leaves), physiological (simultaneous chlorophyll decomposition and synthesis processes, chlorophyll distribution in leaf mesophyll) (Kastori, 1995; ByungJoo et al., 2001; Nikolić, 1997) and abiotic factors (herbicides, temperature, relative humidity, mineral nutrition, quality of light, etc.) (Milivojević and Nikolić, 1998; Kastori, 1995; Anderson et al., 1993) affect its content in plant leaves.

2. OPTICAL ACTIVITY AND CONTENT OF CHLOROPHYLL IN LEAVES

Determination of the optical activity and content of photosynthetic pigments in leaves is one of the key techniques in studying the process of photosynthesis and

measuring plant productivity. Chlorophyll molecules absorb and re-emit light, a characteristic which was the basis for developing two basic methods: absorption and fluorescence monitoring of the optical activity of chlorophyll molecules. The method of chlorophyll fluorescence is used for monitoring photosynthesis *in situ* and *in vivo* and for estimating the impact of various stress factors (abiotic, biotic, xenobiotic) on this crucial process. It makes it possible to differentiate plant genotypes resistant to the aforementioned stressful environmental factors, and also to assess the positive impact of various agricultural measures on plant health (mineral nutrition, use of herbicides, etc.). Methods for nondestructive quantification of chlorophyll in plant leaves have also been developed. They are particularly valuable in assessing nitrogen content in plants because chlorophyll is one of the most important points of its accumulation. Chlorophyll content can also be determined by destructive methods based on the Law of Lambert and Beer (measurement of light absorption by chlorophyll in plant extract).

The light energy absorbed by chlorophyll is mostly converted into chemical equivalents (ATP and $NADPH_2$), which are used in the dark phase photosynthesis for CO_2 fixation and the synthesis of organic molecules (Geiger and Servaites, 1994). A part of the absorbed energy gets lost throught: a) re-emission in the form of heat radiation, b) re-emission of energy in the form of light of longer wavelengths than the absorbed light (fluorescence) (Krause and Weis, 1991; Maxwell and Johnson, 2000; Baker, 2008). The ratio of the energy used and energy lost depends on several abiotic (high/low temperature, salinity and soil pH, drought, presence of phytotoxic elements in the soil, etc.), biotic (phytopathogenic viruses, bacteria, fungi, nematodes, arthropods and other organisms) and xenobiotic factors (pesticides, other phytotoxic chemicals, radionuclides and similar) and their combinations (Demmig-Adams and Adams, 1992; Long et al., 1994; Giardi et al., 1997). All these factors limit the synthesis and utilization of organic matter, and growth and development of plants (Lichtenthaler, 1996; Larcher, 2003). In order to alleviate the negative impact of stress factors, plants limit or reduce the effectiveness of light transformation into chemical energy by enhancing thermal or fluorescent light re-emission (Demmig-Adams and Adams, 1992). The efficiency of (photo)chemical transformation of sunlight is determined by measuring the intensity of re-emitted fluorescence and calculating various relative parameters (Krause and Weis, 1991; Demmig-Adams and Adams, 1992; Maxwell and Johnson, 2000).

2.1. Optical activity and chlorophyll content in leaves of intact plants

2.1.1. Chlorophyll fluorescence - modulated and unmodulated

Chlorophyll fluorescence is a rapid, sensitive and reliable method (van Oorschot and van Leeuven, 1992; Maxwell and Johnson, 2000). Despite the fact that total fluorescence is small (1-2% of absorbed light), its measurement is quick and easy, and its intensity is inversely proportional to the efficiency of photosynthesis (Krause and Weis, 1991). Fluorescence measurement yields relative values because losses of light energy are permanent (Maxwell and Johnson, 2000). When light is absorbed, plastoquinone Qa accepts an electron, which is blocked until transferred to plastoquinone Qb. During that time, RC PS II is „closed", so that the absorbed light energy can be re-emitted in the form of light of higher wavelength than the absorbed one. Proportional closing of RC PS II leads to an overall decrease in the efficiency of photochemical processes and simultaneous increasing of fluorescence yield (when exposing the plants to light, after a period in the dark). In the following minutes the level of fluorescence decreases, which is known as fluorescence quenching (Figures 1a, b; Schreiber et al., 1986).

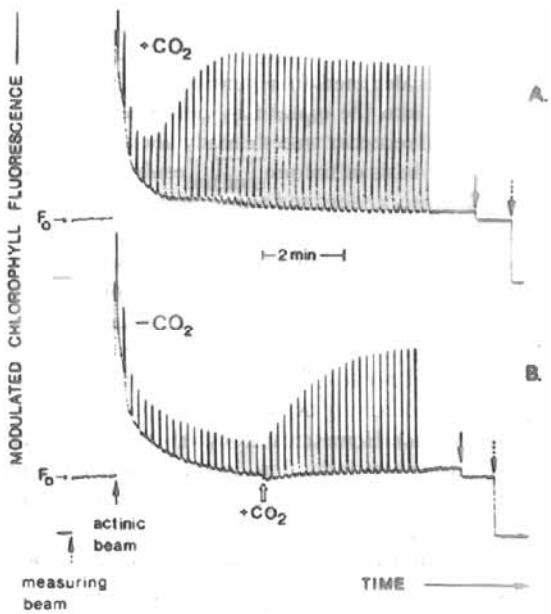

Figure 1. Modulated chlorophyll fluorescence. Induction curves of *P. vulgaris* with repetitive application of saturation pulses depending on the availability of CO_2. A) The leaf was exposed to air with 0.03% CO_2; B) CO_2 was removed from the air stream. An example of plant stress detected by chlorophyll fluorescence method

A decrease in the level of fluorescence can be attributed to the activity of light-activated enzymes involved in the metabolism of carbohydrates and to stoma opening (photochemical quenching), as well as to an energy loss in the form of heat (non-photochemical quenching) (Maxwell and Johnson, 2000). These processes last 15-20 minutes. Fluorescence measurement includes the monitoring of minimum (F_0) and maximum fluorescence (Fm), as well as their difference, i.e. variable fluorescence (Fv). In the mid-1980s, the method of chlorophyll fluorescence was innovated by introducing the PAM system, i.e. the modulation of chlorophyll fluorescence amplitude by double illumination of leaves. The leaf is exposed to continuous actinic light and short (up to 1s) repetitive pulses of high-intensity white light (≥2000 μmol photons m^{-2} s^{-1}). It then comes to a temporary „closure"/saturation of its RC PS II centers and electron acceptors are completely reduced. A part of the energy absorbed by chlorophyll in the PS II can be used in photochemical reactions ($Fv=Fm-F_0$; Gilmore, 1997). Photochemical efficiency of photosynthesis (quantum yield) is calculated from the ratio of Fv/Fm (Schreiber et al., 1986; Genty et al., 1989). The optimum value of Fv/Fm for healthy plants is around 0.8 (Björkman and Demmig, 1987), so this parameter can be used as an indicator of whether plants have been exposed to stress factors (drought, herbicides, etc.), i.e. whether the inhibition of photosynthesis has taken place.

Many researchers have studied the impact of various factors on the process of photosynthesis (Long et al., 1994; Lichtenthaler, 1996; Giardi et al., 1997; Larcher, 2003; Lichtenhaler and Babani, 2004). A particularly interesting aspect of this issue is the inhibition of photosynthesis by herbicides (Nikolić, 1997; Klem et al., 2002; Pavlović, 2005; Nikolić, 2007; Nikolić et al., 2007 a, b, c; Pavlović, 2010). The impact of atrazine has been most closely studied because of its frequent and widespread application in the 1980s (Moreland, 1980; Ali and Machado, 1981; Ahrens et al., 1981; Chodova et al., 1995; Gronwald et al., 1995; Yerkes et al., 1995; Pavlović et al., 2002, 2004, 2005, 2006). Application of that herbicide interferes with photosynthesis (reducing yield of fluorescence) in all sensitive plants. Experiments conducted on *Chenopodium album, Amaranthus retroflexus* and *Abutilon theophrasti* plants have revealed decreases in fluorescence yield 24 hours after the application of atrazine (2, 4 and 8 kg ha^{-1}) (Pavlović, 2005; Pavlović et al., 2006). After herbicide application, the value of Fv/Fm decreased from 0.8 to 0.2, which clearly indicates a blocked electron transport chain and damage to photosynthetic structures. Nikolić et al. (2007 a, b, c) monitored the effect of sulphosate on maize plants (*Zea mays*) in this way. They concluded that the presence of this herbicide caused a reallocation of dry matter from leaves and the root system to the stem, according to a theory of „functional balance" (Lang and Thorpe, 1985). By combining the chlorophyll fluorescence method with weighing of organs biomass of control plants and plants treated with sulphosate, strong correlations were confirmed among assimilates reallocation, photosynthesis, bioproductivity and crop yield. It was also concluded that their interrelation was strongly influenced by environmental factors and plant age (Nikolić et al., 2007 a, b, c; Pavlović et al. 2010). Poorter and Nagel (2000) came to similar results in their research. Those results are significant for practical work because they make it easier to study the effects of herbicides on plants by applying Chl *a* fluorescence as a non-destructive method. It is important to point out that there are plant populations that are able to cope with stress through certain mechanisms without causing a change in fluorescence yield, i.e. in the process of photosynthesis. Particularly important in that context are several studies carried out on cultivated plants. Based on fluorescence yield, a parameter of phytotoxic herbicide effect has been defined as an estimated impact of a herbicide on plant yield and indicator of financial viability of its application. Thus, the effect of glyphosate has been monitored on *Beta vulgaris* (Madsen et al., 1995), clodinafop on *Hordeum murinum* and *Avena sativa* (Abbaspoor and Streibig, 2005), and the effect of diuron on *Triticum aestivum* (Habash et al., 1985). Experiments have shown that the technique of chlorophyll fluorescence is a very reliable and sensitive method of monitoring physiological changes in plants under the impact of herbicides. In addition, chlorophyll fluorescence is used in agronomic practice for solving the problem of weed resistance to herbicides. In plants resistant to herbicides, the Fv/Fo value is always around 0.8, which indicates that the process of photosynthesis has not been compromised. Van Oorschot and van Leeuven (1992) studied the process of photosynthesis by monitoring chlorophyll fluorescence after the application of chlorotoluron on *Alopecurus myosuroides* plants. They recorded a deterioration of some plants, but also partial or full recovery of others. In the latter group, there was a mutation of D_1 protein (in photosystem II), which ensures smooth progress of

photosynthetic electron transport in spite of the present herbicides, inhibitors of photosynthesis (Ahrens et al., 1981; Yerkes et al., 1995; Pavlović, 2005; Pavlović et al., 2006). Some researchers have also used this method to monitor the effects of abiotic factors (humidity, light, mineral nutrition, etc.) on the physiology and yield of cultivated plants and weeds. Adaptation of plants to extreme environmental conditions causes short-term chemical, molecular and physiological, as well as long-term physiological, structural and morphological modifications (Giardi et al., 1997). Changes in activation, transcription and translation of genes often occur during acclimatization of plants and develop into a kind of tolerance to a stressful factor (Lichtenthaler, 1996; Giardi et al., 1997). Oberhuber and Edwards (1993) studied the photosynthetic reactions in plants under conditions of extremely high and low temperatures. They noticed that the light energy absorbed by plants was excessive under low temperatures and bright light, which affected the bioproductivity and/or yield of plants (Oberhuber and Edwards, 1993, Long et al., 1994). Excess energy must be either consumed or eliminated in order to avoid photoinhibition and photooxidation during photosynthesis (Demmig-Adams and Adams, 1992). Plant adaptation to different light intensity conditions was also studied by Lichtenhaler and Babani (2004). Nikolić et al. (2008) showed that variations in light intensity have impact on the photosynthesis of various plants, both cultivated and wild. The method of chlorophyll fluorescence is also used in phytopathology (Nikolić et al., 2011). Higher values of the parameter Fv/Fm were found in leaves of the vine variety Frankovka than in the variety Gamay tenturier infected with a virus. The comparison of Fv/Fm values, obtained for leaves from the middle parts of plants infected with virosis, revealed a greater sensitivity of Gamay tenturier leaves to plant viruses (Nikolić et al., 2011).

2.1.2. High resolution fluorescence imaging systems

In order to analyze and describe changes in the process of photosynthesis and detect stress in plants, various types of indicators of chlorophyll activity were used at the end of the 20th century (Lichtenthaler, 1996; Lichtenthaler et al., 1996). However, all these indicators were based on the principle of „spot measurement", i.e. on photosynthetic activity measured on a small area of a single leaf. Therefore, the representativeness and statistical replication of such measurements were limited. Advanced optical technologies (new types of lenses, sources and detectors of light of different wavelengths) have modernized the fluorescence methods, i.e. it is now possible to monitor the health of entire plants, even plant communities. A method developed by Lichtenthaler (1996) – the high resolution fluorescence imaging system – is based on leaf fluorescence intensity ratios in different parts of the visible spectrum. The fluorescence intensity ratios of red/far-red: F_{690}/F_{740}, blue/far-red: F_{440}/F_{740} and blue/green: F_{440}/F_{520} are quite good indicators of plant health (Lichtenthaler, 1996; Lichtenthaler et al., 1996). This method is valuable because it does not only observe the fluorescence of chlorophyll (red and far-red part of the spectrum: F_{690}, F_{740}) as an indicator of photosynthetic apparatus activity, but also the fluorescence of polyphenols (blue-green and yellow part of the spectrum: F_{440}, F_{520}) as indicators of cell wall condition (Lichtenthaler, 1996). The parametric relations F_{440}/F_{690} and F_{690}/F_{740} are especially suitable for monitoring stress effects caused by environmental factors and for assessing damage cause to the photosynthetic apparatus. The method can generate data on a different fluorescence distribution along the leaf longitudinal and transverse axes after herbicide application (Figure 2; Lichtenthaler, 1996). In this way, we can monitor the uptake and translocation of herbicides in plants, which may further optimize its use in efforts to suppress weeds and increase crop yields. Some researchers have studied the effects of diuron on electron transport. They concluded that herbicides became bound to the Q_B-binding site, which blocks electron transport (Lichtenthaler, 1988; Lichtenhaler and Rinderle, 1988; Szigeti et al., 1996). The result of such inhibition is a decrease in the efficiency of photosynthetic conversion of the absorbed light, and significant increase in the emission of red (F_{690}) and far-red (F_{740}) chlorophyll fluorescence. On the other side, F_{690} and F_{740} in untreated plants have very low values. By monitoring the fluorescence emission it is possible to determine the distribution of weeds within crops after herbicide application (Lichtenhaler, 1996; Lichtenthaler et al., 1996), as well as the health of cultivated plants (Lichtenthaler et al., 1996; Lichtenhaler and Babani, 2004). In addition, there are also other types of chlorophyll fluorescence imaging (Meyer and Genty, 1998; Baker et al., 2001; Leipner et al., 2001; Fryer et al., 2002; Simić et al., 2012), based on the imaging of different chlorophyll fluorescence parameters described in section 2.1.1.

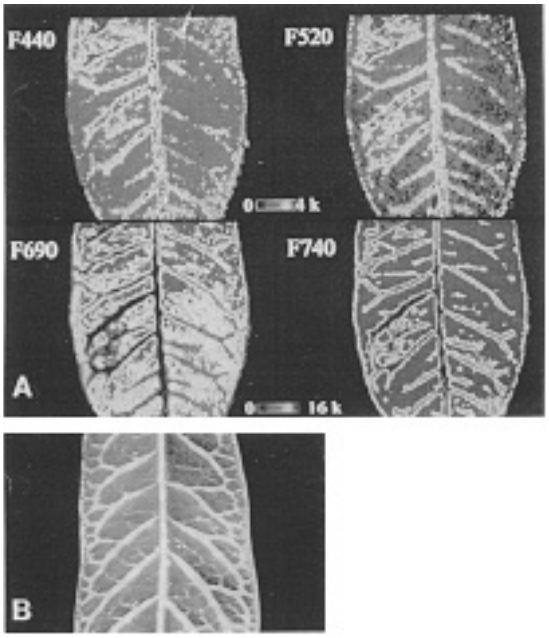

Figure 2. A) False fluorescence images of the blue (F440), green (F520), red (F690) and far-red (F740) fluorescence emission in a young green leaf of *Codiaeum variegatum* L. Fluorescence intensities increase from dark to light shade (see scale in the figure). B) Photograph of a leaf of *C. variegatum*.

2.1.3. Other methods for monitoring the optical activity of chlorophyll

While the two described methods (based on rapid chlorophyll fluorescence) have been widely applied in ecophysiological and agronomic research, methods based on other types of light re-emission such as thermoluminescence (Demeter and Govindjee, 1989), delayed fluorescence (Radenović et al., 1994), ultraweak bioluminescence (Radotić et al., 1998), and the like, have so far been applied only in fundamental researches. However, rapid fluorescence (sections 2.1.1. and 2.1.2.) had itself been initially considered suitable only for fundamental research, until the PAM fluorescence measuring system was introduced in the early 1980s and everything changed therefrom (Schreiber et al., 1986). This method is now considered one of the basic tools in plant ecophysiology (Maxwell and Johnson, 2000; Baker, 2008). In that sense, delayed fluorescence is possibly the most promising method (Radenović et al., 1994; Раденович et al., 2005), accompanied of course by appropriate technical innovations.

2.1.4. Determination of relative chlorophyll content by light transmittance/absorbance: SPAD meter

Determination of relative chlorophyll content using the SPAD meter is a quick, efficient and relatively reliable way of defining symptoms developed during photosynthesis as a result of plant exposure to stress. Measuring chlorophyll content without sacrificing plants enables the monitoring of several parameters in the same plants and obtaining more reliable data. High sensitivity of chloroplasts and chlorophyll grains outside the plant and the possibility of deriving erroneous conclusions support the use of this method. High light intesity induces thicker leaves, higher Chl *a/b* ratios and chlorophyll content per unit area, but lower chlorophyll content per unit weight or volume (Boardman, 1977; Khan et al., 2000; Terashima et al., 2006). Accurate measurement of leaf thickness is difficult and time consuming because leaf blade changes under pressure. Leaf chlorophyll content can be rapidly estimated *in situ* by SPAD (Soil Plant Analysis Development). This technique has been used for measuring the deficit of macro- and microelements in mineral nutrition of plants, changes in chlorophyll contents due to different illumination levels, changes in plant biomass, content of N (Thompson et al., 1996; Gaborick, 2000; Abdelhamid, 2003; Woonho, 2003; Nauš et al., 2010; Chao-Yi and Der-Ming, 2008; Jarrahi et al., 2013) and for measuring the impact of herbicides on photosynthesis (Ferell et al., 2003). Jinwen et al. (2009) showed that leaves became thinner with increasing N application and Chl *a/b* ratios decreased. Also, the sensitivity of SPAD readings of the same leaves at different ages to N rates was assessed through coefficients of variation (CV). The CV of SPAD readings increased from 8.8% to 21.6% during leaf lifetime, which indicates that SPAD readings became more and more sensitive to nitrogen rates as the leaves aged. Pavlović et al. (2004, 2005) studied the secondary effect of atrazine on the process of photosynthesis by measuring relative chlorophyll content with the SPAD meter. In damaged plants of *C. album,* there was a decrease in the relative content of chlorophyll (5 days after application), compared to healthy plants (Pavlović et al., 2002, 2004, 2006). The content of chlorophyll (SPAD readings) in healthy plants ranged from 36.04 relative units (r.u.) (resistant population) to 36.38 r.u. (susceptible population), while the application of atrazine resulted in plant stress and reduction in relative chlorophyll content from 34.89 r.u. (resistant population) to 30.40 r.u. (susceptible population) (Pavlović, 2005, Table 1).

Table 1. Total amount of chlorophyll (r.u.) determined by SPAD meter readings

Weed population	Control plants		5 days after treatment (8 kg ha^{-1} atrazine)			
			Average amount		LSD test	
	R	S	R	S	C:R	C:S
Abutilon teophrasti	24.58	25.27	22.42	23.45	ns	**
Amaranthus retroflexus	22.80	22.17	21.86	18.45	ns	**
Chenopodium album	36.04	36.38	34.89	30.40	**	**

p<0.01**, ns-nonsignificant differences, K-control, R-resistant population, S-susceptible population

The measurements by SPAD meter showed differences in chlorophyll contents among the weed species. From a standpoint of suppression and control of weed species, the obtained results may represent important information. Chlorophyll content (SPAD readings) was in the range of 20.31-25.00 r.u. in *A. retroflexus* plants, while the results ranged from 23.95 to 25.27 r.u. for *A. teophrasti* plants and from 33.14 to 37.99 r.u. for *C. album* plants (Pavlović, 2005; Pavlović et al., 2007a, b). Damaged chloroplasts were clearly evidenced in plants of *Conyza canadensis*, *C. bonariensis* and *Lolium rigidum* after application of glyphosate-trimesium (or sulphosate) (Pavlović, 2010; Pavlović et al., 2013). A simple and quick way of measurement reading showed that chlorophyll contents had changed, which is a good and reliable indicator of the efficiency of the applied herbicide from the aspect of its justified use. The study included cultivated plants as well. Treatments of soybean and maize plants with sulphosate resulted in a slightly decreased chlorophyll content over a time period of 2-6 days after application. Genetically modified soybean plants (GMO) recovered after an initial stress caused by herbicide application, while the content in GMO maize plants was still slightly lower than in the control group 6 days after application. In sensitive plants of both types, the level of chlorophyll over time was constantly lower than in the control group (Pavlović, 2010). However, measurements involving weeds (*L. rigidum* and two *Conyza* species, Table 2) showed that, after a pronounced initial stress (2-4 days after application, Figures 3 and 4), the plants recovered (6 days after application, Figures 3 and 4) from the effects of sulphosate application (Pavlović, 2010).

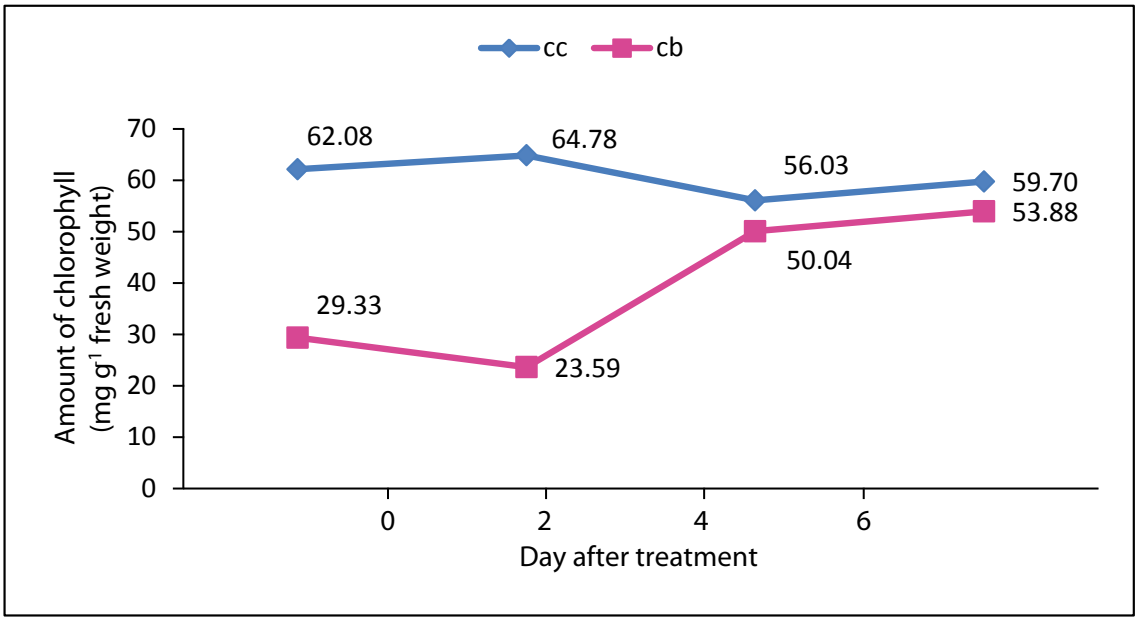

Figure 3. Amount of total Chl (mg g^{-1} fresh weight) of *C. canadensis* (cc) and *C. bonariensis* (cb) vs. day of sampling after treatment with 1 kg a.i. ha^{-1} glyphosate

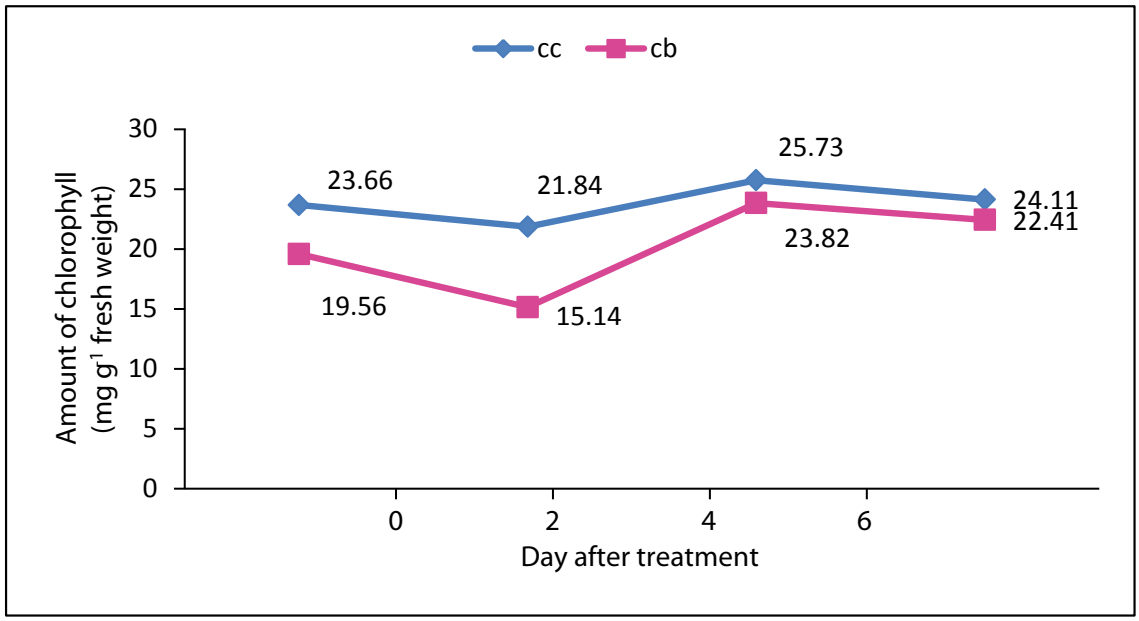

Figure 4. Amount of Chl *a* (mg g^{-1} fresh weight) of *C. canadensis* (cc) and *C. bonariensis* (cb) vs. day of sampling after treatment with 1 kg a.i. ha^{-1} glyphosate

Table 2. Total amount of chlorophyll (r.u.) determined by SPAD meter readings, *Lolium* sp. and *Conyza* sp. populations

Weed population	Control plants		6 days after treatment (1 kg a.i.ha^{-1} glyphosate)			
			Average amount		LSD test	
	R	S	R	S	C-R	C-S
Lolium rigidum	33.43	38.33	37.06	34.69	ns	**
Conyza canadensis	-	48.46	-	50.81	-	Ns
Conyza bonariensis	-	39.17	-	43.06	-	*

p<0.01**, p<0.05*, ns-nonsignificant differences, C-control, R-resistant population, S-susceptible population

Plant recovery from the effects of sulphosate showed that the weed species overcame stress more easily than the cultivated plants by activating various metabolic and biochemical processes. In order to determine weed resistance and improve the quality of application of herbicides, Bozic et al. (2007) measured chlorophyll contents with the SPAD meter and indicated some possible effects of ALS herbicides. In contrast, tests conducted on sunflower plants and *Xanthium strumarium* L. in the field revealed no statistically significant differences in contents between untreated plants and those treated with ALS-inhibiting herbicides (Božić, 2010).

2.1.5. Other optical methods for non-destructive determination of chlorophyll content

In addition to non-destructive determination of chlorophyll content in plant leaves by measuring their transmittance/absorbance (SPAD method), it has been concluded that chlorophyll fluorescence can be used for the same purpose. A group of researchers (Hak et al., 1990; Babani and Lichtenthaler, 1996) came to a conclusion that the ratio of chlorophyll fluorescence intensity, as defined by two maximums (red: F_{690} and far-red: F_{740} or F_{730}), is in inverse hyperbolic proportionality ($y=ax^{-b}$) with total chlorophyll content, so that it is possible to determine total chlorophyll content non-destructively in this way. Also, Björkman and Demmig (1987) found the ratio of total chlorophyll content against the Fv/Fm fluorescence parameter (measured on the same leaf) to have a sigmoidal dependence, which enables an approximate estimate of chlorophyll content in leaves by non-destructive measurement of Chl *a* fluorescence. This approach was also applied by Nikolić et al. (2004, Figure 5).

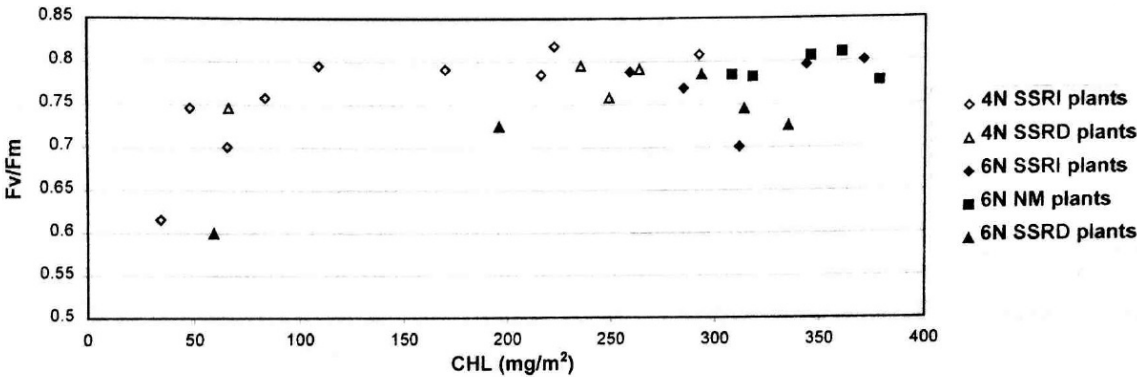

Figure 5. Relationship of total chlorophyll content (mg m^{-2}) and quantum efficacy of photosystem II (Fv/Fm) in young fully developed leaves of four- or six-week old *Z. mays* plants; N - week; SSRI – plants in which the source-sink ratio increases; SSRD – plants in which the source-sink ratio decreases (Nikolić et al., 2004).

2.2. Determination of chlorophyll content in the sample using a destructive method – extraction

The first practical confirmation of the two forms of chlorophyll had been given by Tswett (1903, cit. Nešković et al., 2003) when he separated leaf pigments using a chalk column. A hundred years later, we use spectrophotometry to analyse plant pigments extracted in: 80% (v/v) acetone, dimethylformamide (DMF), methanol or dimethylsulfoxide (DMSO). This method of chlorophyll quantification is reliable but time-consuming and requires great precision. The main disadvantage of the method is that the process of extraction can result in erroneous qualitative and quantitative determination of the content of pigments (due to photochemical reactions, impact of ambient oxygen, chlorophyllase activity, pheophytinization caused by acids from plant tissue, etc.) (Wellburn, 1994; Jelić et al., 1992). The selection of solvent for extraction brings about a dilemma. A number of factors may affect the activity of a solvent: the time required for extraction, amount of plant material, percentage of moisture in plant material, preservation of extract in unchanged form (Jelić et al., 1992; Moran and Porath, 1980), as well as the fact that the extraction of Chl a is a slower process than that of Chl b. Light, an important environmental factor, causes degradation of chlorophyll, so that extraction should be carried out in almost total absence of light (Jelić et al., 1992). Discussing the solubility of chlorophyll in a solvent, Jelić et al. (1992) showed that pigments were most stable in DMF, and least so in DMSO. Their research showed that samples extracted with 80 % acetone and DMF could be stored up to 6 days without significant loss (degradation during that period is less than 5%), while extraction with DMSO required spectrophotometric analysis on the same day already. Using DMF, Jelić et al. (1992) extracted the maximum amount of chlorophyll from leaves of *Glycine max.* (containing 60% water). They came to a conclusion that reduced water content in leaves decreased solvent efficiency and thus extended the time of extraction. In extremely dry samples, regardless of DMF presence, it is necessary to homogenize the tissue, which can then result in the loss of a certain amount of pigment. The authors observed the efficiency of chlorophyll extraction with DMSO and showed that the amount of tissue subjected to extraction did not affect the success of extraction process. The technique is suitable even for working with dry plant material. The material should not be homogenized, but immersed in 5 ml of DMSO at 60^0C in a dry heater and then the extraction can be performed.

Extraction with acetone (or some other non-polar solvent) involves tissue homogenization with quartz sand and rapid obtaining of an extract of all pigments (a few minutes per sample). The extract is then separated from the quartz sand and remaining plant tissue by sample filtration and centrifugation. The procedure with DMF does not require homogenization and further steps, but low temperature (4^0C) during handling because of a high solvent evaporation rate. Leaf samples are left in a specific amount of DMF at 4^0C for 24 h, which is sufficient for chlorophyll (and carotenoids) extraction from young and hydrated leaf tissue. The absorbance of the chlorophylls is then quantitatively determined by spectrophotometry at the wavelengths of maximum Chl *a* and Chl *b* absorption ($\lambda = 647$ nm and $\lambda = 664$ nm, Moran and Porath, 1980), while the actual content of photosynthetic pigments is calculated according to Wellburn's formulas (1994). This procedure

is based on the Lambert-Beer law on linear relationship between absorbance and concentration of pigments within a certain range. Pavlović et al. (2006) noted in *C. album* and *A. retroflexus* plants a significant reduction in total chlorophyll content after atrazine application. The herbicide acted as a stress factor in the process of chlorophyll synthesis, stimulating decomposition. Different amounts of atrazine applied to sensitive and partially sensitive plants led to a statistically significant decrease in chlorophyll content, as compared to control plants. However, there was not a significant decrease in plants that inherited D_1 protein mutations. Similar results were obtained by Chodova et al. (1995) on *Senecio vulgaris* plants.

Methanol extraction is the simplest way to extract chlorophyll. Comminuted plant material (about 0.5 g) is added to methanol (5 ml). Chlorophyll absorption is read at wavelengths λ=653 and λ=666 nm. The content of chlorophyll *a* and *b*, as well as total chlorophyll content, are calculated by Lichtenthaler and Wellburn's formula (1983). Using methanol as a solvent, Pavlović (2010) demonstrated the stressful effect of sulphosate on chlorophyll contents of both cultivated plants and weeds. All measured parameters (total chlorophyll, Chl *a* and *b*) for *G. max* and *Z. mays* showed higher tendencies of decrease over time compared to the control. This information is very important for designing integrated weed control systems for various crops and for obtaining good crop yields.

Difficulties in comparing the results obtained by different extraction techniques sometimes raise the question of validity of research conclusions. A particular problem is posed by the fact that the amounts of chlorophyll measured after extraction with various solvents are hard to compare because different formulae are used for content calculation (Lichtenthaler, 1988). The defined absorption coefficients in these formulae are based on measurements made with outdated or imprecise spectophotometers that are still in use. Therefore, the results obtained by different groups of researchers may differ, even when using the same extraction solvents, and be incomparable for several reasons: (I) differences in spectrophotometer resolutions in the range of red light wavelengths (II) the accuracy of readings of selected wavelengths, and (III) water content in analyzed tissues (Jelić et al., 1992). Wellburn (1994) compared different Chl extraction techniques (extraction using 80% and 100% acetone, chloroform, diethyl ether, dimetyl formamide, dimethyl sulphoxide and methanol) and stressed the importance of interpreting the results and justified their comparison. It was shown that the measured concentration and ratios of photosynthetic pigments derived by two sets of equations were comparable when used with appropriate types of spectrophotometers but less so if inappropriate equations were used. The amount of extracted chlorophyll may provide information on the sensitivity of plants during cultivation and herbicide application, and even indicate the manner of phytotoxic activity of herbicides. This is how Nikolić et al. (2007 a, b, c) monitored the phytotoxic effect of sulphosate on maize plants. After herbicide application, symptoms were observed on plants grown in containers of different volumes. They found the chl a content (extraction with DMF) to be higher or the same in both treated and control plants growing in pots (V-5L) (as early as 4 days after treatment). This led to a conclusion that the volume of substrate, i.e. the state of plant roots, significantly affected the scope of phytotoxic effects of a herbicide. This was attributed to the fact that the root is where the synthesis of cytokinins (phytohormones required for chlorophyll synthesis) takes place (Nikolić, 2007).

3. CONCLUSION

The described biophysical methods, based on fluorescence and absorption of light re-emitted or released by plant leaves, are good tools for monitoring the functional activity of photosynthetic apparatus and for quantification of chlorophyll. By monitoring these parameters, conclusions on plant health can be drawn, and fast preliminary assessments made on the impact of various environmental factors on plant health. Also, a variety of studies on genetic variations important for plant resistance to stressful environmental factors can be performed. All described biophysical methods (based on chlorophyll fluorescence and absorption of light) offer a good choice for quick preliminary studies designed to determine plants' health and their resistance to environmental factors.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for financing the experiments (Grants: TR 31018 and TR 31037). The authors dedicate this article to Academician Prof. Dr. Vaskrsija Janjić on the occasion of his 70th birthday.

REFERENCES

Abbaspoor, M., & Streibig, J.C. (2005). Clodinafop changes the chlorophyll fluorescence induction curve. *Weed Science,* 53(1), 1-9.

Abdelhamid, M., Horiuchi, T., & Oba, S. (2003). Evaluation of the SPAD Value in Faba Bean (*Vicia faba* L.) Leaves in Relation to Different Fertilizer Applications. *Plant Production Science,* 6(3), 185-189. doi:10.1626/pps.6.185

Ahrens, W.H., Arntzen, C.J., & Stoller, E.W. (1981). Chlorophyll Fluorescence Assay for the Determination of Triazine Resistance. *Weed Science,* 29(3), 316-322.

Ali, A., & Machado, S.V. (1981). Rapid detection of 'triazine resistant' weeds using chlorophyll fluorescence. *Weed Research,* 21(3-4), 191-197. doi:10.1111/j.1365-3180.1981.tb00116.x

Anderson, D.M., Swanton, C.J., Hall, J.C., & Mersey, B.G. (1993). The influence of temperature and relative humidity on the efficacy of glufosinate-ammonium. *Weed Research,* 33(2), 139-147. doi:10.1111/j.1365-3180.1993.tb01927.x

Babani, F., & Lichtenthaler, H.K. (1996). Light-induced and Age-dependent Development of Chloroplasts in Etiolated Barley Leaves as Visualized by Determination of Photosynthetic Pigments, CO_2 Assimilation Rates and Different Kinds of Chlorophyll Fluorescence Ratios. *Journal of Plant Physiology,* 148(5), 555-566.

Baker, N.R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis *In Vivo*. *Annual Review of Plant Biology,* 59, 89-113. pmid:18444897

Baker, N.R., Oxborough, K., Lawson, T., & Morison, J.I. (2001). High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. *Journal of Experimental Botany,* 52(356), 615-21. pmid:11373309. doi:10.1093/jexbot/52.356.615

Björkman, O., & Demmig, B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta,* 170(4), 489-504. pmid:24233012. doi:10.1007/BF00402983

Boardman, N.K. (1977). Comparative Photosynthesis of Sun and Shade Plants. *Annual Review of Plant Physiology,* 28(1), 355-377. doi:10.1146/annurev.pp.28.060177.002035

Bozic, D., Vrbnicanin, S., Barac, M.B., & Stefanovic, L. (2007). Determination of johnsongrass [*Sorghum halepense* (L.) Pers.] level of sensibilty to nicosulfuron. *Maydica,* 52(3), 271-275.

Božić, D. (2010). Reakcija populacija i hibrida korova na herbicide inhibitore acetolaktat sintetaze. Beograd: Poljoprivredni fakultet. Doktorska disertacija.

Chao-Yi, L., & Der-Ming, Y. (2008). Potassium Nutrition Affects Leaf Growth, Anatomy, and Macroelements of Guzmania. *HortScience,* 43(1), 146-148.

Chodova, D., Mikulka, J., & Kocova, M. (1995). Comparing some differences of atrazine susceptible and resistant common groundsel (*Senecio vulgaris*). In: International Symposium on Weed and Crop Resistance to Herbicides, Cordoba, Spain. 144-146.

Demeter, S., & Govindjee, (1989). Thermoluminescence in plants. *Physiology of Plants,* 75(1), 121-130. doi:10.1111/j.1399-3054.1989.tb02073.x

Demmig-Adams, B., & Adams, W.W. (1992). Photoprotection and other responses of plants to high light stress. *Annual Review of Plant Physiology and Plant Molecular Biology,* 43(1), 599-626. doi:10.1146/annurev.pp.43.060192.003123

Ferell, J.A., Earl, H.J., & Vencill, W.K. (2003). The effect of selected herbicides on CO2 assimilation, chlorophyll fluorescence, and stomatal conductance in johnsongras (*Sorghum halepense* L.). *Weed Science,* 51(1), 28-31.

Fryer, M.J., Oxborough, K., Mullineaux, P.M., & Baker, N.R. (2002). Imaging of photo-oxidative stress responses in leaves. *Journal of Experimental Botany,* 53(372), 1249-54. pmid:11997373. doi:10.1093/jexbot/53.372.1249

Fuerst, P.E., & Norman, M.A. (1991). Interactions of Herbicides with Photosynthetic Electron Transport. *Weed Science,* 39(3), 458-464.

Gaborick, N. (2000). Chlorophyll concentracion (SPAD values) in leaves of Dactylis glomerata L. as an indicator of nitrogen concentracion and production grassland. *Acta fytotechnica et zootechnica,* 3, 21-24.

Geiger, D.R., & Servaites, J.C. (1994). Diurnal regulation of photosynthetic carbon metabolism in C3 plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* 44, 235-256. doi:10.1146/annurev.pp.45.060194.001315

Genty, B., Briantais, J.M., & Baker, N.R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta - General Subjects,* 990(1), 87-92.

Giardi, M.T., Masojidek, J., & Godde, D. (1997). Effects of abiotic stresses on the turnover of the D1 reaction centre II protein. *Physiologia Plantarum,* 101(3), 635-642. doi:10.1111/j.1399-3054.1997.tb01048.x

Gilmore, A.M. (1997). Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiologia Plantarum,* 99(1), 197-209. doi:10.1111/j.1399-3054.1997.tb03449.x

Habash, D., Percival, M.P., & Baker, N.R. (1985). Rapid chlorophyll fluorescence technique for the study of penetration of photosynthetically active herbicides into leaf tissue. *Weed Research,* 25(5), 389-395. doi:10.1111/j.1365-3180.1985.tb00660.x

Hák, R., Lichtenthaler, H.K., & Rinderle, U. (1990). Decrease of the chlorophyll fluorescence ratio F690/F730 during greening and development of leaves. *Radiation and Environmental Biophysics,* 29(4), 329-36. pmid:2281139. doi:10.1007/BF01210413

Jarrahi, N., Moez Ardalan, M., & Akhlaghi Amiri, N. (2013). Effect of bicarbonate of irrigation water on absorption of some of micro elements and leaf chlorophyll of some citrus rootstocks in hydroponic culture. *International Journal of Agriculture and Crop Sciences,* 6(7), 389-395.

Jelić, G., Bogdanović, M., & Nikolić, B. (1992). Metode za određivanje fotosintetskih pigmenata. *Acta herbologica,* 1(2), 235-242.

Jinwen, L., Jingping, Y., Pinpin, F., Junlan, S., Dongsheng, L., Changshui, G., & Wenyue, C. (2009). Responses of rice leaf thickness, SPAD readings and chlorophyll a/b ratios to different nitrogen supply in paddy field. *Field Crops Research,* 114(3), 426-432.

Kastori, R. (1995). *Fiziologija biljaka*. Novi Sad: Feljton.

Khan, S.R., Rose, R., Haase, D.L., & Sabin, T.E. (2000). Effects of shade on morphology, chlorophyll concentration and chlorophyll fluorescence of four Pacific North west conifer species. *New Forests,* 19(2), 171-186. doi:10.1023/A:1006645632023

Kitchen, L.M., Witt, W.W., & Rieck, C.E. (1981). Inhibition of δ-aminolevulinic acid synthesis by glyphosate. *Weed Science,* 29(5), 571-7.

Klem, K., Spundova, M., Hrabalova, H., Naus, J., Vanova, M., Masojidek, J., & Tomek, P. (2002). Comparison of chlorophyll fluorescence and whole-plant bioassays of isoproturon. *Weed Research,* 42(5), 335-341. doi:10.1046/j.1365-3180.2002.00293.x

Krause, G.H., & Weis, E. (1992). Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology,* 42, 313-349. doi:10.1146/annurev.pp.42.060191.001525

Lang, A., & Thorpe, M.H. (1985). Partitioning of assimilates at the whole plant level. In: Photosynthesis and physiology of the whole plant, OECD Workshop, Braunschweig, Germany. Paris, France: OECD.108-135.

Larcher, W. (1995). Physiological plant ecology. In *Ecophysiology and Stress Physiology of Functional Groups, 4th ed*. Berlin, Heidelberg, Germany: Springer Verlag. in English.

Leipner, J., Oxborough, K., & Baker, N.R. (2001). Primary sites of ozone-induced perturbations of photosynthesis in leaves: identification and characterization in *Phaseolus vulgaris* using high resolution chlorophyll fluorescence imaging. *Journal of Experimental Botany,* 52(361), 1689-96. pmid:11479334. doi:10.1093/jexbot/52.361.1689

Lichtenthaler, H.K. (1988). *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. In H.K. Lichtenthaler (Ed.), *Applications of Chlorophyll Fluorescence*. (pp. 129-142). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Lichtenthaler, H.K. (1996). Vegetation Stress: An Introduction to the Stress Concept in Plants. *Journal of Plant Physiology,* 148(1-2), 4-14. doi:10.1016/S0176-1617(96)80287-2

Lichtenthaler, H.K., & Babani, F. (2004). Light Adaptation and Senescence of the Photosynthetic Apparatus: Changes in Pigment Composition, Chlorophyll Fluorescence Parameters and Photosynthetic Activity. In Papageorgiu E & G.C. Govindjee (Eds.), *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. (pp. 713-736). Dordrecht, The Netherlands: Springer.

Lichtenthaler, H.K., Lang, M., Sowinska, M., Heisel, F., & Miehe, J.A. (1996). Detection of Vegetation Stress Via a New High Resolution Fluorescence Imaging System. *Journal of Plant Physiology,* 148(5), 599-612.

Lichtenthaler, H.K., & Rinderle, U. (1988). The Role of Chlorophyll Fluorescence in the Detection of Stress Conditions in Plants. *CRC Critical Reviews in Analytical Chemistry,* 19(Suppl.), 29-85.

Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents (603rd Meeting, Liverpool). *Biochemical Society Transaction,* 11(5), 591-592.

Long, S.P., Humphries, S., & Falkowski, P.G. (1994). Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology,* 45(1), 633-662. doi:10.1146/annurev.pp.45.060194.003221

Maxwell, K., & Johnson, G.N. (2000). Chlorophyll fluorescence–a practical guide. *Journal of Experimental Botany,* 51(345), 659-68. pmid:10938857. doi:10.1093/jexbot/51.345.659

Madsen, K.H., Heitholt, J.J., Duke, S.O., Smeda, R.J., & Streibig, J.C. (1995). Photosynthetic parameters in glyphosate-treated sugarbeet (Beta vulgaris L.). *Weed Research,* 35(2), 81-88.

Malkin, R., & Nyogi, K. (2000). Photosynthesis. In B.B. Buchanan, W. Gruissem, & R.L. Jones (Eds.), *Biochemistry and Molecular Biology of Plants*. (pp. 568-628). Rockville, MD, USA: ASPP.

Meyer, S., & Genty, B. (1998). Mapping Intercellular CO_2 Mole Fraction (Ci) in *Rosa rubiginosa* Leaves Fed with Abscisic Acid by Using Chlorophyll Fluorescence Imaging. Significance of Ci Estimated from Leaf Gas Exchange. *Plant Physiology,* 116(3), 947-57. pmid:9501127. doi:10.1104/pp.116.3.947

Milivojević, D.B., & Nikolić, B.R. (1998). Effects of diquat on pigment-protein complexes of thylakoid membranes in soybean and maize plants. *Biologia Plantarum,* 41(4), 597-600. doi:10.1023/A:1001804802959

Moran, R., & Porath, D. (1980). Chlorophyll determination in intact tissues using N,N-dimethylformamide. *Plant Physiology,* 65(3), 478-9. pmid:16661217

Moreland, D.E. (1980). Mechanism of action of herbicides. *Annual Review of Plant Physiology,* 31(1), 597-638. doi:10.1146/annurev.pp.31.060180.003121

Nauš, J., Prokopová, J., Rebíček, J., & Spundová, M. (2010). SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. *Photosynthesis Research ,* 105(3), 265-71. pmid:20661644

Nešković, M., Konjević, R., & Ćulafić, L. (2003). *Fiziologija biljaka*. Beograd: NNK International.

Nikolić, B. (1997). Uticaj herbicida metribuzina, linurona i dikvata na sadržaj fotosintetskih pigmenata lista soje (*Glycine max*. Merr.). Beograd: Biološki fakultet. Magistarski rad (M.Sc. thesis).

Nikolić, B. (2007). Inhibicija fotosinteze i rastenja kukuruza (*Zea mays* L.) u uslovima stresa izazvanim herbicidom sulfosatom. Kragujevac: Prirodno-matematički fakultet. Doktorska disertacija (PhD thesis).

Nikolić, B., Dodig, D., Jovanović, V., Janjić, V., & Milićević, Z. (2008). Uticaj godišnjih promena temperature i svetlosti (PAR) na indukciju fluorescencije Chla *in situ* kod *Stellaria media* (L.) i *Plantago maior* (L.). *Acta herbologica,* 17(1), 147-153.

Nikolić, B., Drinić, G., Jovanović, V., Janjić, V., & Stojaković, S. (2007a). Different aspects of inhibition of growth and photosynthesis of maize (*Zea mays* L.) by the phosphonate herbicide sulphosate, 1: Root manipulation. *Acta herbologica,* 16(1), 29-39.

Nikolić, B., Drinić, G., Jovanović, V., Janjić, V., & Marković, A. (2007b). Different aspects of inhibition of growth and photosynthesis of maize (*Zea mays* L.) by the phosphonate herbicide sulphosate, 2: Leaf canopy manipulation. *Acta herbologica,* 16(1), 41-49.

Nikolić, B., Drinić, G., Jovanović, V., Janjić, V., & Marković, A. (2007c). Different aspects of inhibition of growth and photosynthesis of maize (*Zea mays* L.) by the phosphonate herbicide sulphosate, 3: Effect of plant age. *Acta herbologica,* 16(1), 51-62.

Nikolić, B., Ivanović, Ž., Đurović, S., Starović, M., & Milićević, Z. (2011). Preliminarna zapažanja o primeni metode fluorescencije hlorofila u fitopatologiji kod nas. Zaštita bilja, 62(2), 147-152.

Nikolić, B., Janjić, V., Ignjatović, S., & Jovanović, V. (2004). Source-Sink Manipulation in Herbicide Sulfosate Stressed Maize (*Zea mays* L) Plants. In Ivana Maksimović and Maja Čuvardić (Eds.). Proceedings of XXXIV Annual ESNA Meeting, Vol. 2, Novi Sad, Serbia and Montenegro. 478-483.

Oberhuber, W., & Edwards, G.E. (1993). Temperature Dependence of the Linkage of Quantum Yield of Photosystem II to CO_2 Fixation in C_4 and C_3 Plants. *Plant Physiology,* 101(2), 507-512.

Pavlović, D. (2005). Određivanje rezistencije korova prema herbicidima – inhibitorima fotosinteze. Beograd: Poljoprivredni fakultet. Magistarska teza (M.Sc. thesis).

Pavlović, D. (2010). Osetljivost biljaka na glifosat: Morfo-anatomski, fiziološki i biološki aspekti. Beograd: Poljoprivredni fakultet. Doktorska disertacija (PhD thesis).

Pavlović, D., Elezović, I., Jovanović, L., & Mirisavljević, D. (2002). Investigation on atrzine resistance in different populations of *Chenopodium album* L. and *Amaranthus retroflexus* L. using nondestructive methods. *Zaštita bilja (Plant Protection),* 53(4), 181-190.

Pavlović, D., Elezović, I., Jovanović, L., & Marisavljević, D. (2004). Primena nedestruktivnih metoda za određivanje stepena rezistentnosti pepeljuge (*Chenopodium album* l.) na atrazin. *Acta herbologica,* 13(2), 531-536.

Pavlović, D., Nikolić, B., Pfaf-Dolovac, E., Marisavljević, D., Milićević, Z., & Đurović, S. (2010). Hlorofil a kao indikator reakcije biljaka na herbicide. *Zaštita bilja*, 61(2), 67-86.

Pavlović, D., Vrbničanin, S., Božić, D., & Marisavljević, D. (2007a). Testing resistance of *Abutilon theoprasti* Med. to atrazine. In: 14th EWRS Symposium, Hamar, Norway. 157.

Pavlović, D., Vrbničanin, S., Božić, D., & Simončič, A. (2007b). The study of methods for determination of metabolism based resistance of *Abutilon theophrasti* Medic. to atrazine. *Journal of Central European Agriculture,* 8(4), 435-442.

Pavlović, D., Vrbničanin, S., Elezović, I., Jovanović, L., & Marisavljević, D. (2006). Alterations in amount of chlorophyll as indicator of resistance for *Chenopodium album* L. and *Amaranthus retroflexus* L. to atrazine. *Journal of Plant Diseases and Protection,* 20, 131-138.

Pavlović, D., Vrbničanin, S., Elezović, I., Marisavljević, D., & Božić, D. (2005). Fiziološki parametri kao osnova razdvajanja rezistentnih od osetljivih populatija *Chepodium album* L. prema atrazinu. *Pesticidi i fitomedicina,* 20(4), 241-246.

Pavlović, D., Vrbničanin, S., & Reinhardt, C. (2013). Crop response to glyphosate trimesium sulphosate. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 41(2), 582-589.

Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology,* 27(6), 595-607.

Раденович, Ч., Дринич, Г. & Иванович, М. (2005) Элитные инбредные линии кукурузы с вертикально стоящими листями. Характеристика эффективной фотосинтетической модели в селекции. *Кукуруза и сорго*, 3: 19-24.

Radenović, Č.N., Jeremić, M., & Marković, D. (1994). Delayed chlorophyll fluorescence in plants models. *Photosynthetica,* 30(1), 1-24.

Radotić, K., Radenović, C., & Jeremić, M. (1998). Spontaneous Ultraweak Bioluminescence in Plants: Origin, Mechanisms and Properties. *General Physiology and Biophysics ,* 17(4), 289-308. pmid:10191416

Schreiber, U., Schliwa, U., & Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research,* 10(1-2), 51-62. pmid:24435276. doi:10.1007/BF00024185

Simić, M., Hamouzova, K., Soukup, J., Boz, O., Nikolić, A., & Dragičević, V. (2012). Testiranje korovske vrste *Solanum nigrum* L. na rezistentnost prema triazinskim herbicidima. *Acta herbologica,* 21(2), 69-77.

Szigeti, Z., Richter, P., & Lichtenthaler, H.K. (1996). Fluorescence emission spectra of paraquat resistant *Conyza canadensis* during the chlorophyll fluorescence induction as determined by the CCD-OMA system. *Journal of Plant Physiology,* 148(5), 574-578.

Terashima, I., Hanba, Y.T., Tazoe, Y., Vyas, P., & Yano, S. (2006). Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *Journal of Experimental Botany ,* 57(2), 343-54. pmid:16356943

Thompson, J.A., Schweitzer, L.E., & Nelson, R.L. (1996). Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. *Photosynthesis Research,* 49(1), 1-10. pmid:24271528. doi:10.1007/BF00029422

van Oorchot, J.L.P., & van Leeuwen, P.H. (1992). Use of fluorescence induction to diagnose resistance of *Alopecurus myosuroides* Huds. (black-grass) to chlorotoluron. *Weed Research*, 32(6), 473-482. doi:10.1111/j.1365-3180.1992.tb01908.x

WoonHo, Y., ShaoBing, P., JianLiang, H., Sanico, A.L., Buresh, R.J., & Witt, C. (2003). Using leaf color charts to estimate leaf nitrogen status of rice. *Agronomy Journal,* 95(1), 212-217.

Wellburn, A.R. (1994). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology,* 144(3), 307-313.

Yerkes, C.N., Esqueda, V.A., Nourizadeh, S.D., & Weller, S.C. (1995). Comparsion and characterization of two Indiana atrazine resistant weed species. In: Proceedings of Abstracts of International Symposium on weed and crop resitance to herbicides, Cordoba, Spain. 124-127.

Hlorofil kao merilo zdravlja biljaka: agroekološki aspekti

REZIME

Kako je proces fotosinteze osnovni proces tokom kojeg se svetlosna energija apsorbuje i konvertuje u organsku materiju, ključni je značaj postojanja biljnog pigmenta hlorofila (*a* i *b* forma) kao posrednika u transformaciji apsorbovane svetlosne energije i njegove aktivnosti u procesu fotosinteze i sinteze organskih materija kod biljaka. Stoga je u radu dat pregled metoda za praćenje optičke aktivnosti molekula hlorofila, kao i metoda (nedestruktivnih i destruktivnih) kvantifikacije hlorofila u biljkama. Ove metode se primenjuju u proceni uticaja različitih stresnih faktora (abiotskih, biotskih i ksenobiotskih) na efikasnost fotosinteze i bioproduktivnost biljaka, sa ciljem procenjivanja uticaja koji ovi ograničavajući faktori imaju na prinos useva. Takođe, pomenute metode za analizu optičke aktivnosti i/ili sadržaja hlorofila su odgovarajuće i za procenu reakcije korova na različite poljoprivredne prakse (mineralna ishrana, primena herbicida i sl.) i ispitivanje različitih aspekata ekofiziologije korova i procenu njihovog uticaja na prinos useva.

Ključne reči: hlorofili, fotosinteza, zdravlje biljaka