DOI: 10.32615/ps.2019.107 PHOTOSYNTHETICA 58 (SI): 186-193, 2020

Development and aging of photosynthetic apparatus of *Vitis vinifera* L. during growing season

K. SITKO[*,+], S. RUSINOWSKI[**], M. POGRZEBA[**], A. DASZKOWSKA-GOLEC[***], Ż. GIEROŃ[*], H.M. KALAJI[#], and E. MAŁKOWSKI[*,+]

Department of Plant Physiology, University of Silesia in Katowice, Katowice, Poland[*]
Institute for Ecology of Industrial Areas, Katowice, Poland[**]
Department of Genetics, University of Silesia in Katowice, Katowice, Poland[***]
Department of Plant Physiology, Warsaw University of Life Sciences (SGGW), Warsaw, Poland[#]

Abstract

The aim of this study was to examine the development and aging of chosen grapevine leaves *in situ* during the growing season (130 d) using chlorophyll (Chl) *a* fluorescence measurements and determining the changes in pigment contents. During the course of photosystems development, the increase of Chl and decrease of anthocyanin contents in leaves was observed simultaneously. On 28th day, the maximum content of Chl and minimum content of anthocyanins was measured. However, the maximal photosynthetic performance was found one week later, when the content of Chl started to diminish. Our study proved that the achievement of maximal photosynthetic performance of each leaf took about quarter of organ life and this state lasted very shortly. In this work, we described and discussed for the first time the dynamics of Chl, anthocyanins, and flavonols combined with photosynthetic efficiency changes during the leaf life *in situ*.

Additional key words: chlorophyll *a* fluorescence transient; energy flux; photosynthesis; reaction center; senescence.

Introduction

Most of plants growing in temperate climate develop new leaves annually. During the spring, under optimal environmental conditions, plants develop new leaves very quickly, which allow them to reach sunlight and carry out the life cycle. Completing this process successfully depends on both productivity and performance of photosynthetic apparatus. The formation of chloroplasts from proplastids and their ultrastructure specialization, Chl accumulation, and the synthesis of the major protein components of the photosynthetic apparatus proceed almost in parallel during the leaf development. The compilation of these processes in time results in a proportional increase of net photosynthesis (Jiang *et al*. 2006a). At the end of each growing season, the phenomenon of senescence of leaves is manifested through a visible change of their colors. Although many processes related to the beginning of the development and aging of leaves are well explained, there are still questions to be answered.

Grapevine (*Vitis vinifera* L.) is European native species, but cultivated with favor worldwide. This characteristic liana with its round berries, full of anthocyanins, became not only a permanent element of Mediterranean landscape, but also exceptionally interesting object of scientific investigations (Agati *et al.* 2007, Ziliotto *et al.* 2012, Vitulo *et al.* 2014). Taking into account its high economic importance, grapevine genome has been sequenced (Jaillon *et al.* 2007, Velasco *et al.* 2007) and many experiments, aimed to better understand its biology, were performed (Kolb *et al.* 2001, Jiang *et al.* 2006a, Kadir *et al*. 2007, Wright *et al.* 2009, Cerovic *et al.* 2015). The anthocyanin synthesis pathways and genes involved have been identified (Boss *et al.* 1996) and the antioxidative role of seed extract was determined (Jayaprakasha *et al.* 2001). To counteract the progressive desertification of different habitats, research was done to characterize a response of grapevine to drought stress (Haider *et al.* 2017). Research on flavonols

Received 20 February 2019, *accepted* 2 July 2019.
[+]Corresponding author; e-mail: krzysztof.sitko@us.edu.pl, eugeniusz.malkowski@us.edu.pl
Abbreviations: ABS/CS – absorption flux per CS; CS – excited cross section of leaf; DI/CS – dissipation energy flux per CS; ET/CS – electron transport per CS; F_0 – minimal fluorescence, when all PSII RCs are open (at t = 0); F_m – maximal fluorescence, when all PSII RCs are closed; F_t – fluorescence at time t; F_v – maximal variable fluorescence; PI_{ABS} – performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors; RC/CS – percentage of active reaction centers per CS; TR/CS – trapped energy flux per CS; V_t – relative variable fluorescence at time t; δR_0 – probability with which an electron from the intersystem electron carriers will move to reduce the end acceptors at the PSI acceptor side; φD_0 – quantum yield (at t = 0) of energy dissipation; φE_0 – quantum yield of electron transport (at t = 0); φP_0 – maximum quantum yield of primary photochemistry (at t = 0); φR_0 – quantum yield for reduction of end electron acceptors at the PSI acceptor side; ψ_{E0} – probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A.
Authors dedicate the present paper to Professor Dr. Reto Jörg Strasser for his magnificent input to the wide application of chlorophyll *a* fluorescence in physiological research and inestimable inspiration.

in grapevine is limited to grape berries, especially, to the expression of genes involved in biosynthesis pathways (Chaves *et al.* 2010, Hichri *et al.* 2010, Martínez-Lüscher *et al.* 2014).

Chl *a* fluorescence measurements enable monitoring physiological status of plants. It is a common nondestructive and sensitive method to evaluate overall changes in the status of plant bioenergetics. The mobility of measuring devices has influenced the prevalence of this method (Baker 2008, Kalaji *et al.* 2014a, 2017). The same is true for the pigment content measurements, where portable sensors become increasingly used (Cerovic *et al.* 2015, Goltsev *et al.* 2016, Lefebvre *et al.* 2016, Gonzalez-Mendoza *et al.* 2017, Hosseini *et al.* 2017, Sitko *et al.* 2017). Both types of measurements are widely used; *e.g.*, to study physiological status of plants under environmental stress (Sitko *et al.* 2017), effects of fertilization on plant growth (Pogrzeba *et al.* 2017), or phenotyping of mutants (Daszkowska-Golec *et al.* 2017, 2018). It seems obvious that the parameters describing performance of electron transport chain are characterized by inverted U-shape curve during lifespan, but the dynamics of those changes has not been studied in details until now.

Currently, in addition to the content of Chl, modern devices allow to monitor the content of flavonols and anthocyanins in plant tissues. Both these pigment groups belong to flavonoids – a large family of plant secondary metabolites (Skórzyńska-Polit *et al.* 2004, Mattivi *et al.* 2006). Different flavonoid subclasses have been found to possess protective roles in plant tissues and the biosynthesis of flavonoids often increases in response to biotic and abiotic stress factors (Jaakola *et al.* 2004). Anthocyanins play mostly a photoprotective role in leaves, trapping the excessive light radiation, but may also offer an alternative way of reducing the accumulation of hexoses, thus delaying the sugar-induced early senescence of leaves (Landi *et al.* 2015, Lo Piccolo *et al.* 2018). Flavonols have been found to play a protective role as a UV filter, but may also participate in a peroxidase-flavonol quenching system for reactive oxygen species (Jaakola *et al.* 2004, Skórzyńska-Polit *et al.* 2004). Also, the typical pattern of Chl and anthocyanins content changes during lifespan of leaves is well known, nevertheless, the dynamics of those changes is still unspecified. On the other hand, there is a lack of data describing the changes of flavonols content in leaves during the growing season, except the newest paper of Mattila *et al.* (2018), in which authors described the changes of flavonols content in leaves of four tree species during senescence.

The aim of this study was to examine the dynamics of changes of the photosynthetic apparatus in grapevine during the growing season *in situ*. We used nondestructive methods to examine particular phases of electron transport chain domains development and aging during the lifespan of chosen leaves. The measurements of changes in contents of selected pigments during a lifetime of leaves were also conducted. For the first time, we present the summarized changes of chlorophyll *a* fluorescence, chlorophyll, anthocyanins, and flavonols content for leaves during their lifespan.

Materials and methods

Plant material: In this study, six plants of *Vitis vinifera* L. were examined. Plants of age from 10 to 15 years were grown on the courtyard of Faculty of Biology and Environmental Protection of University of Silesia in Katowice, Poland (50°15'17.0"N, 19°01'25.0"E). At the beginning of growing season, five leaves for each plant were selected and marked with plastic band on their petioles. Selected leaves were always the fourth on the new shoot. Measurements started when leaves achieved the optimal size of lamina, about fourth day of their development. At this day, the area of leaves was proper for measurement devices, which covered the entire area of measuring sensors, and the probability of mechanical damage of leaves was relatively low. Plants were kept well-watered during the experiment. The weather data (Fig. 1S, *supplement*) were obtained from ASM 'Andretti', the weather station was located about 10 km from the Faculty.

The fluorescence of Chl *a* and the content of pigments: Measurements were performed for all selected leaves during 130 d (between 10 May and 16 September in 2016) with variable frequency. Through the first three weeks of experiment, measurements were performed every day to examine the dynamics of photosynthetic apparatus development. For next 16 weeks, measurements were performed two times a week. The measurements of the pigments content and fluorescence were performed on the same leaves. The polyphenol and Chl meter *Dualex Scientific+*^{TM} (*Force-A*, France) was used to measure contents of Chl, flavonols, and anthocyanins in leaves. The measurement method of *Dualex* sensor and equations were widely described in Cerovic *et al.* (2015) and Julkunen-Tiitto *et al.* (2015). Chl *a* fluorescence measurements were performed on the same leaves using the plant efficiency analyzer (*Pocket PEA*, *Hansatech Instruments Ltd*., UK). Before fluorescence measurements, each selected leaf was adapted in the dark for 30 min using a dedicated leaf clips. After adaptation, a saturating light pulse of 3,500 μmol(quanta) m^{-2} s^{-1} was applied for 1 s, which closed all of the reaction centers, and the fluorescence parameters were measured. Both pigment contents and Chl fluorescence measurements were performed *in situ* without destruction of plant tissues.

Statistical analysis: Results are shown as the means ± SE. The statistical significance of the differences was determined using one-way analysis of variance (*ANOVA*) and the post hoc *Tukey*'s HSD test (P<0.05). The software used for the statistical analyses was *Statistica v.13.1* (*Dell Inc*., USA). The phenomenological models of energy fluxes through the leaf cross sections were created using *CorelDRAW X8 2017* (*Corel Corp*., Canada).

Results

Chl *a* fluorescence parameters: Changes in OJIP rise kinetics during the growing season were presented as typical fluorescence rise curves and the subtraction

between variable fluorescence curves for each selected day and day 35 of the experiment (ΔV_t). The early stages of development of PSII (1–7 d) were characterized by flattened fluorescence curves, without visible characteristic steps: J, I, and P (Fig. 1*A*). From the day 10, the increase of F_0 value and the appearance of J step was observed. The highest F_m value and the typical kinetic of fluorescence rise curve were observed at day 35 and the curve obtained this day was used as a reference to calculate ΔV_t (Fig. 1*B*). The characteristic peaks appearing in the ΔV_t curves were described in details in Kalaji *et al.* (2014b) and Paunov *et al.* (2018). At the early stages of leaf development, the ΔK and ΔJ steps were visible, which may suggest that during development of photosynthetic apparatus, the biggest changes occurred in oxygen-evolving complex (OEC) and pool of quinone electron transporters (*e.g.*, primary quinone) (Fig. 1*B*). The lack of significant ΔI, ΔH, and ΔG bands may suggest that activity of ferredoxin-$NADP^+$ oxidoreductase (FNR) and other electron acceptors in PSI was at constant high level from the beginning of leaf development (Fig. 1*B*). From day 35 to about day 100, the kinetics of fluorescence curves were comparable (Fig. 2*A*). That was the period of maximal yield of photosystems. From the day 102, the curves became flattened until almost linear at day 130 (Fig. 2*A*). First signals of leaf aging were observed as an appearance of ΔJ and ΔH, which may be related to accumulation of Q_A^- (reduced primary quinone) and decrease of activity of final electron acceptors in PSI, respectively (Fig. 2*B*). The more intensive changes which signalize the ageing of photosynthetic apparatus were observed between days 100 and 116 as the appearance of significant ΔI and ΔH bands. Also, the ΔJ band moved toward ΔK band (Fig. 2*B*). Such a dynamics of senescence suggests that deactivation of PSI may occur before the degradation of PSII.

The phenomenological energy fluxes per the excited cross sections (CS) of the grapevine leaves during growing season are presented in Fig. 3. On day 1 of measurements (about 4th day of leaf development), the leaves were characterized by relatively low energy fluxes, which significantly increased at 7 d. Rapid increase of absorbed energy (ABS/RC) and electron transport per cross section (ET/CS) was observed between 13–35 d (Fig. 3). After reaching the maximal efficiency on day 35, the energy fluxes slowly decreased until day 102. Then, the senescence became more intensive and on day 130, photosynthetic apparatus was deactivated completely. The most sensitive parameter of energy fluxes seems to be RC/CS, which reached the maximal value relatively slowly and started decreasing just right after. The dissipated energy (DI/CS) reached its maximal value relatively fast (about 10 d) and started to decrease at about day 60 of the measurements. Presented data suggest that senescence was a faster process than photosystem development (Fig. 3).

The changes of the most of parameters describing the quantum yield were correlated with the OJIP curves (Figs. 1, 2) and energy fluxes (Fig. 3). Parameters related to OJ phase of fluorescence (ψE_0 and φE_0), as well as F_v, φP_0, and φR_0, reached their maximum on day 35 and minimum at the last day of measurements (Table 1). The quantum yield of energy dissipation (φD_0) was minimal on day 35. The probability with which an electron moves to reduce the acceptors at the PSI acceptor side (δR_0) presented the highest values at the early phase of leaf development (4–13 d) and markedly declined after day 35 of the measurements (Table 1). Two maximal peaks were characteristic for kinetics of PI_{ABS} changes; the first in the phase of leaf development and the second in the phase of

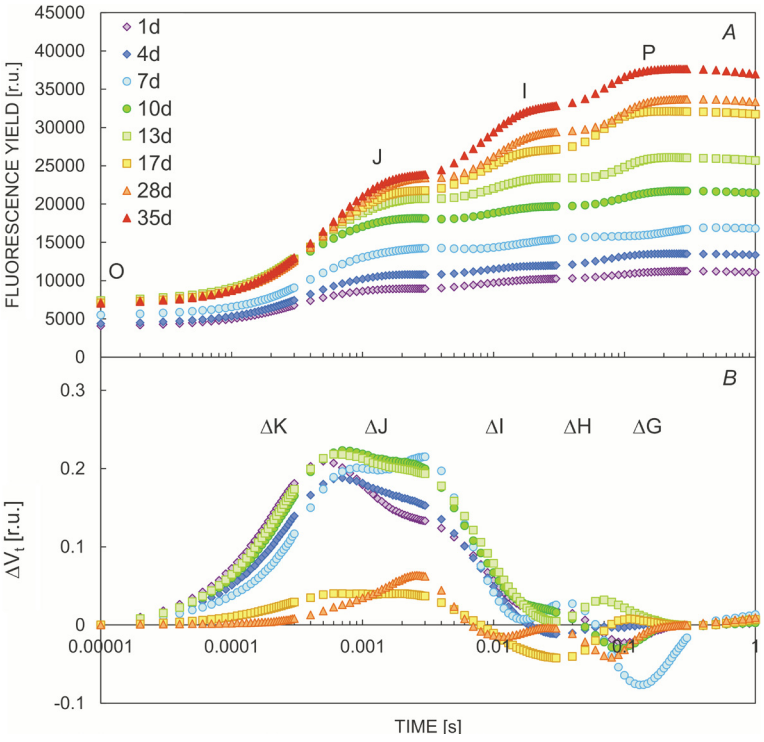

Fig. 1. Chlorophyll *a* fluorescence induction curve of *Vitis vinifera* L. during development of photosynthetic apparatus (*A*) and the effect of development of PSII on the relative variable fluorescence [$\Delta V_t = (F_t - F_0)/F_v - V_{t35d}$] of studied leaves (*B*). For ΔV_t analysis, the fluorescence of leaves on day 35 of the measurements was used as a reference and equalled 0. Values are means ($n = 30$).

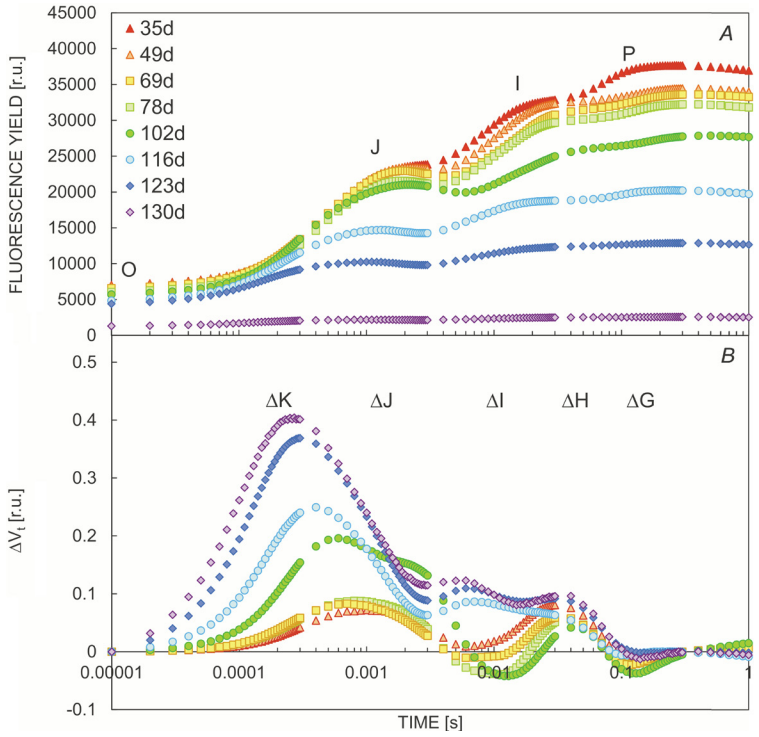

Fig. 2. Chlorophyll *a* fluorescence induction curve of *Vitis vinifera* L. during aging period of growing season (*A*) and the effect of aging of PSII on the relative variable fluorescence [$\Delta V_t = (F_t - F_0)/F_v - V_{t35d}$] of studied leaves (*B*). For ΔV_t analysis, the fluorescence of leaves on day 35 of the measurements was used as a reference and equalled 0. Values are means ($n = 30$).

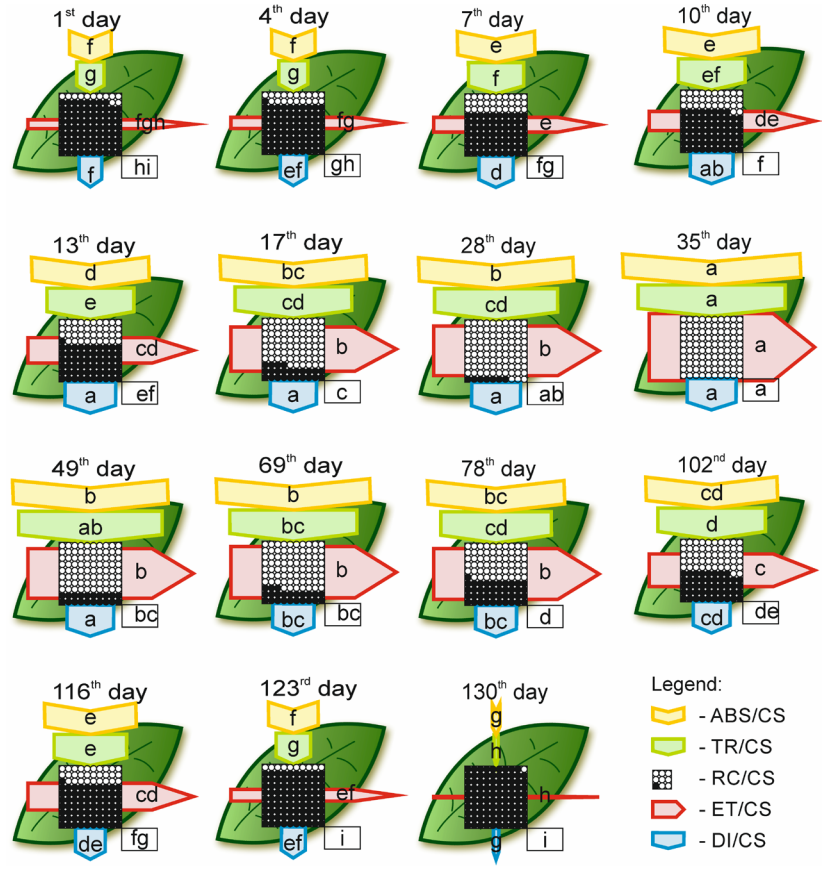

Fig. 3. Leaf models showing the phenomenological energy fluxes per excited cross-sections (CS) of the leaves of *Vitis vinifera* L. during growing season. Each relative value of the measured parameters is the mean ($n = 30$) and the width of each arrow corresponds to the intensity of the flux. ABS/CS – absorption flux per CS approximated; TR/CS – trapped energy flux per CS; ET/CS – electron transport flux per CS; DI/CS – dissipated energy flux per CS; RC/CS – percentage of active/inactive reaction centers. *White circles* inscribed in squares represent reduced Q_A reaction centers (active), *black circles* represent nonreducing Q_A reaction centers (inactive), 100% of the active reaction centers responded with the highest mean value observed in the reference at 35 d. Means followed by *the same letter* for each parameter are not significantly different from each other using the *Tukey*'s HSD test ($P \leq 0.05$). Letters are inscribed into arrows, except for RC/CS where they are placed in a box in the lower right corner of the square with circles.

leaf senescence. On the other hand, the minimal value of this parameter was observed on day 35 (Table 1).

Pigment contents: The kinetics of changes in pigment contents in leaves of grapevine during growing season is

Table 1. Characteristics of photosynthetic apparatus of grapevine during growing season. Presented data are means ± SE (n = 30). Means followed by *the same letter* in a column are not significantly different from each other using the *Tukey*'s HSD test ($P \leq 0.05$). F_v – maximum variable fluorescence; φP_0 – maximum quantum yield of the primary PSII photochemistry; ψE_0 – probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φE_0 – quantum yield for electron transport from Q_A^- to plastoquinone; δR_0 – probability with which an electron from the intersystem electron carriers will move to reduce the end acceptors at the PSI acceptor side; φR_0 – quantum yield for the reduction of the end electron acceptors at the PSI acceptor side; φD_0 – quantum yield (at t = 0) of energy dissipation; PI_{ABS} – performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors.

Day	F_v	φP_0	ψE_0	φE_0	δR_0	φR_0	φD_0	PI_{ABS}
1	7,070 ± 240^{h}	0.64 ± 0.01^{d}	0.32 ± 0.01^{cde}	0.20 ± 0.01^{ghi}	0.44 ± 0.02^{cde}	0.09 ± 0.00^{de}	0.36 ± 0.01^{bc}	13.9 ± 0.5^{bc}
4	8,980 ± 430^{gh}	0.68 ± 0.01^{cd}	0.29 ± 0.01^{ef}	0.20 ± 0.01^{hi}	0.58 ± 0.02^{bc}	0.11 ± 0.00^{bc}	0.32 ± 0.01^{bc}	15.4 ± 0.4^{bc}
7	11,090 ± 560^{fg}	0.66 ± 0.01^{cd}	0.25 ± 0.01^{fg}	0.17 ± 0.01^{ij}	0.52 ± 0.01^{c}	0.09 ± 0.00^{de}	0.37 ± 0.01^{b}	15.7 ± 0.7^{bc}
10	13,880 ± 730^{ef}	0.65 ± 0.01^{cd}	0.22 ± 0.02^{g}	0.15 ± 0.01^{j}	0.80 ± 0.09^{a}	0.09 ± 0.00^{cde}	0.35 ± 0.01^{bc}	24.2 ± 1.8^{a}
13	19,820 ± 970^{d}	0.70 ± 0.01^{c}	0.25 ± 0.02^{fg}	0.18 ± 0.01^{hij}	0.74 ± 0.08^{ab}	0.11 ± 0.00^{cd}	0.31 ± 0.01^{c}	25.1 ± 2.7^{a}
17	23,950 ± 800^{bc}	0.78 ± 0.01^{ab}	0.36 ± 0.01^{bc}	0.28 ± 0.01^{def}	0.49 ± 0.02^{cd}	0.13 ± 0.00^{a}	0.22 ± 0.01^{de}	12.4 ± 0.7^{bc}
28	26,740 ± 550^{b}	0.79 ± 0.01^{ab}	0.40 ± 0.01^{b}	0.31 ± 0.01^{cde}	0.42 ± 0.02^{cde}	0.13 ± 0.00^{ab}	0.21 ± 0.01^{de}	10.6 ± 0.4^{c}
35	30,930 ± 300^{a}	0.82 ± 0.00^{a}	0.46 ± 0.01^{a}	0.38 ± 0.01^{a}	0.34 ± 0.02^{def}	0.13 ± 0.01^{ab}	0.18 ± 0.00^{e}	10.1 ± 0.4^{c}
49	26,890 ± 800^{b}	0.80 ± 0.01^{ab}	0.40 ± 0.01^{b}	0.32 ± 0.01^{cd}	0.21 ± 0.02^{f}	0.06 ± 0.00^{fg}	0.20 ± 0.01^{de}	14.3 ± 0.8^{bc}
69	26,600 ± 430^{b}	0.82 ± 0.00^{a}	0.40 ± 0.01^{b}	0.33 ± 0.01^{bc}	0.24 ± 0.01^{f}	0.08 ± 0.00^{ef}	0.18 ± 0.00^{e}	15.9 ± 1.2^{bc}
78	23,680 ± 730^{bc}	0.80 ± 0.01^{ab}	0.45 ± 0.01^{a}	0.37 ± 0.01^{ab}	0.24 ± 0.02^{f}	0.09 ± 0.01^{def}	0.20 ± 0.01^{de}	14.5 ± 0.8^{bc}
102	22,740 ± 870^{cd}	0.81 ± 0.01^{ab}	0.30 ± 0.01^{d}	0.24 ± 0.01^{fg}	0.43 ± 0.02^{cde}	0.10 ± 0.00^{cd}	0.19 ± 0.01^{de}	27.9 ± 1.6^{a}
116	15,860 ± 1,190^{e}	0.77 ± 0.02^{b}	0.35 ± 0.01^{bcd}	0.27 ± 0.01^{ef}	0.30 ± 0.03^{ef}	0.07 ± 0.00^{ef}	0.23 ± 0.01^{d}	25.2 ± 1.1^{a}
123	8,650 ± 640^{gh}	0.66 ± 0.02^{cd}	0.33 ± 0.01^{cd}	0.22 ± 0.01^{gh}	0.22 ± 0.03^{f}	0.05 ± 0.01^{gh}	0.34 ± 0.02^{bc}	22.9 ± 1.3^{a}
130	1,270 ± 290^{i}	0.46 ± 0.02^{e}	0.30 ± 0.01^{de}	0.14 ± 0.01^{j}	0.22 ± 0.02^{f}	0.03 ± 0.00^{h}	0.54 ± 0.02^{a}	16.7 ± 1.1^{b}

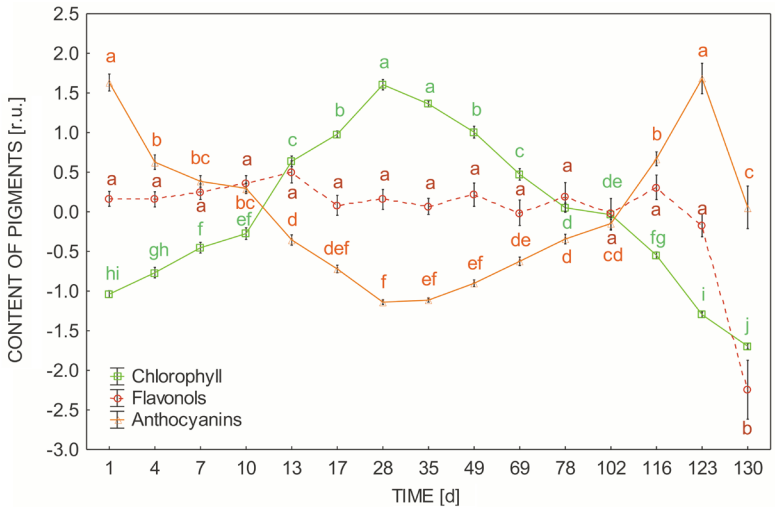

Fig. 4. Changes in content of pigments in studied leaves of *Vitis vinifera* L. during growing season. Values are standardized means ± SE (n = 30). Means followed by *the same letter* for each pigment are not significantly different from each other using the *Tukey*'s HSD test ($P \leq 0.05$).

presented in Fig. 4. The Chl content in leaves increased from the first day of experiment, until reaching maximal value on day 35, then slowly decreased until the end of measurements. The Chl content in leaves was significantly lower at the end of the growing season (130 d) compared to the content at the beginning of leaf development (Fig. 4). Anthocyanins were characterized by the inversed dynamics of the content changes compared to the Chl (Fig. 4). The highest content of anthocyanins was measured on the first day of the experiment. Interestingly, the same content of these pigments was observed the week before abscission. The lowest content of anthocyanins in leaves was observed in parallel with the highest content of Chl at the same time. Flavonols were characterized by the constant content in grapevine leaves, except the last week of measurements, when the content drastically decreased (Fig. 4). During senescence of leaves, especially during the last week, the degradation of all pigments was observed (Fig. 4).

Discussion

Lo Piccolo *et al.* (2018) found that the Chl content increased in mature leaves and significantly decreased toward the end of the experiment. These typical Chl content changes during lifespan of leaves were confirmed by many other authors (Lu *et al.* 2001, 2002; Weng *et al.* 2005,

Mauromicale *et al.* 2006, Ierna 2007, Balazadeh *et al.* 2008) and is also presented in our work (Fig. 4). Moreover, Mattila *et al.* (2018) investigated four tree species and found that Chl content may stay high during the autumn and rapidly decrease for 10 d until abscission (*Acer platanoides*, *Betula pendula*, and *Prunus padus*) or Chl may slowly degrade during the whole autumn (*Sorbus aucuparia*). Our results for grapevine confirmed the latter scenario (Fig. 4). Lo Piccolo *et al.* (2018) found that anthocyanin content showed an inversed course of changes compared to Chl, but did not reach the values of juvenile leaves. However, we found that anthocyanins content both at the beginning and at the end of leaf lifespan were comparable and statistically insignificant (Fig. 4). Differences may be due to the age of the tested leaves and frequency of measurements. Lo Picollo *et al.* (2018) measured the anthocyanins index for senescent leaves, but it is difficult to assess, whether the investigated leaves were before or after reaching the maximum autumn value of these pigments, which we observed. Nevertheless, based on our results, it is clear that the content of anthocyanins in leaves was comparable both at the beginning and at the end of the lifespan of leaves (Fig. 4). There is a lot of data on the flavonol content changes during lifespan of leaves; however, only the paper by Mattila *et al.* (2018) describes the autumnal changes of these pigment contents. Mattila *et al.* (2018) found that the flavonols content in leaves of three tree species slightly increased during the whole autumn and the increase seemed to speed up with a decrease in the Chl content. They also found for *Prunus padus* that the flavonols content did not changed during autumn. We also observed the slight increase (about 7.5%) of flavonols from 35 to 116 d of experiment, but it was insignificant; thus, we observed the similar effect as in *P. padus* (Mattila *et al.* 2018). After this insignificant increase of flavonols, the strong decrease was observed (Fig. 4), which could be due to the relocation of nutrients to still active younger leaves.

The changes of photosynthetic apparatus are naturally associated with the dynamics of Chl content in leaves. Both, Jiang *et al.* (2006a,b) and Lo Piccolo *et al.* (2018) demonstrated the increase of minimal fluorescence (F_0) and maximum quantum yield of primary photochemistry (φP_0) with the young leaves development. Moreover, Jiang *et al.* (2006a) showed that with leaf development, the efficiency that a trapped exciton can move an electron into the electron transport chain further than Q_A (Ψ_0) and the quantum yield of electron transport beyond Q_A (φE_0), increased gradually and rapidly. They also suggest, based on the appearance of the K step, that the OEC might not to be fully connected with PSII at the beginning of leaf growth (Jiang *et al.* 2006a). Our observations confirmed those obtained by Jiang *et al*. (2006a) and Lo Piccolo *et al*. (2018). Moreover, our results expand this knowledge with new data (Table 1, Fig. 1). Here, we showed for the first time that at the earliest stage of leaf development, Ψ_0 and φE_0 significantly decreased, but after the first week of development, the rapid increase of these parameters was measured until they reached the maximum value (Table 1). Jiang *et al.* (2006a) determined the φP_0 of young grapevine leaves to be 90% of the value measured in fully developed leaves. Nevertheless, they found the fluorescence rise from O to J step to be clearly speeded up in young leaves compared to that in fully expanded leaves (Jiang *et al.* 2006a). In our work, much younger leaves were investigated; as a result, the initial φP_0 was lower by 22% when compared to fully developed leaves (Table 1). Also, the fluorescence rise curve from O to J step was speeded up in young leaves, which was shown apparently by increasing the frequency of measurements compared to Jiang *et al.* (2006b) (Fig. 1).

From the moment of reaching the maximum efficiency of photosynthetic apparatus, its decrease was observed, particularly during the last two weeks of leaf senescence (Figs. 2, 3). The process of leaf senescence was widely examined and many authors denoted the decrease of φP_0 (Miersch *et al.* 2000, Lu *et al.* 2001, 2002; Weng *et al.* 2005, Ierna 2007, Tang *et al*. 2015), F_0 (Weng *et al.* 2005, Ierna 2007, Tang *et al.* 2015) or Ψ_0 and φE_0 (Tang *et al.* 2015), which was also observed in our experiment (Table 1, Fig. 2). Tang *et al.* (2015) showed the characteristic flattening of the I step in fluorescence rise curve in aging leaves and the decrease of percentage of active reaction centers per cross sections of leaves (RC/CS). Both observations were confirmed by our experiments (Figs. 2, 3) and enriched by analyses of changes of energy fluxes per cross section. On the basis of presented data (Table 1, Fig. 2), it could be hypothesized that PSI may be degraded faster than PSII in grapevine, as previously suggested by Miersch *et al.* (2000) and Tang *et al.* (2005) by protein analysis in barley and rice, respectively.

In summary, we were able to trace and locate individual processes of development and aging of leaves in time using nondestructive techniques based on chlorophyll fluorescence. It was demonstrated that the maximum efficiency of photosynthetic apparatus was a very short-time phenomenon. In the current study, it was shown for the first time that the maximum chlorophyll content in leaves precedes the maximum performance of photosystems. We found that the dynamics of chlorophyll and anthocyanin content changes was characterized by inversion, but in contrast to chlorophyll, anthocyanin content was comparable at the beginning and at the end of leaf lifespan. For the first time, it was demonstrated that flavonol contents in leaves remained constant for the most of the growing season and rapidly degraded during the last week of senescence. Thus, our research and data from the literature indicate that changes in the content of pigments during aging may be species-specific, and it is necessary to examine a greater number of plant species to precise these relations.

References

Agati G., Meyer S., Matteini P., Cerovic Z.G.: Assessment of anthocyanins in grape (*Vitis vinifera* L.) berries using a noninvasive chlorophyll fluorescence method. – J. Agr. Food Chem. **55**: 1053-1061, 2007.

Baker N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – Annu. Rev. Plant. Biol. **59**: 89-113, 2008.

Balazadeh S., Parlitz S., Mueller-Roeber B., Meyer R.C.: Natural developmental variations in leaf and plant senescence in

Arabidopsis thaliana. – Plant Biol. **10**: 136-147, 2008.
Boss P.K., Davies C., Robinson S.P.: Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. – Plant Physiol. **111**: 1059-1066, 1996.
Cerovic Z.G., Ben Ghozlen N., Milhade C. *et al*.: Nondestructive diagnostic test for nitrogen nutrition of grapevine (*Vitis vinifera* L.) based on Dualex Leaf-Clip measurements in the field. – J. Agr. Food Chem. **63**: 3669-3680, 2015.
Chaves M.M., Zarrouk O., Francisco R. *et al*.: Grapevine under deficit irrigation: hints from physiological and molecular data. – Ann. Bot.-London **105**: 661-676, 2010.
Daszkowska-Golec A., Skubacz A., Marzec M. *et al*.: Mutation in *HvCBP20* (*Cap Binding Protein 20*) adapts barley to drought stress at phenotypic and transcriptomic levels. – Front. Plant Sci. **8**: 942, 2017.
Goltsev V.N., Kalaji H.M., Paunov M. *et al*.: Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. – Russ. J. Plant Physl+ **63**: 869-893, 2016.
Gonzalez-Mendoza D., Mendez-Trujillo V., Grimaldo-Juarez O. *et al*.: Changes of photochemical efficiency and epidermal polyphenols content of *Prosopis glandulosa* and *Prosopis juliflora* leaves exposed to cadmium and copper. – Open Life Sci. **12**: 373-378, 2017.
Haider M.S., Zhang C., Kurjogi M.M. *et al*.: Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq analysis. – Sci. Rep.-UK **7**: 13134, 2017.
Hichri I. Heppel S.C. Pillet J. *et al*.: The basic Helix-Loop-Helix transcription factor MYC1 is involved in the regulation of the flavonoid biosynthesis pathway in grapevine. – Mol. Plant **3**: 509-523, 2010.
Hosseini S.A., Maillard A., Hajirezaei M.R. *et al.*: Induction of barley silicon transporter *HvLsi1* and *HvLsi2*, increased silicon concentration in the shoot and regulated starch and ABA homeostasis under osmotic stress and concomitant potassium deficiency. – Front. Plant Sci. **8**: 1359, 2017.
Ierna A.: Characterization of potato genotypes by chlorophyll fluorescence during plant aging in a Mediterranean environment. – Photosynthetica **45**: 568-575, 2007.
Jaakola L., Määttä-Riihinen K., Kärenlampi S., Hohtola A.: Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. – Planta **218**: 721-728, 2004.
Jaillon O. Aury J.-M. Noel B. *et al.*: The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. – Nature **449**: 463-467, 2007.
Jayaprakasha G.K., Singh R.P., Sakariah K.K.: Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. – Food Chem. **73:** 285-290, 2001.
Jiang C.-D., Jiang G.-M., Wang X. *et al*.: Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (*Ulmus pumila*) probed by the fast fluorescence rise OJIP. – Environ. Exp. Bot. **58**: 261-268, 2006b.
Jiang C.-D., Shi L., Gao H.-Y. *et al*.: Development of photosystems 2 and 1 during leaf growth in grapevine seedlings probed by chlorophyll *a* fluorescence transient and 820 nm transmission *in vivo*. – Photosynthetica **44**: 454-463, 2006a.
Julkunen-Tiitto R. Nenadis N. Neugart S. *et al*.: Assessing the response of plant flavonoids to UV radiation: An overview of appropriate techniques. – Phytochem. Rev. **14**: 273-297, 2015.
Kadir S., Von Weihe M., Al-Khatib K.: Photochemical efficiency and recovery of photosystem II in grapes after exposure to sudden and gradual heat stress. – J. Am. Soc. Hortic. Sci. **132**: 764-769, 2007.
Kalaji H.M., Oukarroum A., Alexandrov V. *et al*.: Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements. – Plant Physiol. Bioch. **81**: 16-25, 2014b.
Kalaji H.M., Schansker G., Brestič M. *et al*.: Frequently asked questions about chlorophyll fluorescence, the sequel. – Photosynth. Res. **132**: 13-66, 2017.
Kalaji H.M., Schansker G., Ladle R.J. *et al*.: Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. – Photosynth. Res. **122**: 121-158, 2014a.
Kolb C.A., Käser M.A., Kopecký J. *et al*.: Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. – Plant Physiol. **127**: 863-875, 2001.
Landi M., Tattini M., Gould K.S.: Multiple functional roles of anthocyanins in plant-environment interactions. – Environ. Exp. Bot. **119**: 4-17, 2015.
Lefebvre T., Millery-Vigues A., Gallet C.: Does leaf optical absorbance reflect the polyphenol content of alpine plants along an elevational gradient? – Alpine Bot. **126**: 177-185, 2016.
Lo Piccolo E., Landi M., Pellegrini E. *et al*.: Multiple consequences induced by epidermally-located anthocyanins in young, mature and senescent leaves of *Prunus*. – Front. Plant Sci. **9**: 917, 2018.
Lu C., Lu Q., Zhang J., Kuang T.: Characterization of photosynthetic pigment composition, photosystem II photochemistry and thermal energy dissipation during leaf senescence of wheat plants growing in field. – J. Exp. Bot. **52**: 1805-1810, 2001.
Lu Q., Lu C., Zhang J., Kuang T.: Photosynthesis and chlorophyll *a* fluorescence during flag leaf senescence of field-grown wheat plants. – J. Plant Physiol. **159**: 1173-1178, 2002.
Martínez-Lüscher J., Sánchez-Díaz M., Delrot S. *et al*.: Ultraviolet-B radiation and water deficit interact to alter flavonol and anthocyanin profiles in grapevine berries through transcriptomic regulation. – Plant Cell Physiol. **55**: 1925-1936, 2014.
Mattila H., Valev D., Havurinne V. *et al*.: Degradation of chlorophyll and synthesis of flavonols during autumn senescence – The story told by individual leaves. – AoB Plants **10**: ply028, 2018.
Mattivi F., Guzzon R., Vrhovsek U. *et al*.: Metabolite profiling of grape: Flavonols and anthocyanins. – J. Agr. Food Chem. **54**: 7692-7702, 2006.
Mauromicale G., Ierna A., Marchese M.: Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. – Photosynthetica **44**: 76-82, 2006.
Miersch I., Heise J., Zelmer J., Humbeck K.: Differential degradation of the photosynthetic apparatus during leaf senescence in barley (*Hordeum vulgare* L.). – Plant Biol. **2**: 618-623, 2000.
Paunov M., Koleva L., Vassilev A. *et al*.: Effects of different metals on photosynthesis: Cadmium and zinc affect chlorophyll fluorescence in durum wheat. – Int. J. Mol. Sci. **19**: 787, 2018.
Pogrzeba M., Rusinowski S., Sitko K. *et al*.: Relationships between soil parameters and physiological status of *Miscanthus* × *giganteus* cultivated on soil contaminated with trace elements under NPK fertilisation *vs.* microbial inoculation. – Environ. Pollut. **225**: 163-174, 2017.
Sitko K., Rusinowski S., Kalaji H.M. *et al*.: Photosynthetic efficiency as bioindicator of environmental pressure in

A. halleri. – Plant Physiol. **175**: 290-302, 2017.

Skórzyńska-Polit E., Drążkiewicz M., Wianowska D. *et al*.: The influence of heavy metal stress on the level of some flavonols in the primary leaves of *Phaseolus coccineus*. – Acta Physiol. Plant. **26**: 247-254, 2004.

Tang G., Li X., Lin L. *et al*.: Combined effects of girdling and leaf removal on fluorescence characteristic of *Alhagi sparsifolia* leaf senescence. – Plant Biol. **17**: 980-989, 2015.

Tang Y., Wen X., Lu C.: Differential changes in degradation of chlorophyll-protein complexes of photosystem I and photosystem II during flag leaf senescence of rice. – Plant Physiol. Bioch. **43**: 193-201, 2005.

Velasco R., Zharkikh A., Troggio M. *et al*.: A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. – PLoS ONE **2**: e1326, 2007.

Vitulo N., Forcato C., Corteggiani Carpinelli E. *et al*.: A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. – BMC Plant Biol. **14**: 99-115, 2014.

Weng X.-Y., Xu H.-X., Jiang D.-A.: Characteristics of gas exchange, chlorophyll fluorescence and expression of key enzymes in photosynthesis during leaf senescence in rice plants. – J. Integ. Plant Biol. **47**: 560-566, 2005.

Wright H., DeLong J., Lada R., Prange R.: The relationship between water status and chlorophyll *a* fluorescence in grapes (*Vitis* spp.). – Postharvest Biol. Tec. **51**: 193-199, 2009.

Ziliotto F., Corso M., Rizzini F.M. *et al*.: Grape berry ripening delay induced by a pre-véraison NAA treatment is paralleled by a shift in the expression pattern of auxin- and ethylene-related genes. – BMC Plant Biol. **12**: 185-200, 2012.