

A Significant Loss in Photosynthetic Activity Associated with the Yellow Vine Syndrome of Cranberry

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Abstract. Numerous observations of yellow vine syndrome of cranberry have been reported from commercial cranberry growers. The molecular mechanism resulting in yellow vine syndrome is unknown. We have previously reported on the shading effect as an approach to explore the mechanisms of yellow vine formation and proposed photoinhibition as a possible cause. To compare the photosynthetic performance of yellow vine-affected and normal cranberry leaves, we conducted chlorophyll fluorescence analyses over 1 period of 1 day and 3 weeks, respectively. Both experimental data sets indicated that the maximum quantum efficiency of photosystem II, the size of the quinone pool, the numbers of reaction centers (RCs) per chlorophyll absorption, and the photosynthesis performance index of the yellow vine samples are substantially lower than those of normal cranberry leaves. These results are in line with the data of yellow vine leaves, having 26% to 28% less in chlorophyll than the normal leaves as measured by spectrometric and high-performance liquid chromatography analysis. We concluded that yellow vine syndrome is associated with poor photosynthetic activity and is likely becoming a threat for the long-term growth and crop production of cranberries.

The American cranberry (*Vaccinium macrocarpon*) contains antioxidants abundant both in quality and quantity because of its significant flavonoid content and phenolic acids. It is popular as a nutritious food and beverage source and has well-documented health benefits for various human diseases, including cardiovascular disease and cancer (Deyhim et al., 2007; Howell et al., 1998; Kalgaonkar et al., 2010; Lipson et al., 2007; Neto, 2007; Neto et al., 2008; Youdim et al.,

2002). Environmental stress plays an important role in the growth and production of agricultural plants. Response mechanisms of plants to stress factors were proposed in the past decade such as nutrition deficiency (Abadia, 1992; Davies and Grossman, 1998; Imsande, 1998), drought stress (Cornic and Massacci, 1996), heat-induced inactivation (Allakhverdiev et al., 2008; Gombos and Murata, 1998; Pshybytko et al., 2008), ultraviolet and visible effects (Kasahara and Wada, 2005; Tevini, 2004), tolerance to salinity (Demetriou et al., 2007; Munns and Tester, 2008), water stress (Bailey-Serres and Voesenek, 2008; Mommer and Visser, 2005; Sack and Holbrook, 2006), and chilling sensitivity (Nishida and Murata, 1996).

The production and quality of cranberries may be affected by long-term or short-term environmental stress such as temperature, humidity, water, nutrient, and light intensity. To produce uniform quality of cranberries, balanced fertilization and irrigation is vital (Savvas et al., 2009). In addition, treatment for storage of cranberries can play a role. The current recommendation for storage of cranberry is 0 to 7 °C and 75% to 82% relative humidity (Forney, 2009). Sunlight is a powerful and abundant energy source, which can be harmful to plants, including cranberry (Bailey

and Grossman, 2008; Kramer, 2010; Takahashi and Murata, 2008; Vener, 2007).

Cranberry growers have observed yellow vine syndrome in the cranberry bog under normal light conditions, which produces yellow color along the leaf margins, whereas the area along the vein remains green (Fig. 1). Almost every year numerous reports of yellow vine syndrome in cranberry are received from the cranberry growers in Massachusetts (DeMoranville et al., 2009). Typically the symptoms appear first in the year-old leaves and then move up the stem into the new growth. The most common time for the symptoms to become severe is around or after fruit set when demand for resources in the plants is high. Nutritional imbalance might be associated with yellow vine development in cranberry (DeMoranville et al., 2009). However, fertilizer management is not the cause of the problem. It is possible that nutrient imbalance is secondary to root problems. Additionally, yellow vine syndrome often worsens in bogs with drainage problems, indicating that water stress may be another factor in the formation of yellow vine in cranberries (DeMoranville et al., 2009). Water stress conditions may lead to poor root development. Alternatively, yellow vine symptom might be a result of root rot, viruses, pathogens, or other biological problems. However, the molecular mechanism causing the yellow vine syndrome in cranberries is unknown.

We have previously reported the shading effect on yellow vine syndrome of cranberry to explore the mechanisms of yellow vine formation and proposed a possible role of photoinhibition to cause the yellow vine syndrome in cranberry plants. A complete understanding of the mechanisms of yellow vine syndrome development in cranberry plants may offer an opportunity to minimize its effect. In this work we use bioanalytical methods, including spectrometry, high-performance liquid chromatography (HPLC), and chlorophyll fluorescence kinetics to examine cranberry leaves with yellow vine syndrome and normal cranberry leaves to provide novel insight into the underlying mechanism causing the syndrome.

Materials and Methods

Cranberry sample preparation. Leaves of cranberry cultivar Stevens were collected from State Bog in East Wareham, MA. Five sets of normal cranberry plant samples (each ≈100 g) and five sets of yellow vine samples (each ≈100 g) were harvested from the different areas in the bog, respectively. These samples were quantified immediately or stored at –80 °C for later use. To extract the pigments from the cranberry leaves, ≈5 g of leaves were shredded completely at 4 °C in a blender. The resulting leaf paste mixtures were extracted with 50 mL of 100% methanol (Sigma) for 1 h in the dark at room temperature. The extract solution was immediately analyzed by spectrometric and HPLC methods or stored in the dark at –80 °C for later use.

Experimental design and statistical analysis. Chlorophyll (Chl) content in plants can be

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easily determined by extraction using organic solvents followed by spectrometric and HPLC methods (Borrmann et al., 2009; De las Rivas et al., 1989; Porra et al., 1989; Shioi et al., 1983). The difference in chlorophyll between the yellow vine and normal leaves was monitored. Chlorophyll fluorescence analysis is widely used to probe the photosynthetic behaviors of plants *in vivo*. It will enable us to examine the difference in photosynthetic activity between the normal and yellow vine leaves. For spectrometric and HPLC analysis, three replicated measurements were made and used to calculate the SDS. For chlorophyll fluorescence analysis, five replicated measurements were conducted for determination of photosynthetic parameters and used to calculate SDS. A *t* test was used to compare the measurement of yellow vine leaves with the measurement of normal cranberry leaves to decide whether they are statistically different (Harries, 2003).

Chlorophyll analysis by ultraviolet-visible absorption spectrometry. The pigment extracts of cranberry leaves were diluted with the extraction solvent to the proper concentration before spectrometric measurement. Typically, 100- μ L extractions were diluted 1000 times with the extraction solvent methanol. The ultraviolet-visible absorption spectra were recorded with Hewlett Packard 8452A diode array spectrophotometer with a 1-cm light path cuvette. The concentration of Chl *a* and Chl *b* was calculated using the equations: Chl *a* = $16.29 A^{665.2} - 8.54 A^{652.0}$ and Chl *b* = $30.66 A^{652.0} - 13.58 A^{665.2}$ according to published procedures (Porra et al., 1989), respectively.

Chlorophyll analysis by high-performance liquid chromatography. A reverse-phase HPLC column (250 \times 4.6 mm, Grace Prevail C18, 5 μ m) with a diode array detector (DAD) (HP 1200 series LC systems; Agilent Inc.) was used for HPLC analysis according to published procedures (De las Rivas et al., 1989). The column was equilibrated with acetonitrile:methanol (7:1 by v/v, mobile phase A). The mobile phase A was pumped at the flow rate of 1 mL·min⁻¹ for 2 min immediately after sample injection. Next, a mixture of acetonitrile:methanol:water:ethyl acetate (7:0.96:0.04:2, mobile phase B) was pumped for 1 min. Finally, acetonitrile:methanol:water:ethyl acetate (7:0.96:0.04:8, mobile phase C) was pumped until all the components were eluted. The doubly distilled water was filtered with a HPLC solvent filter and used in HPLC analysis. The detection wavelength was set at 450 nm. The standard solution of Chl *a* (1.00 ppm in methanol) was prepared using Chl *a* purchased from Sigma and used for HPLC analysis. The quantitative analysis of Chl *b* was conducted using the response factor of Chl *a*. The chromatographic peaks were identified by three different techniques: an internal standard, absorption spectra obtained by the DAD detector, and published HPLC data (De las Rivas et al., 1989).

Chlorophyll fluorescence assay of cranberry leaves in the bog. The Chl fluorescence parameters of cranberry leaves in bogs were determined with portable Pocket PEA Chlorophyll Fluorimeters (Hansatech Instruments Ltd.,

U.K.). Yellow vine syndrome and normal cranberry leaves were collected randomly and used for Chl fluorescence measurements immediately. Typically, five leaves in five different plants located in five different areas in the bog were chosen for the measurements. The *in vivo* Chl fluorescence parameters were determined after a 10-min dark period in the bog with a cover provided by the equipment. The samples were collected using attached leaves. The data from detached and attached leaves were in agreement within the relative error of 10% to 15%. The field experiments were carried out once a week for 3 weeks in Sept. to Oct. 2008. The Chl fluorescence data from an average of five replicated measurements was used to calculate the photosynthetic parameters.

Results and Discussion

Spectrometric and high-performance liquid chromatography analysis. Cranberry leaves with yellow vine syndrome in bogs typically exhibit yellowing along the leaf margins. Chlorophyll content in the plant leaves was easily determined for normal and affected

samples. We anticipated lower Chl content in yellow vine leaves than the normal healthy ones, and this was supported in the analysis of leaf tissue and is shown in Table 1. The content of both Chl *a* and Chl *b* was lower as compared with the normal leaves by \approx 22% to 24%. The lower content of Chl *a* and Chl *b* was confirmed using HPLC analysis (Table 2). The *t* test showed that the differences in Chl *a* and Chl *b* by spectrometric and HPLC analysis are significant at the 95% confidence level. This suggests that the yellow vine is the result of altered Chl biosynthesis (Castelfranco, 2007) or Chl degradation (Kramer, 2010; Porra and Scheer, 2001). Another possibility would be that the lower Chl content might be the direct photodamage of Chl by excess light energy, because the Chl molecule in photosystem II is one of the main targets in photoinhibition (Aro et al., 1993; Barber and Andersson, 1992; Telfer and Barber, 1989). There is good evidence that photoinhibition occurs because of damage to the oxygen-evolving complex of photosystem II (PSII) (Hakala et al., 2005; Nishiyama et al., 2006; Wei et al., 2011).

It is interesting to note that although the Chl content was lower in yellow vine leaves, the



Fig. 1. Images of normal cranberry leaves (left panel) and yellow vine syndrome cranberry leaves (right panel).

Table 1. Spectrometric analytical results of chlorophyll (Chl) *a*, Chl *b*, and Chl *a*/Chl *b* ratio in yellow vine and normal cranberry leaves.^z

	Normal leaves (mg·g ⁻¹ fresh wt)	Yellow vine syndrome leaves (mg·g ⁻¹ fresh wt)	Change (%)
Chl <i>a</i>	1.30 \pm 0.09 ^y	0.99 \pm 0.10	23.8
Chl <i>b</i>	0.93 \pm 0.06	0.72 \pm 0.09	22.6
Chl <i>a</i> /Chl <i>b</i> ratio	1.40	1.40	0

^zThe concentration of Chl *a* and Chl *b* was determined by measuring absorption spectra of the extracts using organic solvent methanol and calculated using the extinction coefficients and equations published elsewhere (Porra et al., 1989).

^yThe numbers were obtained by average of three independent measurements. The statistic analysis (*t* test) showed that the differences in Chl *a* and Chl *b* content are significant at the 95% confidence level.

Table 2. High-performance liquid chromatography analytical results of chlorophyll (Chl) *a*, Chl *b*, and Chl *a*/Chl *b* ratio in yellow vine and normal cranberry leaves.^z

	Normal leaves (relative peak area, %)	Yellow vine syndrome leaves (relative peak area, %)	Change (%)
Chl <i>a</i>	42.9 \pm 4.0 ^y	56.4 \pm 5.0	21.8
Chl <i>b</i>	27.5 \pm 3.0	35.1 \pm 3.0	22.6
Chl <i>a</i> /Chl <i>b</i> ratio	1.56	1.61	3.1

^zThe relative content of Chl *a* and Chl *b* was determined by using a reverse-phase C-18 HPLC column with a diode array detector according to the procedures published (De las Rivas et al., 1989).

^yThe numbers were obtained by average of three independent measurements. The statistic analysis (*t* test) showed that the differences in Chl *a* and Chl *b* content are significant at the 95% confidence level.

ratio of Chl *a* to Chl *b* was almost identical (Tables 1 and 2). We note the Chl *a*/Chl *b* ratio is slightly different using spectrometric (≈ 1.4) and HPLC analysis (≈ 1.6), which may be the result of the lower accuracy and larger error of the spectrometric data. Alternatively, the response factor of Chl *a* and Chl *b* in HPLC profiles may be slightly different. Chlorophyll *a* is primarily in the reaction centers, and Chl *b* is predominantly found in light-harvesting complexes (LHCs) in photosynthetic membrane proteins (Blankenship, 2002). The Chl *a*/*b* ratio is practically unchanged, which indicates that both RC and LHC are declined. We propose that the lower content of Chl in yellow vine leaves may be the result of the smaller number of photosynthetic reaction centers and their intact light-harvesting systems.

Chlorophyll fluorescence analysis. The kinetics of the electron transfer steps in photosynthetic reaction centers has been thoroughly investigated over the complete timescale of femtoseconds to many seconds (Brudvig, 2008; Govindjee and Seibert, 2010; Rappaport and Diner, 2008; Renger and Holzwarth, 2005; Seibert and Wasielewski, 2003; van Grondelle and Gobets, 2004). The early electron transfer occurring in PSII revealed by ultrafast spectroscopy can be divided into several steps: 1) absorption of light quanta by antenna to form excited states of pigments; 2) trapping of excitation energy by the primary electron donor P_{680} in the reaction center on the picosecond time scale; 3) primary charge separation from the singlet excited state of P_{680} to the primary acceptor Pheo in ≈ 3 to 20 ps; 4) stabilization of the separated charges from the radical pair $P_{680}^+ \text{Pheo}^-$ on the acceptor side by electron transfer to Q_A in ≈ 200 ps and to Q_B on the hundreds-of- μs time scale; and 5) on the donor side, an electron is supplied to reduce P_{680}^+ from water through a tyrosine residue (Y_Z) and Mn_4Ca cluster involving a S-state oxygen evolution cycle on the ns- μs time scale (Brudvig, 2008; Debus, 1992; Diner and Babcock, 1996; Kok and Radmer, 1976; Rutherford et al., 1992; Tommos and Babcock, 2000).

The fluorescence by Chl is very sensitive to each step of the PSII electron transfer reactions in vivo. Therefore, Chl fluorescence is proven to be an intriguing tool and reveals information on plant performance and responses through non-intrusive measurements, especially addressing the effects of plant leaves under environmental stress conditions (Adams and Demmig-Adams, 2004; Baker, 2008; Cavender-Bares and Bazzaz, 2004; Krause and Weis, 1991; Strasser et al., 2004). Figure 2 shows typical Chl fluorescence transients, O-I-I-P curves. When the dark-adapted cranberry samples were illuminated by saturating light, the Chl fluorescence was increased in three phases: 1) $O \rightarrow J$ step is associated with the electron transfer from P_{680}^* to Q_A , and Mn_4Ca cluster to P_{680}^+ ; 2) $J \rightarrow I$ step is related to the electron transfer from Q_A to Q_B ; and 3) $I \rightarrow P$ step is the results of the plastoquinone pool reduction (Boisvert et al., 2006; Joly et al., 2010). As shown in Figure 2, the amplitudes in fluorescence (circle symbols in red) for the three steps from the yellow vine

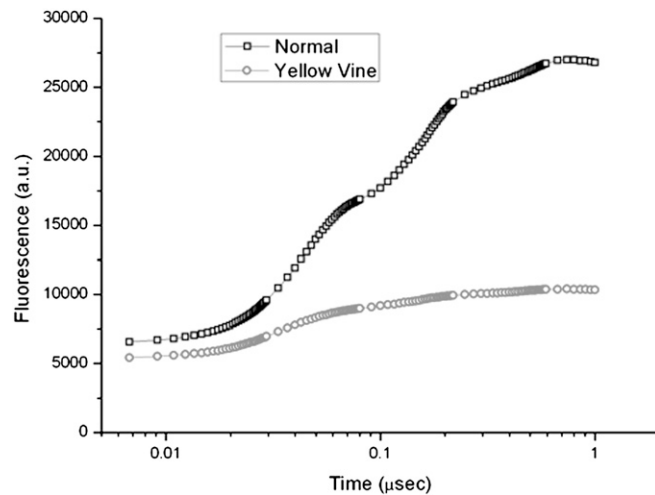


Fig. 2. A representative chlorophyll fluorescence transient curves of normal cranberry leaves (upper, black symbol) and yellow vine syndrome cranberry leaves (lower, gray symbol).

sample were significantly lower than the normal cranberry leaves (square symbols in black), which suggests that the photosynthetic activity in yellow vine samples is dramatically different from the health cranberry leaves.

The interpretation of data in O-I-I-P transient to structural and functional parameters quantifying PSII performance includes initial fluorescence (F_o), variable fluorescence (F_v), maximum fluorescence (F_m), total lifetime constant, which is the time to reach the maximum fluorescence, and PSII maximum quantum yield (F_v/F_m) (Strasser et al., 2004). In addition, the “area,” which is the quantity of fluorescence area above the transient curve, may represent the size of the PSII quinone pool (Strasser et al., 2004). Figure 3 is a typical spider graph, which summarizes the six Chl fluorescence parameters of cranberry leaves. Five of the six values from the yellow vine cranberry leaves (thin lines in red) were smaller than normal samples (broad lines in black), which indicates a substantial loss in photosynthetic activity resulting from the yellow vine syndrome of cranberry. Similar behavior was observed in camellia leaves with development of an energy pipeline model of the photosynthetic apparatus (Kruger et al., 1997; Toth Szilvia et al., 2007). In contrast, the total lifetime constant of PSII in both samples is nearly identical, implying an intact photosynthetic electron transfer in both leaves. This is agreeable with the unchanged organization of photosynthetic machinery judged by the spectrometric and HPLC data of the Chl *a*/Chl *b* ratio.

Table 3 lists the four Chl fluorescence parameters of the yellow vine syndrome and normal cranberry leaves. In addition to F_v/F_m , “area,” the number of RC per chlorophyll absorption (RC/ABS), we calculate the photosynthesis performance index (PI value), which is defined as an indication of a driving force of the primary photosynthetic reaction (Srivastava et al., 1999; Strasser et al., 2004). The PSII maximum quantum yield was decreased by 28%, which is similar to the level of Chl *a* and Chl *b* ($\approx 25\%$). We conclude that

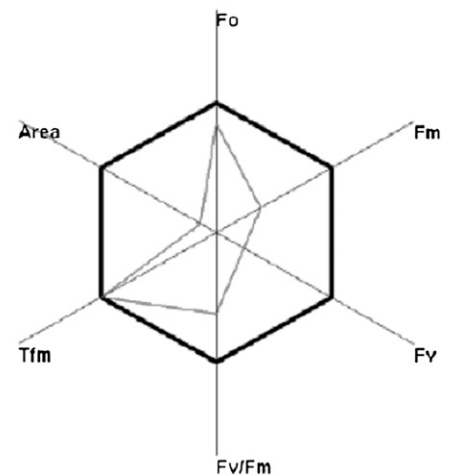


Fig. 3. A typical spider graph of cranberry leaves. Black thick lines stands for the normal leaves and gray thin lines stands for the yellow vine samples.

Table 3. Relative chlorophyll fluorescence parameters of yellow vine syndrome and normal cranberry leaves.^z

	F_v/F_m	Area	RC/ABS	PI
Normal leaf	100 ± 5^y	100 ± 15	100 ± 8	100 ± 25
Yellow vine	72 ± 4	40 ± 6	43 ± 5	11 ± 3

^z F_v/F_m is the maximum quantum yield of photosystem II (PSII); “area” represents the size of quinone pool in PSII; RC/ABS stands for the numbers of photosynthetic reaction center per chlorophyll absorption; PI is the photosynthetic performance index, which is the overall driving force of photosynthetic reaction.

^yThe values were obtained by average of five independent measurements. The statistic analysis (*t* test) showed that the differences in these four parameters are significant at the 95% confidence level.

the numbers of PSII containing RCs and light-harvesting proteins in yellow vine leaves may be lower than that of normal leaves by 25% to 30%. The size of the quinone pool and the

number of RCs per Chl absorption in yellow vine sample were decreased by 57% to 60%. The *t* test showed that the differences in F_v/F_m , area, RC/ABS, and PI are significant at the 95% confidence level. The discrepancy between these numbers and F_v/F_m may be the result of a substantial number of inactive PSII in yellow vine leaves. The content of Chl *a* and Chl *b* is an extrinsic quality and F_v/F_m is an intrinsic property. The F_v/F_m only inform one about how intact centers are performing, not how many there are. Our F_v/F_m data could be the result of the decrease in the quinone:PSII ratio. In addition, the much smaller size of the quinone pool will imply the vulnerability and sensitivity of yellow vine to other environmental stress factors. This is supported by the observation that water stress significantly worsens the symptom in the cranberry bog (DeMoranville, 2006; DeMoranville and Lampinen, 1999). The PI value of yellow vine leaves is almost completely diminished with a loss of $\approx 90\%$. This suggests that the impairment and destruction of the photosynthetic apparatus in yellow vine syndrome of cranberry leaves is multitargeted and complex.

To monitor the photosynthetic behavior of yellow vine leaves in cranberry bogs, we conducted Chl fluorescence measurements on yellow vine syndrome leaves over a period of 3 weeks in Figure 4 and of 1 d in Figure 5. As shown in Figure 4, the three quantities—maximum quantum yield, size of quinone pool, and photosynthesis performance index—in the yellow vine samples were consistently smaller than the normal leaves over the entire 3 weeks. Similar trends were obtained in the 1-d experiment in Figure 5. This observation confirms the previous experimental data and strongly supports the idea that yellow vine syndrome reduces the photosynthetic activity possibly by inhibiting and degrading multiple protein complexes including decreasing the numbers of PSII complexes.

We noted that the variation and error of the maximum quantum yield of PSII over 3 weeks were rather large (relative SD of 19%) in the case of yellow vine samples (Fig. 4). In contrast, the parameter over a period of 1 d showed relatively small experimental error (relative SD of 5%) in Figure 5. This may suggest that the photosynthetic apparatus in yellow vine syndrome is more vulnerable and sensitive to experimental conditions such as changes in temperature, water level, nutrition, humidity, herbicide, light intensity, age of leaves, and disease problems. Alternatively, the variation and large errors may be the result of the sampling of Chl fluorescence experiments, although the data were an average of five independent measurements. However, additional experimental evidence is required to distinguish these possibilities. For example, the effects of temperature, pH, and nutrition on the photosynthetic activity of yellow vine syndrome may support or disprove these hypotheses.

Molecular mechanisms of yellow vine syndrome formation. It is hypothesized that the yellow vine symptoms may be caused by nutritional imbalance and water stress

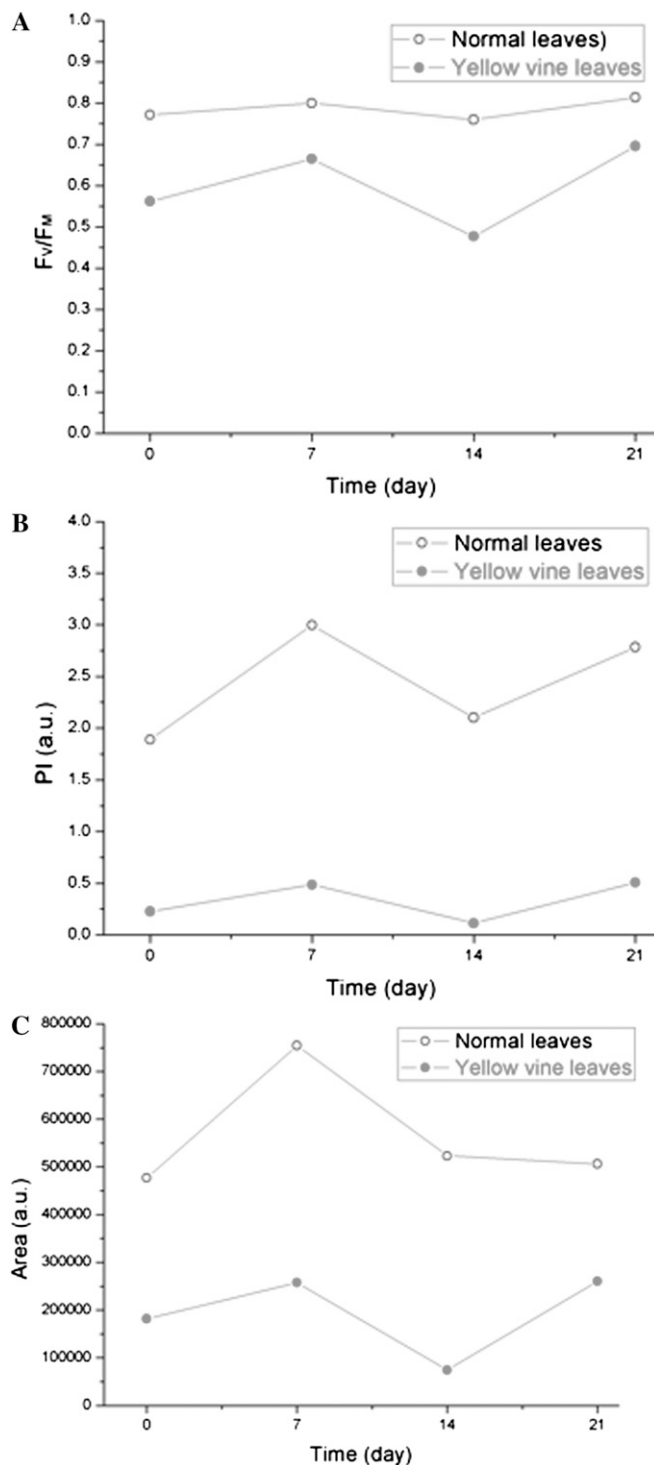


Fig. 4. The chlorophyll fluorescence parameters obtained during a period of 3 weeks. (Upper panel) Photosystem II (PSII) quantum efficiency (F_v/F_m); (midpanel) photosynthesis performance index (PI); (lower panel) quinone pool size (area). The relative SDs are 5% in F_v/F_m , 15% in area, and 25% in PI, respectively.

(DeMoranville et al., 2009). The effect of shade treatment on yellow vine syndrome in cranberry bogs revealed that the shading of cranberry plants appears to reduce the syndrome by improving the photosynthetic activity and increasing the Chl content (Wei et al., 2010). The yellow vine leaves were associated with $11\% \pm 5\%$ and $14\% \pm 5\%$ increase in Chl *a*/Chl *b* ratio after shading, respectively. The

electron transport efficiency in PSII and the size of the quinone pool are increased. In addition, the overall photosynthesis performance index is drastically improved by shading. These results suggest that the shade effect will increase the numbers of PSII in the cells of yellow vine cranberry leaves. Because PSII is the main target of photoinhibition, we suggest a possible role of

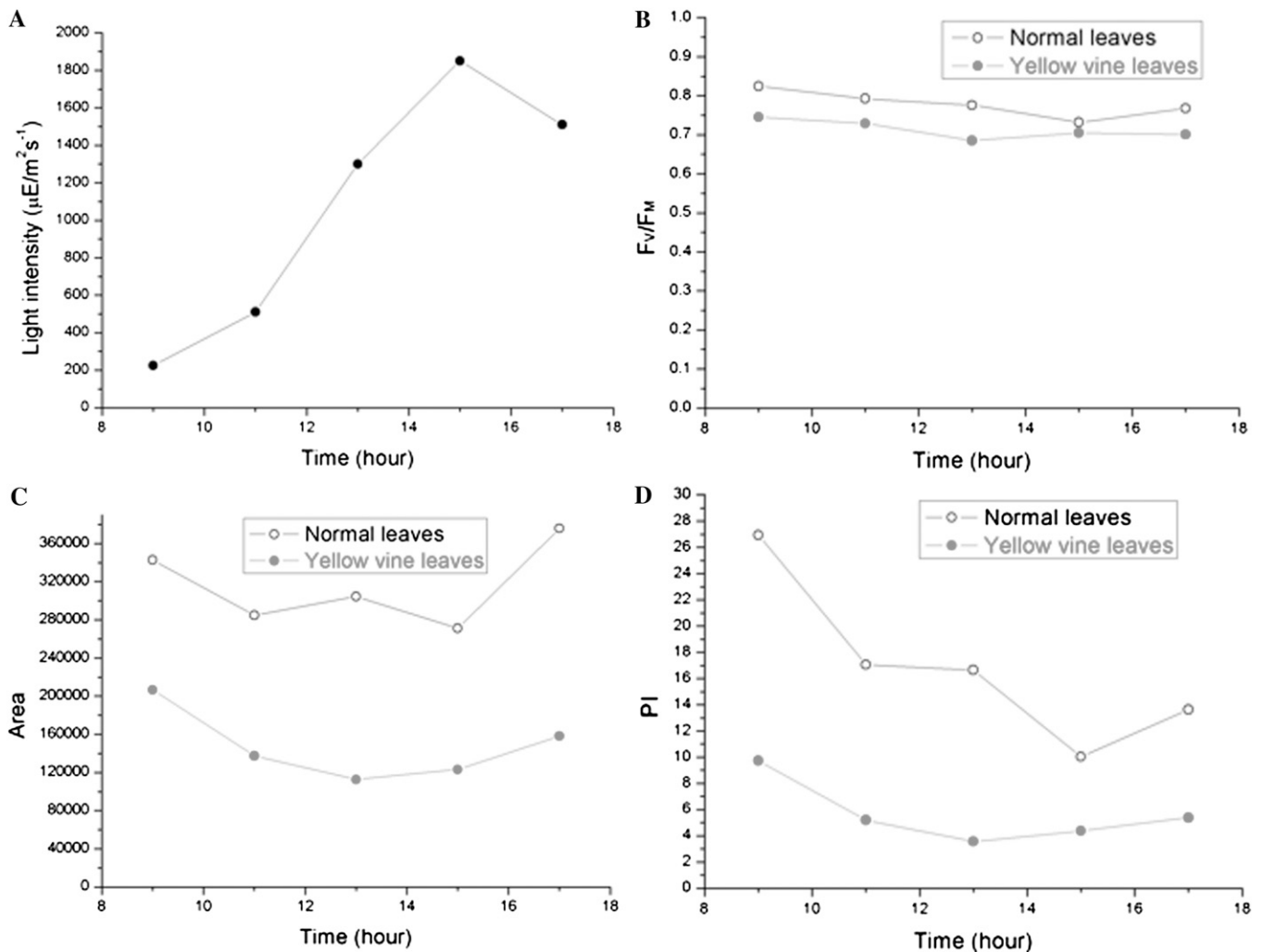


Fig. 5. The chlorophyll fluorescence parameters during a period of 1 d. (Upper left panel) Light intensity during daytime; (upper right panel) photosystem II (PSII) quantum efficiency (F_v/F_m); (lower left panel) quinone pool size (area); (lower right panel) photosynthesis performance index (PI). The relative sds are 5% in F_v/F_m , 15% in area, and 25% in PI, respectively.

photoinhibition is associated with the yellow vine syndrome in cranberry plants.

Yellow vine formation is likely the result of multiple factors. It is reported that the environmental stress may enhance the photoinhibitory effect on photosynthetic machineries (Takahashi and Murata, 2008). We propose that the combination of photoinhibition, water stress, and nutritional imbalance may cause the yellow vine syndrome of cranberries. Based on our experimental data and previous reports, yellow vine syndrome is likely a result of the decrease in the steady concentration and activity of PSII. We proposed a possible model to explain yellow vine syndrome of cranberry. Nutritional imbalance, water stress, or photodamage results in the formation of fewer PSII centers and this phenomenon results in yellow vine syndrome. Chlorophyll biosynthesis may be inhibited and negatively regulated or degradation of Chl may be activated and positively regulated. Alternatively, increased degradation of D1 could also result in yellow vine syndrome. Our data cannot distinguish between these two possibilities.

Concluding remarks. We investigated the molecular mechanisms of yellow vine formation by spectrometric, HPLC, and Chl fluorescence analysis. The performance of yellow vine and normal cranberry leaves by Chl fluorescence analysis over 1 period of 1 d and 3 weeks, respectively, indicated that the photosynthetic parameters of the yellow vine samples are substantially lower than those of the normal cranberry leaves. Spectrometric and HPLC analyses revealed that yellow vine leaves contained 26% to 28% less Chl than normal cranberry leaves. These data sets demonstrated that yellow vine syndrome is associated with a poor photosynthetic activity and is problematic for the long-term growth and crop production of cranberries.

The recovery experiments may provide further insight into the mechanisms of yellow vine syndrome development in cranberry bogs and offer an opportunity to solve the problem. Recent recovery of photoinhibited plant leaves was examined, and PSII mobility in thylakoid membranes may play a key role (Oguchi et al., 2008). We plan to conduct experiments on the recovery from the syn-

drome to establish optimized experimental conditions.

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