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Research Article

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Assessment of vitality *Berberis thunbergii* DC. in Kyiv: Photosynthesis and Phytopathology

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ABSTRACT

The process of functioning of the photosynthetic apparatus is one of the most vulnerable to stress factors. The information about the functional state of plants and the prospects for their use in urban greening can provide methods of fluorescence analysis. The objective of this research was to develop the state of plants *Berberis thunbergii* DC. which grow in the Mariinskyi Park (Kyiv) by the content of photosynthetic pigments and fluorescence induction chlorophyll (FIC). The article presents the results of the evaluation of fluorescence induction (FI) indicators and the content of photosynthetic pigments *B. thunbergii* according to the degree of damage by powdery mildew. Experimental plants were planted in the chernozem layer of 50 cm (humus content 4 %). The plants grow in a group planting of *Picea glauca* 'Conica' and *Chlorophytum comosum* 'Variegatum' directly 1.5–5 m from the highway under the canopy of *Tilia cordata* Mill. Moreover, the variability of FI curves in the absence/presence of plant lesions. The authors investigated the relationship between the content of photosynthetic pigments and FI. The degree of damage to experimental plants by powdery mildew affects the total chlorophyll content (a+b) and decreases by approximately 16 %, 7 %, and 5 % compared to the control. Recorded gradually disappear FI curves in plants with the highest degree of damage which affect photochemical reactions due to the slowing down of the outflow of Calvin cycle enzymes.

KEYWORDS: Induction of chlorophyll fluorescence, Powdery mildew, Chlorophyll *a* and *b*.

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INTRODUCTION

Currently, the global landscaping products market presents a wide variety of cultivars of *Berberis thunbergii* DC. which differ by leaf plate color, size, and shape of the canopy.

The family *Berberidaceae* approx. 500 species (CABI, 2021) and is represented by two genera – *Berberis* L. and *Mahonia* Nutt. In the '80s of the XIX century, considerable work of breeders was focused on dwarfism and various coloring of cultivars of *B. thunbergii* (Gossler *et al.*, 2009; Hatch, 2015). However, it should be noted that plants with a decorative color of the leaf blade lose color under conditions of growth in the shade (Bruns Pflanzen, 2000). The distribution area covers the territories of North America, Europe, Asia, and Australia (CABI, 2018).

It was established (Yakobchuk & Kolesnichenko, 2013) that representatives of the genus *Berberis* L. were introduced to 5 regions of cultivation in Ukraine: Polissya - 15 species, Forest-steppe - 68 species, 11 cultivars, Steppe - 45 species, 5 cultivars, Carpathians - 7 species, 7 cultivars, Crimea (South coast) - 82 species, 9 cultivars.

B. thunbergii is a perennial highly ornamental shrub, 60-90 cm in height, resistant to factors of the urban ecosystem. Stem grayish grooved with a thin crust and bright yellow inner bark and wood. Roots inside yellow. The plants have a purple, red, slightly bluish-green, dark green, and dark reddish-purple color of the leaves, which are pointed, toothless and spatulate, usually obtuse, entire, tapering at the base to a short petiole, 13-32 mm long, arranged whorled. The leaves are gray below. Blooming in late April or May, yellow flowers 6 mm wide with elongated drooping inflorescence. Fruits - ovoid, dry or slightly juicy, hard berries, red, 8-13 mm long, which are well preserved in winter (CABI, 2021).

It is well known that the color of the leaf blade depends on the number of photosynthetic pigments which is variable depending on environmental factors and during the growing season. The processes of photosynthesis and IF in purple

leaves are more intense than green due to the different content of anthocyanin and their ability to absorb photons of light (Close & Beadle, 2005; Li *et al.*, 2009). The nature of the dependence of the functional state of plants on the conditions of their growth, development, and adaptation to environmental factors is closely related to the complex nature of photosynthesis and, above all, the peculiarity of the functioning of the pigment apparatus (Rubyn, 1971). The results of the study of the kinetics of FIC in assimilating plant tissues allow evaluating the efficiency of photosystem II (PS II) which is one of the most important protein complexes in the photosynthetic apparatus of higher plants and is sensitive to external environmental factors which allow us to assess the resistance of plants to a particular factor (Leshchenko *et al.*, 2014; Zhang *et al.*, 2016).

MATERIALS AND METHODS

Plant Material

In 2018, the selected plants for research in the Mariinsky Park (Kyiv) were planted in the amount of 100 pieces in November 2016 in common chernozem (density content 1.4 g/cm^3, pH 6.5, the number of absorbed bases - 34.0 mg-eq./100 g of soil, humus content - 4%, nitrogen - 39 mg/kg, phosphorus - 48 mg/kg, potassium - 310 mg/kg) in a layer of 50 cm. The plants grow in a group planting from *Picea glauca* 'Conica' and *Chlorophytum comosum* 'Variegatum' directly 1.5–5 m from the highway under the canopy of *Tilia cordata* Mill. (Figure 1).

An average monthly temperature indicates a sufficient amount of heat during the growing season, light, and heat for photosynthesis in the leaf blade *B. thunbergii* (Figure 2).

Leaf Blade Damage

The intensity of plant damage was assessed on the scales Geshele (1971) in percentages or points with certain modifications. The percentage scale of intensity of defeat by powdery mildew has the following gradations: 1, 5, 10, 20, 40, 60, 80 % of the leaf surface is occupied by a mycelium of pathogens. To analyze the FI chlorophyll changes in the leaf depending on the nature of the lesion we divided the affected leaves into 3 groups: partially affected (5-20 %), moderately affected (20-40 %), and severely affected (40-80 %).

Photosynthetic Apparatus

The kinetics of changes in the FIC are determined by a portable device (chronoflurometer) "Floratest" (Kytaev *et al.*, 2005). Adaptation of plant leaves to darkness was performed for 5 minutes. Chlorophyll fluorescence strength was obtained for 90-time points with an interval from 3 μs to 300 s with three-time repetition (Korsunskiy & Snegur, 1997) and determined in relative units. The induction curves we constructed on points using software Microsoft Excel, analyzed the amplitude and time parameters of their individual phases (Veselovskiy & Veselova, 1990).

Figure 1: Researched plants *Berberis thunbergii* DC. in Mariinskii park

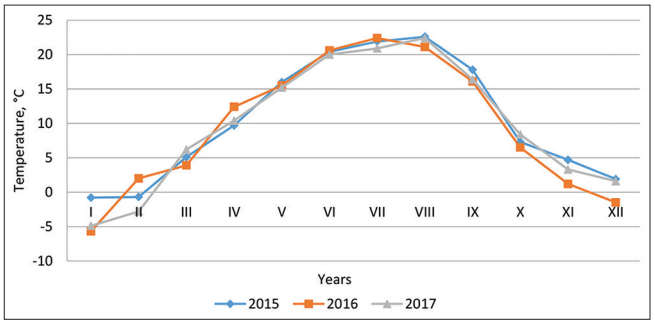

Figure 2: Average monthly temperatures in Kyiv in the period from 2015 to 2017 (Central Geophysical Observatory named after Boris Sreznevsky)

Chlorophyll (chl.) a and Chlorophyll b contents determined by spectrophotometric method (Shlyk, 1971; Hrodzynskyi & Hrodzynskyi, 1973; Yuzbekov, 1990). The concentration (c) of pigments was determined by the following formulas (Wrolstad, 2001): c_a (μg/ml) = 16.72 $A_{665.2}$ – 9.16 $A_{652.4}$, c_b (μg/ml) = 34.09 $A_{652.4}$ – 15.28 $A_{665.2}$ (c_a – concentration chl.*a*; c_b – concentration chl.*b*; $A_{665.2}$ – extract absorbance at a wavelength of 665.2 nm; $A_{652.4}$ – extract absorbance at a wavelength of 652.4 nm).

Data Analysis

The one way analysis of variance (ANOVA) was used to analyze the results of FIC parameters. The linear regression analysis we used to determine the correlation between chlorophyll content and FIC parameters. These dependencies were calculated using MS Exel software (licensed Windows 10 Pro). We used the least significant difference (LSDα0.05) which characterizes the marginal error of the sample averages at a certain number of degrees of freedom for statistical analysis of results of determining chlorophyll content and ratio (0,1-0,4). The results were presented as means ± SD at $p<0,05$. The calculated indicators of the least significant difference were within the permissible deviations.

RESULTS AND DISCUSSION

According to our research, the most common and harmful disease on barberry was powdery mildew. We identified fungi on

B. thunbergii plants. as *Erysiphe berberidis* DC. [=*Microsphaera berberidis* (DC.) Lév.]. Infected plants lost their decorative effect due to the presence of conidia on both parts of the leaves, shoots, and their subsequent deformation (Figure 3). Researchers Cho *et al.* (2018) analyzed infected plants during 2015–2017 at Korea University Herbarium and provided a detailed life cycle description of *E. berberidicola*.

We recorded that the disease appears in May-June on young leaves and shoots in the form of individual spots of white plaque which further spreads and can cover a large area of the leaf blade (Figure 4). Powdery mildew on the surface of the affected plant organs is a mycelium (Figure 5a) and conidial sporulation (Figure 5b, c).

Later, dark, cleistotheca spots appear among the white plaque (Figure 5a) with specific appendages (Figure 5b). There are bags inside the cleistothecia (Figure 5b) with ascospore (Figure 5c).

Our research indicates that *B. thunbergii* plants affected by powdery mildew mainly grew in the shade and is also confirmed by scientists Cho *et al.* (2018).

Certain sections of the FIC curve are indicators of physiological processes in the photosynthesis chain. Disturbances of separate links are caused by exogenous and endogenous factors and demonstrated by characteristic changes of the corresponding sites of the FIC curve Minimal fluorescence ratio (F_0) characterizes the number of inactive chlorophyll that does not transfer excitation energy to the reaction centers, so it sets the initial level of FIC and depends on the excitation energy loss during the migration of pigment matrix (Leshchenko *et al.*, 2014). Maximum indicators F_0 were recorded for experimental plants *B. thunbergii* which were partially affected by powdery mildew (480-488 a.u.) the lowest for very affected plants – 80 a.u. (Table 1).

The parameters dF_{pl} depend on the effect of endo- and exogenous factors on the plant and are determined by

Figure 3: General overview plants *Berberis thunbergii* DC. affected by *Erysiphe berberidis* (2017)

Figure 4: Symptoms of powdery mildew on barberry leaf blades caused by micromycete *Erysiphe berberidis*

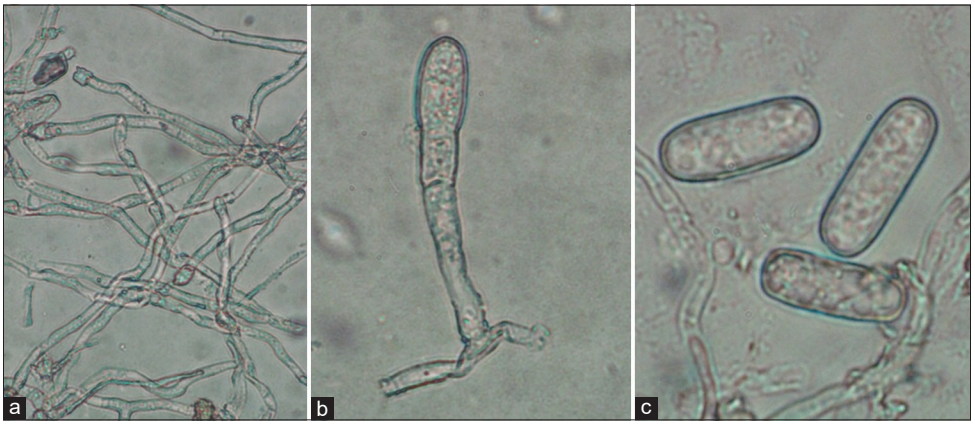

Figure 5: Vegetative structures of micromycetes *E. berberidis*: a) exophytic mycelium, b) conidiophore, c) conidia

the magnitude of the fluorescence increase from F_0 to $F_{pl.}$ Consequently, the parameter dF_{pl} is determined by the period of rapid recovery of the primary acceptor in the complexes PS II and values vary greatly from 496 to16 a.u.

According to Kautzky and Hirsch (1934), under conditions of maximum fluorescence at the point Fp on the induction curve, the productivity of photosynthesis is at a minimum, which is confirmed by the results of studies. Thus, the highest values of the "first maximum" Fp were characteristic of partially affected plants.

Under conditions of maximum fluorescence at the point Fp on the induction curve, the productivity of photosynthesis is minimal. We established the highest values of the "first maximum" Fp for partially affected plants.

The indicators of the "second maximum" Fm on the induction curve should not exceed the indicators Fp with sufficient lighting of plants. The results of the studies show that this indicator for almost all experimental plants was Fp> Fm except for plants where the leaves have lost their purple color and grow under the crowns of trees *T. cordata* Fm> Fp (Figure 6 and 7). Plants with the purple color of the leaf blade are sensitive to light because the synthesis of anthocyanin directly depends on the level of light, so shading can directly impair their synthesis (Michal, 2009).

Variable fluorescence is a physiological indicator that reflects the effect of environmental and experimental factors on the plant (Leshchenko *et al.*, 2014). The highest fluorescence rates F_v *B. thunbergii* plants which are partially affected can be caused by blocking the transport of electrons from the primary acceptors to the electron transport chain of thylakoids or exceeding the light intensity of the saturation level (Schreiber *et al.*, 1998).

Various processes of assimilation in a leaf are dysregulated by powdery mildew because of the harmful physical influence of a mycelial cover on processes of photosynthesis and transpiration (Marçais & Desprez-Loustau, 2014). Researchers established (Kyryk *et al.*, 2011) that the optimal values of parameter dFpl/Fv must not exceed 0.4 which correlates with the defeat of a plant by a viral infection. The results of our studies are consistent with previous studies by scientists (Table 1). We propose to divide plants partially affected by the value of dFpl/Fv in the range of 0.23-0.28 a.u., moderately - 0.3-0.4 a.u. and very affected> 0.5 a.u.

We also assessed the functional status of *B. thunbergii* by the content and ratio of chlorophyll in the leaves which lost their purple color. There is a tendency to decrease the content of chlorophyll with an increasing degree of plants damage (Table 2). Found that the processes of chlorophyll breakdown may prevail over synthesis in the presence of adverse environmental factors and affects the ratio chl. a/b (Sushynska & Korshykov, 2019). Thus, the results of the research indicate that the increase in this indicator is a sign of activation of photosynthesis.

Usually, during the growing season, the ratio of chl. a/b in the leaves of plants is approximately 2.5-4.0 (Richardson *et al.*, 2002). However, the species specificity of plants should be taken into account. Indicators of the ratio chl. a/b allow diagnosing disorders in the functioning of light-harvesting complexes and reaction centers of photosystems in plants. Thus, the results of the ratio (Table 2) indicate dysfunction of the photosynthetic apparatus in the leaves *B. thunbergii* depending on the degree of damage by the micromycete *E. berberidis*. We found that the total content of chl. (a+b) decreases by 16 % compared with the control in very affected plants of *B. thunbergii* powdery mildew, moderate – 7 %., partially – 5 %.

The correlation analysis between chlorophyll content and chlorophyll fluorescence parameters in plants *B. thunbergii* which lost purple color and were affected by *E. berberidis* showed that not all indicators are informative and interrelated (Figure 8).

Table 1: Induction of chlorophyll fluorescence *Berberis thunbergii* DC.

Degree of damage	F_0	F_{pl}	F_p	F_{max}	F_T	dF_{pl}	F_v	dF_{pl}/F_v	F_v/F_p	F_P-F_T/ F_T
Lost purple color										
Partially	448	944	2208	2144	608	496	1760	0,28	0,80	3,63
Moderately	272	512	944	1136	352	240	672	0,36	0,71	1,68
Severely	80	96	112	112	96	16	32	0,50	0,29	0,17
With purple color										
Partially	480	800	1872	1856	1280	320	1392	0,23	0,74	1,46
Moderately	80	176	336	320	128	96	256	0,38	0,76	2,53
Severely	80	96	112	112	96	16	32	0,50	0,29	0,17

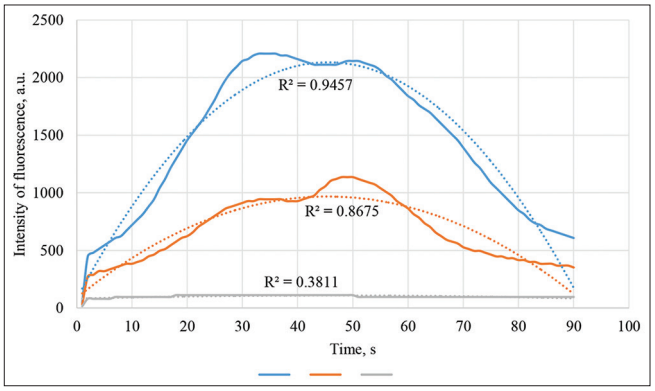

Figure 6: Induction curve of chlorophyll fluorescence for plants *Berberis thunbergii* DC. affected by *Erysiphe berberidis* (lost purple color)

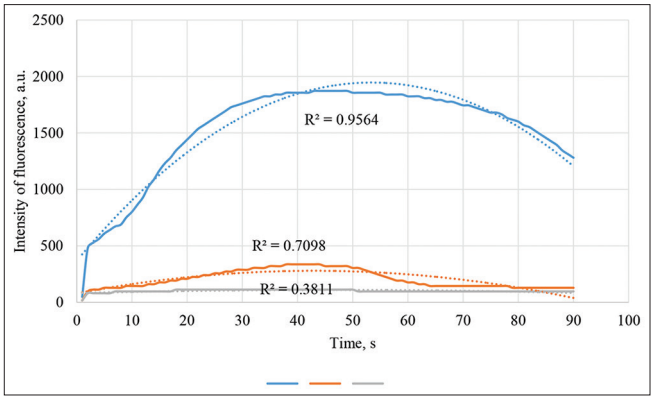

Figure 7: Induction curve of chlorophyll fluorescence for plants *Berberis thunbergii* DC. affected by *Erysiphe berberidis* (with purple color)

Table 2: Chlorophyll content and ratio in plants *Berberis thunbergii* DC. (lost purple color), p<0,05

Degree of damage	Chlorophyll, mg/g				Chlorophyll, mg/dm²			
	a	в	a+в	a/в	a	в	a+в	a/в
Control	0.98±0.03	0,25±0.01	1.23±0.04	3.92	1.70±0.05	0.44±0.01	2.14±0.06	3.86
Partially	0.95±0.02	0.22±0.01	1.17±0.03	4.31	1.68±0.03	0.42±0.01	2.10±0.05	4
Moderately	0.92±0.03	0.23±0.01	1.15±0.04	4.00	1.62±0.05	0.41±0.01	2.03±0.06	3.95
Severely	0.84±0.03	0.20±0.01	1.04±0.03	4.20	1.49±0.05	0.36±0.01	1.85±0.06	4.14

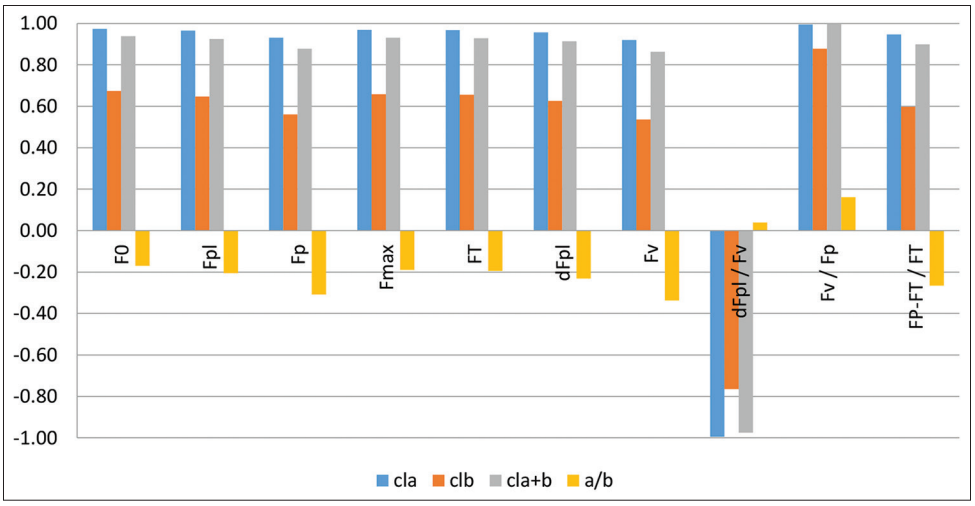

Figure 8: Correlation between chlorophyll content and chlorophyll fluorescence parameters in plants *Berberis thunbergii* DC. (lost purple color)

From the above conclusions, the high informativeness for the study of the functional state of plants can be reached by the parameters of the photosynthetic apparatus chl. a, chl. (a+b) with FI indicators (correlation value 0.86-1.0). On the other hand, the correlation analysis showed the coefficients were largely increased between Fv/Fp value and chl. b compared to other FI indicators (0.88). Furthermore, we found a very strong relationship between chl. a, chl. b, chl. (a+b) chl. a and Fv/Fp (0.99-0.88-1.00). From the above conclusions, the ratio chl.a/b and dF_{pl}/F_v had slight interrelation and accompanied by low or negative correlation.

The scientists found that biotrophic fungi that cause disease reduce the amount of chlorophyll which leads to serious damage in the functioning of the photosynthetic apparatus in various plant species (Ingram, 1981; Lindenthal *et al.*, 2005; Mandal *et al.*, 2009). To our opinion, in pathogen-infected leaves, the total chlorophyll content decreases may be caused by disruption of the photosynthetic apparatus, light-collecting complexes, and enzymatic processes of carbon uptake.

CONCLUSION

The results of studies indicate the dependence of the degree of plant damage *B. thunbergii* by the micromycete *E. berberidis* and their functional state with FIC indicators. Infected plants lose their decorative effect due to the presence of conidia on both parts of the leaves, shoots, and their subsequent deformation. The results show that *B. thunbergii* plants affected by powdery mildew mainly grew in the shade under tree canopies. We found the high sensitivity of FIC parameters *B. thunbergii* leaves to the influence of endogenous and exogenous factors. It is recorded the disappearance of induction curves in severely affected plants. In our opinion, this is the result of inhibition of the photochemical reactions due to the slowing down of the outflow of Calvin cycle enzymes and the presence of powdery mildew infection. We recorded that the parameter Fv/Fm shows destructive changes in chlorophyll molecules that occur in infected *B. thunbergii* cells. This hypothesis is consistent with the data of scientists Moriondo *et al.* (2005) and Mandal *et al.* (2009), where the Fv/Fm index in healthy plant cells is stable. The correlation analysis demonstrate a close relationship between the indicators of chlorophyll content and FIC. The results the ratio of chl. a/b indicates dysfunction of the photosynthetic apparatus *B. thunbergii* leaves depending on the degree of damage by the micromycete *E. berberidis*.

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