The effects of para-hydroxybenzoic acid treatment on photosynthetic parameters of *Populus × euramericana* “Neva”

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Abstract

As a common tree species in northern China, *Populus × euramericana* “Neva” has an important practical value for the study of continuous cropping obstacles in poplar cultivation. Plant allelopathy is the main reason for continuous cropping obstacles, which are caused by allelochemicals, such as para-hydroxybenzoic acid (p-HB). The objective of this study was to investigate the effects of p-HB on the photosynthesis of poplar. Photosynthetic parameters of *Populus × euramericana* “Neva” poplar were determined in a pot culture experiment where five p-HB concentrations were used (0, 1, 2, 4, and 6 mmol L⁻¹). Each seedling was treated with 4 L of p-HB solution every seven days, ten times in total. p-HB inhibited the photosynthesis of poplar significantly, as shown by a clear decline in the net photosynthetic rate. Our results indicated nonstomatal limitation responsible for the photosynthesis reduction.

Additional key words: chlorophyll fluorescence; cropping obstacle; gas exchange; plant allelopathy; poplar.

Introduction

Plant allelopathy was initially proposed in 1937 and since then it has been addressing more and more concerns in the academic community (Makoi and Ndakidemi 2007). In the practice and research of agricultural production, it has been found that many crops, which suffer from continuous cropping obstacles, release allelochemicals resulting in an autotoxic effect. So far, most of the discovered allelochemicals are secondary metabolic substances, of which low molecular-mass organic acids, phenolic compounds, and terpenoids are the most common compounds (Rice 1984, Li *et al.* 2002). Patrick (1971) found that phenolic acids have stronger allelopathic activity and strong toxicity to trees (Kuiters and Sarink 1986) and can cause soil ecological environment changes, resulting in a decline in soil fertility. Indeed, phenolic acids are considered to be an important cause of the continuous decline of cropping soil fertility in plantations (Weidenhamer and Romeo 2004).

Photosynthesis is essential for plant survival, growth, and production (Chen *et al.* 2013). Studies of the relationship between photosynthesis and physiological processes involving different phenolic acids at various concentrations could provide in-depth understanding of plant allelopathy, encourage a deeper understanding of the adaptive capacity of different plants in continuous cropping environments, and provide physiological evidence for research on the mechanism underlying continuous cropping obstacles.

Poplar is one of the most important, fast-growing tree species in the world, an important source of paper-making raw materials with a wide distribution and a high economic value (Wang 1995, Zhao *et al.* 1995). Continuous cropping is one of the most common production modes for poplars, often leading to serious declines in soil fertility and productivity, occasionally resulting in “plant deserts”.

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Abbreviations: AQY – apparent quantum yield; C_a – air CO₂ concentration; C_i – intercellular CO₂ concentration; Chl – chlorophyll; E – transpiration rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; LCP – light-compensation point; L_s – stomatal limitation; LSP – light-saturation point; NPQ – nonphotochemical quenching; p-HB – para-hydroxybenzoic acid; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; q_p – photochemical quenching coefficient; R_D – respiration rate; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

The rhizosphere soil of different continuous cropping black poplar plantation were analyzed with high performance liquid chromatography (HPLC), indicating that allelochemicals had accumulated in the rhizosphere soil. The p-HB content in the second generation of a poplar forest rhizosphere soil increased by 245.5% from the first generation, while the content of both cinnamic acid and benzoic acid increased by 64.2% (Tan *et al.* 2008). Thus, p-HB is the main phenolic allelochemical produced by continuous cropping of poplars. Benzoic acid and cinnamic acid inhibited the growth of watermelon radicles, characterized by shorter lengths, browning root caps, less root hairs, and underdeveloped lateral roots (Sun *et al.* 2006). Phenolic allelochemicals, such as vanillic acid, coumaric acid, caffeic acid, and clove phenol acid also inhibited the growth of bean axial roots and lateral roots (Vaughan and Ord 1990). In addition, cinnamic acid,

benzoic acid, and para-hydroxybenzoic acid affected the length of the radicle and plumule of the Chinese fir seedling (Wang *et al.* 2002). However, only a few studies on the relationship between p-HB and poplar photosynthesis have been reported.

P. × euramericana “Neva” is one of the most common poplar species in northern China, and its wide distribution and rich resources make it an important protection and commercial forest tree species in the plains and deserts of northern China. In addition, the Euramerican poplar has an important practical value for the study of poplar allelopathy. The objective of this study was to investigate the effects of para-hydroxybenzoic acid on photosynthesis of *P. × euramericana* “Neva”. Our results could provide important information on the influence of allelochemicals on poplar cultivation.

Materials and methods

Materials and treatments: In March 2015, one-year-old healthy branches of *P. × euramericana* “Neva”, planted at the forestry ecostation of Shandong Agricultural University (Tai'an, Shandong, China), were cut from the middle and lower part of the crown. The branches were cut to a length of about 20 cm and a diameter of about 2 cm and were then inserted into a plastic container. Under field conditions, the cuttings were further grown under hydroponic conditions until roots appeared. In early April 2015, when roots appeared, the cuttings were transferred to an earthen basin with quartz sand as a cultivation matrix. Then, the cuttings were grown under natural conditions with regular watering.

Starting from middle of June, the poplar plants were treated with different concentrations of p-HB. Plants with similar growth status were selected and divided into five groups, with each group containing 24 seedlings. The groups were treated with 4 L of p-HB solutions (mixed with 0.2% ethanol solution; the original liquid concentration was 10 mmol L⁻¹) of different final concentrations (1, 2, 4, and 6 mmol L⁻¹) with zero concentration as a control every seven days for ten weeks. In late August, three typical experimental plants from each group were selected as the test plants and were then divided in three groups with each group containing five plants. The photosynthetic gas exchange and chlorophyll (Chl) fluorescence parameters of the leaves were measured. Each group was measured daily (repeated three times) and all measurements were completed in three days.

Photosynthetic gas-exchange parameters: A portable photosynthesis system (CIRAS-2, Amesbury, MA, USA) was used to measure photosynthetic light-response parameters. During the measurements, the CO₂ concentration of the leaf chamber was maintained at 365 ± 5 μmol m⁻², the air temperature was maintained at 26 ± 1.5°C, and the relative humidity was maintained at 60 ± 4.0%. For

measuring light-response parameters *via* the portable photosynthesis system, 15 PAR [2,000; 1,800; 1,600; 1,400; 1,200; 1,000; 800; 600; 400; 200; 150; 100; 60; 30, and 0 μmol(photon) m⁻² s⁻¹] were supplied by a cold light-emitting diode [LED; range 0–2,000 μmol(photon) m⁻² s⁻¹; peak emission wavelength of red light (90%): 620–630 nm, and white light (10%): 425–625 nm]. Each PAR was measured for 120 s and was repeated three times. The portable photosynthesis system automatically recorded PAR, net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO₂ concentration (C_i), and air CO₂ concentration (C_a). The water-use efficiency (WUE) was calculated as $WUE = P_N/E$ and the stomatal limitation (L_s) was calculated as $L_s = 1 - C_i/C_a$ (Berry and Downton 1982, Nijs *et al.* 1997).

Chl fluorescence parameters: A portable pulse amplitude-modulated chlorophyll FMS-2 fluorometer (Hansatech, King's Lynn, UK) was used to measure Chl fluorescence parameters, including minimum Chl fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), steady-state fluorescence yield (F_s), minimum fluorescence of the light-adapted state (F_0'), and maximal fluorescence yield of the light-adapted state (F_m'). All measurements were done on 3–4 leaves from the middle of a plant. Additional Chl fluorescence parameters were calculated according to the following equations. Maximal quantum yield of PSII photochemistry (F_v/F_m) = $(F_m - F_0)/F_m$; photochemical quenching coefficient (q_p) = $(F_m' - F_s)/(F_m' - F_0')$; nonphotochemical quenching (NPQ) = $(F_m - F_m')/F_m'$; and effective quantum yield of PSII photochemistry (Φ_{PSII}) = $(F_m' - F_s)/F_m'$ (Krause *et al.* 1991, Nijs *et al.* 1997, Xu 2002, Zhu *et al.* 2011).

Response curves (P_N -PAR) of P_N to light intensity: Current research has shown that the rectangular hyperbolic

correction model is an accurate response curve (P_N -PAR) of the net photosynthetic rate to light intensity (Ye and Yu 2007, Lang *et al.* 2011, Chen *et al.* 2012a, Chen *et al.* 2012b). *SPSS v. 17.0* (IBM SPSS, Chicago, IL, USA) was used to analyze the parameters of the light-response process. The calculation formulae of the rectangular hyperbolic correction model are as follows:

$$P_N = \Phi \frac{1 - \beta \text{PAR}}{1 + \gamma \text{PAR}} \text{PAR} - R_D \quad (1)$$

$$\text{LSP} = \frac{\sqrt{(\beta + \gamma)/\beta} - 1}{\gamma} \quad (2)$$

$$P_{N\max} = \Phi \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right)^2 - R_D \quad (3)$$

The calculation formula of quantum yield at light-compensation point (Φ_c), intrinsic quantum yield (Φ_0), and the absolute value of the slope of the light-response curve between PAR = 0 and PAR = $I_c(\Phi_0)$ are as follows:

$$\Phi_c = P'_N(\text{PAR} = I_c) = \alpha \frac{1 + (\gamma - \beta)I_c - \beta \gamma I_c^2}{(1 + \gamma I_c)^2} \quad (4)$$

$$\Phi_0 = P'_N(\text{PAR} = 0) = \Phi \quad (5)$$

$$\Phi_{c0} = \left| \frac{R_D}{I_c} \right| \quad (6)$$

where Φ is the apparent quantum yield (AQY; mol mol^{-1}); PAR is the photosynthetic active radiation intensity [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]; LSP is the light-saturation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$); R_D is the respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); β and γ are the model coefficients; P_N is the net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); $P_{N\max}$ is the maximum net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); and I_c is the light-compensation point (LCP; $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Data processing: *SPSS* and the *Excel* were used to perform one-way analysis of variance (*ANOVA*), using the least significant difference method (LSD). The results of different treatments were examined at the 5% level. The response characteristics of photosynthetic parameters of poplar to different p-HB concentrations were based on the leaf gas-exchange data obtained under the fixed light intensity.

Results

Light-response parameters: With increasing p-HB concentration, LCP and R_D clearly increased, while $P_{N\max}$, LSP, and AQY declined markedly (Fig. 1, Table 1).

Gas-exchange parameters: The photosynthetic para-

eters of poplar leaves in response to p-HB concentrations under the fixed PAR [$1,200 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] are shown in Table 2. At different concentrations of p-HB and under a fixed light intensity, P_N , E , g_s , C_i , L_s , and WUE changed significantly, while with concentration of p-HB,

Fig. 1. Photosynthetic rate-light response curves and stimulation of *Populus × euramericana* “Neva” under different phenol concentrations. Values are expressed as the mean of three replicates $\pm$ SD. P_N – net photosynthetic rate; PAR – photosynthetically active radiation.

Table 1. Responses of apparent quantum yield (AQY), light-saturation point (LSP), light-compensation point (LCP), light-saturated net photosynthetic rate (P_{Nmax}), and respiration rate (R_D) of *Populus × euramericana* “Neva” to different para-hydroxybenzoic acid concentrations. R^2 – coefficient of determination; Φ_0 – intrinsic quantum yield; Φ_c – quantum yield at light-compensation point; Φ_{c0} – absolute value of slope of light-reponse curve between PAR = 0 and PAR = I_c .

Para-hydroxybenzoic acid concentration [mmol L ⁻¹]	Φ_0	Φ_c	Φ_{c0}	LSP	P_{Nmax}	LCP	R_D	R^2
0	0.0146	0.0138	0.0135	1356	15.83	5.86	0.178	0.9998
1	0.0134	0.0130	0.0128	1184	9.82	16.74	0.476	0.9990
2	0.0128	0.0124	0.0121	990	7.68	29.67	0.805	0.9999
4	0.0122	0.0109	0.0101	846	5.65	47.21	1.176	0.9990
6	0.0115	0.0102	0.0099	754	3.83	60.03	1.358	0.9998

P_N , E , g_s , and L_s decreased significantly (Table 2). However, with increasing p-HB concentration, the C_i increased significantly (Table 1), but WUE increased at first and then declined. Higher WUE values were observed at the concentrations of 0, 1, and 2 mmol L⁻¹.

Discussion

With increasing world population, the pressure for economic crop and grain production is also increasing. Plant allelopathy is a main reason for the decline in economic crop and grain production and has aroused the attention of the scholars all over the world. Meanwhile, phenolic acids, allelochemicals, which promote plant growth and stress resistance and also serve as pigments and defense and signal molecules, are gradually arousing concern (Makoi and Ndakidemi 2007). In agricultural ecosystems, allelochemicals such as phenolic acids are destructive for annual crop growth and soil activity and are an important cause of continuous cropping obstacles (Rice 1984, Singh *et al.* 2001).

Photosynthesis is an important basis for plant growth and development (Wang *et al.* 2010b). Currently, as a rapid, sensitive technique with nondestructive photosynthesis probes, Chl fluorescence has been applied in research for the detection of heavy metals, toxic substances, and adversity stress effects on plant photosynthesis (Brack and Frank 1998, Su *et al.* 2002, Ouzounidou *et al.* 2008, El-Hassani *et al.* 2009, Wang *et al.* 2010a).

With the increase of p-HB concentration, light-response parameters showed that AQY, LSP, and P_{Nmax} declined markedly, while LCP and R_D increased clearly. The decline of AQY implies that the conversion efficiency of low-light energy decreased (Farquhar and Sharkey 1982, Richard 2001). Meanwhile, the decrease in P_{Nmax} and increase in R_D indicated a lower rate of photosynthesis and higher respiration rate. The large decline in P_{Nmax} after the 1 mmol L⁻¹ treatment indicated that the photosynthesis of *P. × euramericana* “Neva” was very sensitive to p-HB stress.

In this study, our results of photosynthetic gas

Chl fluorescence parameters: Under different concentrations of p-HB, the F_v/F_m , Φ_{PSII} , q_p , and NPQ of poplar leaves clearly changed (Table 3). As the p-HB concentration increased, the F_v/F_m and Φ_{PSII} decreased, while $1 - q_p$ increased. NPQ increased at a lower concentration, under 2 mmol L⁻¹, then decreased at higher concentrations.

exchange showed that p-HB significantly inhibited the photosynthesis of *P. × euramericana* “Neva”. The higher the p-HB concentration was the greater the inhibition effect occurred, which was mainly caused by nonstomatal factors. According to studies on plant photosynthesis, as stress factor aggravating, there was a transition from stomatal limitation to nonstomatal limitation factors, which was the main reason for the reduction in photosynthesis (Wang *et al.* 2010a, Qian *et al.* 2011). However, in this study, poplar showed a nonstomatal limitation of photosynthesis, even at a low concentration of p-HB (1 mmol L⁻¹); compared with the control, P_N , E , g_s , LSP, and P_{Nmax} decreased significantly. Therefore, a concentration range of 0–1 mmol L⁻¹ was shown to be a critical threshold at which a change from stomatal to nonstomatal factors caused a reduction in photosynthesis. However, further study is needed to determine the precise critical concentration.

P_N and E might be affected by other physical characteristics, such as g_s and C_i . Inorganic ions could be transported to different groups of plants along with upward water transport by transpiration. At the same time, CO₂ was absorbed in by stomata with H₂O lost to the surrounding air. p-HB damaged stomatal function and prevented the exchange of H₂O and CO₂, which affected the P_N and E values. Along with the rapid decrease in E and P_N , WUE first increased (0–1 mmol L⁻¹) and then decreased (1–6 mmol L⁻¹), implying that the decline of P_N was slighter than that of E at the lower concentration of p-HB (0–1 mmol L⁻¹). The increase of LCP and decline of LSP suggest that the light-use efficiency (under both strong and weak light) declined, which is in accordance with the results of F_v/F_m and Φ_{PSII} .

Table 2. Response of photosynthetic parameters of *Populus × euramericana* “Neva” to different para-hydroxybenzoic acid concentrations under the same photosynthetically active radiation [$1,200 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. Values are expressed as the mean of three replicates $\pm$ SD. Means in columns within the different concentrations of para-hydroxybenzoic acid that are followed by *different letters* are significantly different (LSD test, $P < 0.05$). P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; L_s – stomatal limitation; WUE – water-use efficiency.

Para-hydroxybenzoic acid concentration [mmol L ⁻¹]	P_N [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	E [mmol m ⁻² s ⁻¹]	g_s [mmol m ⁻² s ⁻¹]	C_i [$\mu\text{mol mol}^{-1}$]	L_s	WUE [$\mu\text{mol mmol}^{-1}$]
0	15.60 $\pm$ 1.07 ^a	4.03 $\pm$ 0.30 ^a	61.13 $\pm$ 6.29 ^a	236.61 $\pm$ 6.23 ^a	0.35 $\pm$ 0.02 ^a	3.87 $\pm$ 0.03 ^a
1	9.80 $\pm$ 0.87 ^b	2.47 $\pm$ 0.22 ^b	36.32 $\pm$ 4.6 ^b	253.85 $\pm$ 4.25 ^{ab}	0.30 $\pm$ 0.02 ^{ab}	3.96 $\pm$ 0.10 ^a
2	7.50 $\pm$ 0.79 ^{bc}	2.10 $\pm$ 0.17 ^{bc}	27.24 $\pm$ 3.03 ^c	261.04 $\pm$ 6.12 ^{bc}	0.28 $\pm$ 0.02 ^{bc}	3.57 $\pm$ 0.11 ^b
4	5.20 $\pm$ 0.58 ^{cd}	1.59 $\pm$ 0.15 ^{cd}	26.77 $\pm$ 2.80 ^c	280.20 $\pm$ 5.35 ^c	0.23 $\pm$ 0.02 ^c	3.26 $\pm$ 0.08 ^c
6	3.40 $\pm$ 0.40 ^d	1.31 $\pm$ 0.14 ^d	25.18 $\pm$ 2.42 ^c	320.91 $\pm$ 9.78 ^d	0.12 $\pm$ 0.01 ^d	2.60 $\pm$ 0.05 ^d

Table 3. Effects of phenol concentrations on chlorophyll fluorescence parameters of *Populus × euramericana* “Neva.” Values are expressed as the mean of three replicates $\pm$ SD. Means in columns within different concentrations of para-hydroxybenzoic acid that are followed by *different letters* are significantly different (LSD test, $P < 0.05$). F_v/F_m – maximal quantum yield of PSII; NPQ – nonphotochemical quenching; q_p – photochemical quenching coefficient; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Para-hydroxybenzoic acid concentration [mmol L ⁻¹]	F_v/F_m	1- q_p	NPQ	Φ_{PSII}
0	0.824 $\pm$ 0.025 ^a	0.252 $\pm$ 0.026 ^d	0.863 $\pm$ 0.062 ^c	0.544 $\pm$ 0.050 ^a
1	0.760 $\pm$ 0.023 ^b	0.818 $\pm$ 0.071 ^c	1.069 $\pm$ 0.063 ^b	0.328 $\pm$ 0.037 ^b
2	0.733 $\pm$ 0.022 ^c	1.020 $\pm$ 0.059 ^{bc}	1.210 $\pm$ 0.055 ^a	0.264 $\pm$ 0.031 ^c
4	0.672 $\pm$ 0.018 ^d	1.045 $\pm$ 0.065 ^b	0.954 $\pm$ 0.061 ^c	0.236 $\pm$ 0.029 ^d
6	0.630 $\pm$ 0.012 ^e	1.400 $\pm$ 0.076 ^a	0.883 $\pm$ 0.062 ^d	0.137 $\pm$ 0.027 ^e

Chl fluorescence parameters represent the activity of antenna pigments for the transport of light to the reaction centers of PSII that shows the maximum efficiency of the absorption of light energy used in photochemical reactions by the photosynthetic apparatus, also with respect to the degree of photoinhibition (Chen *et al.* 2008).

Our study showed that with an increase in the p-HB concentration, 1 – q_p increased, but Φ_{PSII} and F_v/F_m decreased (Table 3). The decline of F_v/F_m and Φ_{PSII} under increasing p-HB concentration indicated that the potential activity of PSII decreased. The decrease of q_p (or increase of 1 – q_p) also represents a decline in the activity of the PSII reaction centers (Piñol and Simón 2009, Zhu *et al.* 2011). The Φ_{PSII} value gives an estimation of efficiency (Baker and Rosenqvist 2004), with a decline in Φ_{PSII} representing weaker photochemical efficiency (Maxwell and Johnson 2000). The decrease in F_v/F_m reflects a reduction in light-use efficiency (Baker 2008; Li *et al.* 2015), which is in accordance with the decline of LSP and increase of LCP observed in this study. Taken together, our results showed that p-HB inhibited the activity and efficiency of PSII in *P. × euramericana* “Neva” leaves.

Our results indicated that p-HB could destroy the PSII reaction centers gradually, lead to higher restriction on the effective transfer of excited electrons and reduction of the actual light energy used for the photochemical reactions of PSII (Hang and Zhao 2010). However, with increasing

p-HB concentration, NPQ first increased and then decreased (Table 3). At lower concentrations of p-HB ($\leq 2 \text{ mmol L}^{-1}$), NPQ increased, and at the same time, 1 – q_p and Φ_{PSII} decreased, indicating that thermal dissipation by PSII increased, which might help to alleviate the damage to PSII reaction centers (Brack *et al.* 1998; Liang *et al.* 2010; Liu *et al.* 2012). However, with p-HB at higher concentrations ($> 4 \text{ mmol L}^{-1}$), NPQ decreased, which indicated that the energy used for the heat dissipation of antenna pigments in PSII decreased (Xu 2002, Liang *et al.* 2010). P_N decreased at the same time, which indicated that the high p-HB concentration might destroy some enzymes related to the xanthophyll cycle, particularly, violaxanthin de-epoxidase, which might lead to a decline in heat dissipation dependent on the xanthophyll cycle (Chen *et al.* 2008).

The responses of different plants to p-HB have been recently studied. Wang *et al.* (2014) reported that after treatment of *Malus hupehensis* Rehd. seedlings with 0.4 mmol L^{-1} p-HB, the chlorophyll content, PSII center activities, and photosynthetic capacity decreased. The reduction in P_N under p-HB stress was thought to be caused by nonstomatal restrictions. As both *P. × euramericana* “Neva” and *M. hupehensis* Rehd. belong to the xylophyta family, we expected similar effects of p-HB in both species.

In this study, when the para-hydroxybenzoic acid concentration reached 2 mmol L⁻¹, P_N and E decreased to 49.8 and 49.5%, respectively. Therefore, in the continuous cropping of *P. × euramericana* “Neva”, the para-hydroxybenzoic acid concentration in soil should be lower than 2 mmol L⁻¹, otherwise the physiological activity,

growth, and development of the plants significantly decrease. Whether a concentration of 6 mmol L⁻¹ is the limiting para-hydroxybenzoic acid concentration, which *P. × euramericana* “Neva” can tolerate in order to maintain normal photosynthetic activities, it is difficult to determine from our present results.

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