DOI: 10.32615/ps.2020.071 PHOTOSYNTHETICA 58 (5): 1210-1216, 2020

Phenolic acids inhibit the photosynthetic productivity of poplar

K. LI**, T. ZHANG**, H. LI*,+, L.D. ZHANG***, and F. LI#

*College of Agriculture and Forestry Science, Linyi University, 276000 Lin'yi, China**
*Taishan Forest Ecosystem Research Station/Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, 271018 Tai'an, Shandong, China***
*Station for State-owned Forest Farm Management of Shandong Province, 250014 Ji'nan, Shandong, China****
State-owned Yishan Forest Farm of Yishui County, 276400 Yi'shui, Shandong, China#

Abstract

Barriers to continuous cropping of poplar plantations are closely related to the accumulation of phenolic acids in the soil. The purpose of this study was to explore the mechanism through which phenolic acid stress affects poplar productivity. The results showed that phenolic acids had a significant inhibitory effect on the photosynthesis of poplar. The inhibition of photosynthesis due to phenolic acids occurred mainly because, during electron transfer at the PSII reaction center, the primary quinone acceptor of PSII (Q_A) in the oxidized state was reduced, and excess light energy was lost in the form of heat dissipation; thus, poplar productivity decreased. At low phenolic acid concentrations ($0X$–$1.5X$), the activity of the PSII reaction center was temporarily inactivated, mainly because of stomatal limiting factors. At high phenolic acid concentrations ($1.5X$–$3.0X$), the PSII reaction centers were damaged, and photoinhibition occurred, mainly because of nonstomatal limiting factors.

Keywords: chlorophyll fluorescence; continuous cropping; gas exchange; nonphotochemical quenching; poplar.

Introduction

Poplar is an important industrial timber species (Griu and Lunguleasa 2016), and owing to its fast growth rate and broad adaptability, it is widely cultivated in many countries, such as those in Europe, America, and China, and plays a large role in meeting the demand for wood (Heilman 1999, Kang *et al.* 2015). However, owing to the shortage of land resources, continuous cropping is widely applied in poplar plantations in many countries, which brings about a series of problems, such as reduced soil nutrient contents, severe pests, and diseases, and significantly lowered forest productivity (Afas *et al.* 2008, Wang and Xin 2016). Throughout the years, the relationship between the decline and the allelopathic effects of continuously cropped plantations has received widespread attention. Studies have shown that an important reason for the decline in poplar productivity is the accumulation of certain allelochemicals in the soil. Phenolic acids, which compose a class of substances with strong allelopathic activity, are recognized as the main autotoxic substances responsible for plantation decline (Weidenhamer and Romeo 2004, Liu *et al.* 2018). Specifically, benzoic acid, vanillin, ferulic acid, *p*-hydroxybenzoic acid, cinnamic acid, *etc.*, are considered to be the most important phenolic acids (Wang *et al.* 2016).

Since the accumulation of phenolic acids in the soil after several generations of continuous poplar forests was detected (Wang *et al.* 2016), phenolic acid research has continuously increased (Xie *et al.* 2018). Phenolic acids are excreted from plant root systems and exert an inhibitory growth effect on adjacent rhizospheres (Kefeli *et al.* 2003). Phenolic acids affect the morphological development and several physiological and biochemical characteristics of the roots of poplar seedlings, and in turn, this effect has a significant inhibitory effect on the growth and development

---

Received 12 July 2020, *accepted* 5 October 2020,

+Corresponding author; fax: +865397258722, e-mail: huili@lyu.edu.cn

Abbreviations: C_a – air CO_2 concentration; ChlF – chlorophyll fluorescence; C_i – intercellular CO_2 concentration; 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; HPLC – high performance liquid chromatography; LCP – light-compensation point; L_s – stomatal limitation; LSP – light-saturation point; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; PQ – plastoquinone; Q_A – primary quinone acceptor of PSII; Q_B – secondary quinone acceptor of PSII; q_p – photochemical quenching coefficient; R_D – respiration rate; RuBP – ribulose-1,5-bisphosphate; WUE – water-use efficiency; Φ – apparent quantum efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Acknowledgements: This work was supported by grants from National Natural Science Foundation of China (31700553) and the Science and Technology Fund Project of Shandong Provincial Department of Agriculture and Rural Affairs (2019LY005).

Conflict of interest: The authors declare that they have no conflict of interest.

of the whole plant (An *et al.* 2006). Phenolic acids can also inhibit antioxidant enzyme activities in roots and reduce root activity (Vaughan and Ord 1990, Wang *et al.* 2019). Moreover, phenolic acids in the soil affect the activity of soil microorganisms, inhibiting the fixation and transformation of nitrogen and other processes, which subsequently could lead to nitrogen deficiency in plants and failure to grow normally (Castaldi *et al.* 2009, Castells *et al.* 2003). Thus far, research on the effects of phenolic acids on poplar trees has focused mainly on the effects on soil physical and chemical properties and the rhizosphere (Wang *et al.* 2016). Xie *et al.* (2018) examined the effects of three different concentrations of phenolic acids on the photosynthesis characteristics and growth of poplar plants growing in vermiculite. Phenolic acids also can accumulate within the chloroplasts of plant cells and regulate cell elongation (Kefeli *et al.* 2003). Previous studies have shown that phenolic acids have powerful allelopathic effects and inhibit leaf photosynthesis (Mersie and Singh 1993, Fu *et al.* 2019). However, there is still little information on the effects of phenolic acids on the productivity of poplar due to the lack of studies both on the characteristics of phenolic acid substances in the soil and on the photosynthesis-response characteristics of poplar, including chlorophyll (Chl) fluorescence dynamics, leaf gas exchange, and biomass. To closely mimic the conditions in field plantations, this study used I-107 poplar (*Populus* × *euramericana* 'Neva') as the test material in a potted plant simulation experiment to determine the response of poplar in terms of their photosynthesis-related physiology and growth indicators in response to different phenolic acid concentrations. This study was designed to determine the effects of soil-based phenolic acids stress on the growth and photosynthesis of poplar and lay a plant physiology-based foundation for solving the problems associated with continuous cropping of poplar.

Materials and methods

Experimental design and treatments: The experimental site is located in the integrated experimental station of the Forestry College of Shandong Agricultural University (36°11'N, 117°08'E), Shandong Province, China. The area lies in a warm temperate zone with a semi-humid continental monsoon climate. The frostless season lasts approximately 202 d. The mean annual temperature is approximately 12.9°C, and the annual cumulative temperature greater than 10°C is 4,213°C. The mean altitude is 150 m, and the mean annual precipitation is 741.8 mm, with more than 74% of the precipitation falling from June to September. The mean annual relative humidity is 65%. The soil is classified as brown soil, with a sandy loam texture, and the mean soil bulk density is 1.29 g cm^{-3} (Liu *et al.* 2017, Xie *et al.* 2018).

Harwood cuttings (1.5 cm in diameter, 10 cm in length) of I-107 poplar were obtained from the middle sections of the stems of one-year-old plants. In late March 2018, the cuttings were cultivated in water within containers to initiate rooting. After two weeks, 56 plants of similar size were selected, and individual plants were assigned randomly to ceramic pots (52 cm in diameter and 33 cm in height) filled with soil (86 kg of soil sprayed with a phenolic acids combination per pot) following a single-factor randomized block design (eight replications per treatment); there were seven phenolic acid combinations, and continuous water supply was provided throughout the experiment. To account for the variation in phenolic acid concentrations caused by soil colloid adsorption and microbial degradation after treatment, based on the research by Wang *et al.* (2013), 86 kg of soil was weighed and put into the corresponding pots, after which a standard solution of the corresponding concentration of phenolic acids was applied *via* injection; a 1-cm infiltration layer was maintained in each pot. The phenolic acid solution in the culture dish was changed daily to maintain the concentration of phenolic acids in the soil at a relatively stable concentration. The concentration used for spraying phenolic acids was determined during preliminary experiments based on the high-performance liquid chromatography (HPLC) methods of Wang *et al.* (2016).

In the mid of April 2018, we established seven treatments, 0*X*, 0.5*X*, 1.0*X*, 1.5*X*, 2.0*X*, 2.5*X*, and 3.0*X* (*see* text table), based on the actual concentration of phenolic acids in the soil of a second-generation continuously cropped poplar plantation, which was used as the reference concentration (*X*) (Li *et al.* 2018). Before the start of the test, the phenolic acids were sprayed onto each pot. At 130 d after treatment, no symptoms of toxicity, such as necrosis, chlorosis, and leaf fall, were observed on plants that survived the treatments of phenolic acids. Photosynthesis and fluorescence parameters of the poplar leaves were subsequently measured.

Photosynthetic gas-exchange parameters: The gas-exchange parameters of poplar were measured *via* a portable photosynthesis system (*CIRAS-2*, *PP Systems*, Amesbury, MA, USA) on 16 July 2018. Four robust, healthy trees displaying the same growth potential (equal height and ground diameter) were selected from each treatment, after which four fully developed mature leaves from the middle part of the canopy of each seedling were carefully selected and marked. The gas-exchange parameters were measured three times for each selected leaf. All the measurements were carried out from 08:00–11:00 h on sunny days. During the measurements, an artificial light source (light-emitting diode, LED) was used to supply PAR at 14 different intensities: 2,000; 1,600; 1,400; 1,200; 1,000; 800, 600, 400, 200, 150, 100, 60, 30, and 0 μmol(photon) m^{-2} s^{-1}. Each measurement spanned 120 s under each light intensity, and each measurement was repeated three times at a consistent CO_2 concentration (370 ± 5.0 μmol mol^{-1}), temperature (26 ± 1.5°C), and relative humidity (65 ± 5%). The instrument recorded the PAR, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and net photosynthetic rate (P_N). The response of the net photosynthetic rate to light intensity (P_N–PAR) was plotted. The photosynthetic light-saturation point (LSP) and the maximum net photosynthetic rate

		p-hydroxybenzoic acid	Vanillin	Ferulic acid	Benzoic acid	Cinnamic acid
Phenolic acid concentrations in the rhizosphere soil of a second-generation poplar plantation [μg ml^{-1}]		152.00	10.40	6.50	20.60	1.95
Experimental soil phenolic acid concentration [μg·ml^{-1}]	0*X*	0	0	0	0	0
	0.5*X*	76.00	5.20	3.25	10.30	0.98
	1.0*X*	152.00	10.40	6.50	20.60	1.95
	1.5*X*	228.00	15.60	9.75	30.90	2.93
	2.0*X*	304.00	20.80	13.00	41.20	3.90
	2.5*X*	380.00	26.00	16.25	51.50	4.88
	3.0*X*	456.00	31.20	19.50	61.80	5.85

(P_{Nmax}) were estimated according to the trend of the light-response curve. During the low light phase [PAR of 200 μmol(photon) m^{-2} s^{-1} or lower], the light response data from linear regression analysis were used to determine the apparent quantum efficiency (Φ), light-compensation point (LCP), and dark respiration rate (R_D). The stomatal limitation value (L_s) was calculated as follows: $L_s = 1 - C_i/C_a$.

Chl fluorescence parameters: Chl fluorescence (ChlF) parameters and photosynthetic gas-exchange parameters were measured during the same morning. Four robust, healthy trees with the same growth potential were selected from each treatment, and six well-developed leaves were selected from the middle and upper parts of the plants under the different treatments. The ChlF parameters were measured once for each selected leaf *via* an *FMS-2* pulse-modulated fluorometer (*Hansatech*, Norfolk, UK). This allows comparison of the minimal ChlF (F_0 in dark-adapted state and F_0' in light-adapted state) and maximal ChlF (F_m in the dark-adapted state and F_m' in light-adapted state) during the initial redox state of the primary quinone acceptor of PSII (Q_A) and when Q_A is fully reduced at the end of a saturating flash, respectively. The following ChlF parameters were calculated (Nijs *et al.* 1997, Dąbrowski *et al.* 2015, Yao *et al.* 2018): 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: $NPQ = (F_m - F_m')/F_m'$; effective quantum yield of PSII photochemistry: $\Phi_{PSII} = (F_m' - F_s)/F_m'$.

Statistical analysis: Before the analyses, *Shapiro-Wilk*'s and *Levene*'s tests were performed to check all the data for normality and homogeneity of variances, respectively. The measured variables were subjected to one-way analysis of variance (*ANOVA*), assuming equal variance, and the separation of means was carried out according to the mean post hoc least significant difference (LSD) test, with differences considered significant at $P<0.05$. *SPSS 24.0* (*IBM*, Chicago, USA) was used to analyze the data, and *Origin* software (*2019b*, *OriginLab*, USA) was used to construct figures.

Results

Light-response parameters: As shown in Fig. 1, the photosynthesis light response of poplar leaves differed significantly in response to different phenolic acids concentrations. When the PAR was ≤ 200 μmol(photon) m^{-2} s^{-1}, the P_N under all the treatments increased linearly with an increase in PAR; when the PAR was ≥ 200 μmol(photon) m^{-2} s^{-1} but ≤ 1,200 μmol(photon) m^{-2} s^{-1}, the P_N gradually increased; and when the PAR was > 1,200 μmol(photon) m^{-2} s^{-1}, the P_N at the 0*X*, 0.5*X*, 1.0*X*, 1.5*X*, and 2.0*X* concentrations tended to be stable, whereas the P_N at the 2.5*X* and 3.0*X* concentrations significantly decreased. The results showed that phenolic acids could inhibit the photosynthesis of poplar; the greater the concentration of phenolic acids, the stronger the inhibitory effect.

With an increase in phenolic acid concentration, the poplar LCP increased (Table 1). Compared with that

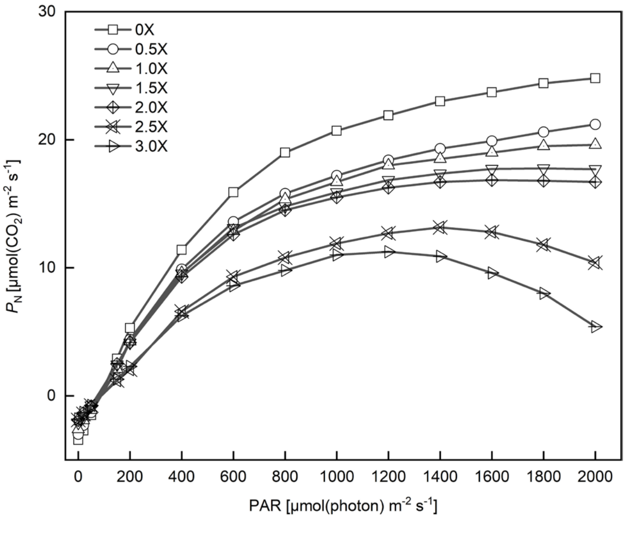

Fig. 1. Photosynthesis light-response curves of poplar subjected to different phenolic acids concentrations. The values are the means of three replications. P_N – net photosynthetic rate. PAR – photosynthetically active radiation.

in the control group, the LCP at the different phenolic acid concentrations ($0.5X$, $1.0X$, $1.5X$, $2.0X$, $2.5X$, $3.0X$) increased by 2.9, 3.8, 5.2, 5.9, 11.8, and 9.7%, respectively, showing that phenolic acids could reduce poplar's ability to use light and increase the use of low light. At the different phenolic acid concentrations, Φ was 17.3, 33.6, 39.9, 44.8, 54.7, and 56.1% lower, respectively, than that in the control group. P_{Nmax} is an indicator of maximum photosynthesis efficiency (Zhu *et al.* 2020). Compared with that in the control group, the P_{Nmax} at the different phenolic acid concentrations ($0.5X$, $1.0X$, $1.5X$, $2.0X$, $2.5X$, $3.0X$) decreased by 14.5, 21.0, 28.4, 32.1, 47.0, and 54.6%, respectively. The phenolic acids influenced poplar photosynthesis and decreases in the P_{Nmax} and Φ in the presence of phenolic acids could lead to photoinhibition. Compared with that in the control group, the R_{D} at the $0.5X$, $1.0X$, $1.5X$, $2.0X$, $2.5X$, and $3.0X$ concentrations decreased by 15, 28.3, 38.9, 44.7, 49.4, and 51.9%, respectively. The phenolic acids affected the R_{D} of poplar, and the greater the phenolic acid concentration was, the stronger the inhibition.

Gas-exchange parameters: As shown in Table 2, the P_{N} of poplar leaves decreased significantly with increasing phenolic acid concentration, indicating that phenolic acids could inhibit poplar productivity; moreover, the greater the concentration of phenolic acids was, the stronger the inhibitory effect. At the concentrations from $0X$ to $1.5X$, with an increase in the concentration of phenolic acids, the C_{i} decreased, and the $\mathrm{L_s}$ increased. However, from the $1.5X$ to $3.0X$ concentrations, the C_{i} increased, and the $\mathrm{L_s}$ decreased. The g_{s} of the plants at phenolic acid concentrations of $0X$–$3.0X$ decreased, as measured by the Farquhar and Sharkey limit criterion (Farquhar and Sharkey 1982), suggesting that at phenolic acid concentrations of $0X$–$1.5X$, stomatal opening decreased, the CO_2 supply decreased, and stomatal factors caused the decline in photosynthesis of poplar. At phenolic acid concentrations of $1.5X$ to $3.0X$, the C_{i} increased. The main limitation are nonstomatal factors. And the critical turning point of PAR was not observed with the change from stomatal limit to nonstomatal limit, which requires a deep-going research at present.

Fluorescence parameters of poplar: As shown in Table 3, the $\mathrm{F_0}$ at increasing phenolic acid concentrations indicated deactivation or destruction of the PSII reaction center. However, the increasing trend of $\mathrm{F_0}$ differed under increasing phenolic acid concentrations. These results suggested that, at phenolic acid concentrations $< 1.5X$,

Table 1. Responses of the photosynthesis quantum efficiency (Φ), light-saturation point (LSP), maximum net photosynthetic rate (P_{Nmax}), light-compensation point (LCP), and respiration rate (R_{D}) of poplar to different phenolic acid concentrations. R^2 – coefficient of determination. The values are means ± SD. The means in the columns within different concentrations of phenolic acids followed by *different letters* are significantly different (LSD test, $P<0.05$).

Experimental concentration	LSP [μmol m^{-2} s^{-1}]	Φ	P_{Nmax} [μmol m^{-2} s^{-1}]	LCP [μmol m^{-2} s^{-1}]	R_{D} [μmol m^{-2} s^{-1}]	R^2
$0X$	≥ 1,500	0.0446 ± 0.0046^{a}	24.80 ± 1.10^{a}	80.61 ± 3.76^{d}	3.60 ± 0.10^{a}	0.9996
$0.5X$	≥ 1,500	0.0369 ± 0.0044^{b}	21.20 ± 1.00^{b}	82.98 ± 4.17^{cd}	3.06 ± 0.12^{b}	0.9993
$1.0X$	≥ 1,500	0.0296 ± 0.0038^{c}	19.60 ± 0.40^{c}	83.68 ± 3.51^{cd}	2.58 ± 0.10^{c}	0.9994
$1.5X$	≥ 1,400	0.0268 ± 0.0040^{cd}	17.76 ± 0.37^{d}	84.80 ± 3.29^{bc}	2.20 ± 0.09^{d}	0.9991
$2.0X$	≥ 1,400	0.0246 ± 0.0037^{d}	16.85 ± 0.42^{d}	85.39 ± 3.61^{bc}	1.99 ± 0.10^{e}	0.9991
$2.5X$	≥ 1,300	0.0202 ± 0.0049^{e}	13.15 ± 0.74^{e}	90.10 ± 3.66^{a}	1.82 ± 0.08^{f}	0.9989
$3.0X$	≥ 1,200	0.0196 ± 0.0032^{e}	11.25 ± 0.81^{f}	88.44 ± 3.01^{ab}	1.73 ± 0.10^{f}	0.9993

Table 2. Response of photosynthesis parameters of poplar to different phenolic acid concentrations under the same PAR [1,200 μmol(photon) m^{-2} s^{-1}]. The values are means ± SD. The means in the columns within different concentrations of phenolic acids followed by *different letters* are significantly different (LSD test, $P<0.05$). C_{i} – intercellular CO_2 concentration; g_{s} – stomatal conductance; $\mathrm{L_s}$ – stomatal limitation; P_{N} – net photosynthetic rate.

Experimental concentration	P_{N} [μmol(CO_2) m^{-2} s^{-1}]	g_{s} [mmol(H_2O) m^{-2} s^{-1}]	C_{i} [μmol(CO_2) mol^{-1}]	$\mathrm{L_s}$
$0X$	25.28 ± 1.10^{a}	379.75 ± 9.11^{a}	329.25 ± 7.41^{a}	0.12 ± 0.02^{a}
$0.5X$	21.43 ± 1.00^{b}	335.75 ± 15.06^{b}	292.00 ± 11.17^{b}	0.22 ± 0.06^{b}
$1.0X$	18.85 ± 0.40^{c}	293.25 ± 12.37^{c}	237.00 ± 7.87^{d}	0.36 ± 0.02^{c}
$1.5X$	17.40 ± 0.37^{d}	250.50 ± 10.25^{d}	210.00 ± 9.83^{e}	0.43 ± 0.03^{d}
$2.0X$	16.55 ± 0.42^{d}	206.50 ± 8.35^{e}	242.25 ± 6.99^{d}	0.35 ± 0.02^{e}
$2.5X$	12.68 ± 0.74^{e}	191.00 ± 13.74^{e}	269.50 ± 7.51^{c}	0.27 ± 0.02^{f}
$3.0X$	9.60 ± 0.81^{f}	172.25 ± 14.55^{f}	296.25 ± 8.14^{b}	0.20 ± 0.02^{f}

the PSII reaction center was still in its inactive state, and at concentrations > 1.5*X*, the PSII reaction centers might have suffered damage, decreasing the absorption of the energy transferred to PSII by the antenna of the accessory pigments and leading to excess energy dissipation in the form of fluorescence, *etc.*, to protect the neighboring reaction centers from damage.

The F_m decreased by 2.0, 4.4, 7.0, 11.1, 17.0, and 31.3% with increasing phenolic acid concentrations of 0.5*X*, 1.0*X*, 1.5*X*, 2.0*X*, 2.5*X*, 3.0*X*, respectively, compared with that in the control group (Table 3). This indicated that phenolic acids had a certain influence on electron transfer in poplar photosynthesis. With the increase in phenolic acid concentration (0.5*X*, 1.0*X*, 1.5*X*, 2.0*X*, 2.5*X*, 3.0*X*), the F_v/F_m significantly decreased by 0.8, 1.6, 2.7, 4.6, 6.9, and 13.3%, respectively, compared with that in the control group. These results indicated that phenolic acids had a stress effect on the photosynthesis of poplar and that the stress effect was stronger at relatively high concentrations of phenolic acids. Moreover, phenolic acids can cause photochemical inhibition in poplar leaves and can reduce the potential maximum light energy conversion efficiency of poplar. When the concentration of phenolic acids was > 1.5*X*, the decreasing degree of F_v/F_m was enhanced, and the photoinhibition effect substantially increased.

With the increase in the concentration of phenolic acids, Φ_{PSII} significantly decreased (Table 3). At phenolic acid concentrations from 1.0*X* to 3.0*X*, this reduction was obviously greater than that at the concentrations from 0*X* to 1.0*X*, which indicated that there was a concentration of phenolic acids between 1.0*X* and 1.5*X* that caused the actual light energy conversion rate of poplar leaves to decrease rapidly. The specific concentration threshold needs further study. Moreover, the Φ_{PSII} is affected by carbon assimilation, photorespiration and oxygen-dependent electron flow in plants. Combined with the data shown in Table 2, the effects of phenolic acids on gas-exchange parameters (carbon assimilation) of poplar trees suggest that the concentration of phenolic acids leading to the switch of photosynthesis-inhibiting factors from stomatal ones to nonstomatal ones was between 1.0*X* and 1.5*X*.

Compared with that in the control group, q_p in the treatment groups decreased significantly (by 5.8–58.0%) with increasing phenolic acid concentrations (Table 3). Moreover, the decrease in q_p at phenolic acid concentrations of 1.0*X*–3.0*X* was significantly greater than that at phenolic acid concentrations of 0*X*–1.0*X*. This indicated that phenolic acids can not only reduce the ability of captured quantum energy to be used in photochemical reactions but also reduced the proportion of open reaction centers of PSII. Moreover, a high concentration of phenolic acids makes the electron accumulation capacity of PQ stronger than that at low concentrations of phenolic acids, and the efficiency of conversion into chemical energy is lower than that at low concentrations of phenolic acids. Poplar counteracts the phenolic acids damage by increasing heat dissipation in leaves (increasing NPQ; Table 3). At the same time, it also indicated that, with the increase in phenolic acids concentration, poplar tree self-protection ability was enhanced. To avoid damage to their photosynthesis mechanism, plants dissipate excess light energy in the form of heat. However, there is a close relationship between plant heat dissipation and the xanthophyll cycle, so the effect of phenolic acids on the xanthophyll cycle in poplar needs to be further studied.

Discussion

As a class of allelochemicals secreted by plants, phenolic acids enter the soil through roots; phenolic acids not only damage the soil environment but also reduce the Chl content (Fu *et al.* 2019), affecting plant growth and development (Zhu *et al.* 2018). P_{Nmax} and Φ can indicate the operation of photosynthetic apparatus (Xia *et al.* 2014); when Φ and P_{Nmax} are high, the plant photosynthesis is in good condition. This study found that, with increasing concentration of phenolic acids, both the leaf P_{Nmax} and Φ of poplar decreased to different degrees. Continuous cropping reduces the P_{Nmax} and Φ of plant leaves and then damages the operation of photosynthetic apparatus (Zhang *et al.* 2013). In this study, the P_N decreased with the increase of phenolic acid concentration at 0*X*–3.0*X* treatment. These results were similar to those of a previous study in which at 0*X*–1.0*X* phenolic acid concentrations, with an increase in the concentration of phenolic acids, the

Table 3. Chlorophyll *a* fluorescence parameters of poplar seedlings at different concentrations of phenolic acids. The values are means ± SD. The means in the columns within different concentrations of phenolic acids followed by *different letters* are significantly different (LSD test, $P<0.05$). F_0 – initial fluorescence; F_m – maximum fluorescence; F_v/F_m – maximal quantum yield of PSII; Φ_{PSII} – effective quantum yield of PSII photochemistry; q_p – photochemical quenching coefficient; NPQ – nonphotochemical quenching.

Experimental concentration	F_0	F_m	F_v/F_m	Φ_{PSII}	q_p	NPQ
0*X*	404 ± 6^{e}	$2,367 \pm 26^{a}$	0.827 ± 0.005^{a}	0.53 ± 0.01^{a}	0.69 ± 0.01^{a}	0.50 ± 0.01^{e}
0.5*X*	412 ± 7^{de}	$2,319 \pm 31^{b}$	0.823 ± 0.005^{ab}	0.50 ± 0.02^{ab}	0.65 ± 0.02^{ab}	0.56 ± 0.01^{d}
1.0*X*	417 ± 5^{cd}	$2,264 \pm 19^{c}$	0.816 ± 0.007^{bc}	0.47 ± 0.01^{b}	0.63 ± 0.01^{b}	0.58 ± 0.01^{d}
1.5*X*	425 ± 3^{c}	$2,201 \pm 16^{d}$	0.805 ± 0.003^{c}	0.39 ± 0.02^{c}	0.51 ± 0.02^{c}	0.63 ± 0.02^{c}
2.0*X*	442 ± 4^{b}	$2,104 \pm 26^{e}$	0.786 ± 0.005^{d}	0.33 ± 0.02^{d}	0.45 ± 0.01^{d}	0.70 ± 0.01^{b}
2.5*X*	448 ± 4^{ab}	$1,965 \pm 35^{ef}$	0.777 ± 0.007^{e}	0.24 ± 0.01^{e}	0.35 ± 0.01^{e}	0.71 ± 0.01^{b}
3.0*X*	457 ± 5^{a}	$1,627 \pm 23^{f}$	0.714 ± 0.008^{f}	0.18 ± 0.02^{f}	0.29 ± 0.01^{f}	0.74 ± 0.01^{a}

poplar P_N also significantly decreased (Li *et al.* 2018, Xie *et al.* 2018). Some scholars use Hoagland's nutrient solution for cultivation. Continuous cropping obstacles in poplar have been found in response to 0*X*–1.0*X* phenolic acid concentrations. Both stomatal and nonstomatal limitation affected photosynthetic rate under phenolic acids stress (Fu *et al.* 2019). In the present study, with the increase in the concentration of phenolic acids, the P_N decreased and the C_i increased, indicating that the main cause of this phenomenon involves stomatal factors (Li *et al.* 2018). Moreover, at 0*X*–1.5*X* phenolic acids concentrations and with further increase in the concentration of phenolic acids (when both the P_N and C_i decreased, and the L_s increased), the degree of stomatal opening gradually decreased, and the CO_2 supply was continuously blocked, which was similar to the results of Fu *et al.* (2019). Stomatal factors are also the main factors leading to reduced photosynthesis of poplar trees. However, the phenolic acid concentration in the study of Li *et al.* (2018) was only 1.0*X*, so whether the results in response to a phenolic acid concentration of 1.5*X* would be similar to the results of this study needs to be further verified. In the present study, at concentrations from 1.5*X*–3.0*X*, the C_i increased with increasing phenolic acid concentration, whereas the P_N, L_s, and g_s decreased. The main reasons for the decrease in photosynthesis in poplar involved nonstomatal factors. Nonstomatal factors cause photosynthesis to decrease because of a decrease in the photosynthetic activity of mesophyll cells. Rubisco acts as a rate-limiting enzyme of photosynthesis and plays a decisive role in the plant photosynthetic rate (Yasumoto *et al.* 2018). The leaf photosynthetic rate is closely related to Rubisco activity (Bernacchi *et al.* 2003), and the degradation rate of Rubisco in leaves is significantly accelerated when plants are stressed (Li *et al.* 2016), which affects the normal photosynthesis process. Thus, further research is needed to determine whether the high concentration of phenolic acids has a certain influence on Rubisco in poplar and thus affects poplar productivity.

Changes in plant photosynthesis can be explored based on fluorescence parameters (Dąbrowski *et al.* 2019). F_0 is the fluorescence emitted by fully dark-adapted leaves under very low light conditions. This component of the fluorescence is emitted by the antenna Chl before the excitation energy in the antenna is captured by the reaction center. After full dark adaptation, the PSII electron acceptors in green plant organs are fully oxidized, and their ability to accept electrons is the strongest. The PSII reaction center is open fully at this time, and the fluorescence emitted by the plant under light is minimal. In the present study, as the concentration of phenolic acids increased, the F_0 increased, but the increases differed between low concentrations and high concentrations. When the concentration of phenolic acids was < 1.5*X*, the F_0 increased gradually with the increasing concentration, and the PSII reaction center was inactivated. However, when the phenolic acid concentration was > 1.5*X*, the F_0 increased rapidly with increasing phenolic acid concentration; the PSII reaction center may have been damaged. The increase in F_0 indicated that the PSII reaction center was inactivated until it was destroyed, and the plant was photoinhibited (Dąbrowski *et al.* 2016). At the same time, with the increase in phenolic acid concentration, the Φ_{PSII} significantly decreased and the NPQ significantly increased. According to the principle of the distribution of plant-absorbed light energy (Li *et al.* 2016), the results showed that, when the poplar plants were subjected to phenolic acid stress, to protect themselves from damage, most of the absorbed light energy was lost in the form of heat dissipation. When plants experience photoinhibition, they show a decrease in F_v/F_m and an increase in NPQ (Yao *et al.* 2018). In the present study, as the concentration of phenolic acids increased, the F_v/F_m decreased, and the NPQ increased. Whether phenolic acids cause photoinhibition of poplar needs further study.

When plants are subjected to severe phenolic acid stress, the heat dissipation pathway cannot completely consume all of the excess light energy. Therefore, the excess light energy can easily induce the accumulation of singlet oxygen and reactive oxygen species, reduce the electron transfer efficiency, hinder both the reduction of NADP and the synthesis of ATP, and further reduce the rate of carbon assimilation, affecting the accumulation of assimilates in poplar. In the present study, with the increase in phenolic acid concentration, the F_m, F_v/F_m, Φ_{PSII}, and q_P decreased to different extents; the electron transfer of the PSII reaction center was inhibited and the photochemical efficiency decreased. This led to a decrease in the productivity of poplar. As the concentration of phenolic acids increased, the F_m, F_v/F_m, Φ_{PSII}, and q_P decreased, and the F_0 and NPQ increased (Table 3). Li *et al.* (2016) also studied the effects of phenolic acids in wastewater on the photosynthesis-related physiological parameters of weeping willow leaves and found that phenolic acids stress can reduce the F_v/F_m and Φ_{PSII}. Overall, this indicated that the ChlF parameters of plants change accordingly when they are subjected to unfavorable stress conditions.

Conclusion: With an increase in phenolic acid concentration, the productivity of poplar decreased significantly. At low phenolic acid concentrations (0*X*–1.5*X*), the main reason for the decrease in photosynthesis of poplar were stomatal factors. However, at high concentrations of phenolic acids (1.5*X*–3.0*X*), nonstomatal factors were the main reason for the decrease in the photosynthesis of poplar.

Low phenolic acid concentrations can temporarily inactivate the PSII reaction center of poplar leaves, while high phenolic acid concentrations can damage the PSII reaction center and cause photoinhibition of poplar. Under phenolic acids stress, the electron transfer of PSII and photochemical efficiency were blocked, plants can keep their normal growth and development by promoting heat dissipation through xanthophyll cycle.

References

Afas N.A., Marron N., Van Dongen S. *et al.*: Dynamics of biomass production in a poplar coppice culture over three rotations (11 years). – Forest Ecol. Manag. **255**: 1883-1891, 2008.

An Y., Shen Y.B., Wu L.J., Zhang Z.X.: A change of phenolic acids content in poplar leaves induced by methyl salicylate and methyl jasmonate. – J. Forestry Res. **17**: 107-110, 2006.

Bernacchi C.J., Pimentel C., Long S.P.: *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. – Plant Cell Environ. **26**: 1419-1430, 2003.

Castaldi S., Carfora A., Fiorentino A. *et al.*: Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by *Arbutus unedo*. – Plant Soil **315**: 273-283, 2009.

Castells E., Peñuelas J., Valentine D.W.: Influence of the phenolic compound bearing species *Ledum palustre* on soil N cycling in a boreal hardwood forest. – Plant Soil **251**: 155-166, 2003.

Dąbrowski P., Baczewska A.H., Pawluśkiewicz B. *et al.*: Prompt chlorophyll *a* fluorescence as a rapid tool for diagnostic changes in PSII structure inhibited by salt stress in Perennial ryegrass. – J. Photoch. Photobio. B **157**: 22-31, 2016.

Dąbrowski P., Baczewska-Dąbrowska A.H., Kalaji H.M. *et al.*: Exploration of chlorophyll *a* fluorescence and plant gas exchange parameters as indicators of drought tolerance in perennial ryegrass. – Sensors-Basel **19**: 2736, 2019.

Dąbrowski P., Pawluśkiewicz B., Baczewska A.H. *et al.*: Chlorophyll *a* fluorescence of perennial ryegrass (*Lolium perenne* L.) varieties under long term exposure to shade. – Zemdirbyste **102**: 305-312, 2015.

Farquhar G.D., Sharkey T.D.: Stomatal conductance and photosynthesis. – Ann. Rev. Plant Physio. **33**: 317-345, 1982.

Fu Y.H., Quan W.X., Li C.C. *et al.*: Allelopathic effects of phenolic acids on seedling growth and photosynthesis in *Rhododendron delavayi* Franch. – Photosynthetica **57**: 377-387, 2019.

Griu T., Lunguleasa A.: The use of the white poplar (*Populus alba* L.) biomass as fuel. – J. Forestry Res. **27**: 719-725, 2016.

Heilman P.E.: Planted forests: poplars. – New Forest. **17**: 89-93, 1999.

Kang M., Zhang Z., Noormets A. *et al.*: Responses of energy partitioning and surface resistance to drought in a poplar plantation in northern China. – Biogeosciences Discuss. **12**: 345-388, 2015.

Kefeli V.I., Kalevitch M.V., Borsari B.: Phenolic cycle in plants and environment. – J. Cell Mol. Biol. **2**: 13-18, 2003.

Li H., Xie H.C., Du Z.L. *et al.*: The effects of phenolic acid on nitrogen metabolism in *Populus* × *euramericana* 'Neva'. – J. Forestry Res. **29**: 925-931, 2018.

Li H., Zhang G.C., Xie H.C. *et al.*: [The effect of phenol concentration on photosynthetic physiological parameters of *Salix babylonica*.] – Chin. Bull. Bot. **51**: 31-39, 2016. [In Chinese] doi: 10.11983/CBB15028.

Liu S., Qin F., Yu S.: *Eucalyptus urophylla* root-associated fungi can counteract the negative influence of phenolic acid allelochemicals. – Appl. Soil Ecol. **127**: 1-7, 2018.

Liu X.M., Ma F.Y., Zhu H. *et al.*: Effects of magnetized water treatment on growth characteristics and ion absorption, transportation and distribution in *Populus* × *euramericana* 'Neva' under NaCl stress. – Can. J. Forest Res. **47**: 828-838, 2017.

Mersie W., Singh M.: Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. – J. Chem. Ecol. **19**: 1293-1301, 1993.

Nijs I., Ferris R., Blum H. *et al.*: Stomatal regulation in a changing climate: A field study using free air temperature increase (FATI) and free air CO_2 enrichment (FACE). – Plant Cell Environ. **20**: 1041-1050, 1997.

Vaughan D., Ord B.: Influence of phenolic acids on morphological changes in roots of *Pisum sativum*. – J. Sci. Food Agr. **52**: 289-299, 1990.

Wang J.Y., Xin L.J.: Effects of poplar plantations on the physical and chemical properties of soils: a case study in the North China Plain. – J. Res. Ecol. **7**: 352-359, 2016.

Wang X.Q., Du G.D., Lu X.F. *et al.*: Characteristics of mitochondrial membrane functions and antioxidant enzyme activities in strawberry roots under exogenous phenolic acid stress. – Sci. Hortic.-Amsterdam **248**: 89-97, 2019.

Wang Y.P., Li C.R., Wang Q.K. *et al.*: Environmental behaviors of phenolic acids dominated their rhizodeposition in boreal poplar plantation forest soils. – J. Soils Sediments **16**: 1858-1870, 2016.

Wang Y.P., Wang H.T., Xu T. *et al.*: [Effects of exogenous phenolic acid on soil nutrient availability and enzyme activities in a poplar plantation.] – Chin. J. Appl. Ecol. **24**: 667-674, 2013. [In Chinese] doi: 10.13287/j.1001-9332.2013.0209.

Weidenhamer J.D., Romeo J.T.: Allelochemicals of *Polygonella myriophylla*: chemistry and soil degradation. – J. Chem. Ecol. **30**: 1067-1082, 2004.

Xia J.B., Zhang G.C., Zhang S.Y. *et al.*: Photosynthetic and water use characteristics in three natural secondary shrubs on Shell Islands, Shandong, China. – Plant Biosyst. **148**: 109-117, 2014.

Xie D.F., Zhang G.C., Xia X.X. *et al.*: The effects of phenolic acids on the photosynthetic characteristics and growth of *Populus* × *euramericana* cv. 'Neva' seedlings. – Photosynthetica **56**: 981-988, 2018.

Yao J., Sun D., Cen H. *et al.*: Phenotyping of *Arabidopsis* drought stress response using kinetic chlorophyll fluorescence and multicolor fluorescence imaging. – Front. Plant Sci. **9**: 603, 2018.

Yasumoto K., Sakata T., Yasumoto J. *et al.*: Atmospheric CO_2 captured by biogenic polyamines is transferred as a possible substrate to Rubisco for the carboxylation reaction. – Sci. Rep.-UK **8**: 17724, 2018.

Zhang X.H., Lang D.Y., Zhang E.H. *et al.*: Diurnal changes in photosynthesis and antioxidants of *Angelica sinensis* as influenced by cropping systems. – Photosynthetica **51**: 252-258, 2013.

Zhu T.T., Li J., Liu Y.Y. *et al.*: Leaf photosynthetic light response of summer maize: comparison of models and analysis of parameters. – Photosynthetica **58**: 19-28, 2020.

Zhu W.R., Wang Q.T., Liu M.L. *et al.*: [The difference in fine root lifespan between successive rotations of poplar plantation and the dominant causal factors.] – Acta. Ecol. Sin. **38**: 226-235, 2018. [In Chinese] doi: 10.5846/stxb201612202624.