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Photosynthetic performance of walnut leaves during the occurrence of leaf scorch

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Abstract

To clarify the effect of leaf scorch on walnut leaf photosynthesis, photosynthetic parameters were measured in *Juglans regia* 'Wen185' and 'Xinxin2' symptom trees (WS, XS) and symptomless trees (WH, XH). At the early stage of infection and under the low-grade leaf scorch, WS showed significantly lower net photosynthetic rate (P_N), stomatal limitation (L_s), and higher intercellular CO_2 concentration (C_i) than those of WH. However, at the mid to late stage of infection and under the high-grade leaf scorch, WS showed significantly lower P_N, C_i, the maximal quantum yield of PSII photochemistry and higher L_s, minimal fluorescence yield of the dark-adapted state, nonphotochemical quenching than those of WH, which would occur once *Juglans regia* 'Xinxin2' was infected. The above results indicated the effect of leaf scorch on walnut leaf photosynthesis was related to the walnut varieties and the duration and severity of the disease. Under the influence of leaf scorch, the decline in photosynthesis of *Juglans regia* 'Wen185' leaves changed from stomatal to nonstomatal restriction, while *Juglans regia* 'Xinxin2' leaves showed always nonstomatal restriction.

Keywords: chlorophyll fluorescence; daily variation; gas exchange; seasonal changes; stomatal regulation.

Introduction

A leaf scorch disorder of unknown etiology in walnut (*Juglans regia*) has been reported since 2008 in Xinjiang, China (Xu 2017). Currently, walnut leaf scorch occurs in a large area in the southern Xinjiang basin, and no effective control measures have been found. Almost all scholars believe walnut leaf scorch is a physiological disease (Zhang *et al*. 2012, Liang *et al*. 2014, Li *et al*. 2022). Through Koch's rule and molecular detection, we preliminarily determined that the walnut leaf scorch was caused by *Xylella fastidiosa*.

Highlights

- The leaf scorch reduced the photosynthetic capacity of *Juglans regia*
- There are differences in photosynthetic restriction pathways between the two *J. regia* varieties
- *J. regia* 'Xinxin 2' is more affected by leaf scorch than *J. regia* 'Wen185'

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Abbreviations: C_i – intercellular CO_2 concentration; F_0 – minimal fluorescence yield of the dark-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_0 – maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; Φ_{PSII} – actual photochemical efficiency of PSII.

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As the main organ of plant photosynthesis, the quality of leaves determines the strength of photosynthetic ability. Stable photosynthesis is considered necessary for healthy plant growth and development (Muhammad *et al.* 2021). There were different opinions on how the photosynthesis of plant leaves changed after suffering from a disease. Most studies showed that the disease would lead to the reduction of plant photosynthesis, such as in zucchini (*Cucurbita pepo*) infected with silver leaf disease (Hao *et al.* 2010), sweet potato (*Ipomoea batatas*) infected with a scab (Stone *et al.* 2007), grape (*Vitis vinifera*) infected with downy mildew (Moriondo *et al.* 2005), and rice (*Oryza sativa*) infected with flax leaf spot (Dallagnol *et al.* 2011). On the contrary, it was also reported that plant photosynthesis increased after disease stress, such as in soybean (*Glycine max*) infected with the soybean mosaic virus Sg strain (Lu and Chen 1992). On the other hand, other studies had shown that there was no significant change in plant photosynthesis after disease stress, such as in eucalyptus (*Eucalyptus robusta*) infected with rust (Alves *et al.* 2011). These contradictory results forced researchers to conduct in-depth research on stomatal conductance and photosynthetic system and found that the relationship between disease and plant photosynthesis was affected by many factors.

The most important factor was the plant species and disease type. Many pathogenic bacteria could forcibly open the stomata of plants to facilitate their entry and exit into cells. The toxin of *Pseudomonas syringae* could promote stomatal opening through interaction with the COI1 factor (Melotto *et al.* 2006). However, more studies showed that the infection of pathogenic fungi significantly inhibited the opening of stomata of host plants, resulting in the decrease of stomatal conductance of pine (*Pinus*) and Alaska shrubs (Rohrs-Richey *et al.* 2011, Hubbard *et al.* 2013, Aguadé *et al.* 2015). After eucalyptus (*Eucalyptus robusta*) was infected with rust (Alves *et al.* 2011), sugarcane (*Saccharum officinarum*) was infected with citrus rust (Zhao *et al.* 2011), and pea (*Pisum sativum*) was infected with pea mosaic virus (Kyseláková *et al.* 2011), the stomatal conductance of leaves decreased in a short time. At present, there was a great controversy about the maximal quantum yield of PSII photochemistry (F_v/F_m) by diseases. Many studies showed that after the plants were infected, the F_v/F_m decreased, such as in summer orange (*Citrus* cv. Olinda) infected with green spot algae (Wang *et al.* 2006), douglas pine (*Pinus douglasiana*) infected with fungi (Manter 2002), loquat (*Eriobotrya japonica*) infected with anthracnose (Zheng 2001), chestnut (*Castanea mollissima*) infected with *Phytophthora*, and zucchini (*Cucurbita pepo*) infected with silverleaf disease (Hao *et al.* 2009, Dinis *et al.* 2011). It had also been reported that the F_v/F_m did not change after the infection of diseases, such as in tobacco (*Nicotiana tabacum*) infected with tobacco mosaic virus (Dinis *et al.* 2011) and eucalyptus (*Eucalyptus robusta*) infected with rust (Alves *et al.* 2011).

There were few studies on the transmission and utilization of photoelectrons, maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII (F_v/F_0), and actual photochemical efficiency of PSII (Φ_{PSII}) of plant leaves after disease infection. Even though most studies showed that these parameters had a downward trend, some studies still showed that the duration and severity of the disease affected the relationship between disease and plant photosynthesis. At the early stage of grape (*Vitis vinifera*) powdery mildew infection, the leaf F_v/F_m increased significantly, and the increase of F_v/F_m gradually decreased over time (Moriondo *et al.* 2005). After 5% of summer orange (*Citrus* cv. Olinda) was infected by green spot algae, Φ_{PSII} showed no obvious change but increasing the infection degree, Φ_{PSII} significantly decreased (Wang *et al.* 2006). Moreover, the leaves at different development stages had different responses to the disease. After being infected with *Cacoecimorpha pronubana*, the F_v/F_0 values of mature leaves and young leaves of sugar beet (*Beta vulgaris*) were significantly reduced, however, the F_v/F_0 of old leaves showed an increasing trend (Retuerto *et al.* 2006).

To summarize, the effect of disease on plant photosynthesis involved the species and leaf age of plant varieties, and the type, duration, and intensity of the disease, but the research on each category of the system was relatively lacking. At present, walnut leaf scorch has occurred in a large area in Xinjiang, China, and no one studied the harm of walnut leaf scorch from the perspective of photosynthetic physiology. Because of this, this study took two main walnut varieties in the southern Xinjiang Basin as research materials to systematically explore the photosynthesis performance of walnut after the leaf scorch occurrence to clarify the relationship between leaf scorch and walnut photosynthesis, and enrich the theoretical system of the effect of disease on plant photosynthesis.

Materials and methods

Experimental site and plant materials: This study was conducted in three walnut production orchards in 2021, in southwest Xinjiang, China (78°23'12"–80°01'30"E, 40°43'46"–41°51'05"N), with an average altitude of approximately 1,200–2,000 m. It is arid and rainless and has a warm temperate continental climate. The annual average temperature, precipitation, and evaporation are 10.4°C, 91.5 mm, and 2,003.8 mm, respectively. Annual sunshine hours are 2,679–2,750 h, and the frost-free period is 211–250 d. The management conditions of the three orchards were consistent. The main walnut variety was *Juglans regia* 'Wen185', and the pollination variety was *Juglans regia* 'Xinxin2'. They were planted in an east-west row, with spacings of 5 × 6 m.

We selected six *Juglans regia* 'Wen185' trees and six *Juglans regia* 'Xinxin2' trees with an age of ten years, plant height of 6–6.5 m, and ground diameter of about 25 cm. These trees were divided into four groups with three plants in each group: *Juglans regia* 'Wen185' without leaf scorch symptoms was named as WH group, *Juglans regia* 'Wen185' with leaf scorch symptoms was named as WS group, *Juglans regia* 'Xinxin2' without leaf scorch symptoms was named as XH group, and *Juglans regia* ‘Xinxin2’ with leaf scorch symptoms was named as XS group.

Assessment of walnut leaf scorch symptoms: To provide a basis for studying the effects of different grades of leaf scorch on the photosynthesis of walnut leaves, we established a grading standard of walnut leaf scorch based on previous studies and the symptoms of walnut leaf scorch. Walnut leaves with leaf scorch symptoms were divided into five grades.

Leaf scorch grade	Representative value	Grading standards
Grade I	b0	The scorched area accounted for 0% of the total leaf area
Grade II	b1	The scorched area accounted for 1~25% of the total leaf area
Grade III	b2	The scorched area accounted for 26~50% of the total leaf area
Grade IV	b3	The scorched area accounted for 51~75% of the total leaf area
Grade V	b4	The scorched area accounted for 76~100% of the total leaf area

Determination of photosynthetic parameters: Seasonal variations of photosynthetic parameters (leaf gas-exchange parameters and chlorophyll fluorescence parameters) of walnut leaves in four groups were collected at approximately 15-d intervals (13 June, 28 June, 15 July, 2 August, and 20 August) from 11:00–13:00 h. Diurnal variations of photosynthetic parameters were collected from 8:00–19:00 h, once every hour in late August, and, at the same time, the photosynthetic parameters of leaves b0, b1, b2, b3, and b4 in WS and XS groups were collected.

We chose sunny and windless days to collect photosynthetic parameters. Five mature leaves with the same height and leaf position outside the crown were measured for each plant, and the mean value of the measured values of five leaves was one repetition, and the three trees in each group were three repetitions.

Leaf gas-exchange parameters were determined by the non-vein and normal green portion of the leaf by using an *LI-6400 XT* portable photosynthesis system (*LI-COR*, Nebraska, USA). During measurement, the flow rate in the sample room was set to 500 μmol s^{-1}, and the CO_2 concentration in the sample room and the reference room was matched every 20 min. Chlorophyll (Chl) fluorescence parameters were determined by a modulated fluorometer (*FMS-2*, *Hansatech*, UK). After dark adaptation for 20 min, the minimal fluorescence yield of the dark-adapted state (F_0) was measured with a weak measuring light. Thereafter, a strong flash [6,000 μmol(photon) m^{-2} s^{-1}] was applied with a pulse time of 0.7 s, and the maximal fluorescence yield of the dark-adapted state (F_m) was measured. After 20 min of adaptation to natural light, the steady-state fluorescence yield (F_s) of leaves was measured. A strong flash was applied again [6,000 μmol(photon) m^{-2} s^{-1}], with a pulse time of 0.7 s, and the maximal fluorescence yield of the light-adapted state (F_m') was measured again. The variable fluorescence (F_v), F_v/F_m, F_v/F_0, and nonphotochemical quenching (NPQ) of PSII were calculated according to the method of Demmig-Adams *et al.* (1996): $F_v = F_m - F_0$, $F_v/F_m = (F_m - F_0)/F_m$, $NPQ = (F_m - F_m')/F_m'$.

Statistical analysis: Two-way multivariate analysis of variance (*MANOVA*) was conducted to test whether time, groups, and their interactions had significant effects on photosynthetic parameters. One-way analysis of variance (*ANOVA*) was used to evaluate the difference in walnut leaf scorch incidence index at different time points, and to evaluate whether there are significant differences in photosynthetic parameters between leaves of the same variety with different grades. *Duncan*'s multiple comparisons were used in the backtesting, and it was significant at $\alpha = 0.05$ level. *SPSS 22.0* software was used for statistical analysis, Microsoft *Excel 2016* was used for data processing and calculation, and *Origin 9.8* software was used for drawing.

Results

Symptoms of walnut leaf scorch: The results of our previous field investigation showed that walnut leaf scorch appears in late May. At the initial stage, the leaflet margins of odd-pinnate compound leaves on individual branches of walnut trees first showed chlorosis, and the leaf apex emerged with brown spots and slightly shrunk (Fig. 1). Then the spots grew up, like scorched ones, and spread inward in a flame-like shape along the leaf margins. The scorched parts continued to curl inward. A yellow halo boundary was at the junction of the scorched and the healthy parts, however, the leaf veins remained green (Fig. 1). In mid-July, walnut leaf scorch entered a high incidence period, the scorched parts grew symmetrically or asymmetrically with the main leaf vein as the boundary,

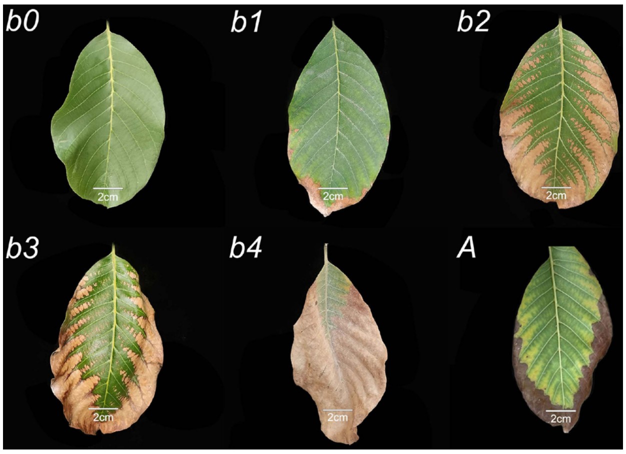


Fig. 1. The same leaf with different severity of leaf scorch symptoms. b0–b4 degrees of damage, corresponding to 0% (healthy leaves), 0–25%, 26–50%, 51–75%, 76–100% leaf area affected. *A* – abaxial surface of walnut leaves infected with leaf scorch disease.

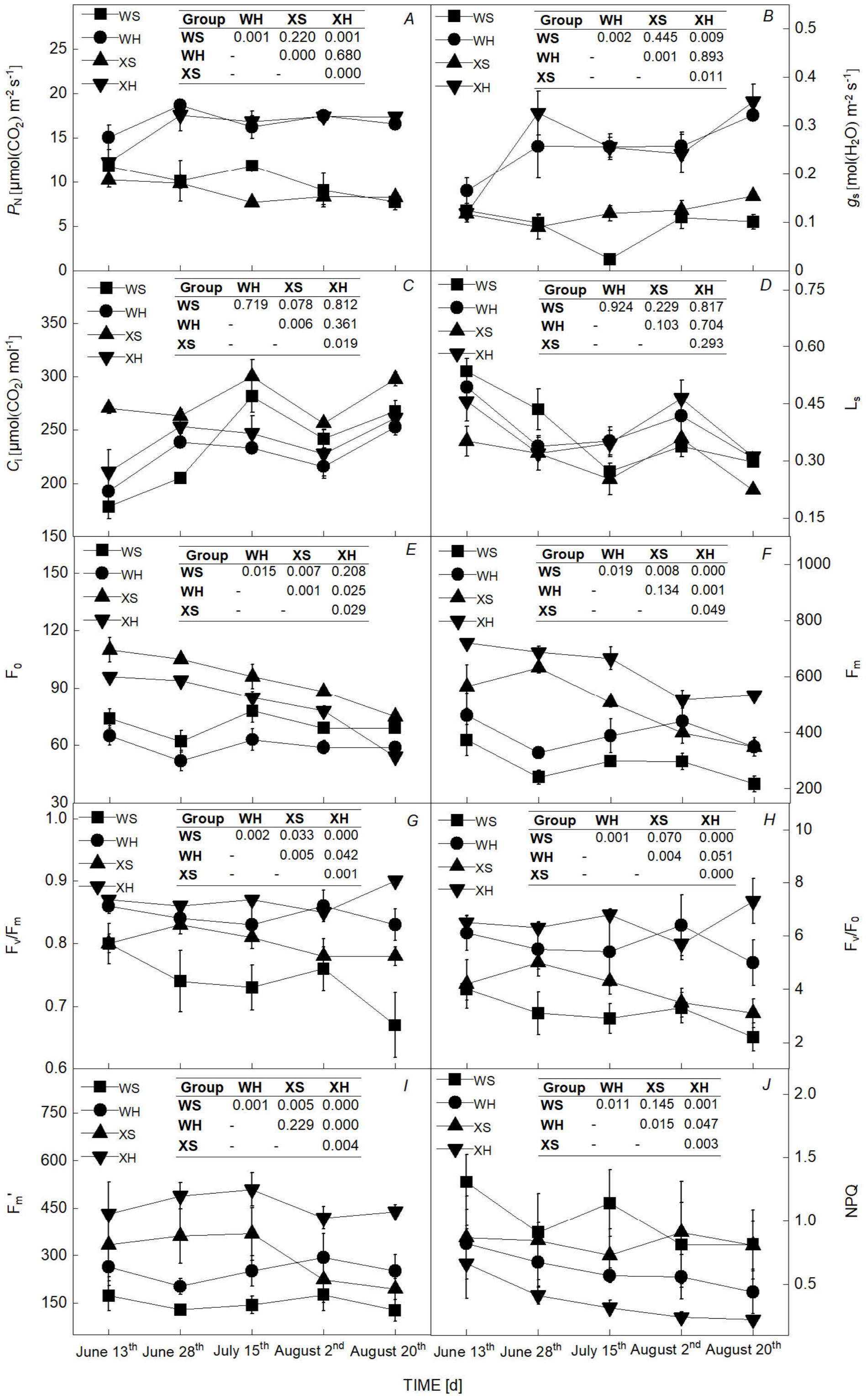
and the leaves became thicker (Fig. 1). When it was serious, the whole leaf turned dry and atrophic, even the leaves of the whole tree were scorched in the late August (Fig. 1). However, the new leaves showed no signs of scorching. No black powder, white powder, rust, mold, bacterial pus, or other symptoms could be found on the leaf surface (Fig. 1).

Effects of leaf scorch on the seasonal variation of photosynthetic parameters of walnut leaves: The result of *MANOVA* (Table 1) showed that the effects of group on the seasonal variation of photosynthetic parameters of walnut leaves were statistically significant, indicating that P_N, g_s, C_i, L_s, and F_0, F_m, F_v/F_m, F_v/F_0, F_m', and NPQ varied greatly between different groups. Thus, leaf scorch had a significant effect on the seasonal dynamic of photosynthetic parameters.

The seasonal variation of gas-exchange parameters (Fig. 2) showed that WH and XH remained significantly higher P_N [> 12 μmol(CO_2) m^{-2} s^{-1}] and g_s [0.15–0.35 mol(H_2O) m^{-2} s^{-1}] than those for WS and XS [P_N < 12 μmol(CO_2) m^{-2} s^{-1}], g_s [0.02–0.15 mol(H_2O) m^{-2} s^{-1}]. On 20 August, the P_N of WS and XS was 53.4 and 52.4% lower than those of WH and XH, respectively. However, the C_i of WH and XH was significantly lower than those of WS and XS, except for the C_i of WH that was significantly higher than that of WS in June. Although there was no significant difference between the L_s of XS and XH in June, the L_s of XS in August was significantly lower than that of XH. Contrarily, the L_s of WS was

Table 1. Two-factor multi-response variable analysis of variance for the effect of time and different groups on the seasonal variation of leaf photosynthetic parameters. C_i – intercellular CO_2 concentration; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_v/F_0 – maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; MS – mean square; P_N – net photosynthetic rate; SS – sum of squares of deviations.

Response variable	Source of variation	df	SS	MS	*F* value	*P* value
P_N	time (T)	4	23.617	5.904	3.702	0.000
	group (G)	3	666.385	222.128	139.288	0.000
	T × G	12	123.592	10.299	6.458	0.000
g_s	T	4	0.065	0.016	20.301	0.000
	G	3	0.337	0.112	141.076	0.000
	T × G	12	0.096	0.008	10.074	0.000
C_i	T	4	25,828.408	6,457.102	49.567	0.000
	G	3	22,777.667	7,592.556	58.283	0.000
	T × G	12	12,337.515	1,028.126	7.892	0.000
L_s	T	4	0.236	0.059	57.517	0.000
	G	3	0.068	0.023	22.271	0.000
	T × G	12	0.083	0.007	6.709	0.000
F_0	T	4	3,404.433	851.108	34.319	0.000
	G	3	10,808.983	3,602.994	145.282	0.000
	T × G	12	3,616.767	301.397	12.153	0.000
F_m	T	4	185,103.067	46,275.767	15.081	0.000
	G	3	913,765.650	304,588.550	99.266	0.000
	T × G	12	101,403.600	8,450.300	2.754	0.008
F_v/F_m	T	4	0.007	0.002	1.590	0.196
	G	3	0.147	0.049	42.815	0.000
	T × G	12	0.026	0.002	1.878	0.068
F_v/F_0	T	4	4.151	12.922	1.189	0.330
	G	3	110.478	237.182	42.207	0.000
	T × G	12	12.668	0.584	1.210	0.310
F_m'	T	4	29,209.767	7,302.442	1.366	0.263
	G	3	739,477.917	246,492.639	46.103	0.000
	T × G	12	75,501.167	6,291.764	1.177	0.332
NPQ	T	4	0.893	0.223	3.134	0.025
	G	3	3.155	1.052	14.766	0.000
	T × G	12	0.750	0.062	0.877	0.576



Group	WH	XS	XH
WS	0.001	0.220	0.001
WH	-	0.000	0.680
XS	-	-	0.000

Group	WH	XS	XH
WS	0.002	0.445	0.009
WH	-	0.001	0.893
XS	-	-	0.011

Group	WH	XS	XH
WS	0.719	0.078	0.812
WH	-	0.006	0.361
XS	-	-	0.019

Group	WH	XS	XH
WS	0.924	0.229	0.817
WH	-	0.103	0.704
XS	-	-	0.293

Group	WH	XS	XH
WS	0.015	0.007	0.208
WH	-	0.001	0.025
XS	-	-	0.029

Group	WH	XS	XH
WS	0.019	0.008	0.000
WH	-	0.134	0.001
XS	-	-	0.049

Group	WH	XS	XH
WS	0.002	0.033	0.000
WH	-	0.005	0.042
XS	-	-	0.001

Group	WH	XS	XH
WS	0.001	0.070	0.000
WH	-	0.004	0.051
XS	-	-	0.000

Group	WH	XS	XH
WS	0.001	0.005	0.000
WH	-	0.229	0.000
XS	-	-	0.004

Group	WH	XS	XH
WS	0.011	0.145	0.001
WH	-	0.015	0.047
XS	-	-	0.003

Fig. 2. Seasonal variation of gas-exchange parameters and chlorophyll fluorescence parameters of walnut leaves in different groups: net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO$_2$ concentration (C_i) (*C*), stomatal limitation (L_s) (*D*), minimal fluorescence yield of the dark-adapted state (F_0) (*E*), maximal fluorescence yield of the dark-adapted state (F_m) (*F*), maximal quantum yield of PSII photochemistry (F_v/F_m) (*G*), maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII (F_v/F_0) (*H*), maximal fluorescence yield of the light-adapted state (F_m') (*I*), nonphotochemical quenching (NPQ) (*J*). The table inserted in the figure showed the *T*-test results of the mean value of the seasonal variation of each parameter, it was significant at α=0.05 level. Data are means ± SE (n = 3).

significantly higher than that of WH in June, and it was significantly lower than WH from the middle of July to late August.

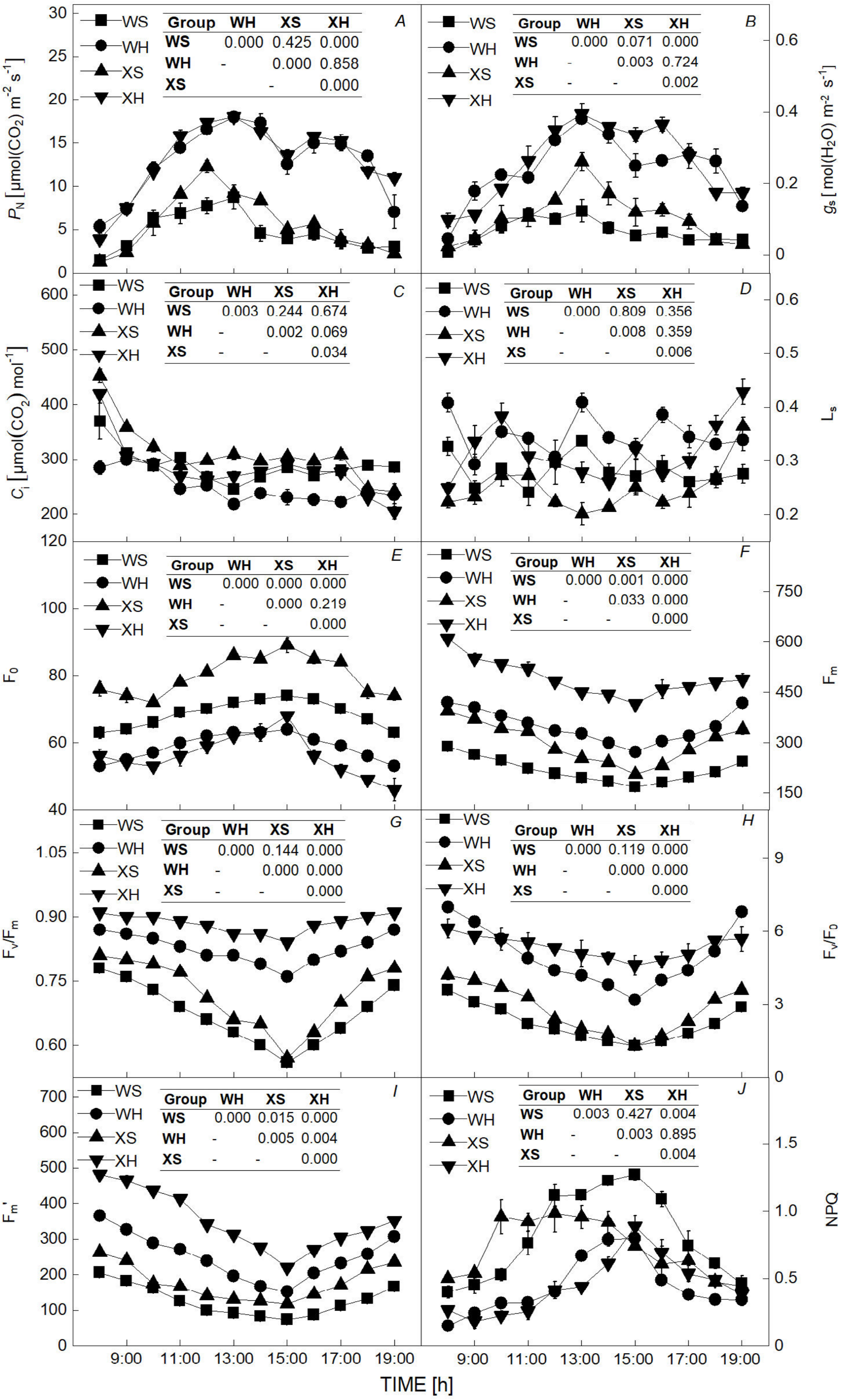
The seasonal variation of Chl fluorescence parameters (Fig. 2) showed that WH and XH had significantly higher F_m, F_v/F_m, F_v/F_0, F_m', and lower F_0 and NPQ than those of WS and XS. Compared to WH, the F_m and F_m' of WS decreased by an average of 27.9 and 40.6%, respectively. And the mean values of F_m and F_m' of XS were significantly lower than those of XH by 22.2 and 35.8%, respectively. WH and XH had significantly higher F_v/F_m (> 0.85) and F_v/F_0 (> 5.00) than those of WS and XS ($F_v/F_m < 0.85$, $F_v/F_0 < 5.00$). However, the mean value of F_0 of WH and XH was significantly lower than that of WS and XS by 18.1 and 18.2%, respectively. The mean value of NPQ of WS and XS (Fig. 2*J*) was significantly higher than that of WH and XH.

Effect of leaf scorch on diurnal variation of photosynthetic parameters in walnut leaves: The result of *MANOVA* (Table 2) showed that the effects of the group on the diurnal variation of photosynthetic parameters of walnut leaves were statistically significant, indicating that P_N, g_s, C_i, L_s, and F_0, F_m, F_v/F_m, F_v/F_0, F_m', and NPQ varied greatly between different groups. So, leaf scorch had a significant effect on the diurnal variation of photosynthetic parameters.

The diurnal variation of gas-exchange parameters (Fig. 3) showed that WH and XH had significantly higher P_N [3–18 µmol(CO$_2$) m^{-2} s^{-1}] and g_s [0.15–0.40 mol(H$_2$O)

Table 2. Two-factor multi-response variable analysis of variance for the effect of time and different groups on daily variation of leaf photosynthetic parameters. C_i – intercellular CO$_2$ concentration; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_v/F_0 – maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; MS – mean square; P_N – net photosynthetic rate; SS – sum of squares of deviations.

Response variable	Source of variation	df	SS	MS	*F* value	*P* value
P_N	time (T)	11	1,323.978	120.362	151.926	0.000
	Group (G)	3	2,284.419	761.473	961.165	0.000
	T × G	33	268.640	8.141	10.275	0.000
g_s	T	11	0.624	0.057	87.431	0.000
	G	3	0.982	0.327	504.779	0.000
	T × G	33	0.194	0.006	9.068	0.000
C_i	T	11	70,540.196	6,412.745	45.606	0.000
	G	3	91,611.444	30,537.148	217.172	0.000
	T × G	33	174,711.733	5,294.295	37.652	0.000
L_s	T	11	0.127	0.012	27.635	0.000
	G	3	0.246	0.082	196.979	0.000
	T × G	33	0.316	0.010	23.005	0.000
F_0	T	11	2,844.576	258.598	88.033	0.000
	G	3	12,604.243	4,201.414	1,430.269	0.000
	T × G	33	612.007	18.546	6.313	0.000
F_m	T	11	307,532.472	27,957.497	224.033	0.000
	G	3	1,428,206.139	476,068.713	3,814.908	0.000
	T × G	33	25,622.028	776.425	6.222	0.000
F_v/F_m	T	11	0.338	0.031	567.035	0.000
	G	3	1.007	0.336	6,194.957	0.000
	T × G	33	0.083	0.003	46.342	0.000
F_v/F_0	T	11	142.141	12.922	296.298	0.000
	G	3	711.547	237.182	5,438.575	0.000
	T × G	33	19.263	0.584	13.385	0.000
F_m'	T	11	437,858.472	39,805.316	788.332	0.000
	G	3	1,013,770.806	337,923.602	6,692.477	0.000
	T × G	33	69,702.028	2,112.183	41.831	0.000
NPQ	T	11	5.410	0.492	86.919	0.000
	G	3	3.966	1.322	233.636	0.000
	T × G	33	2.556	0.077	13.688	0.000



Group	WH	XS	XH
WS	0.000	0.425	0.000
WH	-	0.000	0.858
XS		-	0.000

Group	WH	XS	XH
WS	0.000	0.071	0.000
WH	-	0.003	0.724
XS	-	-	0.002

Group	WH	XS	XH
WS	0.003	0.244	0.674
WH	-	0.002	0.069
XS	-	-	0.034

Group	WH	XS	XH
WS	0.000	0.809	0.356
WH	-	0.008	0.359
XS	-	-	0.006

Group	WH	XS	XH
WS	0.000	0.000	0.000
WH	-	0.000	0.219
XS	-	-	0.000

Group	WH	XS	XH
WS	0.000	0.001	0.000
WH	-	0.033	0.000
XS	-	-	0.000

Group	WH	XS	XH
WS	0.000	0.144	0.000
WH	-	0.000	0.000
XS	-	-	0.000

Group	WH	XS	XH
WS	0.000	0.119	0.000
WH	-	0.000	0.000
XS	-	-	0.000

Group	WH	XS	XH
WS	0.000	0.015	0.000
WH	-	0.005	0.004
XS	-	-	0.000

Group	WH	XS	XH
WS	0.003	0.427	0.004
WH	-	0.003	0.895
XS	-	-	0.004

Fig. 3. Effect of leaf scorch on diurnal variation of gas-exchange parameters and chlorophyll fluorescence parameters in walnut leaves: net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO$_2$ concentration (C_i) (*C*), stomatal limitation (L_s) (*D*), minimal fluorescence yield of the dark-adapted state (F_0) (*E*), maximal fluorescence yield of the dark-adapted state (F_m) (*F*), maximal quantum yield of PSII photochemistry (F_v/F_m) (*G*), maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII (F_v/F_0) (*H*), maximal fluorescence yield of the light-adapted state (F_m') (*I*), nonphotochemical quenching (NPQ) (*J*). The table inserted in the figure showed the *T*-test results of the average value of the diurnal variation of each parameter, it was significant at α=0.05 level. Data are means ± SE (n = 3).

m^{-2} s^{-1}] than those for WS and XS [P_N: 0–12 μmol(CO_2) m^{-2} s^{-1}; g_s: 0.01–0.25 mol(H_2O) m^{-2} s^{-1}]. The diurnal variation of P_N of WH and XH were both bimodal curves, with an obvious phenomenon of 'midday depression of photosynthesis'. The L_s of WH and XH were significantly higher than that of WS and XS. However, the C_i of WH and XH was significantly lower than those of WS and XS.

The diurnal variation of Chl fluorescence parameters (Fig. 3) showed that leaf scorch caused a decrease in F_m, F_m', F_v/F_0, and F_v/F_m and an increase in F_0 and NPQ in leaves. Compared to WH, the F_m and F_m' of WS decreased by an average of 37.7 and 49.4%, respectively. The mean values of F_m and F_m' of XS were significantly lower than those of XH by 39.2 and 49.4%, respectively. Compared to those of WH, the F_v/F_0 and F_v/F_m values of WS were significantly reduced by 61.2 and 21.1%, respectively, and the mean values of F_m and F_m' of XS were significantly lower than those of XH by 75.3 and 32.1%, respectively. However, the mean value of F_0 of WH and XH was significantly lower than that of WS and XS by 14.5 and 30.0%, respectively. The mean value of NPQ of WS and XS (Fig. 3*J*) was significantly higher than that of WH and XH.

Effects of leaf scorch on photosynthetic parameters of walnut leaves with different leaf scorch grades: With the increased leaf scorch grades (Fig. 4), the P_N, g_s, and L_s significantly decreased, and the C_i significantly increased. From b0 to b4, the P_N decreased from 12 (b0) to 0 μmol(CO_2) m^{-2} s^{-1} (b4). At the same grade, the P_N and L_s of *Juglans regia* 'Wen185' were always higher than those of *Juglans regia* 'Xinxin2', however, there was no significant difference between *Juglans regia* 'Wen185' and *Juglans regia* 'Xinxin2' in g_s. From b0 to b4, the C_i increased from 200 (b0) to 450 μmol(CO_2) mol^{-1} (b4), and the C_i of *Juglans regia* 'Wen185' was always lower than that of *Juglans regia* 'Xinxin2' at the same grade.

With the increased leaf scorch grades (Fig. 5), the F_m, F_m', F_v/F_0, and F_v/F_m of *Juglans regia* 'Wen185' and *Juglans regia* 'Xinxin2' significantly decreased, and the F_0 significantly increased. At the same leaf scorch grade, the F_0 of *Juglans regia* 'Xinxin2' was significantly higher than that of *Juglans regia* 'Wen185'. The NPQ of *Juglans regia* 'Xinxin2' significantly increased from b0 to b4, however, the NPQ of *Juglans regia* 'Wen185' first increased and then declined, and the NPQ of b0–b2 in *Juglans regia* 'Xinxin2' were significantly lower than those of *Juglans regia* 'Wen185', except for b3 and b4. There was no significant difference between b3 and b4 in F_m of *Juglans regia* 'Wen185', but they were significantly lower than those of b0–b3, and the same was true for F_m with different leaf scorch grades of *Juglans regia* 'Xinxin2'. At the same leaf scorch grade, the F_m of *Juglans regia* 'Xinxin2' was significantly higher than that of *Juglans regia* 'Wen185'. Although no significant difference was discovered between *Juglans regia* 'Wen185' and *Juglans regia* 'Xinxin2' under b0 and b1, the F_v/F_m and F_v/F_0 of *Juglans regia* 'Wen185' were significantly higher than

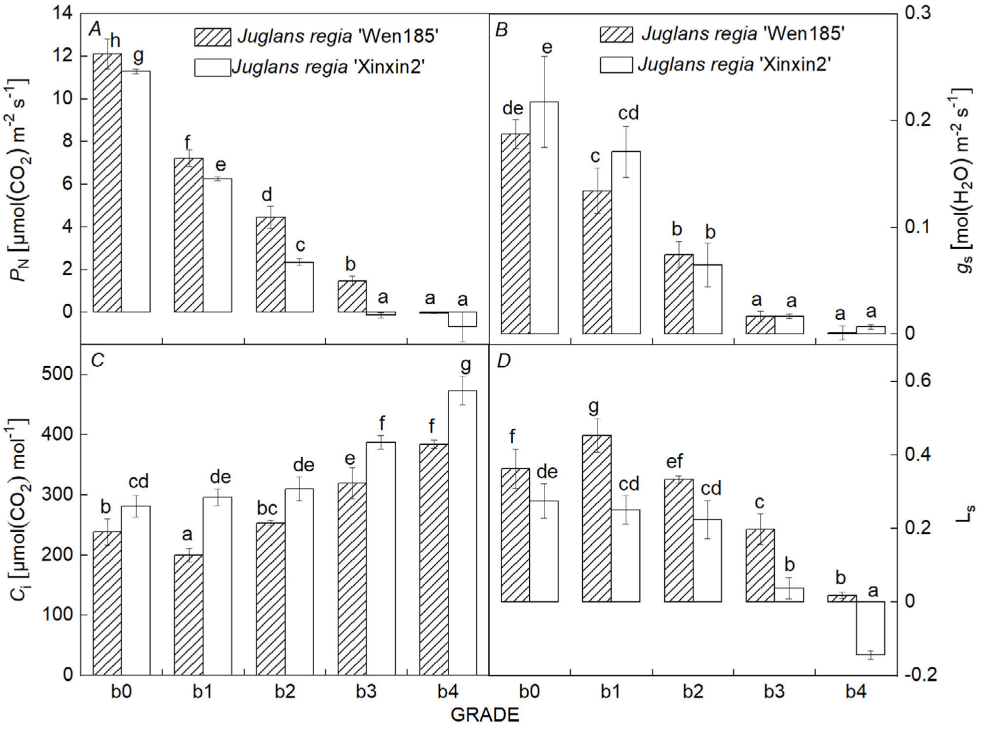


Fig. 4. Gas-exchange parameters of walnut leaves with different severity of leaf scorch: net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), stomatal limitation (L_s) (*D*). Data are means ± SE ($n = 3$). In the illustration, *different lowercase letters* represent significant differences between different treatments ($P<0.05$). *Duncan*'s multiple comparisons were used in the backtesting.

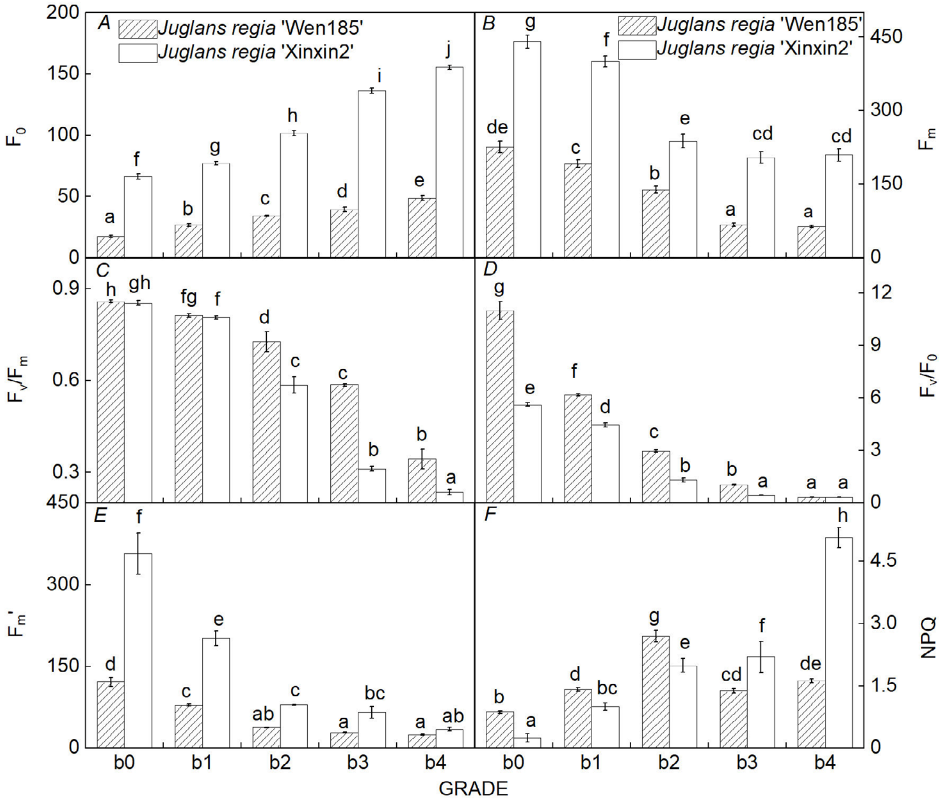


Fig. 5. Changes of chlorophyll fluorescence parameters in leaves with different severity of leaf scorch: minimal fluorescence yield of the dark-adapted state (F_0) (*A*), maximal fluorescence yield of the dark-adapted state (F_m) (*B*), maximal quantum yield of PSII photochemistry (F_v/F_m) (*C*), maximum ratio of quantum yields of photochemical and concurrent nonphotochemical processes in PSII (F_v/F_0) (*D*), maximal fluorescence yield of the light-adapted state (F_m') (*E*), nonphotochemical quenching (NPQ) (*F*). Data are means ± SE ($n = 3$). In the illustration, *different lowercase letters* represent significant differences between different treatments ($P<0.05$). *Duncan*'s multiple comparisons were used in the backtesting.

those of *Juglans regia* 'Xinxin2'. Contrarily, the F_m' of *Juglans regia* 'Wen185' was significantly lower than that of *Juglans regia* 'Xinxin2' at the same grade.

To summarize, compared with symptomless trees (Fig. 6*A*), the P_N and F_v/F_m of walnut trees with leaf scorch symptoms decreased significantly, and the P_N and F_v/F_m of symptom trees decreased to varying degrees with the duration of disease infection. With the increase in disease grade (Fig. 6*B*), leaf scorch led to a significant decrease in P_N and F_v/F_m of symptom trees. Under the influence of leaf scorch (Fig. 6*C*), P_N and F_v/F_m of the two varieties decreased, and P_N and F_v/F_m of leaves of *Juglans regia* 'Xinxin2' decreased more than that of leaves of *Juglans regia* 'Wen185'.

Discussion

Stomata are the channels through which CO_2 enters the mesophyll cells of plants. The opening and closing of stomata have a decisive effect on the photosynthetic efficiency of plants. If the decrease in photosynthesis is caused by the decrease of stomatal opening affecting the entry of CO_2 into mesophyll cells, this restriction is called the stomatal restriction of photosynthesis (Xu 1995). In addition, photosynthetic efficiency is also related to Rubisco activity, photochemical activity, and other factors. If the external environment affects the assimilation ability of mesophyll cells, photosynthesis is still weak even if the concentration of CO_2 in the cell gap is high. This restriction is called the nonstomatal restriction of photosynthesis (Farquhar and Sharkey 1982). Whether the main reason for the decrease of photosynthetic rate is stomatal limitation depends on the correlation between C_i and L_s. If C_i decreases while P_N decreases and L_s increases, it can be determined that the decrease of P_N at that time is caused by stomatal closure, which belongs to the limitation of stomatal factors; if at the same time as P_N decreases, L_s decreases and C_i increases, then the decrease of P_N is mainly caused by nonstomatal limitation factors (Gao *et al*. 2018).

In the seasonal variation process, *Juglans regia* 'Wen185' with leaf scorch symptoms showed significantly lower P_N, g_s, L_s, and higher C_i than those of healthy plants, except for their lower C_i and higher L_s before the middle of July. According to Farquhar and Sharkey (1982), only when C_i decreased and L_s increased, the reduction of photosynthetic rate could be caused by the reduction of stomatal conductance. Therefore, we inferred that, at the

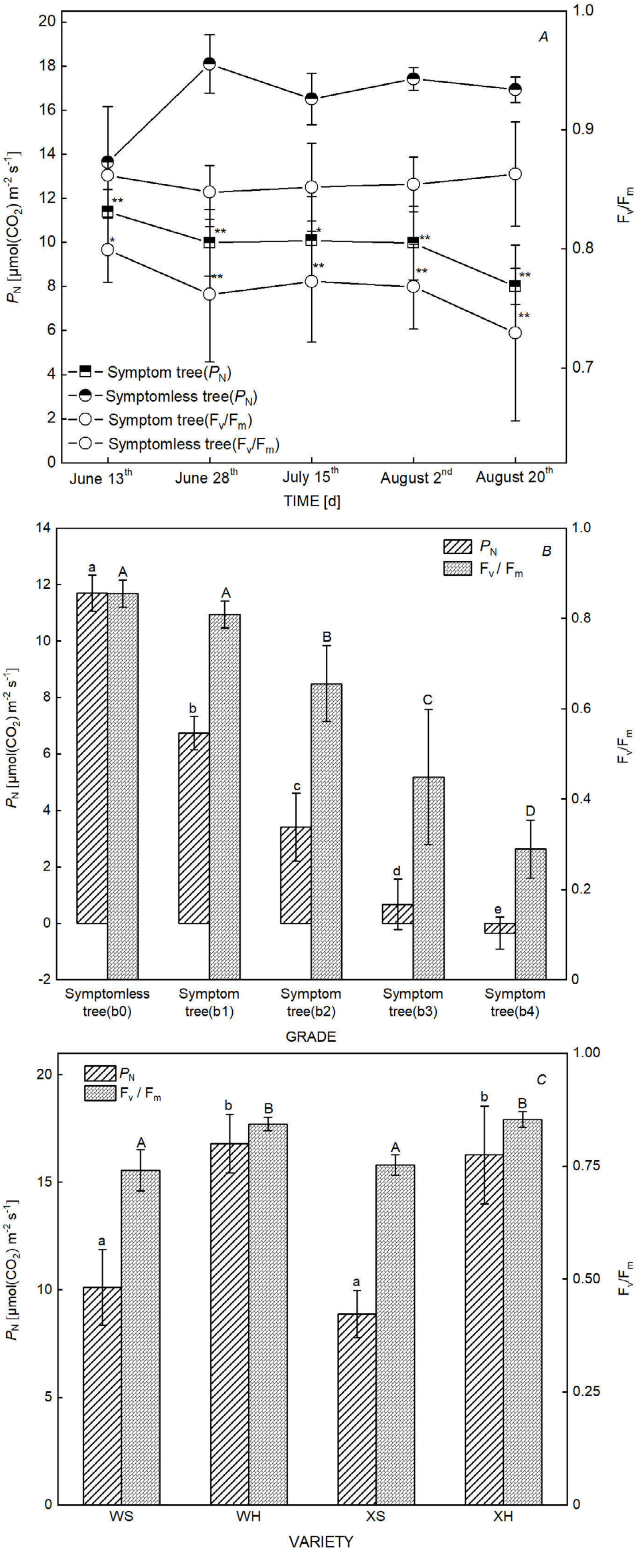


Fig. 6. Changes in the photosynthetic capacity of walnut leaves affected by leaf scorch infection time (*A*), leaf scorch infection grade (*B*), and different varieties (*C*). (*A*) * indicates that the difference between different treatments at the same time point and under the same index is significant ($P<0.05$, *T*-test), ** indicates that the difference between different treatments at the same time point and under the same index is extremely significant ($P<0.01$, *T*-test). (*B*) *Different lowercase letters* indicate that there is a significant difference in net photosynthetic rate (P_N) value between leaves with different leaf scorch severity ($P<0.05$), and *different uppercase letters* indicate that there is a significant difference in maximal quantum yield of PSII photochemistry (F_v/F_m) value between leaves with different leaf scorch severity ($P<0.05$). (*C*) *Different lowercase letters* indicate that there are significant differences in net photosynthetic rate (P_N) values between different groups ($P<0.05$), and *different uppercase letters* indicate that there are significant differences in PSII photochemical maximum quantum yield (F_v/F_m) values between different groups ($P<0.05$). *Duncan*'s multiple comparisons were used in the backtesting.

early stage of infection with leaf scorch, the reduction of photosynthesis belonged to stomatal limitation. After entering July, *Juglans regia* 'Wen185' with leaf scorch symptoms showed significantly higher F_0, NPQ and lower F_m, F_m', F_v/F_m, and F_v/F_0, which indicated that the light-harvesting pigment complex near the PSII reaction center was damaged, the electron transfer efficiency of susceptible plants was reduced, and the PSII reaction centers were destroyed. That was why, with the extension of leaf scorch duration, the photosynthetic mechanism of *Juglans regia* 'Wen185' with leaf scorch symptoms was seriously damaged, resulting in increased stomatal diffusion resistance of mesophyll cells and changes in CO_2-fixation enzyme activity, thereby inhibiting photosynthesis. At this time, the nonstomatal restriction has become the main reason for the decline in the photosynthetic rate (Hajiboland *et al.* 2017). It was also speculated that *Juglans regia* 'Wen185' had a certain self-regulation ability at the early stage of infection with leaf scorch.

However, the photosynthesis of *Juglans regia* 'Xinxin2' was more affected by leaf scorch. In the seasonal variation process, *Juglans regia* 'Xinxin2' with leaf scorch symptoms showed significantly lower P_N, g_s, L_s, and higher C_i than those of healthy plants. We can speculate that the decrease in the photosynthetic rate of *Juglans regia* 'Xinxin2' was caused by nonstomatal limiting factors. Moreover, during the whole process of walnut infection with leaf scorch, *Juglans regia* 'Xinxin2' with leaf scorch symptoms showed significantly higher F_0, NPQ, and lower F_m, F_m', F_v/F_m, and F_v/F_0, which indicated that the photosynthetic apparatus of the leaves of *Juglans regia* 'Xinxin2' with leaf scorch symptoms was damaged in the early stage of infection, which was consistent with the research results of Wang Daping on the infection of summer orange (*Citrus* cv. Olinda) leaves by green spot algae (Wang *et al.* 2006). After walnut was continuously infected by leaf scorch for a long time, the reduction ranges of *Juglans regia* 'Xinxin2' in the diurnal variation (late August) were higher than those of *Juglans regia* 'Wen185' in F_m, F_m', F_v/F_m, and F_v/F_0, indicating that *Juglans regia* 'Xinxin2' had relatively weak photochemical activity to the distribution of light energy between the two light systems (Feng *et al.* 2010). The increase of F_0 of *Juglans regia* 'Xinxin2' with leaf scorch symptoms was greater than that of *Juglans regia* 'Wen185' with leaf scorch symptoms, probably the PSII reaction centers of *Juglans regia* 'Xinxin2' variety were greatly destroyed.

With the increase of the leaf scorch grade, P_N and g_s in the leaves of the two varieties showed a significant downward trend, which was similar to the previous research results on blueberry (*Vaccinium uliginosum*) leaf spot (Roloff *et al.* 2004) and cherry (*Prunus cerasus* 'Montmorency') leaf spot (Gruber *et al.* 2012). The C_i of *Juglans regia* 'Wen185' showed first descent (b0–b1) and then increase (b2–b4), and the trend of L_s was just the opposite, indicating the decrease of P_N in *Juglans regia* 'Wen185' was caused by stomatal restriction under the low-grade leaf scorch, and nonstomatal restriction under the high-grade leaf scorch. However, with the increase of the leaf scorch grade, the continuous rising C_i and falling L_s of *Juglans regia* 'Xinxin2', indicated the decrease of P_N in *Juglans regia* 'Xinxin2' was always caused by nonstomatal restriction, which may be due to the weak resistance of *Juglans regia* 'Xinxin2' to the leaf scorch, which was similar to the previous research results on different varieties of tobacco (*Nicotiana tabacum*) infected with brown spot (Yang *et al.* 2015). With the increase of the leaf scorch grade, PSII of the two varieties were damaged to varying degrees, which could be judged from the increase of F_0 and a decrease of F_m, F_m', F_v/F_m, and F_v/F_0. The higher the leaf scorch grade, the more seriously the PSII would be damaged. The damage in the early stages of the disease (b1 and b2) was small; however, that in the later stages (b3 and b4) was greater. Moreover, the higher leaf scorch grade significantly reduced the original light energy conversion efficiency of the PSII and inhibited the initial reaction process of photosynthesis, resulting in the loss of the function of the PSII reaction center and the destruction of photosynthetic structures (Chen *et al.* 2011). NPQ in *Juglans regia* 'Wen185' increased first and then decreased, however, NPQ in *Juglans regia* 'Xinxin2' continuously decreased, indicating the damage of the light reaction center of *Juglans regia* 'Xinxin2' was more serious than in *Juglans regia* 'Wen185', thus it could not transmit electrons and the absorbed light energy could only be lost through heat, which was consistent with the research results of Luo *et al.* (2019).

Conclusion: In this study, the effect of leaf scorch on walnut leaf photosynthesis was related to the duration of disease infection, the severity of the disease, and walnut varieties. There was a transition between stomatal and nonstomatal restriction in the limitation of leaf photosynthesis by leaf scorch in *Juglans regia* 'Wen185'. At the early stage of infection and under the low-grade leaf scorch, the reduction of photosynthesis was caused by the stomatal restriction. With the extension of leaf scorch duration and under the high-grade leaf scorch, the photosynthetic system in the leaves was seriously damaged, and the photosynthesis-restricting pathway was converted to nonstomatal restriction. Compared with *Juglans regia* 'Wen185', leaf scorch had a greater impact on *Juglans regia* 'Xinxin2' photosynthesis, and the path of photosynthesis restriction was always nonstomatal restriction, which was related to the low resistance of *Juglans regia* 'Xinxin2' to leaf scorch.

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