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Effects of leaf-to-fruit ratio on chlorophyll fluorescence parameters of walnut (*Juglans regia* L.) leaves

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Abstract

In this study, we investigated maximal quantum yield of PSII photochemistry (F_v/F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), and nonphotochemical quenching (NPQ) of walnut (*Juglans regia* 'Xinxin2') leaves with different leaf-to-fruit ratios (LFRs). The results indicated that the increasing LFR increased the values of F_v/F_m, Φ_{PSII}, and NPQ in leaves on the girdled shoot with one and two leaves, and decreased the values of F_v/F_m and Φ_{PSII} in leaves on the girdled shoot with five leaves, whereas had no effect on the chlorophyll (Chl) fluorescence in leaves on the girdled shoot with three and four leaves. These results indicate that the effects of LFR on Chl fluorescence depend on a LFR range and show a transitional trend transition, and that excessive fruit load accelerates leaf senescence resulting in the destruction of the reaction center in PSII.

Additional key words : defoliation; fruit tree; photochemical efficiency of PSII.

Introduction

Effects of LFR on vegetative growth, fruit development, biomass productivity, photosynthetic characteristics of leaves have been studied in many fruit varieties, *e.g*., olive (*Olea europaea* L.) (Dag *et al*. 2010, Martín-Vertedor *et al*. 2011, Naor *et al*. 2012), European plum (*Prunus domestica* L.) (Seehuber *et al*. 2011), apricot (*Prunus armeniaca* L.) (Roussos *et al*. 2011), peach (*Prunus persica* L. Batsch) (Li *et al*. 2007), and grape (*Vitis vinifera* L.) (Rossouw *et al*. 2017). However, carbohydrates and energy, which are used for vegetative growth, fruit development, and biomass productivity are both the products of photosynthesis in source leaves. Thus, effects of LFR on photosynthesis have always been the hot topics.

In fruit trees, LFR is an important factor probably to influence light energy capture and transportation, and photosynthate distribution, and then finally to influence the plant productivity (Zhu *et al*. 2015). Chl fluorescence is tightly related to photosynthesis and can reflect the actual state of photosynthetic apparatus of leaves under abiotic and biotic stress (Schreiber *et al*. 1995). Thus, studying the effects of LFR on Chl fluorescence is important to understand the regulatory mechanism of photosynthesis and fundamental plant physiology. Knowledge of the mechanism may also be useful for improving plant productivity.

There are studies indicating that LFR has significant effects on Chl fluorescence. For example, study on peach has shown that the remaining leaves after defoliation have significantly lower net photosynthetic rate (P_N), maximal fluorescence (F_m), and F_v/F_m than the leaves initally covered with bags and uncovered at different time (Li *et al*. 2007). Study on olive indicates that flower thinning decreases $\Phi_{PSII,}$ but increases NPQ in a short term. The reduction in Φ_{PSII} might be the main reason for the depression of P_N in the long-term response of Chl fluorescence to sink source relationship (Zhu *et al*. 2015).

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Abbreviations: Chl – chlorophyll; DAF – days after full bloom of female flowers; 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/F_m – maximal quantum yield of PSII photochemistry; LFR – leaf-to-fruit ratio; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; OTL – LFRs with one and two leaves; TFL – LFRs with three and four leaves; FL – FRs with five leaves; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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Therefore, the above-mentioned examples have suggested that a low sink demand results in the decreased activity of PSII reaction centers.

There are also studies indicating that the effects of LFR on Chl fluorescence are not significant. For example, data from olive leaves which were monitored at pit hardening and fruit ripening shows that crop load has no significant effect on Chl fluorescence parameters (Haouari *et al*. 2013). Study with citrus shows that increase in minimal fluorescence (F_0) after defruiting is observed on relatively warm days, however, F_v/F_m which is measured at midday only presents small and probably transient response to crop load (Syvertsen *et al*. 2003). Therefore, the above mentioned examples have suggested that the change in a source–sink relation has no significant effect on Chl fluorescence.

The inconsistent information from previous studies has tended to obscure the effect of LFR on Chl fluorescence. Thus, there must be enough studies to demonstrate such an effect. Furthermore we are not quite clear about the effects of LFR on Chl fluorescence of walnut (*Juglans regia* L.) leaves. Thus, we put forward the hypothesis that: the effect of LFR on Chl fluorescence depends on the variation range of LFR. In this study, the different LFRs of walnut trees were artificially altered using a variety of manipulations, including defoliation, fruit thinning, and girdling. After manipulation, Chl fluorescence parameters of leaves on girdled shoots with different LFRs were investigated to evaluate the long-term response of Chl fluorescence to LFR, and to determine their relationship during the growing season. This study will provide a deeper understanding of the effect of LFRs on Chl fluorescence.

Materials and methods

Experimental site and plants: The experiment was carried out in a walnut orchard, located in southwest of Xinjiang, China (41°11′06.31″–41°12′47.74″N, 79°12′12.76″–79°13′57.87″E; 1,394 m a. s. l.). The climate is a warm temperate continental arid environment with a mean annual temperature of 9.4℃. It receives an average rainfall of 91.5 mm. Uniform 10-year-old walnut *(J.* 'Xinxin2') trees were grown at a spacing of 5.0 × 6.0 m in east-west rows in anthropogenic-alluvial soil.

Leaf-to–fruit ratio manipulation: After fruit set, 15 LFRs were applied to sun-exposed and girdled shoots with fully expanded leaves and developing fruit by removing fruit or leaves on the southern side of homogenous trees. LFRs used in this study reflected the bearing habit found in walnut trees under natural conditions and was defined as the ratio of a number of leaves to the number of fruits. The measurement of Chl fluorescence parameters was performed with six trees; fifteen girdled shoots of each tree were subjected to all of the following 15 LFR treatments (Fig. 1):

girdled shoots with one fruit and one leaf	1L:1F (Fig. 1*A*)
one leaf and two fruits	1L:2F (Fig. 1*B*)
one leaf and three fruits	1L:3F (Fig. 1*C*)
girdled shoots with two leaves and one fruit	2L:1F (Fig. 1*D*)
two leaves and two fruits	2L:2F (Fig. 1*E*)
two leaves and three fruits	2L:3F (Fig. 1*F*)
girdled shoots with three leaves and one fruit	3L:1F (Fig. 1*G*)
three leaves and two fruits	3L:2F (Fig. 1*H*)
three leaves and three fruits	3L:3F (Fig. 1*I*)
girdled shoots with four leaves and one fruit	4L:1F (Fig. 1*J*)
four leaves and two fruits	4L:2F (Fig. 1*K*)
four leaves and three fruits	4L:3F (Fig. 1*L*)
girdled shoots with five leaves and one fruit	5L:1F (Fig. 1*M*)
five leaves and two fruits	5L:2F (Fig. 1*N*)
five leaves and three fruits	5L:3F (Fig. 1*O*)

Girdling was applied at the shoot base after defoliation or defruiting in order to prevent the exchange of carbohydrates between treated shoots and other parts of the tree. The girdles were preserved for the entire growing season by discarding any scar tissue at 15-d intervals. Immature leaves and the apical and auxiliary buds were removed from the treated shoots to ensure that the assimilates mainly flowed to the fruits.

Chl fluorescence parameters: During the 2016 growing seasons, Chl fluorescence parameters were measured once a day (11:00−14:00), on five cloudless days at 7, 22, 52, 82, and 107 d after initiating LFR manipulation (*i.e*., 30, 45, 75, 105, and 130 d after full bloom of female flowers). Two fully developed leaves per girdled shoot close to the developing fruit were selected for the Chl fluorescence measurement, which was assessed using a fluorescence monitoring system (*FMS-2*, *Hansatech*, England). The selected leaves were placed in the dark for 20 min, and then a low-intensity [< 5 μmol(photon) m^{-2} s^{-1}] red-measuring light was applied first to obtain minimum fluorescence, F_0. Then, maximum fluorescence, F_m, was

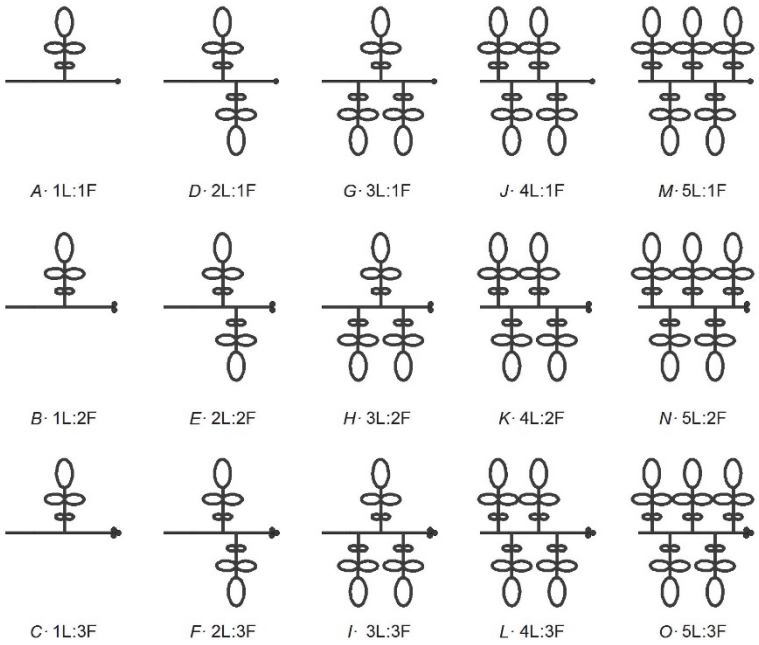

Fig. 1. Different leaf-to-fruit ratio manipulations on the girdled shoots of walnut.

obtained under a saturating light [< 5 μmol(photon) m^{-2} s^{-1}] red-measuring light was applied first to obtain minimum fluorescence, F_0. Then, maximum fluorescence, F_m, was obtained under a saturating light pulse (0.8 s) of 6,000 μmol(photon) m^{-2} s^{-1}. The same leaves after dark measurement, were first exposed to normal light for 30 min and then were illuminated by actinic light [700 μmol(photon) m^{-2} s^{-1}] for 150 s to determine the steady-state fluorescence (F_s), and then maximum fluorescence in the light-adapted state (F_m') could be measured by applying saturating white light pulse (0.8 s) of 6,000 μmol(photon) m^{-2} s^{-1}. F_v/F_m and $\Phi_{PS\,II}$ were calculated by a *FMS-2* instrument. Nonphotochemical quenching, NPQ, was calculated as follows:

$$NPQ = (F_m - F_m')/F_m' \quad (1)$$

Statistical analysis: Data were statistically analyzed using *SPSS ver. 22.0* statistical software. Firstly, repeated-measures analysis was used to examine the effects of LFR, growth stage, and their interaction on the profiles of Chl fluorescence parameters. Then profile analysis was used to determine whether the Chl fluorescence parameters differed significantly between two different LFRs. $\mu_1' = (\mu_{11},\mu_{12},\mu_{13},\mu_{14},\mu_{15})$ and $\mu_2' = (\mu_{21},\mu_{22},\mu_{23},\mu_{24},\mu_{25})$, respectively, represented mean vectors of two different LFR crossed five growth stages. Each profile was the average values of one Chl fluorescence parameter across five growth stages for the same treatment from six replicated samples. Profile analysis addressed two questions.

(*1*) Whether the two profiles were parallel or not?

$$H_{01}: \mu_{1i} - \mu_{1,i-1},\ i = 2,3,4,5 \quad (2)$$

The hypothesis H_{01} was tested by the result of multivariate tests. $P<0.05$ indicated hypothesis H_{01} was rejected, that was, there was significant difference between the two LFRs of test takers. $P>0.05$ indicated hypothesis H_{01} was accepted, and then the hypothesis H_{02} (coincidence profile test) was tested.

(*2*) Whether the two profiles were coincident or not?

$$H_{02}: \mu_{1i} - \mu_{2i},\ i = 2,3,4,5 \quad (3)$$

The hypothesis H_{02} was tested by the result of tests of between subject effects. $P<0.05$ indicated hypothesis H_{02} was rejected, that was, there was significant difference between the two LFRs of test takers. $P>0.05$ indicated hypothesis H_{02} was accepted and there was no significant difference between the two LFRs of test takers (Kruskal 1964).

General linear regression analysis was applied to determine the relationship between LFR and Chl fluorescence parameters.

Results

According to the result of repeated-measures analysis (Table 1), the effects of LFR, growth stage, and their interaction on the Chl fluorescence parameters were statistically significant. The values of three Chl fluorescence parameters varied greatly with different LFR and growth stages. And the change trend of the values with growth stages was affected by LFR.

Table 1. Repeated-measures analysis of Chl fluorescence parameters profiles. F_v/F_m – maximal quantum yield of PSII photochemistry, Φ_{PSII} – photochemical efficiency of PSII photochemistry, NPQ – nonphotochemical qunching; F – the statistic value of analysis of variance; *P* – the probability values under the corresponding *F* values.

		F_v/F_m	Φ_{PSII}	NPQ
Growth stage (S)	*F*	2,095.024	762.824	1,437.453
	P	0.000	0.000	0.000
Treatments with different leaf-to-fruit ratios (T)	*F*	511.292	174.594	247.589
	P	0.000	0.000	0.000
T×S	*F*	55.694	4.119	19.101
	P	0.000	0.000	0.000

LFRs with one and two leaves (OTL): In the girdled shoots with one and two leaves (Fig. 2), F_v/F_m , Φ_{PSII}, and NPQ decreased significantly with increasing fruit load. Integrated over the growing season, LFRs with one fruit (1L:1F and 2L:1F) had larger values of F_v/F_m, Φ_{PSII}, and NPQ than LFRs with three fruit (1L:3F and 2L:3F), with 1L:2F and 2L:2F showing intermediate values. On the basis of profile analysis, any two LFRs for F_v/F_m and Φ_{PSII} showed up as a significantly distinct profile since they were not coincident, although F_v/F_m and Φ_{PSII} varied curvilinearly across the growing season in all LFRs. However, LFR made some difference in the patterns of NPQ.

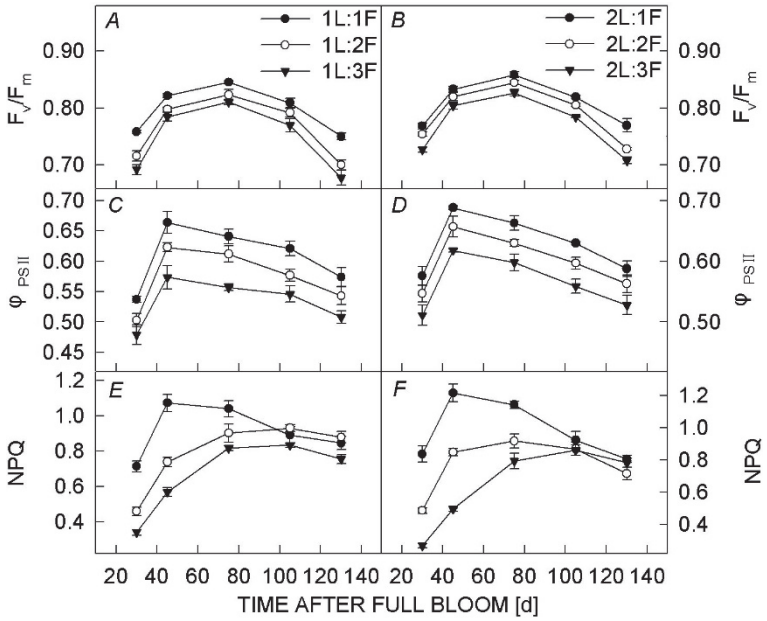

Fig. 2. Changes in Chl fluorescence parameters in response to leaf-to-fruit ratios (LFRs) with one and two leaves. Data are presented as means ± SD (n = 6). (*A*,*B*) the maximal quantum yield of PSII photochemistry (F_v/F_m) of LFRs with one leaf and two leaves, respectively; (*C*,*D*) effective quantum yield of PSII photochemistry (Φ_{PSII}) of LFRs with one leaf and two leaves, respectively; (*E*,*F*) nonphotochemical quenching (NPQ) of LFRs with one leaf and two leaves, respectively.

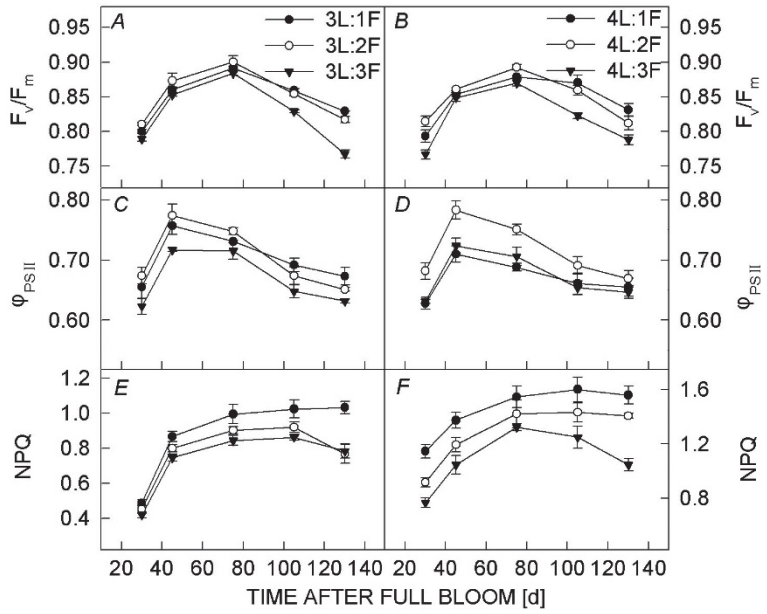

Fig. 3. Changes in Chl fluorescence parameters in response to leaf-to-fruit ratios (LFRs) with three and four leaves. Data are presented as means ± SD (n = 6). (*A*,*B*) the maximal quantum yield of PSII photochemistry (F_v/F_m) of LFRs with three leaves and four leaves, respectively; (*C*,*D*) effective quantum yield of PSII photochemistry (Φ_{PSII}) of LFRs with three leaves and four leaves, respectively; (*E*,*F*) nonphotochemical quenching (NPQ) of LFRs with three leaves and four leaves, respectively.

LFRs with three and four leaves (TFL): In the girdled shoots with three and four leaves (Fig. 3), NPQ decreased significantly with increasing fruit load. LFRs with one fruit (3L:1F and 4L:1F) showed the highest annual mean NPQ values. However, a similar response in LFRs in F_v/F_m and Φ_{PSII} was observed for all the LFRs. Results of profile analysis showed that there were no significant differences in F_v/F_m and Φ_{PSII} between any two LFRs, with the exception of 3L:3F and 4L:2F.

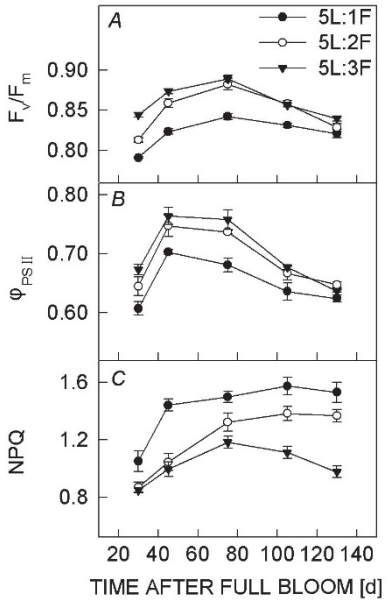

Fig. 4. Changes in Chl fluorescence parameters in response to leaf-to-fruit ratios (LFRs) with five leaves. Data are presented as means ± SD (n = 6). (*A*) The maximal quantum yield of PSII photochemistry (F_v/F_m) of LFRs with five leaves; (*B*) effective quantum yield of PSII photochemistry (Φ_{PSII}) of LFRs with five leaves; (*C*) nonphotochemical quenching (NPQ) of LFRs with five leaves.

LFRs with five leaves (FL): In the girdled shoots with five leaves (Fig. 4), the values of F_v/F_m and Φ_{PSII} decreased significantly with increasing LFR. Integrated over the growing season, 5L:3F had larger values of F_v/F_m and Φ_{PSII} than 5L:1F, with 5L:2F showing intermediate values (Fig. 4*A*,*B*). NPQ decreased significantly with the increasing fruit load. 5L:3F showed the lowest annual mean NPQ value, which was respectively 14.6 and 27.9% lower than that for 5L:2F and 5L:1F.

Table 2. The relationship between leaf to fruit ratio (x) and chlorophyll fluorescence parameters (y). The relationships are indicated by the linear regression equations of the form y = mx +c, where x and y are independent and dependent variables, respectively, and m and c are slope and intercept, respectively. R^2 – correlation coefficient for each relationship. Levels of statistical significance are: *P<0.05, **P<0.01. "-" indicates no correlation between variables. F_v/F_m – maximal quantum yield of PSII photochemstry; Φ_{PSII} – photochemical efficiency of PSII photochemstry; NPQ – nonphotochemical quenching; OTL – leaf-to-fruit ratio (LFR) with one and two leaves; TFL – LFR with three and four leaves; FL – LFR with five leaves.

LFR	Parameter	Linear regression equation	R^2
OTL	F_v/F_m	y = 0.035 x + 0.748 y	0.831**
	Φ_{PSII}	y = 0.052 x + 0.536 y	0.814**
	NPQ	y = 0.189 x + 0.620 y	0.697*
TFL	F_v/F_m	-	-
	Φ_{PSII}	-	-
	NPQ	-	-
FL	F_v/F_m	y = –0.120 x + 0.879 y	0.980**
	Φ_{PSII}	y = –0.015 x + 0.727 y	0.997**
	NPQ	-	-

Correlation between LFR and Chl fluorescence parameters: When relationships of the data of F_v/F_m, Φ_{PSII}, and NPQ with the data of LFR were investigated using mean values of the data, it was found that OTL were positively correlated with F_v/F_m, Φ_{PSII}, and NPQ, whereas the FL showed negatively significant correlations with F_v/F_m and Φ_{PSII}. TFL showed no correlation with all the Chl fluorescence parameters (Table 2).

Discussion

The induced Chl fluorescence as a kinetic parameter plays an important role in research of photosynthetic physiological conditions and studying the mechanism of photosynthesis due to its celerity and undamage to plants (Hazrati *et al*. 2016, Mouradi *et al*. 2016). In this study, LFR was manipulated in walnut trees in the absence of perturbation of other functional sinks such as roots and branches. To study the effect of LFR on Chl fluorescence, ninety girdled shoots with fifteen LFR were implemented during the entire growing season, and three Chl fluorescence parameters (F_v/F_m, Φ_{PSII}, and NPQ) were analyzed.

F_v/F_m as the maximum photochemical quantum yield of PSII is obtained under the conditions of all photosystem reaction centers being in an open state, and it reflects the intrinsic efficiency of energy conversion in the PSII reaction center (Oxborough and Baker 1997, Gorbe and Calatayud 2012). Φ_{PSII} reflects PSII activities and evaluates the transfer rate of electrons transporting from PSII to PSI (Krall and Edward 1992). Nonphotochemical quenching (NPQ) is a photoprotective process that removes excess excitation energy in the form of heat dissipation, which is closely related to xanthophyll cycling and prevents the likelihood of formation of damaging reactive oxygen species (ROS). Production of large amounts of ROS is inevitable during photosynthesis process, *e.g*., singlet-excited oxygen and hydroxyl radical, which can oxidize surrounding bimolecular. If the

nonradiative energy dissipation of the xanthophyll cycle cannot completely dissipate excess light energy, which will form ROS and cause damage to the photosynthetic mechanism (Apel and Hirt 2004, Demmig-Adams 1990, Pinnola *et al*. 2013). Under optimal circumstances, the Chl in plant leaves dissipate absorbed light by photosynthetic electron transport, Chl fluorescence emission, and heat dissipation. There is a reciprocal relationship among these three processes. Changes in photosynthesis and heat dissipation causes the corresponding changes in fluorescence (Zheng and Shangguan 2006).

In the girdled shoots with one and two leaves, LFRs were positively correlated with F_v/F_m, Φ_{PSII}, and NPQ (Table 2). The decrease in values of F_v/F_m, Φ_{PSII}, and NPQ with increasing fruit load indicated that extremely low LFRs (1L:2F, 1L:3F, 2L:2F, and 2L:3F) reduced the efficiency of energy conversion, actual photosynthetic efficiency, and heat dissipation. On the basis of our results, we deduced that there were less absorbed light allocated to these three approaches. It means that there was less light energy captured by Chl in the leaf. Compared to LFRs with more leaves, under the same light conditions, the lower values of F_v/F_m, Φ_{PSII}, and NPQ observed at OTL indicated that leaves in OTL had a poor ability to capture light energy. It is probably caused by abnormal photosynthetic function due to the underdeveloped leaves. The photosynthates produced by plant leaves are initially utilized to meet leaf own physiological needs, and then the surplus photosynthates is exported to other sinks (Lv and Zhang 2000). However, serious scarcity of photosynthates caused by precious extremely low LFR seriously affected the development of leaves and their photosynthetic apparatus (Fang *et al*. 2001), which might explain why most F_v/F_m values were below 0.8. The serious scarcity of photosynthates would further impede the development of photosynthetic apparatus. In addition, the effect of girdling on the leaf development should not be ignored. Girdling blocks the flow of nutrients transported from roots through phloem to leaves, which is not conducive to the development of leaves (Fang *et al*. 2001). It has been proven that girdling reduced F_v/F_m (Nebauer *et al*. 2011) and extremely low LFR resulted in a decrease in photosynthesis (Fang *et al*. 2001). Thus, we deduced that in OTL, the depressing effect of girdling on Chl fluorescence was stronger than the positive effect of the decrease in LFR on Chl fluorescence, which led to the positive correlation between LFR and Chl fluorescence.

In the girdled shoots with five leaves, LFRs showed negatively significant correlations with F_v/F_m and Φ_{PSII}, which is consistent with the reports for other plants (Li *et al*. 2007, Zhu *et al*. 2015). The greater values of Φ_{PSII} indicated that photosynthetic apparatus had a better ability to convert light energy. In the present study, the leaves of the highest LFR (5L:1F) showed a loss of Φ_{PSII} (Fig. 3*A*), which implied that if supply exceeded demand, then the light-use efficiency for photosynthesis must decrease (van Rooijen *et al*. 2015). The increase in F_v/F_m and decrease in NPQ with the increasing fruit load indicated that fruit showed a strong demand for photosynthate (Fischer *et al*. 2013), which improved the solar energy conversion efficiency of PSII and reduced the thermal energy dissipation. The similar results were observed in peach (Duan *et al*. 2008) and cherry (Layne and Flore 1993). On the basis of our results, we deduced that under the same light conditions, high fruit load consumes a large portion of absorbed light energy by photosynthetic electron transport. Thus, a relatively little portion of absorbed light energy is consumed by heat dissipation. In the present study, the lowest value of F_v/F_m and Φ_{PSII} and the highest value of NPQ were observed in 5L:1F (Fig. 4). Previous studies suggest that leaf photosynthesis is affected by the light availability and the demand for photosynthates from the other sink (Adams III *et al*. 2015). Removal of fruit leads to the accumulation of carbohydrate in the leaves and the downregulation of photosynthesis, which is accompanied by significantly greater decreases in F_v/F_m and Φ_{PSII} and increase in NPQ (Duan *et al*. 2008, Cheng *et al*. 2009). High LFR also contributed to the accumulation of carbohydrates when the plant's other sinks fail to utilize the photosynthate by phloem girdling. Thus, we deduced that in FL, the positive effect of the decrease in LFR on Chl fluorescence was stronger than the depressing effect of girdling on Chl fluorescence, which led to the negative correlation between LFR and Chl fluorescence (F_v/F_m and Φ_{PSII}).

In the girdled shoots with three and four leaves, LFRs showed no correlation with all the Chl fluorescence parameters. In the present study, there were no differences in F_v/F_m and Φ_{PSII} between any two LFRs, with the exception of 3L:3F and 4L:2F, which indicated the leaf development and carbohydrate accumulation in leaves on the shoot with three and four leaves were not affected by girdling and LFR, in comparison to OTL and FL. Stable value for Φ_{PSII} is due to the balance between supply-side (the processes of formatting excited states of Chl a in PSII) and demand-side (the processes of dissipating excited states of Chl *a*) (Genty *et al*. 1989). Thus, we deduced that in TFL, the positive effect of the decrease in LFR on Chl fluorescence might be cancelled out by the depressing effect of girdling on Chl fluorescence. When supply-side processes in source and demand-side processes in sink reach a balanced state, LFR has no significant effect on Chl fluorescence. However, LFRs with three fruit showed the lowest values of F_v/F_m, Φ_{PSII}, and NPQ with the exception of NPQ in 4L:3F. It is probably the combination of high fruit load, relatively few leaves, and girdling affects the leaf development, and further impedes the development of photosynthetic apparatus. On the basis of our results, we deduced that TFL could be considered as a transient state from OTL to FL, and that LFRs has no effect on Chl fluorescence.

In the present study, it was interesting to note that the seasonal patterns of every Chl fluorescence parameter in all LFRs were similar, and these general patterns seemed to be independent on the change in LFR. It was probably

due to the changes in environmental conditions. The results from citrus suggested changes in P_N of the treatments with different LFR depended on the change in the environment conditions, which is tightly related to the leaf temperature (Nebauer *et al*. 2011). In the present study, the relationship between the Chl fluorescence and environment conditions remains for further study. In addition, it was also interesting to note that at 105-130 d after full bloom, a reduction in three Chl fluorescence parameters (F_v/F_m, Φ_{PSII}, and NPQ) were observed for all the LFRs with three fruit. It was probably because excessive load accelerated leaf senescence resulting in the destruction of the reaction center in PSII. It has been reported that the inhibition of leaf photosynthesis by the high availability of photosynthetic products in the absence of fruit depends on the growth stage (Nii 1993, Syvertsen *et al*. 2003).

Conclusion: The Chl fluorescence characteristics of leaves at different LFRs showed in this study indicated that the effects of LFR on Chl fluorescence depended on LFR range and showed a transitional trend transition. The positive correlation between LFR (with one and two leaves) and Chl fluorescence is a consequence of the inhibition of leaf development caused by girdling, and the negative correlation between LFR (with five leaves) and Chl fluorescence (F_v/F_m and Φ_{PSII}) is due to a large portion of absorbed light energy consumed by photosynthetic electron transport caused by high fruit load, whereas the LFR has no significant effects on Chl fluorescence when there is a balance supply and demand of photosynthates between source leaf and sink fruit (LFR with three and four leaves). Additionally, excessive fruit load accelerates leaf senescence resulting in the destruction of the reaction center in PSII.

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