Photosynthesis of young apple trees in response to low sink demand under different air temperatures

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Summary Gas exchange, chlorophyll fluorescence, photosynthetic end products and related enzymes in source leaves in response to low sink demand after girdling to remove the root sink were assessed in young apple trees (Malus pumila) grown in two greenhouses with different air temperatures for 5 days. Compared with the non-girdled control in the low-temperature greenhouse (diurnal maximum air temperature <32 °C), low sink demand resulted in lower net photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (E) but higher leaf temperature on Day 5, while in the high-temperature greenhouse (diurnal maximum air temperature >36 °C), Pn, gs and E declined from Day 3 onwards. Moreover, gas exchange responded more to low sink demand in the high-temperature greenhouse than in the low-temperature greenhouse. Decreased Pn at low sink demand was accompanied by lower intercellular CO2 concentrations in the low-temperature greenhouse. However, decreased maximal photochemical efficiency, potential activity, efficiency of excitation capture, actual efficiency and photochemical quenching, with increased minimal fluorescence and non-photochemical quenching of photosystem II (PSII), were observed in low sink demand leaves only in the high-temperature greenhouse. In addition, low sink demand increased leaf starch and soluble carbohydrate content in both greenhouses but did not result in lower activity of enzymes involved in metabolism. Thus, decreased Pn under low sink demand was independent of a direct effect of end-product feedback but rather depended on a high temperature threshold. The lower Pn was likely due to stomatal limitation in the low-temperature greenhouse, but mainly due to non-stomatal limitation in the high-temperature greenhouse.

Keywords: chlorophyll fluorescence, end products, enzyme activity, gas exchange, source–sink regulation.

Introduction

Fruits represent the most important assimilate sink in mature fruit trees, while roots are the main sink in young trees. Girdling the trunk can stop transport of assimilates from source leaves to roots via the phloem while allowing water flow via the xylem (Zhou and Quebedeaux 2003, Cheng et al. 2008). Removal of the sink demand of roots generally results in decreased leaf photosynthesis in many fruit species (Iglesias et al. 2002, Cheng et al. 2008).

In general, photosynthetic end products, soluble sugars and starch accumulate in leaves following reduced sink demand (Goldschmidt and Huber 1992, Iglesias et al. 2002, Urban et al. 2004). End-product inhibition, or so-called direct feedback, has been used to explain the decline in net photosynthesis rate after weakening of the sink (Layne and Flore 1995, Paul and Foyer 2001, Iglesias et al. 2002). For this hypothesis, high concentrations of end products may inhibit enzyme activity and subsequently decrease photosynthesis (Guinn and Mauney 1980, Herold 1980). However, there are still questions about whether lower sink strength reduces photosynthesis by direct feedback through end-product accumulation (Chaumont et al. 1994, Damatta et al. 2008), even though the direct feedback hypothesis was suggested nearly a century ago (Neales and Incoll 1968).

Recent studies showed that decreased photosynthesis due to low sink demand was independent of a direct feedback effect of end products in peach (Li et al. 2007) and coffee (Da-Matta et al. 2008) trees. Moreover, in parallel with reduced photosynthesis, low sink demand resulted in decreased stomatal conductance (gs; Tan and Buttery 1986, Chaumont et al. 1994, Schubert et al. 1996). Therefore, decreased gs was proposed as the first response after a reduction in sink demand (Li et al. 2001, Li et al. 2005). Decreasing gs reduces
Figure 1. Response of average daily net photosynthesis rate ($P_n$, A and F), stomatal conductance ($g_s$, B and G), transpiration rate ($E$, C and H), intercellular CO$_2$ concentration ($C_i$, D and I) and leaf temperature ($T_{leaf}$, E and J) to low sink strength by removing root demand (open circles), compared with the control (filled circles) in young apple trees in low-temperature (left panels) and high-temperature (right panels) greenhouses. Changes in photosynthetic photon flux (PPF, multiplication symbols) are given in panels A and F. Each data point represents the mean of four times of measurement with four replicates at each time. Vertical bars represent ±SE, and *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, between removed root demand and the control in each greenhouse.
the rate of CO₂ entry into leaves and transpiration via stomata, which results in stomatal limitation of photosynthesis (Setter and Brun 1980, Cheng et al. 2008, Wu et al. 2008) and a subsequent increase in leaf temperature (T_leaf). When leaf temperature is above optimum, PSII is damaged and net photosynthetic rate is reduced, leading to non-stomatal limitation. This non-stomatal limitation frequently takes place in field-grown trees and mostly under high photosynthetic photon flux (PPF) and low sink demand (Li et al. 2005, 2007, Wu et al. 2008). Thus, it was suggested that decreased gs might be considered as the trigger or promoter and increased T_leaf as the regulator of photosynthesis under low sink demand (Li et al. 2001).

Ambient micro-environmental conditions can regulate T_leaf due to exchange of energy between trees and the surrounding atmosphere. In the current study, the photosynthetic response of young apple trees to reduced sink demand was investigated under two different air temperatures. The objective was to evaluate the role of T_leaf, regulated by environmental conditions, and modified source–sink relationships on photosynthesis, focusing on stomatal and non-stomatal limitations by interpreting the combined data from gas exchange and chlorophyll fluorescence measurements. Moreover, the effect of source–sink manipulation on the level of end products and activity of related enzymes was investigated to better understand carbon metabolism in the regulation of photosynthesis.

Materials and methods

Experimental sites and plant material

Experiments were carried out in August 2006 in Haidian District, Beijing. One-year-old micropropagated ‘Gala’ apple trees (Malus pumila Mill) with one new shoot were grown in pots in a greenhouse. When the new shoots reached ~20 cm, uniform trees were transferred into black glass containers (15 cm × 20 cm × 80 cm) containing a modified Hoagland nutrient solution, with 0.5 mM KNO₃, 0.3 mM Mg(NO₃)₂, 0.11 mM Ca(NO₃)₂, 0.5 mM CaCl₂, 0.5 mM MgSO₄, 0.5 mM KH₂PO₄, 3.9 μM ZnSO₄, 2.6 μM CuSO₄, 4.6 μM MnCl₂, 0.44 μM EDTA·Fe·Na, 0.25 μM H₃BO₃ and 0.33 mM (NH₄)₆Mo₇O₂₄·4H₂O. The glass containers were covered to maintain a dark environment for the roots, and air was continuously pumped into the solutions for aeration. Water was added daily to compensate for solution loss through transpiration. The solution was completely changed at 3-day intervals throughout the experiments. Trees with uniform vegetative growth at about 30 cm in height were selected for study after cultivation in nutrient solution for 20 days.

Treatments

The selected trees were topped by removing the shoot tip sink and divided into two groups, which were transferred to two separate greenhouses. The air temperature was controlled in one greenhouse, with the highest daytime air temperature maintained at <32 °C (low-temperature house, Figures 2E and 3E), and that in the other greenhouse was not controlled and was >36 °C (>40 °C on some days; high-temperature house, Figures 2J and 3I) throughout the experiment. Trees received one of two treatments, low sink demand or control, in each greenhouse. The low sink demand treatment (LT in the low-temperature greenhouse and HT in the high-temperature greenhouse) was realized by removing the root sink by girdling the stem phloem through removal of a 3-mm-wide band of bark at the base of the trunk. For controls (LC in the low-temperature greenhouse and HC in the high-temperature greenhouse), a 3-mm-wide band of bark was also removed from the same part of the trees, but longitudinally. One tree was used for sink–source manipulation and a randomized complete block design was used in this study.

Measurement of gas exchange and chlorophyll fluorescence

A total of four trees per treatment (i.e., four replicates, one tree per replicate) were randomly selected for measurement of gas exchange in each greenhouse. Gas exchange was measured every 2 h on one fully expanded leaf per tree using a portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) between 1000 h and 1600 h on 19–23 August (all sunny days), corresponding to 1–5 days after treatments were applied. The gas exchange parameters, including PPF, gs, transpiration rate (E), intercellular CO₂ concentration (Cᵢ), T_leaf and Pₘ were measured at ambient CO₂.

On the same leaves used for photosynthetic measurements, chlorophyll fluorescence parameters were also measured at intervals of 2 h from 1000 h to 1600 h using a portable pulse amplitude modulation fluorometer (FMS-2, Hansatech, King’s Lynn, Norfolk, UK), but only on the fifth day. Maximal fluorescence in the dark-adapted (F_m') and light-adapted (F_m) states was measured following a 1.4-s saturating flash of ~3000 μmol photons m⁻² s⁻¹. Minimal fluorescence (F_o), F_m' and maximum photochemical efficiency of PSII (Fₘ'/Fₘ) were measured by keeping the leaf in the dark 30 min before measurement. All measurements of F_o and minimal fluorescence during illumination (Fₘ') were performed with the measuring beam set to a frequency of 600 Hz, whereas all measurements of F_m and F_m' were performed with the measuring beam automatically switching to 20 kHz during the saturating flash. The efficiency of excitation capture by open PSII reaction centers (Fₘ'/Fₘ'), actual efficiency of PSII (Φₚₛᵢᵢ) and photochemical quenching coefficient (qP) were measured. Non-photochemical quenching (NPQ) was calculated from (Fₘ'/Fₘ') + 1 (Bilger and Björkman 1990).

Leaf sampling for carbohydrate and enzyme activity analyses

Source leaves were harvested daily at midday from each treatment, i.e., three replicates for each treatment (60 trees in each greenhouse). The midrib of each leaf was removed. One half of the leaf was analyzed for carbohydrates and the
Figure 2. Diurnal variation in net photosynthesis rate \( (P_n, \ A \ \text{and} \ F) \), stomatal conductance \( (g_s, \ B \ \text{and} \ G) \), transpiration rate \( (E, \ C \ \text{and} \ H) \), intercellular CO\(_2\) concentration \( (C_i, \ D \ \text{and} \ I) \) and leaf temperature \( (T_{leaf}, \ E \ \text{and} \ J) \) at low sink strength by removing root demand (open circles) and the control (filled circles) in young apple trees on Day 3 after initiating source–sink manipulation in low-temperature (left panels) and high-temperature (right panels) greenhouses. Changes in photosynthetic photon flux \( \text{PPF} \) (multiplication symbols) and air temperature \( (T_{air}, \ \text{filled stars}) \) are given in panels A and F, and E and J, respectively. Vertical bars represent ±SE \( (n = 4) \), and * and ** indicate significant differences at \( P < 0.05 \) and \( P < 0.01 \), respectively, between removed root demand and the control in each greenhouse.
Figure 3. Diurnal variation in net photosynthesis rate ($P_n$, A and F), stomatal conductance ($g_s$, B and G), transpiration rate ($E$, C and H), intercellular CO$_2$ concentration ($C_i$, D and I) and leaf temperature ($T_{leaf}$, E and J) at low sink strength by removing root demand (open circles) and the control (filled circles) in young apple trees on Day 5 after initiating source–sink manipulation in low-temperature (left panels) and high-temperature (right panels) greenhouses. Changes in photosynthetic photon flux (PPF, multiplication symbols) and air temperature ($T_{air}$, filled stars) are given in panels A and F, and E and J, respectively. Vertical bars represent ±SE ($n = 4$), and * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively, between removed root demand and the control in each greenhouse.

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other was used for enzyme analyses. Leaf samples were immediately frozen in liquid nitrogen, lyophilized and kept at −80 °C until analysis.

Carbohydrate analysis, enzyme extraction and assay
Soluble carbohydrates from about 1.5 g fresh mass of leaves for each replication were extracted three times with 10 ml 80% ethanol in a water bath at 80 °C for 10 min, followed by centrifugation at 2000g. The supernatants were evaporated to dryness in a water bath at 85 °C, after which 2 ml of double-distilled water was added. This solution was used for sorbitol, glucose, fructose and sucrose measurement by high-performance liquid chromatography (Dionex P680; Dionex Corp., CA, USA) as documented previously (Li et al. 2007). After removing soluble carbohydrates, starch in the pellet was quantified by the glucose–peroxidase method using a test kit (Müller et al. 1994).

For extracting and assaying enzymes [aldose-6-phosphate reductase (A6PR EC 1.1.1.200), sorbitol dehydrogenase (SDH, EC 1.1.1.14), sucrose phosphate synthase (SPS, EC 2.4.1.14), adenosine diphosphate–glucose pyrophosphorylase (ADP–GPPase, EC 2.7.7.27), acid invertase (AI, EC 3.2.1.26) and total amylase (AM, EC 3.2.1.1 and EC 3.2.1.2)], 1 g of frozen leaf tissue per replication was used as previously described (Li et al. 2007).

Statistical analysis
In this experiment, the effect of air temperature was not statistically analyzed due to the absence of a replicate treatment. Therefore, a t-test was used for determining if significant differences occurred only between the low sink demand and control within the same temperature greenhouse.

Results
Evolution of photosynthesis after initiating source–sink manipulation
The effect of low sink demand on average \( P_n \) and other photosynthetic parameters depended on temperature in the greenhouse (Figure 1). In the low-temperature greenhouse, no significant effect of low sink demand treatment on \( P_n \) was observed until Day 5 after removing the root sink (Figure 1A), while in the high-temperature greenhouse low sink demand resulted in significantly decreased \( P_n \) from Day 3 onwards, and the magnitude of the differences between HC and HT treatments became greater from Days 3 to 5 (Figure 1F). Low sink demand (LT and HT) resulted in decreased \( g_s \) and \( E \) compared with controls (Figure 1B–C and G–H). However, the effect appeared earlier and was of greater magnitude in the high-temperature greenhouse than in the low-temperature greenhouse.

Compared with LC during Days 3–5, \( C_i \) of LT declined and the differences between LT and LC increased as the experiment progressed (Figure 1D). However, a significant difference between LT and LC appeared only at Day 5 after initiating sink–source manipulation. Unlike the response of LT, \( C_i \) in HT was lower than in HC for the first 2 days and higher on Days 3 and 4, although there were no significant differences for the average on most days (Figure 1I).

LT resulted in higher \( T_{leaf} \) than LC only on Day 3 (Figure 1E). In contrast to the responses of \( P_n \), \( g_s \) and \( E \), \( T_{leaf} \) increased in HT compared to HC (Figure 1J).

Diurnal variations in leaf gas exchange
Diurnal variations in leaf gas exchange, \( T_{leaf} \) and air temperature (\( T_{air} \)) in the greenhouses during Days 3 and 5 after initiating source–sink manipulation are shown in Figures 2 and 3. The response of leaf gas exchange to low sink demand was different between these 2 days in the same greenhouse. There were no significant differences in \( g_s \) and \( E \) between LT and LC, and between HT and HC on Day 3 in both greenhouses (Figure 2B–C and G–H), while the low sink demand treatment generally resulted in significantly decreased \( g_s \) and \( E \) from 1000 to 1400 h on Day 5 compared with its control in each temperature greenhouse (Figure 3B–C and G–H). For \( P_n \), a marked decrease was found for HT compared to HC for 2 days (Figures 2F and 3F). However, \( P_n \) in LC was significantly higher than LT only at noon on Day 5 (Figure 3A). The response of \( C_i \) to low sink demand in the low-temperature greenhouse differed from that in the high-temperature greenhouse on these 2 days (Figures 2D, I and 3D, I). Significantly lower \( C_i \) was measured in LT than in LC at 1200 h and 1400 h on Day 5 (Figure 3D), while significantly higher \( C_i \) was found in HT at 1400 h and 1600 h on Day 3 (Figure 2I). HT resulted in significantly higher \( T_{leaf} \) at most times on Day 5 and at 1400 h on Day 3 compared to HC (Figure 3E and J). However, significantly higher \( T_{leaf} \) was observed in LT than in LC at 1000 h on Day 3 and 1400 h on Day 5 (Figure 2E and J).

Content of soluble sugars and starch in source leaves
The two low sink demand treatments, LT and HT, resulted in accumulation of soluble sugars and starch after initiating source–sink manipulation, compared with their controls (Figure 4). However, this effect was much more pronounced for starch (Figure 4A and F) than for all soluble sugars (Figure 4B–E and G–J). Starch gradually increased in LT and HT leaves, and a significant difference between HT and HC was found from Days 2 to 5 (Figure 4F) and between LT and LC on Days 3 and 5 (Figure 4A). A significant difference in sorbitol content between the low sink demand and control was observed on Day 5 in low-temperature (Figure 4B) and on Day 4 in high-temperature (Figure 4G) greenhouses. There were also significant differences in the content of other sugars between the low sink treatment and the control, e.g., in the low-temperature greenhouse for sucrose on Day 5 (Figure 4C) and for fructose on Day 3 (Figure 4E), and in the high-
Figure 4. Evolution of starch (A and F), sorbitol (B and G), sucrose (C and H), glucose (D and I) and fructose content (E and J) at midday in source leaves at low sink strength by removing root demand (open circles) and the control (filled circles) in young apple plants over 5 days after initiating treatments in low-temperature (left panels) and high-temperature (right panels) greenhouses. Vertical bars represent ±SE ($n = 3$), and * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively, between removed root demand and the control in each greenhouse.
Figure 5. Activity of adenosine diphosphate–glucose pyrophosphorylase (ADP–GPPase, A and G), amylase (B and H), aldose-6-phosphate reductase (A6PR, C and I), sorbitol dehydrogenase (SDH, D and J), sucrose phosphate synthase (SPS, E and K) and acid invertase (AI, F and L) at midday in source leaves of low sink strength by removing root demand (open circles) and the control (filled circles) in young apple trees in low-temperature (left panels) and high-temperature (right panels) greenhouses. Vertical bars represent ±SE (n = 3), and * indicates significant difference at P < 0.05 between removed root demand and the control.
Changes in enzyme activity related to carbohydrate metabolism were similar in leaves of low sink treatments and their controls in both low- and high-temperature greenhouses (Figure 5). There were no significant differences in the activities of any enzymes between low sink demand treatments and their controls, except that HT resulted in increased ADP–GPPase on Day 5 (Figure 5G) and decreased AI on Day 4 (Figure 5L) compared to HC.

Changes in chlorophyll fluorescence of source leaves

The response of chlorophyll fluorescence of source leaves to low sink demand in the low-temperature greenhouse differed from that in the high-temperature greenhouse on Day 5 (Figures 6 and 7). There were no differences in any parameters of chlorophyll fluorescence between LT and LC (Figures 6A–C and 7A–D). However, HT resulted in increased $F_o$ (Figure 6D) and NPQ (Figure 7H), but decreased $F_v/F_m$ (Figure 6E), $F_v/F_o$ (Figure 6F), $F_v^'/F_m^'$, $\Phi_{PSII}$ and qP (Figures 7E–G), compared to HC. Moreover, the differences between HT and HC were significant at most times of measurement during the day.

Discussion

Girdling is a standard method used in studies of the $P_n$ response to modified source–sink relationships, although it can result in damage to the xylem. However, Wu et al. (2008) showed that the effect of water transport from phloem removal had a negligible effect on $g_s$ and $E$ in their study of $P_n$ response to modified source–sink relationships in
well-irrigated peach trees. Hence, the girdling effect on root hydraulic conductivity should be negligible in the present study. In addition, some conditions, such as extreme high temperature in the high-temperature house and low CO2 concentration in both greenhouses might have been an important influence on $P_n$ and gas exchange responses. These conditions may explain, for example, why much higher $g_s$ were observed on Day 1 and then fell to a relatively constant value on the remaining days, and $P_n$ was highest on Day 1 but lower on all other days throughout the experiment in the low-temperature greenhouse (Figure 1). However, these different responses of $P_n$ and $g_s$ from one day to another might be ignored when studying the source–sink relationships carried out in the same greenhouse, other than that comparing $P_n$ and gas exchange responses of the different days or between the lower and higher temperature greenhouses.

Figure 7. Diurnal variation in efficiency of excitation capture by the open PSII reaction centers ($F_v'/F_m'$, A and E), actual PSII efficiency ($\Phi_{PSII}$, B and F), photochemical quenching coefficient ($qP$, C and G) and non-photochemical quenching coefficient (NPQ, D and H) at low sink strength by removing root demand (open circles) and the control (filled circles) in young apple trees 5 days after initiating treatments in low-temperature (left panels) and high-temperature (right panels) greenhouses. Vertical bars represent ±SE ($n = 4$), and *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, between removed root demand and the control.
Accumulation of end products in leaves is usually cited as a major regulatory process involved in direct feedback modulation of photosynthesis in source leaves under reduced sink demand (Herold 1980, Layne and Flore 1995). Consistent with this hypothesis, photosynthesis and the activity of related key biosynthetic enzymes should decrease when end products accumulate. Similar to the results of previous reports (Krapp and Stitt 1995, Myers et al. 1999, Zhou and Quebedeaux 2003, Urban et al. 2004, Vemmos 2005), low sink strength by removing root sinks increased starch and soluble carbohydrates compared with controls in trees in both greenhouses in the 5-day period of this study (Figure 4). However, the two low sink treatments, in both low- and high-temperature greenhouses, did not result in declining activity of enzymes involved in metabolism of end products, as measured in vitro (Figure 5). This indicates that the activity of the enzymes had at least similar potential under different sink–source relationships and ambient temperatures. Li et al. (2007) and DaMatta et al. (2008) obtained similar results in field-grown peach and coffee trees, respectively, showing no effect of decreased sink demand on the activity of related enzymes. Goldschmidt and Huber (1992) suggested that cycling of soluble sugars in the cytosol could result in down-regulation of the Calvin cycle that might affect some enzymes. Cheng et al. (2008) also indicated that decreased rates of photosynthesis resulting from limited sink demand were partly caused by a reduction of RuBCase activity; however, DaMatta et al. (2008) showed that RuBCase activity was unresponsive to source–sink manipulation in coffee trees. In any case, differences in \( P_n \) under low sink demand in both low- and high-temperature greenhouses should be independent of a direct effect of end-product feedback on down-regulation of the activity of enzymes related to end products. However, the influence of the accumulation of sugars in source leaves under low sink demand on Calvin cycle enzymes should be further studied.

Our previous work suggested that stomatal aperture might be the trigger in reduced \( P_n \) after sink strength was reduced in field-grown peach trees (Li et al. 2001, Li et al. 2005). Decreased \( g_s \), results in a lower flux of CO\(_2\) into stomata and reduced leaf \( E \), which results in increased leaf temperatures. The former leads to stomatal limitation (Cheng et al. 2008, Wu et al. 2008) and the latter to non-stomatal limitation of \( P_n \) if \( T_{leaf} \) is above a critical value (Li et al. 2005, 2007, Duan et al. 2008, Wu et al. 2008).

In this study, the response of \( P_n \) to decreased sink strength after removing root demand in young apple trees in the low-temperature greenhouse differed from that in the high-temperature greenhouse, although \( g_s \) and \( E \) tended to show a similar response in both greenhouses (Figure 1). The response of \( P_n \) was earlier and greater in the high-temperature greenhouse than in the low-temperature greenhouse. This difference in \( P_n \) response was also associated with \( C_i \), \( T_{leaf} \) and chlorophyll fluorescence parameters between the low- and high-temperature greenhouses. On Day 5 after initiating source–sink manipulation in the low-temperature greenhouse, decreased \( P_n \) in LT was associated with lower \( g_s \) and \( C_i \) (Figures 1D and 3D). Moreover, there were no differences in chlorophyll fluorescence parameters between LT and LC at the end of the experiment. These results suggest that the reduced \( P_n \) on Day 5 in the low-temperature greenhouse with weakened sink strength (by removing root demand) should be due to stomatal limitation.

As in the response of \( P_n \) in the high-temperature greenhouse, there was higher \( C_i \) in HT (Figure 2I) or no significant difference in \( C_i \) between HT and HC (Figure 1I). However, \( F_v/F_m \), \( F_o/F_m \), \( F_v/F_m' \), \( \Phi_{PSII} \) and \( qP \) in HT markedly decreased in parallel with increased \( F_o \) and NPQ compared with HC, indicating that reduced \( P_n \) in HT was mainly due to non-stomatal limitation. Increased \( T_{leaf} \) following decreased \( g_s \) and \( E \) should be the main factor in regulating \( P_n \) under the low sink treatment in the high-temperature greenhouse. First, optimum leaf temperature is very important to maintaining high \( P_n \). Sharp declines in \( P_n \) occur if leaf temperature exceeds an optimal level (Crews et al. 1975, Li et al. 2001, Wu et al. 2008). The response of \( P_n \) to \( T_{leaf} \) showed the sharp decrease if \( T_{leaf} \) was greater than a critical value (around 38 °C in this experiment), and the optimal \( T_{leaf} \) for photosynthesis of young ‘Gala’ apple trees was 29–36 °C (Figure 8). Second,
high $T_{\text{leaf}}$ should reduce the activity of related key biosynthetic enzymes for end products in vivo, although there were no significant differences in enzyme activity measured in vitro (Figure 5). In fact, enzyme activity should decrease under low sink demand in vitro due to the high $T_{\text{leaf}}$. Finally, NPQ of leaves at low sink demand increased only in the high-temperature greenhouse (Figure 7H). Thermal dissipation is a mechanism of the photosynthetic apparatus to prevent over-excitation of reaction centers (Ivanov and Edwards 2000). To prevent damage, NPQ (based on xanthophyll-dependent thermal dissipation) increased following increased $T_{\text{leaf}}$ (Duan et al. 2008). High $T_{\text{leaf}}$ leaves in the low sink treatment used a smaller fraction of the absorbed light in electron transport, and there was more thermal dissipation of excitation energy, thus removing excess excitation energy. All of the factors mentioned above may reduce the maximal efficiency of PSII photochemistry ($F_v/F_m$, Figure 6E). Consequently, lower quantum efficiency of PSII at low sink demand in the high-temperature greenhouse appeared ($\Phi_{\text{PSII}}$, Figure 7F) as a result of both decreases in the efficiency of excitation energy capture by open PSII reaction centers ($F_v'/F_m'$, Figure 7E) and in the photochemical quenching coefficient ($q_P$, Figure 7G).

In conclusion, regulating $P_n$ under low sink demand in young apple trees depended on air temperature in this 5-day study. In the low-temperature greenhouse, in which the highest daytime air temperature was maintained at $<32$ °C, low sink demand resulted in decreased $g_s$, $E$ and $C_i$, and lower $P_n$ was due to stomatal limitation. However, low sink demand in the high-temperature greenhouse (the highest daytime air temperature was $>36$ °C and even $>40$ °C on some days) led to decreased $g_s$ and $E$, but increased $T_{\text{leaf}}$. High $T_{\text{leaf}}$ following reduced $g_s$ and $E$ should be the most important factor for regulating $P_n$, and the reduced $P_n$ mainly resulted from non-stomatal limitation (Figure 9).

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