

Accumulation of End Products in Source Leaves Affects Photosynthetic Rate in Peach via Alteration of Stomatal Conductance and Photosynthetic Efficiency

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ADDITIONAL INDEX WORDS. *Prunus persica*, source-sink relationship, leaf temperature, fluorescence response, enzyme activities

ABSTRACT. In ‘Beijing 24’ peach [*Prunus persica* (L.) Batch] trees, a series of source leaves with differing levels of end products were created by retaining fruit (“+fruit”), removing fruit (“-fruit”), or reducing the light period. To alter the light period, leaves were covered with a bag made of brown inner paper and outer silver paper, which was then removed at different times the next day. The highest level of end products were obtained by fruit removal, while reducing the light period resulted in a lower level than “+fruit.” Net photosynthetic rate (P_n) and stomatal conductance (g_s) decreased, but leaf temperatures (T_{leaf}) increased, following an increase in end product levels in leaves. After the “-fruit” treatment, reduced P_n was correlated with lower g_s , and T_{leaf} increase was concomitant with decreases in maximal quantum yield of photosystem II (F_v/F_m), actual photochemical efficiency of photosystem II (Φ_{PSII}), and photochemical quenching, and with an increase in nonphotochemical quenching. However, there were no significant differences in chlorophyll fluorescence between “+fruit” and the two treatments reducing the light period. The Φ_{PSII} decreased following an increase in foliar sorbitol level, and it linearly decreased as sucrose and starch increased. Although fruit removal resulted in a significant accumulation of sucrose, sorbitol, and starch in leaves throughout the day, the extractable activities of several important enzymes involved in carbohydrate leaf storage and translocation did not decrease. Therefore, instead of feedback regulation by the accumulation of end products in source leaves, a high T_{leaf} induced by decreased stomatal aperture may play a key role in regulation of photosynthesis by limiting the photochemical efficiency of the PSII reaction centers under high levels of the end products in peach leaves.

Crop yield and fruit quality in fruit trees are highly dependent on efficient capture of solar energy and subsequent allocation of photoassimilate. Source-sink relationships are important factors influencing these allocation patterns. Fruit constitute the main sink for photoassimilates during the fruit growth period. Removing or retaining fruit has often been used in studies of plant source-sink relationships (Syvertsen et al., 2003; Vaast et al., 2005). Reduced photosynthetic rates under

low sink demand have often resulted in end product accumulation. This accumulation of end products has been often cited as the reason for the decline of P_n (Iglesias et al., 2002; Paul and Foyer, 2001; Wu et al., 2008; Zhou and Quebedeaux, 2003). This influence of end product accumulation has been considered a direct feedback mechanism regulating photosynthesis (Paul and Pellny, 2003). However, this conclusion is controversial (Eliezer and Huber, 1992; Stitt, 1991). For example, photosynthesis was not influenced in some species, although there was accumulation of end products in source leaves in response to low sink demand (Lunn and Hatch, 1995; Nautiyal et al., 1999; Roper et al., 1988). In addition, some recent studies with peach (Li et al., 2007) and coffee (*Coffea arabica* L.) trees (DaMatta et al., 2008) showed that low sink demand created by removing fruit reduced P_n with accumulation of the end products, but there was no influence on in vitro activity of key enzymes in the biosynthetic pathways of the end products.

Received for publication 3 Apr. 2009. Accepted for publication 10 Nov. 2009. The research was supported by the National Natural Science Foundation of China (No. 30370986) and by the Science Foundation of the President of the Chinese Academy of Sciences. We thank Prof. Douglas D. Archbold, Univ. of Kentucky, and Prof. W. Loescher, Michigan State Univ. for critical review of the manuscript.

We thank Yanfeng Xing (Director, Fruit Office, Pinggu District, Beijing, China) for the use of the experimental orchard facilities.

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However, there have been few studies on the influence of end product accumulation on photosynthetic efficiency and activities of related enzymes.

Source-sink relationships can be also modified by reducing or prolonging the light period. Reducing the light period decreased end product amount in source leaves, and this was similar to the effect of increasing sink demand (Li et al., 2007). By removing fruit and reducing the light period, respectively, it may be possible to create a range of source leaves with differing end product levels. This would be a valuable method for investigating the possible feedback effect of end product levels on photosynthetic efficiency and the relationship between the end products and activities of related enzymes.

A positive linear relationship was observed between stomatal conductance (g_s) and P_n in previous studies, showing that stomatal closure was an important factor in the decline in photosynthesis with low sink demand (Cheng et al., 2008; Li et al., 2001). Increased T_{leaf} following a reduction in stomatal aperture has been proposed as a critical factor in regulating photosynthesis under low sink demand in fruit trees (Li et al., 2005). These changes in T_{leaf} have also been related to reduced F_v/F_m (Jifon and Syvertsen, 2003). This article presents the results of studies on the short-term effect of end product accumulation on peach leaf P_n , focusing on $\Phi PSII$ and the activities of related enzymes. Also, the responses of P_n , g_s , and $\Phi PSII$ to end product levels in leaves were analyzed.

Materials and Methods

EXPERIMENTAL SITES AND MATERIALS. The experiment was conducted in Pinggu District, Beijing, China, during the 2006 growing season. The peach cultivar used was Beijing 24, which matures during early September. Trees, grafted on wild peach rootstocks, were planted 2 m apart within rows and 5 m apart between rows in Spring 1998. They were trained to a “Y” training system and were pruned by the long pruning method in winter (Li et al., 1994).

SOURCE-SINK MANIPULATION. During the final stage of rapid fruit growth (on 22 Aug. 2006), 1-year-old shoots located on the southwest and southeast sides of 15 to 20 trees in the outer part of the canopy were used as the units for source-sink manipulation. These previous-season shoots were 1.4 to 1.7 m above-ground, and were selected according to their uniformity in length (40–50 cm) and growth status (at least one new shoot > 30 cm).

Each 1-year-old shoot, supporting one fruit and one new shoot of 10 fully developed leaves retained by topping and removal of smaller basal leaves, was considered a plot. The export of assimilates was strictly controlled by girdling the base and top parts of the previous-season shoots to control the exchange of assimilates between treated and nonexperimental parts of the tree.

Four treatments were applied in this study: 1) “+fruit” treatment (control), 2) “-fruit” treatment, 3) a shoot supporting a fruit, covered with a bag made of brown inner paper and outer silver paper in the late afternoon of 22 Aug. with the bags removed at 0930 HR (“0930 HR bag removal”) on 23 Aug., and 4) a shoot supporting a fruit, covered with the same bag as used in the previous treatment, but with the bags removed at 1130 HR (“1130 HR bag removal”) on 23 Aug. A total of five plots for each treatment was used for the measurements of photosyn-

thetic parameters and chlorophyll fluorescence, and 15 to 20 plots were used for the measurements of sugar content and enzyme activities.

MEASUREMENT OF PHOTOSYNTHETIC PARAMETERS AND CHLOROPHYLL FLUORESCENCE. Photosynthetic gas exchange and chlorophyll fluorescence response of five leaves per treatment (one leaf per replicate) were measured between 0700 and 1700 HR on an hourly basis on 23 Aug. 2006, the day after initiating source-sink manipulation. For the “0930 HR bag removal” and “1130 HR bag removal” treatments, measurements were made 30 min after bag removal to allow for light adaptation by the leaves.

P_n was measured with a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE) and photosynthetically active radiation (PAR), g_s , transpiration rate (E), intercellular carbon dioxide concentration (C_i), and T_{leaf} were obtained simultaneously when P_n was measured.

Chlorophyll fluorescence parameters were measured with a plant efficiency analyzer [PEA (Hansatech, Norfolk, UK)] and a fluorescence monitoring system (FMS-2; Hansatech). After a 30-min dark adaptation period, minimum fluorescence (F_0), maximum fluorescence (F_m), the steady-state fluorescence (F_s), and F_v/F_m , were measured by the PEA. The PEA was also used to estimate the predawn value of F_m . The FMS-2 was used to determine the maximum fluorescence level in the light-adapted state (F_m'), the minimal fluorescence level in the light-adapted state (F_0'), and the efficiency of excitation capture by open PSII reaction centers (F_v'/F_m').

The following calculations were made: (1) photochemical quenching (qP) = $(F_m' - F_s)/(F_m' - F_0')$, and nonphotochemical quenching (NPQ) = $(F_m/F_m') - 1$; (2) $\Phi PSII$ = $(F_m' - F_s)/F_m'$ (Genty et al., 1989).

CARBOHYDRATE ANALYSIS AND ENZYME ASSAY. Leaves from five plots of each treatment were harvested every 2 h between 0700 and 1700 HR (for the bagging treatments, leaves were sampled starting at 1000 or 1200 HR, respectively), and the midrib of each leaf was removed. One half of the leaf was used for carbohydrate analysis and the other half was used for enzyme extraction and biochemical analyses. Leaf samples were frozen immediately in liquid nitrogen and were kept at -80°C until analysis.

For soluble carbohydrate extraction, about 1.5 g of fresh weight of leaves was ground to a fine powder in liquid nitrogen using a mortar and pestle. The powder was extracted three consecutive times in 10 mL of 80% ethanol in a water bath at 80°C for 10 min each time, and solutions were centrifuged (Sigma Laborzentrifugen, Osterode, Germany) at 19,118 g_n for 10 min after each extraction. The combined ethanol supernatants for each sample were evaporated to dryness in a water bath at 85°C , 1.5 mL of double distilled water was added, and samples were placed in a rotary shaker (THZ-C-1; Taicang Instrument, Jiangsu, China) for 1 h (4°C). The solution was then centrifuged at 19,118 g_n for 10 min, the supernatant was passed through a SEP-C18 cartridge (Supelclean ENVI C18 SPE; Supelco, Bellefonte, PA) and filtered through a 0.22- μm filter, and the filtered solution was taken for the measurement of soluble carbohydrates.

Sorbitol, glucose, fructose, and sucrose were separated by high-performance liquid chromatography [HPLC (Dionex, Sunnyvale, CA)], and they were detected using a Shodex RI-101 refractive index detector with reference cell maintained at 40°C (Dionex). A Transgenomic CARB Sep Coregel 87C

column (300 × 7.8 mm i.d., 10 μm particle size; Dionex) with a guard column cartridge was used. The column was maintained at 85 °C with a TCC-100 thermostatted column compartment (Dionex). Degassed, distilled, deionized water was used at a flow-rate of 0.6 mL·min⁻¹ as the mobile phase. The injection volume was 10 μL. The Chromeleon chromatography data system (Dionex) was used to integrate peak areas according to external standard solution calibrations (sugar reagents were purchased from Sigma-Aldrich, St. Louis). Starch remaining in the tissue was extracted and measured according to Li and Li (2005).

The enzyme extracts were obtained as follows: 1.0 g of frozen leaf tissue was ground to a fine powder in liquid nitrogen with quartz sand. Extraction buffer was added to the ground sample, and the slurry was ground to mix thoroughly. The extraction buffer contained 50 mmol·L⁻¹ N-2-hydroxyethylpiperazine-N-ethane-sulphonic acid [Hepes-NaOH (pH 7.5, Sigma-Aldrich)], 10 mmol·L⁻¹ magnesium chloride hexahydrate [MgCl₂ (Sigma-Aldrich)], 1.0 mmol·L⁻¹ disodium ethylenediamine tetraacetate [EDTANa₂ (Sigma-Aldrich)], 2.5 mmol·L⁻¹ dithiothreitol [DTT (Sigma-Aldrich)], 0.05% *t*-octylphenoxypolyoxyethanol (Triton X-100, Sigma-Aldrich), and 0.1% bovine serum albumin [BSA (Sigma-Aldrich)]. The homogenate was filtered through four layers of gauze, and the filtrate was centrifuged at 13,000 *g*_n for 10 min at 2 °C. The supernatant was dialyzed immediately by a 10-fold volume of diluted extraction buffer (minus Triton X-100) for 20 h at 4 °C, and the dialyzate was changed one time. In these analyses, leaves were not allowed to thaw before grinding in extraction buffer, and particular care was taken to work rapidly once the material had been taken up in buffer at 4 °C. The enzyme extracts after dialysis were used for all enzyme assays.

Aldose-6-phosphate reductase [A6PR (EC 1.1.1.200)] activity was assayed in the direction of synthesis following the oxidation of nicotinamide adenine dinucleotide phosphate [NADPH (reduced form, Sigma-Aldrich)] in the presence of glucose-6-P at 340 nm, as described by Merlo and Passera (1991). The reaction mixture contained 0.1 mol·L⁻¹ Tris (hydroxymethyl) aminomethane [Tris-HCl (pH 8.8, Sigma-Aldrich)], 0.1 mmol·L⁻¹ NADPH, 50 mmol·L⁻¹ glucose-6-P, and 50 μL of extract. One unit of A6PR is defined as the amount of enzyme catalyzing the oxidation of 1.0 μmol·min⁻¹ NADPH at 25 °C under standard assay conditions. The activity of sorbitol dehydrogenase [SDH (EC 1.1.1.14)] was assayed by following the reduction of nicotinamide adenine dinucleotide [NAD⁺ (Sigma-Aldrich)] in the presence of D-sorbitol at 340 nm, according to the method described by Negm and Loescher (1979). The reaction mixture contained 0.1 mol·L⁻¹ Tris-HCl (pH 9.0), 1.0 mmol·L⁻¹ NAD⁺, 0.5 mol·L⁻¹ D-sorbitol, and 0.1 mL of extract. Sorbitol and glucose-6-phosphate [G-6-P (Sigma-Aldrich)], dissolved in the same buffer as used in the assay, were used to start the reactions.

Sucrose-phosphate synthase [SPS (EC 2.4.1.14)] activity was determined by measuring the conversion rate of fructose-6-phosphate [F-6-P (Sigma-Aldrich)] to sucrose-phosphate (S-6-P; Sigma-Aldrich), according to the method described by Huber and Israel (1982) with some changes. The assay mixtures (140 μL) contained 50 mmol·L⁻¹ Hepes-NaOH (pH 7.5), 10 mmol·L⁻¹ MgCl₂, 5 mmol·L⁻¹ sodium fluoride (NaF), 25 mmol·L⁻¹ uridine 5'-diphosphoglucose disodium salt [UDPG (Sigma-Aldrich)], 10 mmol·L⁻¹ F-6-P, and 80 μL of extract. The reaction was stopped after 40 min at 25 °C by adding 140

μL of 1.0 mol·L⁻¹ NaOH. Unreacted F-6-P was destroyed by placing the tubes in boiling water for 10 min. After cooling, 0.5 mL of 0.1% resorcinol in 95% ethanol and 1.5 mL of 30% HCl were added, and the tubes were incubated at 80 °C for 8 min and A₅₂₀ was measured.

ADP glucose-pyrophosphorylase [ADPGPPase (EC 2.7.7.27)] activity was determined by measuring pyrophosphate-dependent glucose-1-phosphate formation from adenosine 5'-diphosphoglucose disodium (ADPG; Sigma-Aldrich) at 25 °C in a total of 1 mL of solution, according to the procedures described by Rufty et al. (1983). The reaction was started by addition of ADPG to a final concentration of 5 mmol·L⁻¹ and production of NADPH was monitored at 340 nm.

The activities of acid invertase [AI (EC 3.2.1.26)] and neutral invertase [NI (EC 3.2.1.26)] were assayed in a reaction mixture (1 mL) containing 0.1 mol·L⁻¹ Na-acetate (pH 4.8), 0.1 mol·L⁻¹ sucrose, and 0.2 mL of extract. After incubation of the mixture for 40 min at 37 °C, the reactions were stopped by addition of 1 mL of dinitrosalicylic acid reagent (DNS; Beijing Chemical Reagent Corporation, Beijing, China), and reducing sugars released from sucrose were determined according to the method of Li and Li (2005). Total amylase activity [(EC 3.1.1.1) and (EC 3.1.1.2)] was assayed in a reaction mixture (1 mL) containing 0.1 mol·L⁻¹ Na-acetate (pH 6.5), 1.5 mmol·L⁻¹ NaF, 5 mmol·L⁻¹ Ca (NO₃)₂, 0.5% soluble starch, and 0.2 mL of extract. After incubation at 30 °C for 40 min, the reaction was ended, and the release of reducing groups was determined as in the invertase assay. In invertase and amylase assays, blanks containing reaction mixtures were incubated with DNS.

DATA ANALYSIS. A completely randomized design was used with five replicates for each treatment. The data were subjected to analysis of variance (ANOVA), and means were compared by Duncan's new multiple range test or *t* test (when there were only two groups) using SPSS (version 13.0 for Windows; SPSS, Chicago). Each value of the mean and standard error in the figures represents five replicates of each treatment. Unless otherwise indicated, significant differences among means are given at *P* < 0.05.

Results

GAS EXCHANGE. Fruit removal significantly reduced P_n from 1000 to 1500 HR and g_s from 9000 to 1600 HR, respectively, compared with the "+ fruit" and after removing the bags covering leaves (Fig. 1, A and B). Moreover, the two "bag removal" treatments resulted in significantly higher P_n and g_s from 1300 to 1500 HR than "+ fruit." A significantly lower E was observed at 0900 HR and 1200 to 1600 HR in the "-fruit" treatment than the other three treatments. However, there were no significant differences in E between "+ fruit" and the two "bag removal" treatments (Fig. 1C).

Opposite to the effect on P_n, g_s and E, fruit removal significantly increased T_{leaf} from 1100 to 1500 HR in general compared with the other three treatments. Moreover, a lower T_{leaf} was observed for "bag removal" treatments at 1100, 1300, and 1500 HR than "+ fruit" (Fig. 1D).

C_i changed slowly from 0700 to 1300 HR except for the "-fruit" treatment. For the "-fruit" treatment, the highest C_i appeared at 1400 HR and then decreased gradually. However, no significant differences in C_i were found among the treatments until 1400 HR. The C_i of the "-fruit" treatment was significantly

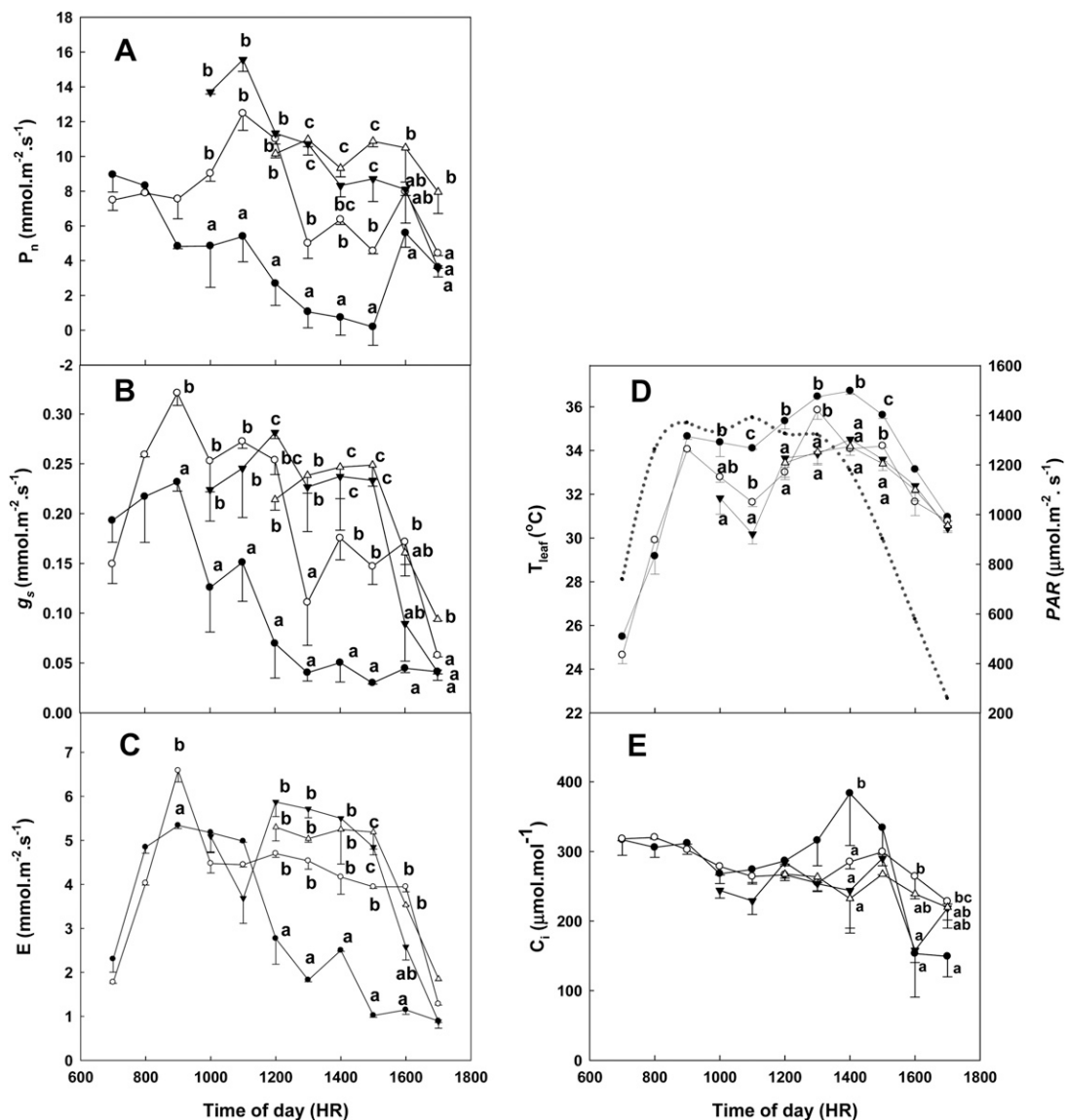


Fig. 1. (A) Diurnal net photosynthetic rate (P_n), (B) stomatal conductance (g_s), (C) transpiration (E), (D) leaf temperature (T_{leaf}) and photosynthetically active radiation (PAR), and (E) intercellular carbon dioxide concentration (C_i) in response to fruit removal (“-fruit”, ●—), “bag removal at 0930 HR” of fruiting shoots (—▼—), “bag removal at 1130 HR” of fruiting shoots (—△—), and fruit-bearing shoots (“+fruit”, —○—) in ‘Beijing 24’ peach trees. The vertical bar represents \pm SE of each mean ($n = 5$). Means with different letters at the same time point in the figure are significantly different among the treatments at $P < 0.05$ based on Duncan’s new multiple range test or t test (when there were only two treatments).

higher than those of other treatments at 1400 HR, but was lower than other treatments at 1600 and 1700 HR (Fig. 1E).

CHLOROPHYLL FLUORESCENCE. The “-fruit” treatment significantly decreased F_v/F_m (Fig. 2A) from 1100 or 1200 HR to the end of the day, Φ PSII (Fig. 2B) at 0900, 1100, and 1400 to 1600 HR, qP (Fig. 2C) at 1000 to 1400 HR, and F_v'/F_m' at 1400 HR (Fig. 2D), but significantly increased F_0 (Fig. 2E) from 1100 HR to the end of the day and NPQ (Fig. 2F) at 1300 to 1700 HR compared with the other three treatments. However, except for Φ PSII and qP , the other chlorophyll fluorescence parameters showed no response in general to “+fruit” and the two “bag removal” treatments. Φ PSII and qP of the “+fruit” treatment were significantly higher than that of the “930 bag removal” treatment at 1100 HR and 1000 to 1100 HR, respectively (Fig. 2, E and F).

SOLUBLE SUGARS AND STARCH. Fruit removal and “bag removal” treatments altered the content of soluble sugars and starch

in leaves (Fig. 3). The “-fruit” generally resulted in the highest foliar content of soluble sugars and starch among the treatments, and the contents of soluble sugars and starch in “+fruit” leaves were higher than those in the two ‘bag removal’ treatments. Moreover, the later time of bag removal lowered the content of soluble sugars and starch more than the earlier removal time. The differences in sorbitol and starch content among the treatments remained unchanged throughout the day generally (Fig. 3, A and C). As regards sucrose, fructose and glucose content in the leaves, the magnitude of the differences among treatments sharply decreased after 1300 or 1500 HR (Fig. 3, B, D, and E).

ACTIVITIES OF RELATED ENZYMES OF CARBOHYDRATE METABOLISM. Compared with “+fruit”, “-fruit” significantly increased A6PR and SDH activities at 1100 HR and from 0900 to 1100 HR, respectively (Fig. 4). However, “bag removal” treatments did not alter A6PR and SDH activities. As regards

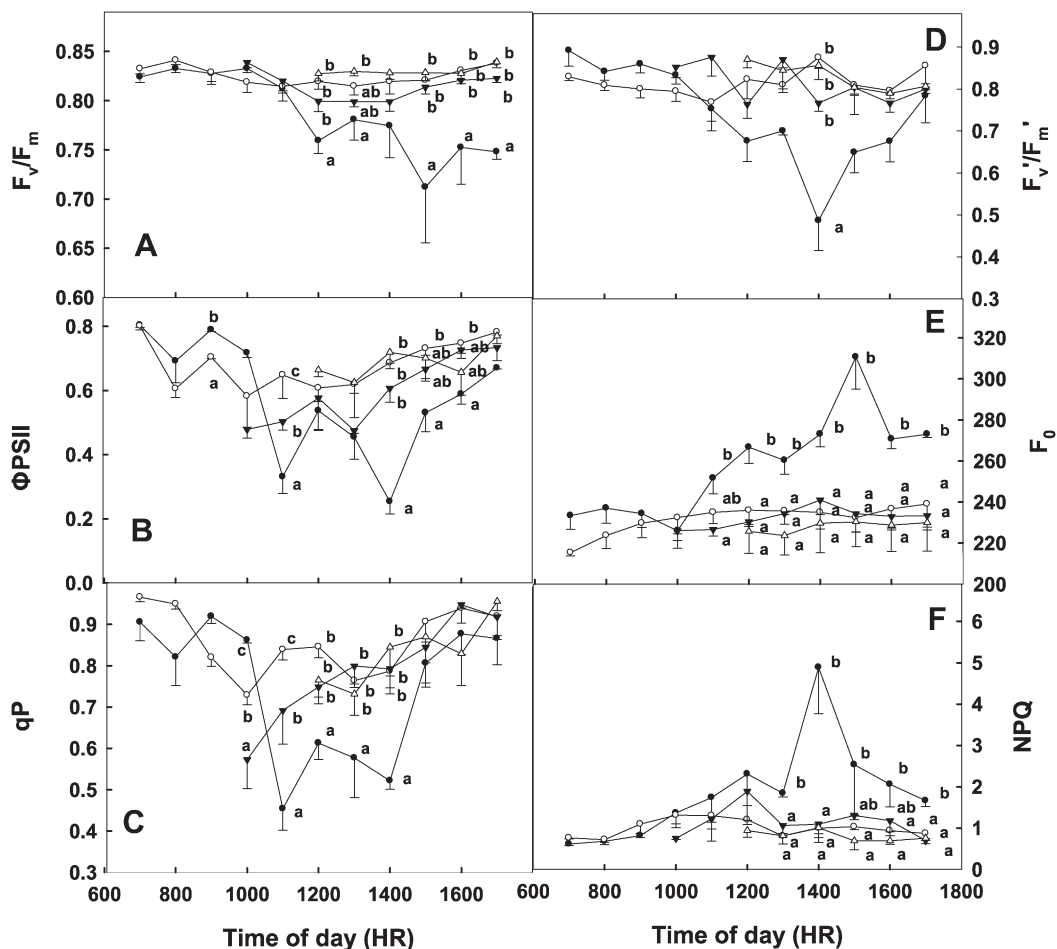


Fig. 2. Diurnal changes in (A) maximal quantum yield of photosystem II (F_v/F_m), (B) actual photochemical efficiency of photosystem II (Φ_{PSII}), (C) photochemical quenching (qP), (D) actual quantum yield of photosystem II (F_v/F_m'), (E) minimal fluorescence level after dark-adaptation (F_0), and (F) nonphotochemical quenching (NPQ) in response to fruit removal (“-fruit”, —●—), “bag removal at 0930 HR” of fruiting shoots (—▼—), “bag removal at 1130 HR” of fruiting shoots (—△—), and fruit-bearing shoots (“+fruit”, —○—) in ‘Beijing 24’ peach trees. The vertical bar represents \pm SE of each mean ($n = 5$). Means with different letters at the same time point in the figure are significantly different among the treatments at $P < 0.05$ based on Duncan’s new multiple range test or t test (when there were only two treatments).

the activities of the other related enzymes of carbohydrate metabolism, no obvious effect of the “-fruit” and “bag removal” treatments were observed (data not shown).

RESPONSE OF P_n TO G_s AND T_{leaf} . P_n increased with increased T_{leaf} until about 30 °C, remained high between 30 and 34 °C, and then sharply decreased when T_{leaf} continued to increase (Fig. 5A). Moreover, when T_{leaf} was greater than a critical temperature of about 34 °C, P_n was significantly correlated with g_s ($r = 0.79^{***}$) (Fig. 5B).

RESPONSE OF P_n , G_s , Φ_{PSII} , AND NPQ TO FOLIAR END PRODUCT AMOUNT. There were significant relationships between P_n and foliar amounts of end products including sorbitol, sucrose, and starch (Fig. 6). P_n decreased following an increase in sorbitol, sucrose, and starch content, indicating that the P_n change was somehow affected by their accumulation. The accumulation of the end products was also correlated to g_s (Fig. 7). A high content of sorbitol, sucrose, and starch in leaves was related to lower g_s , implied that high content of end products changed g_s , presumably by altering osmotic pressure in gourd cell or signal transduction (Fig. 7, A–C), and then lower g_s may have resulted in P_n decrease. However, significant relationships were only found between g_s and foliar content in starch.

The response of Φ_{PSII} to end product amount is shown in Fig. 8. When foliar sorbitol content increased, Φ_{PSII} decreased (Fig. 8A). Φ_{PSII} also decreased linearly with the sucrose increase (Fig. 8B). However, a relationship between Φ_{PSII} and starch content was not apparent (Fig. 8C).

Opposite to Φ_{PSII} , NPQ increased following an increase in foliar sorbitol content (Fig. 9A), and NPQ was significantly correlated with sucrose and starch content (Fig. 9, B and C).

Discussion

Sorbitol, sucrose, and starch are the end products of photosynthesis in many Rosaceae species (Loescher, 1987). In this study, fruit removal resulted in accumulation of end products in leaves, while reducing the light period (“bag removal” treatments) decreased end product levels (Fig. 3). The short-term effects of weakening sink strength by fruit removal in the present study significantly decreased P_n . By contrast, removal of the bags covering the leaves had an effect similar to increasing sink strength, resulting in increased P_n (Fig. 1A). Those effects of varying sink demand on P_n , g_s , and T_{leaf} were similar to those reported in peach trees by Li et al. (2007) and Wu et al. (2008).

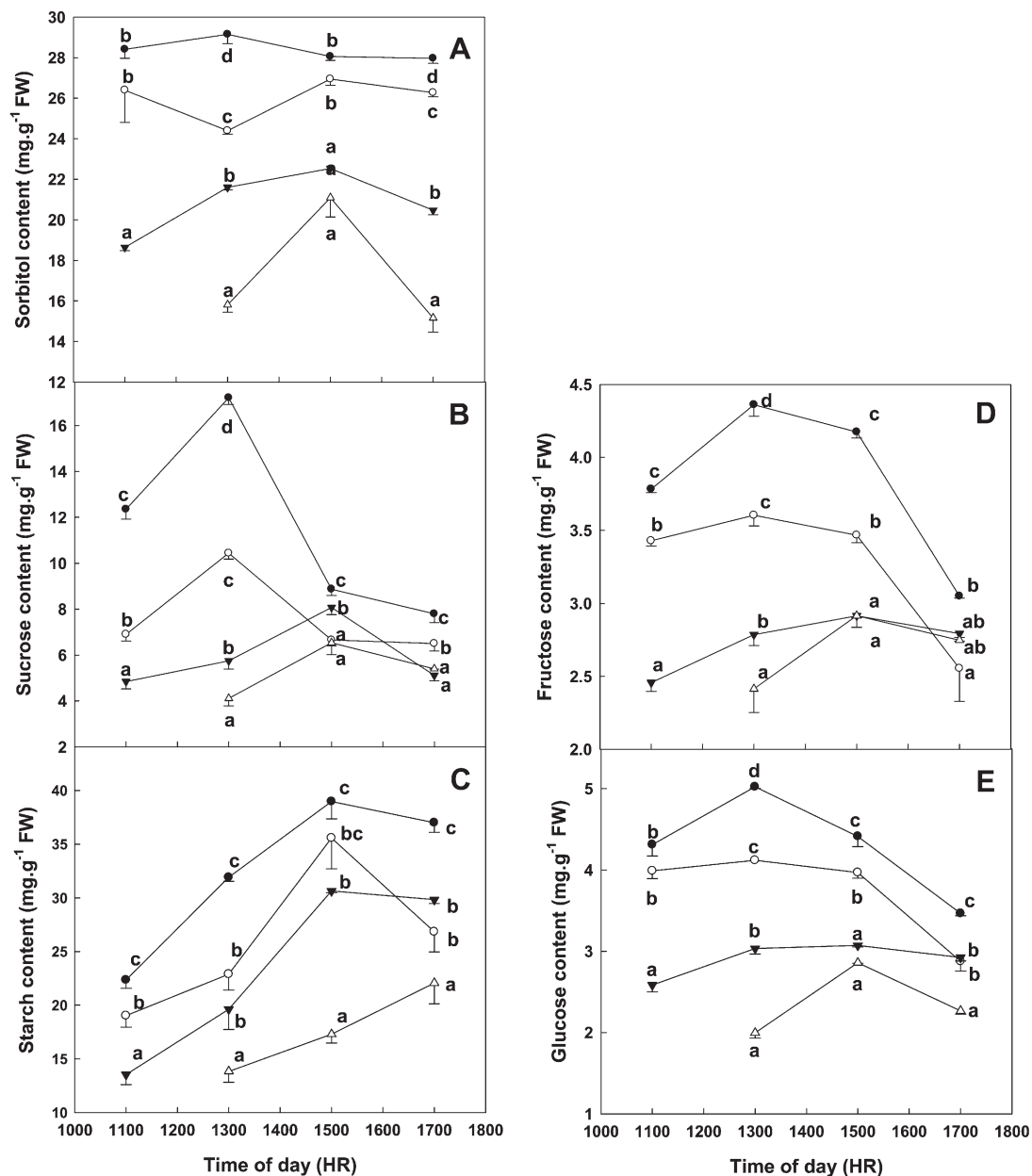


Fig. 3. Diurnal changes in foliar content of (A) sorbitol, (B) sucrose, (C) starch, (D) fructose, and (E) glucose in response to fruit removal (“-fruit”, —●—), “bag removal at 0930 HR” of fruiting shoots (—▼—), “bag removal at 1130 HR” of fruiting shoots (—△—), and fruit-bearing shoots (“+fruit”, —○—) in ‘Beijing 24’ peach trees. Data were recorded on a fresh weight basis (mg.g⁻¹). The vertical bar represents ±SE of each mean (n = 5). Means with different letters at the same time point in the figure are significantly different among the treatments at *P* < 0.05 based on Duncan’s new multiple range test.

P_n was related to the foliar amounts of the three end products in this study, decreasing as foliar end product levels increased (Fig. 6). Accumulation of end products in leaves is usually cited as the principal reason for lower photosynthesis under reduced sink demand, as a direct feedback mechanism (Iglesias et al., 2002; Layne and Flore, 1995). Consistent with this hypothesis, photosynthesis and the activity of related key biosynthetic enzymes should be depressed by end product accumulation with low sink demand (“-fruit”) or enhanced when end products are low (“bag removal”). However, the present results indicate that key extractable enzyme activities did not change correspondingly. These enzyme activities did not decrease, and A6PR and SDH even increased at certain times during the day

with low sink demand and high end product content (Fig. 4). In prior studies of peach (Li et al., 2007) and coffee (DaMatta et al., 2008), differences in P_n also seemed unlikely to have been caused by photochemical impairments or a direct end product-mediated feedback down-regulation of photosynthesis. Given that *in vitro* enzyme activities were not influenced by foliar end product content, the modified photosynthetic rates created by source-sink manipulation may therefore be regulated by a mechanism other than direct feedback effects on related biosynthetic enzymes.

There was a positive linear relationship between g_s and P_n in peach in previous studies (DeJong, 1986; Li et al., 2005, 2007; Tan and Buttery, 1986), as in the present study (Fig. 5B). Li

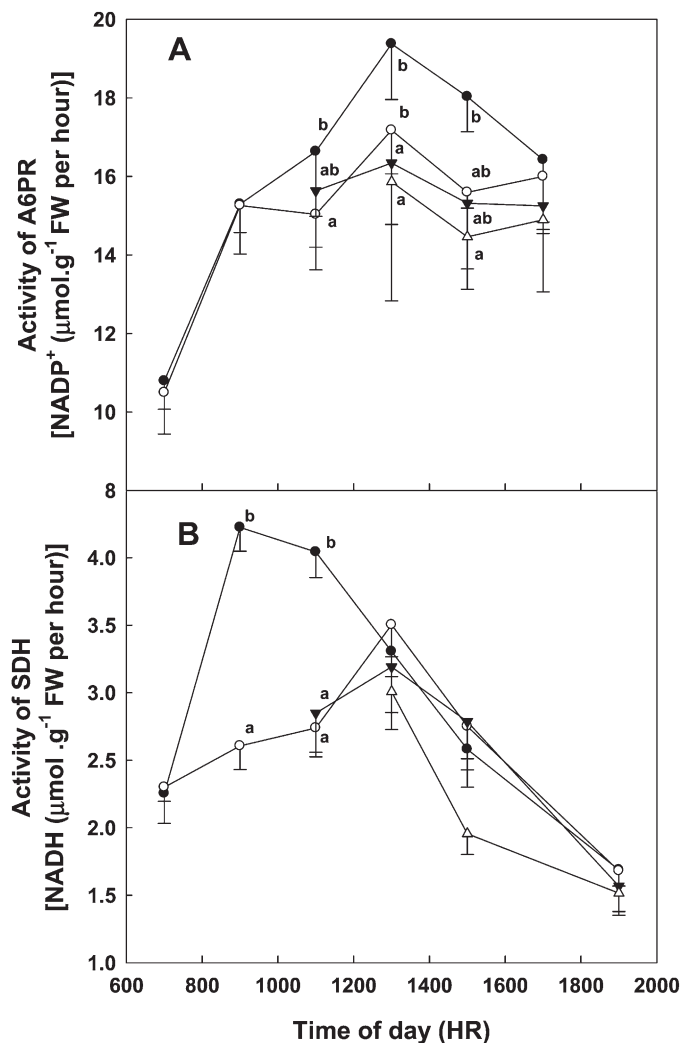


Fig. 4. Diurnal changes in activities of (A) aldose-6-phosphate reductase [NADP⁺ (mmol·g⁻¹ FW per hour)] (A6PR) and (B) sorbitol dehydrogenase [NADH (mmol·g⁻¹ FW per hour)] (SDH) in response to fruit removal (“-fruit”, —●—), “bag removal at 0930 HR” of fruiting shoots (—▼—), “bag removal at 1130 HR” of fruiting shoots (—△—), and fruit-bearing shoots (“+fruit”, —○—) in ‘Beijing 24’ peach trees. Data were recorded on a fresh weight basis. The vertical bar represents ±SE of each mean (n = 5). Means with different letters at the same time point in the figure are significantly different among the treatments at *P* < 0.05 based on Duncan’s new multiple range test or *t* test (when there were only two treatments).

et al. (2001) suggested that stomatal aperture may be the trigger or promoter and leaf temperature the critical factor regulating photosynthesis when modifying source-sink relationships. In the present study, the different sink demands affected g_s and E (Fig. 1, B and C). g_s was negatively correlated with foliar end product content, especially that of starch (Fig. 7, A–C). Low g_s decreases leaf transpiration and therefore increases leaf temperature, which would influence photosynthesis (Li et al., 2001; Li et al., 2005). In the “-fruit” treatment, the high T_{leaf} was above the optimal level for the photosynthesis (Fig. 5A). High T_{leaf} results in damage to the photosynthesis apparatus and cell structure by the generation and accumulation of reactive oxygen species such as hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) (Duan et al., 2008). Damage to the photosynthetic apparatus and cell structure was confirmed by chlorophyll fluorescence results. Low sink demand by fruit

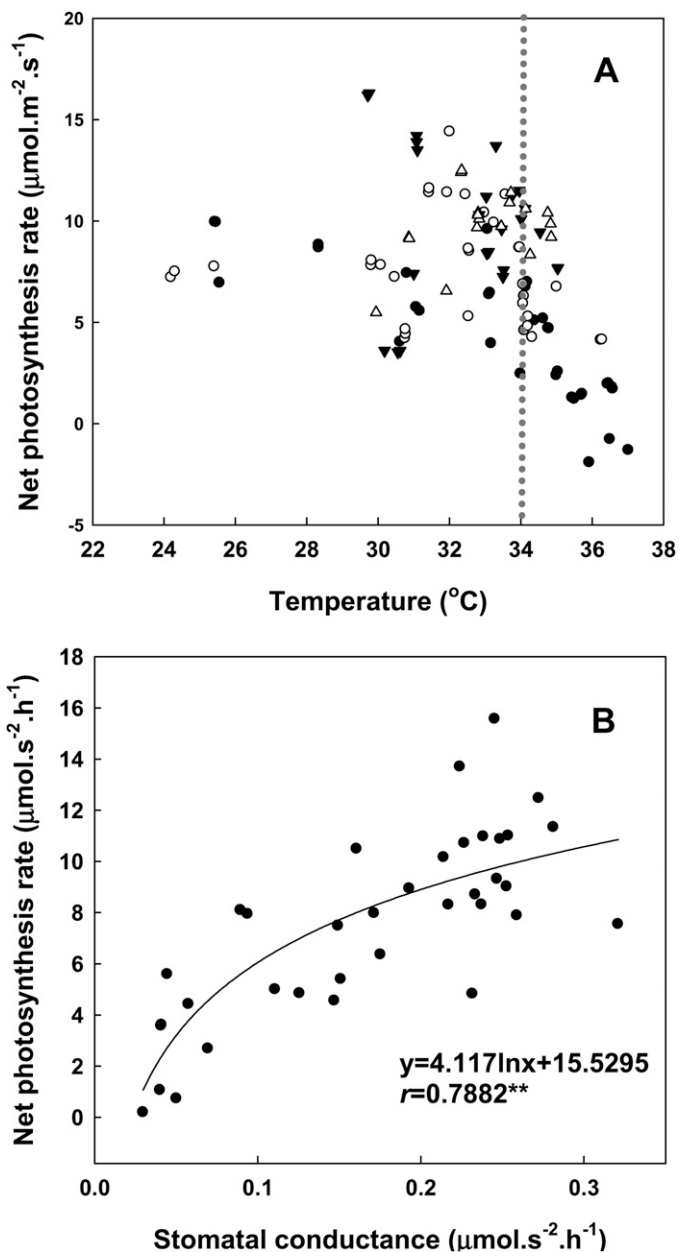


Fig. 5. Net photosynthetic rate (P_n) in response to (A) leaf temperature (T_{leaf}), and (B) g_s when leaf temperature exceeded 34 °C in ‘Beijing 24’ peach trees. Symbols in A indicate fruit removal (“-fruit”, —●—), “bag removal at 0930 HR” of fruiting shoots (—▼—), “bag removal at 1130 HR” of fruiting shoots (—△—), and fruit-bearing shoots (“+fruit”, —○—); ** indicates significant correlation at *P* < 0.01.

removal increased F_0 throughout the day when compared with the “+fruit” and “bag removal” treatments (Fig. 2E), indicating damage to the PSII reaction center complex. F_v/F_m represents electron transport efficiency and photochemical efficiency in the PSII reaction center. Layne and Flore (1995) reported that continuous lighting significantly reduced F_v and F_v/F_m within 1 d in sour cherry (*Prunus cerasus* L.). The decline in F_v/F_m observed in “-fruit” leaves (Fig. 2A) was presumably related to increased dissipation of excess energy.

P_n was significantly correlated with g_s (Fig. 5B), and the latter was correlated with end product levels (Fig. 7), indicating that end product accumulation may affect P_n via altering g_s . In

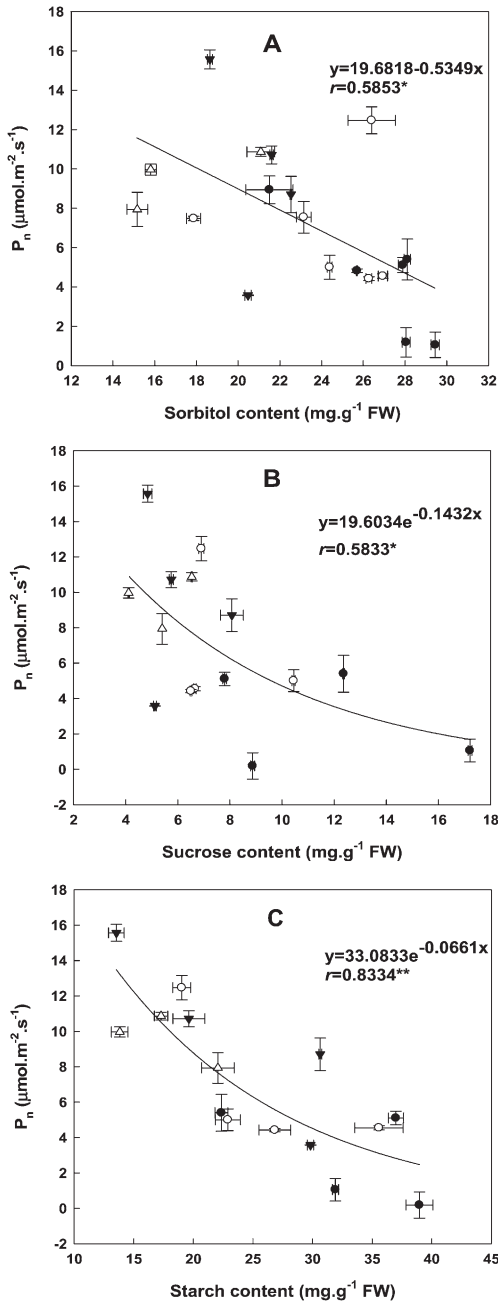


Fig. 6. Response of net photosynthetic rate (P_n) to foliar content of (A) sorbitol, (B) sucrose, and (C) starch in 'Beijing 24' peach trees. Symbols indicate fruit removal ("–fruit", —●—), "bag removal at 0930 HR" of fruiting shoots (—▼—), "bag removal at 1130 HR" of fruiting shoots (—△—), and fruit-bearing shoots ("++fruit", —○—). End product content data were recorded on a fresh weight basis ($\text{mg}\cdot\text{g}^{-1}$ FW). Each point is mean \pm SE for foliar content of individual end products (horizontal, $n = 5$) and P_n (vertical, $n = 5$); * or ** indicate significant correlation at $P < 0.05$ or $P < 0.01$, respectively.

addition, end product accumulation may also affect photochemical efficiency. ΦPSII decreased as end products increased (Fig. 8), while NPQ increased (Fig. 9). High P_n at low end product content could be explained by high ΦPSII and low NPQ. ΦPSII is the product of qP and F_v'/F_m' in open PSII centers (Genty et al., 1989), so increased ΦPSII could be due to an increase in photochemical quenching and F_v'/F_m' . NPQ has usually been used as an indicator of the appearance in the

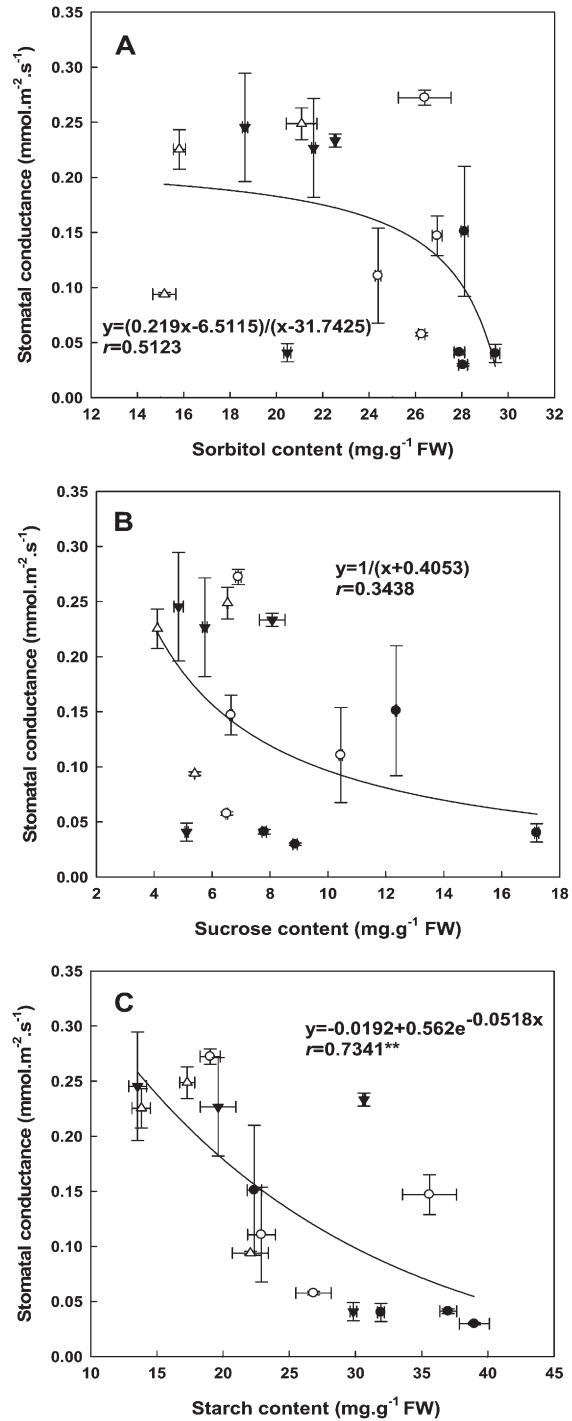


Fig. 7. Stomatal conductance (g_s) response to foliar content of (A) sorbitol, (B) sucrose, and (C) starch in 'Beijing 24' peach trees. Symbols indicate fruit removal ("–fruit", —●—), "bag removal at 0930 HR" of fruiting shoots (—▼—), "bag removal at 1130 HR" of fruiting shoots (—△—), and fruit-bearing shoots ("++fruit", —○—). End product content data were recorded on a fresh weight basis ($\text{mg}\cdot\text{g}^{-1}$ FW). Each point is mean \pm SE for foliar content of individual end products (horizontal, $n = 5$) and g_s (vertical, $n = 5$); ** indicates significant correlation at $P < 0.01$.

photosynthetic apparatus of mechanisms to prevent overexcitation of reaction centers (Ivanov and Edwards, 2000). Leaves with lower end product levels had higher photosynthetic capacity, so they use a bigger fraction of the absorbed light in

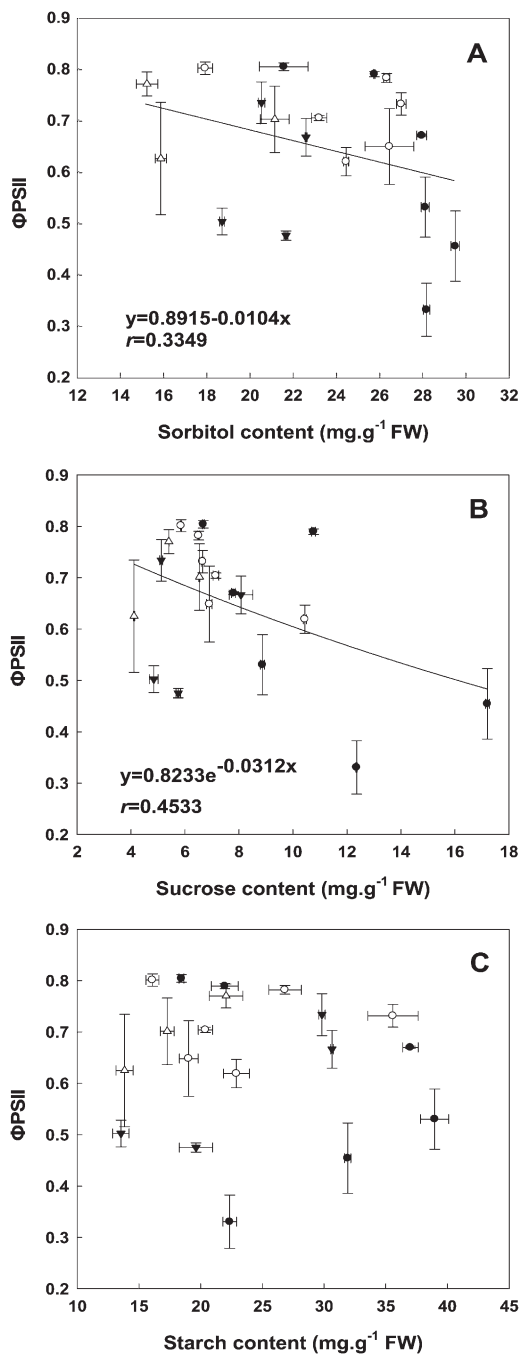


Fig. 8. Actual photochemical efficiency of PSII in response to foliar content of (A) sorbitol, (B) sucrose, and (C) starch in 'Beijing 24' peach trees. Symbols indicate fruit removal ("–fruit", —●—), "bag removal at 0930 HR" of fruiting shoots (—▼—), "bag removal at 1130 HR" of fruiting shoots (—△—), and fruit-bearing shoots ("+fruit", —○—). End product content data were recorded on a fresh weight basis ($\text{mg}\cdot\text{g}^{-1}$). Each point is mean \pm SE for foliar content of individual end product (horizontal, $n=5$) and ΦPSII (vertical, $n=5$).

electron transport. As a result, there was less thermal dissipation of excitation energy.

In conclusion, accumulation of end products in leaves decreased P_n , g_s , and E , but increased T_{leaf} in peach leaves. However, high foliar end product content had no direct feedback effect on the activities of related enzymes of carbon metabolism *in vitro*. Thus, changes in P_n in response to accumulation of

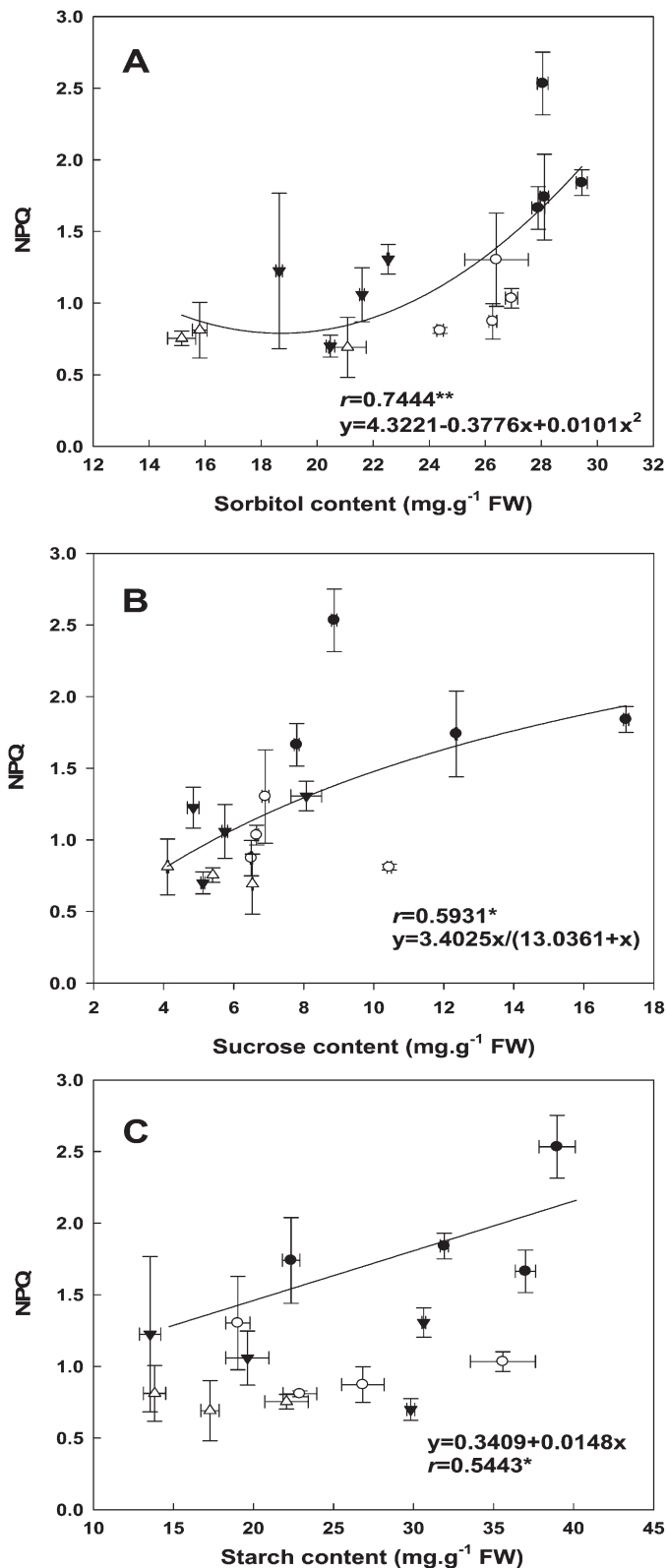


Fig. 9. Response of nonphotochemical quenching (NPQ) to foliar content of (A) sorbitol, (B) sucrose, and (C) starch in 'Beijing 24' peach trees. Symbols indicate to fruit removal ("–fruit", —●—), "bag removal at 0930 HR" of fruiting shoots (—▼—), "bag removal at 1130 HR" of fruiting shoots (—△—), and fruit-bearing shoots ("+fruit", —○—). End product content data were recorded on a fresh weight basis ($\text{mg}\cdot\text{g}^{-1}$). Each point is mean \pm SE for foliar content of individual end product (horizontal, $n=5$) and NPQ (vertical, $n=5$); * or ** indicate significant correlation at $P < 0.05$ or $P < 0.01$, respectively.

end products may result from a negative effect on photosynthetic efficiency. Stomatal aperture may start to close as the initial response and thus affect P_n . Accumulated soluble sugar and starch in leaves may change osmotic pressure of the guard cells, resulting in such a g_s change. End products may also act as signals, with crosstalk with phytohormones such as abscisic acid (ABA) (Gibson, 2005; Lloyd and Zakhleniuk, 2004; Roitsch, 1999). End product accumulation may also affect photochemical efficiency, changing F_0 , F_v/F_m , and Φ_{PSII} . However, it will be necessary to focus on the mechanism of end product alteration of stomatal movement for understanding the effect on P_n . Also, the possibility that accumulation of end products may down-regulate photosynthesis in peach leaves by deactivating some of the key enzymes in carbon fixation, such as Rubisco, should be studied in the future.

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