Trees (2016) 30:1523–1534
DOI 10.1007/s00468-016-1386-5

ORIGINAL ARTICLE

Photosynthetic characteristics of leaves and fruits of Hickory (*Carya cathayensis* Sarg.) and Pecan (*Carya illinoensis* K.Koch) during fruit development stages

Qinyi Xu[1,2] · Jianfeng Wu[1,2] · Yirun Cao[2] · Xianyou Yang[1,2] · Zhengjia Wang[1,2] · Jianqin Huang[1,2] · Guohua Xia[1,2] · Qixiang Zhang[1,2] · Yuanyuan Hu[1,2]

Received: 7 December 2015 / Accepted: 7 March 2016 / Published online: 23 March 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Key message **Fruit photosynthesis in both hickory and pecan significantly contribute to the carbon requirements of late growth stage (corresponding to seed development).**

Abstract Plant parts other than leaves can perform photosynthesis and contribute to carbon acquisition for fruit development. To determine the role of fruit photosynthesis in fruit carbon acquisition in hickory (*Carya cathayensis* Sarg.) and pecan (*Carya illinoensis* K.Koch), we studied changes in dry mass, surface area and CO_2 exchange rate in these fruits during fruit development. Fruit development was divided into two phases: phase one involves the rapid increase of fruit size (from 0 to 59 days after pollination (DAP) for hickory; from 0 to 88 DAP for pecan); phase two involves seed development (from 59 to 121 DAP for hickory; from 88 to 155 DAP for pecan). The net photosynthetic rate (P_n) in hickory leaves decreased by 48.5 % from 76 to 88 DAP, while the P_n in pecan leaves decreased by 32.3 % from 88 to 123 DAP. The gross photosynthetic rate (P_g) in hickory fruit was significantly greater than that of the leaf during the late stage (88 to 121 DAP) of fruit development. Pecan fruit had a significantly higher P_g than leaves during ontogeny. The contribution of fruit photosynthesis to fruit carbon requirements increased during fruit development, which was estimated by the gross fruit photosynthesis divided by respiration and increased dry mass. The contribution of fruit photosynthesis to pecan carbon requirements was significantly greater than that of hickory. Fruit photosynthesis in both hickory and pecan significantly contribute to the carbon requirements of late growth stage.

Keywords Different growth stages · Fruit photosynthesis · Surface area · Carbon requirement

Introduction

Hickory (*Carya cathayensis* Sarg.) and pecan (*Carya illinoensis* K.Koch), belonging to the Juglandaceae family, are important and widely planted crops in Zhejiang and Anhui provinces. Hickory is popular because its seeds have a high nutritional value, good taste, and a unique flavor. The seeds of hickory are relatively small (length 35.4 ± 1.04 mm; width 31.1 ± 2.25 mm), but pecan seeds are large (length 40.4 ± 2.13 mm; width 36.0 ± 0.85 mm). Pecan, which originated in America, has been cultivated in large areas of China because of its wide adaptability and its suitability. The market demand for hickory and pecan nuts now exceeds the supply. Therefore, increasing the nut yields of hickory and pecan tree is would be very useful for the *Carya* industry.

Although green leaves are the main sites of photosynthate production, other parts of plants, such as greenish flowers, stem or developing fruits can be photosynthetically active (Blanke and Lenz 1989; Ogawa et al. 1995; Weiss et al. 1988; Kocurek et al. 2015). Photosynthesis of non-foliar green plant tissue can provide an important additional contribution to carbon requirements (Aschan and Pfanz 2003).

Communicated by D. Treutter.

✉ Yuanyuan Hu
hyy_1985@zafu.edu.cn

[1] The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, Zhejiang 311300, China

[2] School of Forestry and Biotechnology, Zhejiang A&F University, Lin'an, Zhejiang 311300, China

Analysis of the time-course of photosynthetic rate in both leaves and fruits has the potential to quantitatively reveal temporal changes in carbon requirements for fruit development (Hieke et al. 2002; Imai and Ogawa 2009). For example, *Lychee* fruit contribute about 3 % of the total carbon requirement during fruit development (Hieke et al. 2002). Many studies demonstrate a significant photosynthetic contribution by developing fruits to their own carbon requirement for growth and maintenance (Pavel and DeJong 1993; Blanke and Whiley 1995; Marcelis and Hofman-Eijer 1995).

Although the photosynthetic characteristics of leaves of hickory and pecan have been studied (Crews et al. 1980; Li et al. 2007; Lombardini et al. 2009; Huang et al. 2011; Ling et al. 2014), there is little information about the photosynthesis of fruits. The fruits of hickory and pecan are light green or green before maturity, and contain chlorophyll which can capture light energy to perform photosynthesis. In this study, we investigated the photosynthetic characteristics of hickory and pecan fruit and document how they contribute to the carbon requirements during the fruit development stages. We determined the changes in the surface area and dry/fresh mass ratio of fruits of hickory and pecan during the different days after pollination (DAP). We also studied photosynthetic light response curves in leaves and fruits of hickory and pecan during fruit development. We estimated the contribution of fruit photosynthesis to fruit carbon requirement using the gross total photosynthesis, total respiration, and dry mass of fruits. The results will help provide a theoretical basis for achieving higher yields using fruit photosynthesis.

Materials and methods

Plant materials

Six hickory (*Carya cathayensis* Sarg.) grafting trees (2-year hu-nan hickory as rootstock and 1 year hickory as scion grafting in 2008, began bearing fruit since 2012) were studied at Zhejiang Agricultural and Forestry University, Lin'an, China (30°12′N, 119°20′E). Six pecan (*Carya illinoensis* K.Koch) trees (2-year pecan as rootstock and 1 year ''mahan" pecan as scion grafting in 2007) were studied at Lianhua Village, Jiande, Zhejiang province, China (29°29′N, 119°22′E). All trees were grown using standard practices. Blooming season of both 'Hickory' and 'Pecan' occurred from late April to early May. The male inflorescences were removed before pollen dispersal. Female inflorescences were hand pollinated on May 1st (hickory) and May 3rd (pecan) in 2014. Pollen of hickory and pecan was collected from Linlong Mountain in Lin'an city. Prior to experiment initiation, fruits of 'hickory' and 'pecan' were thinned to one fruit per cluster and labelled on June 5th (hickory) and July 5th (pecan) in 2014. Materials for the measurements of gas exchange and the other physiological parameters were collected randomly from the outer part of the crown at a height of around 1.5–2.0 m. Experiments were conducted from May 1st to August 28th (hickory) and May 3rd to October 5th (pecan).

Surface area and biomass

The length (L), width (W) and thickness (T) of fruits were measured with a digital caliper. According to Jindal and Mohsenin (1978), the geometric average diameter (Dg) can be expressed as:

$$Dg = (\mathrm{LWT})^{1/3} \quad (1)$$

The surface area of fruits was determined by the Eq. (2) (Baryeh 2001).

$$S = \pi \times Dg^2 \quad (2)$$

The fresh and dry biomass (oven-dried at 60 °C for 48 h to constant weight) of the fruit was weighed with an electronic balance. The surface area and biomass of each fruit was measured after their gas exchange measurement.

Gas exchange

Gas exchange of fruit and leaves was measured with LI-6400 portable photosynthesis system (Li-COR, Lincoln, NE). Readings were taken from 08:00 to 11:00 h and 14:00 to 16:00 h. A conifer chamber (6400-22, LI-COR) with a 18-RGB light source (LI-COR, Lincoln, NE, USA) was used for fruits and a normal 2 × 3 cm chamber with a 6400-02 (LI-COR) LED light source was used for leaves. The fruits remained attached to the stem during measurement. Leaves or fruits enclosed in the chamber were first exposed to photosynthetically active radiation (PAR) of 1300 μmol m^{-2} s^{-1} for 20 min. At 20 min, the PAR was decreased in step-wise fashion to zero to measure the net photosynthetic rate (P_n) at twelve and eleven PAR values for leaves and fruits, respectively. During the measurements, the chamber temperature was controlled at 28–30 °C and was monitored at an ambient CO_2 concentration of about 400 μmol mol^{-1}. *Carya* fruits had high respiration rates, even in the presence of light. For calculation of the gross photosynthetic rate (P_g) of fruits, the rate of respiration in darkness (R_d) was subtracted from the respiration rate in light (R_l) of fruits (fruits respired more CO_2 in dark than in light). The gross photosynthetic rate (P_g) of leaves was calculated as P_n minus R_d, algebraically. For leaves, we used the P_n and R_d per leaf area, in which the leaf area was treated as one-half the surface area of leaf. Thus, P_g was divided by half of the surface area of fruit when expressed as per

surface area. The rates of P_g and R_d were estimated on the basis of the fruit surface area (μmol CO_2 m^{-2} s^{-1}) or per individual fruit (μmol CO_2 $fruit^{-1}$ s^{-1}).

Modelling of the photosynthesis-PAR curve

The light saturation point (LSP) and maximum assimilation rate ($P_{(n,\ max)}$) of leaf and fruits areas were determined using AQ response curve analysis software (Version 1.0, LI-COR, 2/2008). On a fruit basis, the light response model for photosynthesis determined the relation between P_n and PAR (Charles-Edwards 1981):

$$P_n = \frac{b\mathrm{PAR}}{1 + \alpha\mathrm{PAR}} - r \quad (3)$$

Coefficient α is the reciprocal of PAR at 50 % of the asymptotic rate, coefficient b is the gradient of the saturation curve of Eq. 3 at the origin or the maximum quantum yield. The maximum P_g at light saturation ($P_{(g,\ max)}$) was determined as b/α. The CO_2 re-fixation (%) at light saturation was approximately $P_{(g,\ max)}/r$ (Linder and Troeng 1981).

RuBPC activity and soluble protein content of leaves

Leaf discs (14.38 mm diameter) were excised from the leaves at 40, 50, 59, 76, 121 DAP for hickory and 66, 78, 88, 123, 155 DAP for pecan, respectively. Enzyme extraction was performed, with slight modifications, according to Sayre et al. (1979). Leaf discs (about seven discs per sample) were ground with a mortar and pestle (at 4 °C) containing a small amount of sand and 1.0 mL of grinding media consisting of 0.1 M Tris–HCl (pH 8), 100 mM $MgCl_2$, 1 mM EDTA, 20 mM mercaptoethanol, 100 kg m^{-3} glycerin, and 10 kg m^{-3} polyvinylpyrrolidone. Ground material was centrifuged at 15,000g for 10 min at 4 °C, and the supernatant was used for enzyme assays. RuBPC (Ribulose-1,5-bisphosphate carboxylase) activity was assayed by the method of Racker (1962). The following enzyme extract was added to the reaction mixture: 100 mM Tris–Hcl (pH 8.0), 100 mM $MgCl_2$, 1 mM EDTA, 50 mM dithiothreitol (DTT), 2 mM NADH, 50 mM ATP, 200 μM $NaHCO_3$, 2 units per mL creatine phosphokinase, 4 units per mL each of NAD-dependent glyceraldehyde-3-P-dehydrogenase and 3-P-glycerate kinase in a final volume of 3.2 mL. The mixture was incubated at 25 °C for 5 min. Reactions were initiated by the addition of 9 mM RuBP. The soluble protein content of leaves was measured by a spectrophotometer at 595 nm according to the Coomassie brilliant blue G250 method (Read and Northcote 1981).

Chlorophyll content of leaves and fruits

The chlorophyll content of leaves was determined using leaf discs cut with a calibrated metal borer (14.38 mm diameter). The leaf discs of leaves (one disc per sample) were ground in the ceramic mortar, transferred to a centrifuge tube with 5 mL of 95 % (v/v) ethanol (100 %, Sinopharm Chemical Reagent Company, Shanghai, China) in the dark for 24 h at 25 °C until it was blanched (no green color in the leaf tissue). Absorbance of the supernatant was measured with a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) at 649, 664 and 470 nm after centrifugation. The total chlorophyll (Chlt), chlorophyll a (Chla), chlorophyll b (Chlb), and total Carotenoid (Car) contents were determined according to Lichtenthaler (1987).

Estimation of carbon balance in fruits

The total carbon requirement was calculated as the sum of the cumulative total respiration (Σ_r, expressed in mg carbon) and the carbon equivalent of the increment in fruit dry matter during an interval of time. The total contribution of fruit photosynthesis to the fruit carbon requirement was calculated by the cumulative total gross fruit photosynthesis (Σ_{gp}, expressed in mg carbon) divided by the total carbon requirement (expressed in g carbon) for a short period. We assumed that the daily Σ_{gp} or Σ_r is constant and the value can be determined by the mean of the values on the first day and on the last day during an interval of time.

The Σ_r was and Σ_{gp} per fruit per day was calculated as following Eqs. 4 and 5, respectively (Bazzaz and Carlson 1979).

$$\Sigma_r = (R_d(30^\circ C) \times 14\mathrm{h} + R_d/2\,(20^\circ C) \times 10\mathrm{h}) \quad (4)$$

$$\Sigma_{gp} = ((R_d - R_l)14\mathrm{h}) \quad (5)$$

Night-time respiration [R_d (20 °C)] was calculated from respiration measured during the day respiration [R_d (30 °C)], assuming a Q_{10} of 2 (Lloyd et al. 1995) and a mean day temperature of 30 °C and a mean night temperature of 20 °C. The conversion of fruit dry mass to carbon equivalents was calculated as dry weight multiplying by 0.436 for barely tops (Biscoe et al. 1975). The molecular weight ratio of carbon in one carbohydrate molecule ($C_6H_{10}O_5$) is 0.444. To convert Σ_r or Σ_{gp} from μmol CO_2 $fruit^{-1}$ s^{-1} to mg CO_2 g^{-1} h^{-1}, values were multiplied by 158.4 and divided by the dry mass (μmol CO_2 $fruit^{-1}$ s^{-1} = 44 × 3600/1000 mg CO_2 g^{-1} h^{-1}/dry mass). The equivalent carbon content (mg g^{-1} h^{-1}) was calculated by dividing by 3.6(CO_2/C = 44/12 = 3.6). The P_g was selected when the PAR was 1300 μmol m^{-2} s^{-1}.

Data analysis

Data were subjected to analysis of variance (ANOVA) using SPSS statistical software (version 16.0, IBM, New York, USA). The data are presented as the mean ± SE. Differences at $p \leq 0.05$ were considered significant.

Results

Fruit development

The fruits of hickory and pecan studied during their fruit development were as shown in Fig. 1. The surface area of hickory fruit was very small after fruit-set, then it rapidly increased by more 3.37-folds by 59 DAP compared with that at 40 DAP (at rate of 72–73 mm^2 day^{-1} $fruit^{-1}$) (Fig. 2a). After 59 DAP it slowly increased during the late growth stage of fruit development (24–29 mm^2 day^{-1} $fruit^{-1}$, Fig. 2a). The surface area of pecan fruit increased rapidly from 78 to 112 DAP (69–84 mm^2 day^{-1} $fruit^{-1}$) after which it slowly increased to the maximum value (Fig. 2a).

The fruit dry mass and fresh mass of both hickory and pecan showed a somewhat more linear increase from fruit-set to fruit-maturation (Fig. 2b, c). The fresh mass of hickory fruit increased rapidly from 50 to 59 DAP, then increased slowly for about 17 days, followed by another increase until fruit-maturation (Fig. 2b). Pecan had a rapid increase of fresh mass from 66 to 112 DAP, then a slow increase until fruit-maturation. The fruit dry mass of hickory increased rapidly from 76 to 88 DAP, with a mean of 137 mg day^{-1} $fruit^{-1}$. Pecan had a rapid increase from 88 to 112 DAP, followed by a stasis of about 11 days and then another increase until fruit-maturation (Fig. 2c). The mean fruit dry mass/fresh mass ratio of hickory decreased to its lowest value at 59 DAP, increased until 88 DAP, and then maintained a constant level (Fig. 2d). For pecan, the mean fruit dry mass/fresh mass ratio decreased to its lowest value at 112 DAP and then increased until 155 DAP (Fig. 2d).

CO_2 exchange of leaves and fruits in hickory and pecan

Leaves

The photosynthetic light response curves in leaves and fruits of hickory and pecan during their fruit development stages were as shown in Fig. 3. The light saturation point (LSP) of hickory and pecan leaves rose at the beginning (40 to 76 DAP for hickory; 66 to 88 DAP for pecan); attained peaks at 76 (816 μmol photons $m^{-2}s^{-1}$) and 88 (1157 μmol photons m^{-2} s^{-1}) DAP, respectively (Figs. 3a, 4a). After the peaks, the values of LSP for leaves in both hickory and pecan sharply decreased with age (Figs. 3a, b, 4a). For hickory, the highest value for $P_{(n,\ max)}$ occurred at 76 DAP, after which it decreased and remained almost constant after 88 DAP (Figs. 3a, 4b). The leaves of pecan reached a peak in $P_{(n,\ max)}$ (9.62 μmol CO_2 m^{-2} s^{-1}) at about 88 DAP, followed by a rapid decrease with increasing fruit ages (Figs. 3b, 4b). For hickory, the lowest R_d, on a leaf area basis, was observed at 50 DAP, which was approximately 2.1 % of that of $P_{(n,\ max)}$ at 50 DAP. By comparison, the two peaks of R_d on a leaf area basis in pecan were observed at 88 and 123 DAP. Beyond 88 DAP, the $P_{(n,max)}$ of pecan was significantly greater than that of hickory (Fig. 4c).

Fruits

The LSP in fruits of hickory and pecan decreased at first, followed by an increase, and then a rapid decrease with age (Fig. 5a). Figure 5b shows maximum photosynthesis CO_2 re-fixation at light saturation as a percentage of dark respiration. Fruits of hickory had a low re-fixed rate at 40 DAP (about 19 % of respired CO_2), then reached a maximum re-fixed value of 66 % of respired CO_2 at 59 DAP. After that time the value decreased and then remained almost constant from 88 DAP. The maximum photosynthesis CO_2 re-fixation in fruits of pecan is achieved near 88 DAP (about 86 % of respired CO_2), maintained at this rate for about 20 days and then declines.

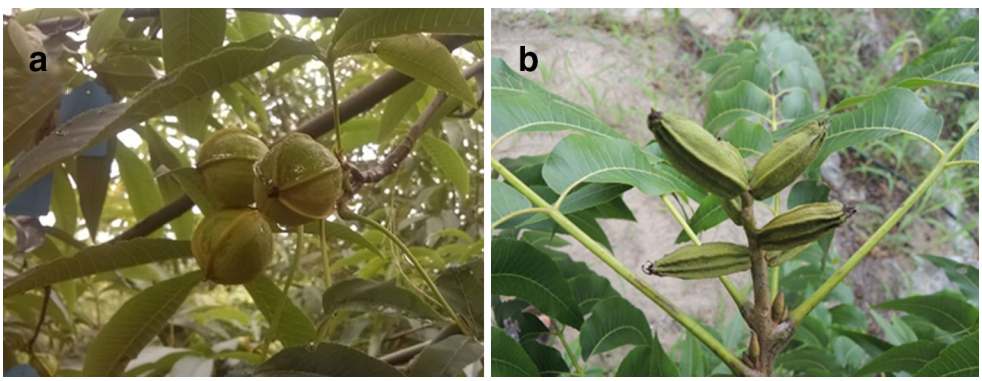

Fig. 1 Fruits of hickory (*Carya illinoensis* Sarg.) (**a**) and pecan (*Carya illinoensis* K.Koch) (**b**)

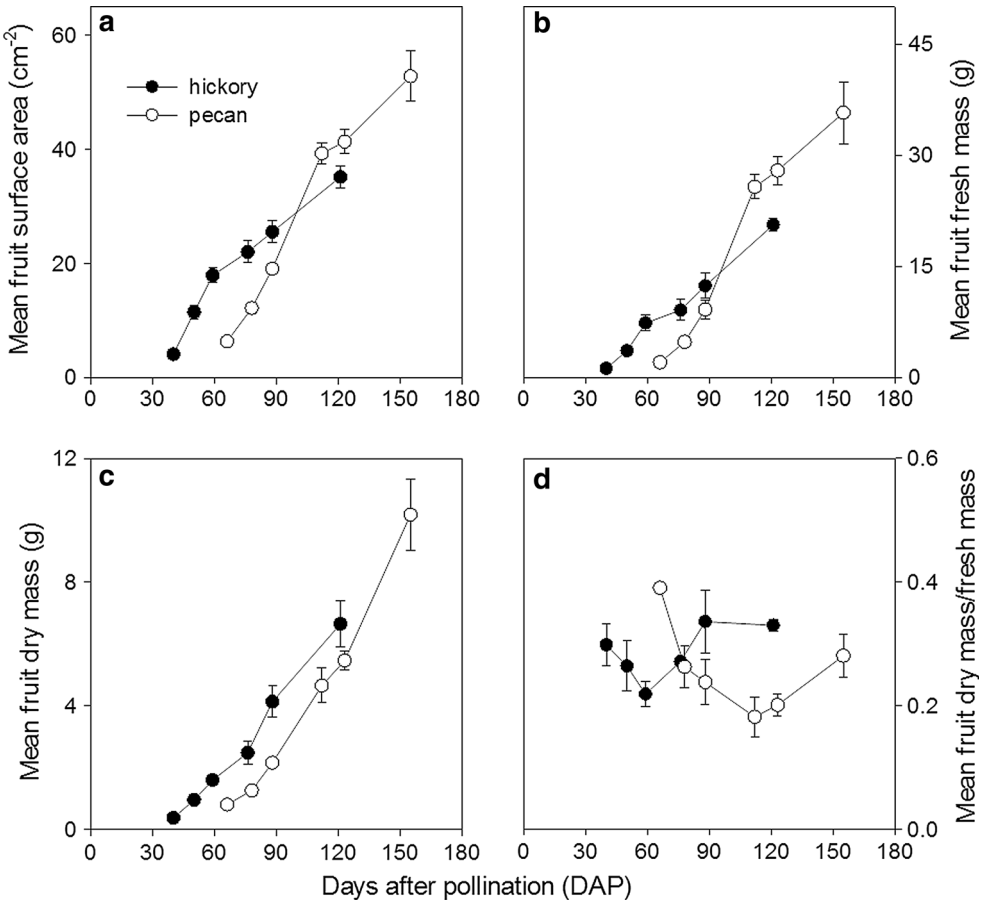

Fig. 2 The changes in the fruit dry mass (**a**), fruit fresh mass (**b**), fruit dry mass/fresh mass and surface area per fruit of hickory and pecan during the fruit development. Data are mean ± SE, $n = 6$

Both the maximum $P_{(g,\ max)}$ per area in fruits of hickory and pecan showed an increasing trend at first, then followed by a decrease trend with fruit development proceeded (Fig. 5c). The $P_{(g,\ max)}$ per fruit of both hickory and pecan showed a significant increasing trend during fruits development (Fig. 5d). On a fruit surface area basis, the value of dark respiration (R_d) in fruit of hickory initially decreased, followed by a small increase, and then had a rapid decrease with age (Fig. 5e). Similarly, the highest R_d in pecan fruit occurred at the beginning, followed by a rapid decrease, and then a slight increase at the end of the fruit development. On a whole fruit basis, the value of R_d in fruits of hickory showed a rapid increase from 40 to 88 DAP, and the R_d in fruits of pecan showed a continuous increase trend during the fruit growth (Fig. 5f).

Chl content in leaves and fruits

The Chl content in leaves and fruits of both hickory and pecan showed a significant initial increase followed by a rapid decrease with age. The leaves of hickory reached a Chl peak at 50 DAP, and stayed at this level for 9 days and then declined. It decreased by 22 % at 88 DAP (Fig. 6a). The highest Chl content in pecan leaves occurred at about 88 DAP, remaining at that level for 35 days (Fig. 6a). The Chl content of hickory and pecan fruits rose initially; and attained peaks at 50 and 78 DAP, respectively. Chl content based on fruit area hickory was significantly higher than the leaves from 40 to 59 DAP (Fig. 6a). However, a dramatic reduction of Chl content in fruits of hickory was observed at 88 DAP compared to 50 DAP. In hickory, the Chl content in fruits was significantly lower than in leaves at the end of the fruit development stage. In contrast, pecan fruit maintained significant higher Chl content than leaves during growth (Fig. 6b).

RuBPC activity and soluble protein content in leaves

On a fresh weight and area basis, RuBPC activity in hickory leaves showed an initial increase, followed by a rapid decrease during fruit development (Fig. 7). The reduction in RuBPC activity of hickory from 76 DAP to 121 DAP was 26 % on an area basis and 19.4 % on a fresh weight basis, respectively (Fig. 7a, b). On a fresh weight basis, leaves of pecan reached a peak of RuBPC activity at 78 DAP, then declined with age and was reduced by 20 % at 155 DAP (Fig. 7a). By comparison, pecan maintained stable RuBPC activity on an area basis during growth (Fig. 7b).

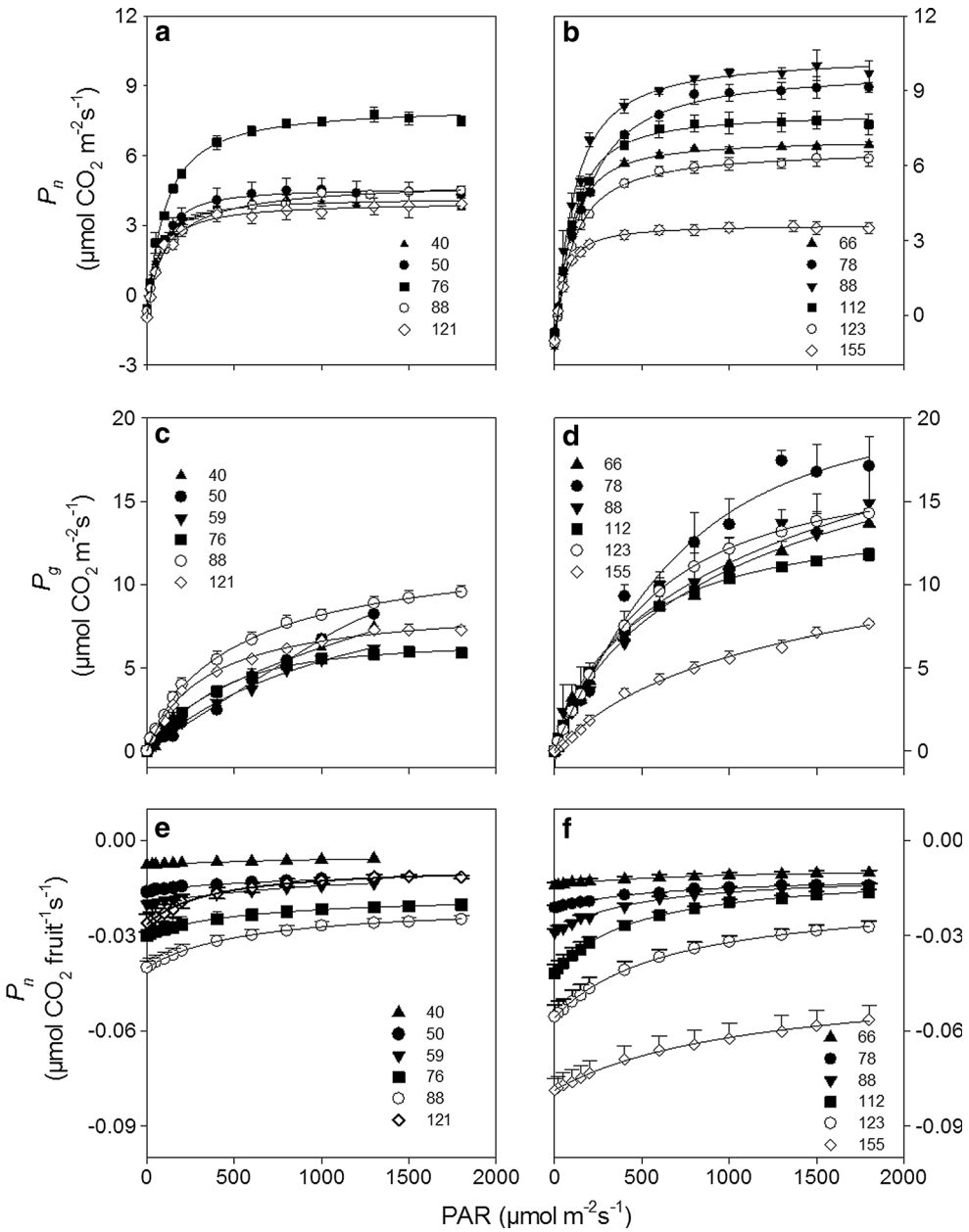

Fig. 3 Photosynthetic light response curves in leave (**a**, **b**) and fruit (**c**, **d**, **e**, **f**) at the different fruit developmental stages. **a**, **c**, **e**: hickory; **b**, **d**, **f**: pecan. Data are mean ± SE, $n = 6$

The soluble protein content in leaves of both hickory and pecan showed a pronounced increase at first, followed by a rapid decreased with ages. The soluble protein content of leaves reached a peak at 50 DAP for hickory and 123 DAP for pecan (Fig. 7c, d).

Fruits and leaf photosynthesis at equal light intensity

Hickory leaves reached a peak in gross photosynthetic rate (10.04 μmol CO_2 m^{-2} s^{-1}) at about 50 DAP (Fig. 8a). After 50 DAP, the rate declined with age and was 51.5 % at 88 DAP (Fig. 8a). By comparison, the gross photosynthetic rate in hickory fruit decreased at first, then increased followed by a rapid decrease with age (Fig. 8a). The gross photosynthetic rate of fruit on an area basis was significantly higher than that of leaves during the late stage of fruit development (88 to 121 DAP) (Fig. 8a). Pecan leaves reached a gross photosynthetic rate peak (10.7 μmol CO_2 m^{-2} s^{-1}) at 88 DAP (Fig. 8b). Peaks of the gross photosynthetic rate occurred at 78 (17.6 μmol CO_2 m^{-2} s^{-1}) and 123 (13.2 μmol CO_2 m^{-2} s^{-1}) DAP in pecan fruits of (Fig. 8b). Pecan fruits maintained a significantly higher gross photosynthetic rate than leaves during growth (Fig. 8b).

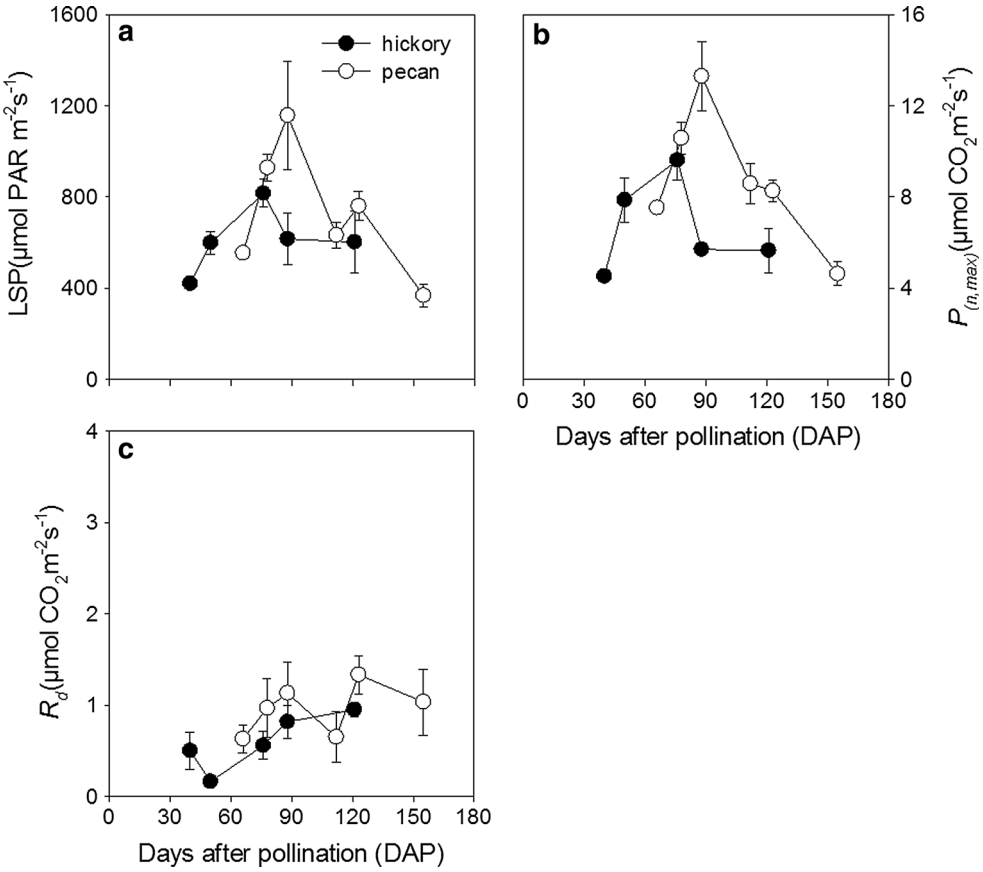

Fig. 4 Changes in the LSP (**a**), $P_{(n,\ max)}$ (**b**) and R_d (**c**) of leaves in hickory and pecan during the fruit development. Hickory, *filled circles*; pecan, *open circles*. Data are mean ± SE, $n = 6$

The contribution of fruit photosynthesis to the fruit carbon requirement

Contributions of fruit photosynthesis to the fruit carbon requirement during the fruit growth stages are shown in Table 1. The maximum value of the contribution of hickory fruit to its fruit carbon requirement occurred from 88 to 121 DAP. Pecan fruit reached a peak in contribution of fruit photosynthesis to fruit carbon requirement from 112 to 123 DAP. Contributions of pecan fruit photosynthesis to the fruit carbon requirement were always higher than those of hickory.

Discussion

Fruit development

The dry mass/fresh mass ratio of fruits can be used to assess the different phases of fruit growth (Imai and Ogawa 2009). In present study, fruit development of hickory can be divided into two phases according to the fruit growth pattern. Phase one is characterized by increasing fruit size (such as surface area, Fig. 2a) and this occurred from early May to early July (0 to 59 DAP). During this period, the mean fruit dry mass/fresh mass ratio decreased to its lowest value (Fig. 2d), indicating that the fruit water content increased. Phase two, from early July to late August (59 to 121 DAP), is characterized by a slowing fruit growth and a rapid increase in fruit dry mass. From 59 to 88 DAP, the mean fruit dry mass/fresh mass ratio gradually increased because of seed development. From 88 to 121 DAP, the mean fruit dry mass/fresh mass ratio of hickory fruits reached a plateau accompanied by rapidly increasing dry mass. This indicated kernel filling and nutrient accumulation accompanied by a decrease in water content. These results are similar to the observations of Xie et al. (2008) who found that hickory had fastest growth from early May to early July, followed by kernel development and nutrient filling during early July until late August. Pecan fruits had slower increases in surface area and dry fruit from 0 to 76 DAP (early May to mid July), followed by a dramatic increase from 76 DAP until maturity. This is consistent with the findings of Diver et al. (1984). The trend of the mean fruit dry mass/fresh mass ratio during pecan fruit development, indicated that the majority of fruit growth and seed development occurred from 0 to 112 DAP and from 112 to 155 DAP, respectively (Fig. 2d).

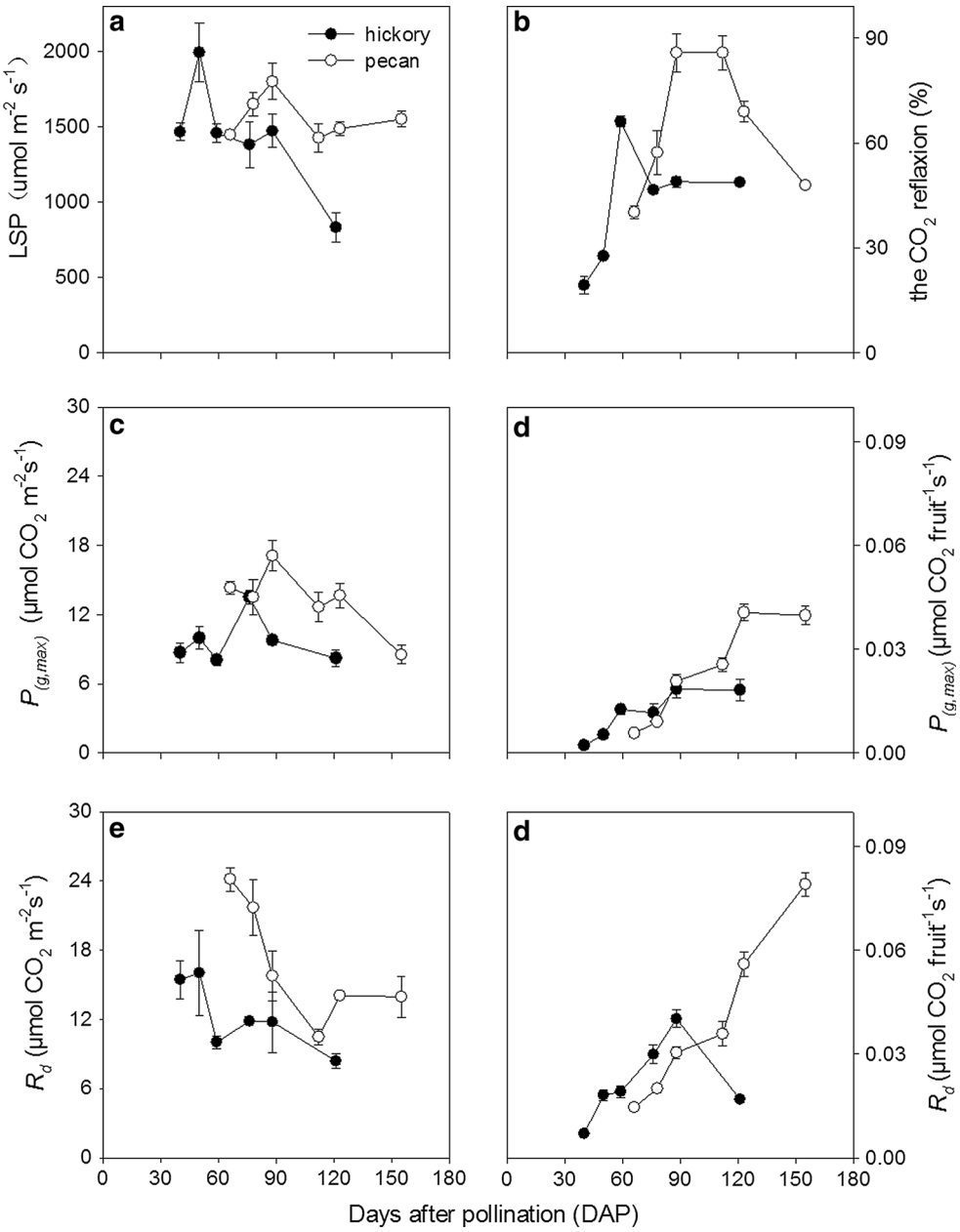

Fig. 5 Changes in the light saturation point (LSP, **a**), the CO_2 refixation (**b**), the maximum gross photosynthetic rate ($P_{(g,\ max)}$, **c** and **d**) and dark respiration (R_d, **e** and **f**) per surface area or individual fruit for fruit of hickory and pecan. Hickory, *filled circles*; pecan, *open circles*. Data are mean ± SE $n = 6$

Photosynthetic characteristics in hickory and pecan leaves during fruit development

Leaves are the main source of photosynthesis in plants. We found a significant decrease in LSP and $P_{(n,\ max)}$ in leaves of hickory and pecan after 76 and 88 DAP, (Fig. 4a, b). In addition, the $P_{(n,\ max)}$ in hickory leaves decreased by 40.7 % from 76 to 88 DAP, while the $P_{(n,\ max)}$ in pecan leaves decreased by 37.8 % from 88 to 123 DAP. Chl content in hickory leaves decreased by 9.3 % from 76 to 88 DAP, while there was little change in Chl content in pecan leave from 88 to 123 DAP (Fig. 6a, b). Degradation of Chl is a characteristic of leaf senescence (Kura-Hotta et al. 1987). Thus, a smaller decrease in $P_{(n,\ max)}$ and Chl content in pecan leaves indicates a slower senescence than hickory during its fruit development stages.

Variation in photosynthetic rate has been attributed to the concentration and activities of RuBPC (Lawlor et al. 1989). We found that $P_{(n,\ max)}$ (Fig. 4b) and RuBPC activity (Fig. 7b) per area in hickory leaves significantly increased from 50 to 76 DAP, while the soluble protein per unit area rapidly decreased. This suggested that the increase in $P_{(n,\ max)}$ was related to the increasing RuBPC activity. In addition, the RuBPC activity per unit area in pecan leaves remained stable from 66 to 88 DAP while the soluble protein content per unit area significantly increased

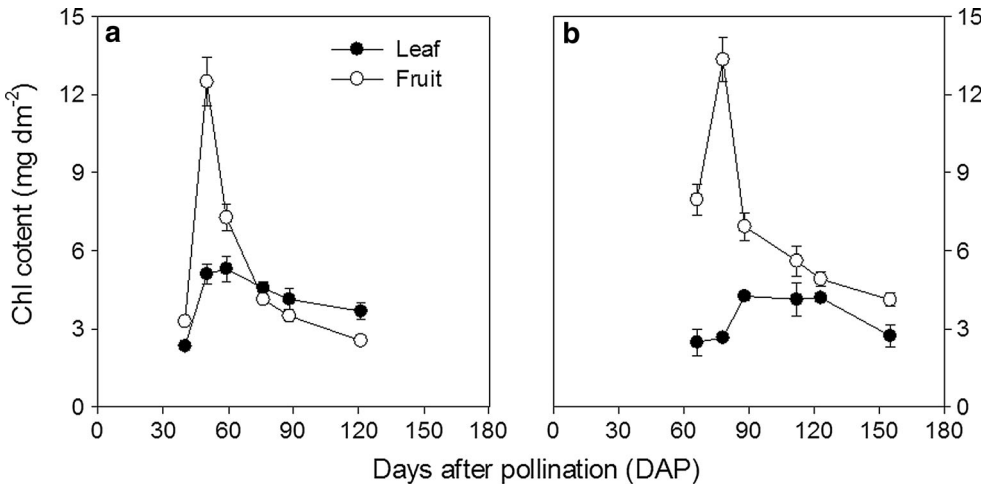

Fig. 6 Changes in total chlorophyll (Chl) content of leaves and fruits expressed on the basis of surface area of hickory (**a**) and pecan (**b**) during the fruit maturity. Leaf, *filled circles*; fruit, *open circles*. Data are mean ± SE, $n = 6$

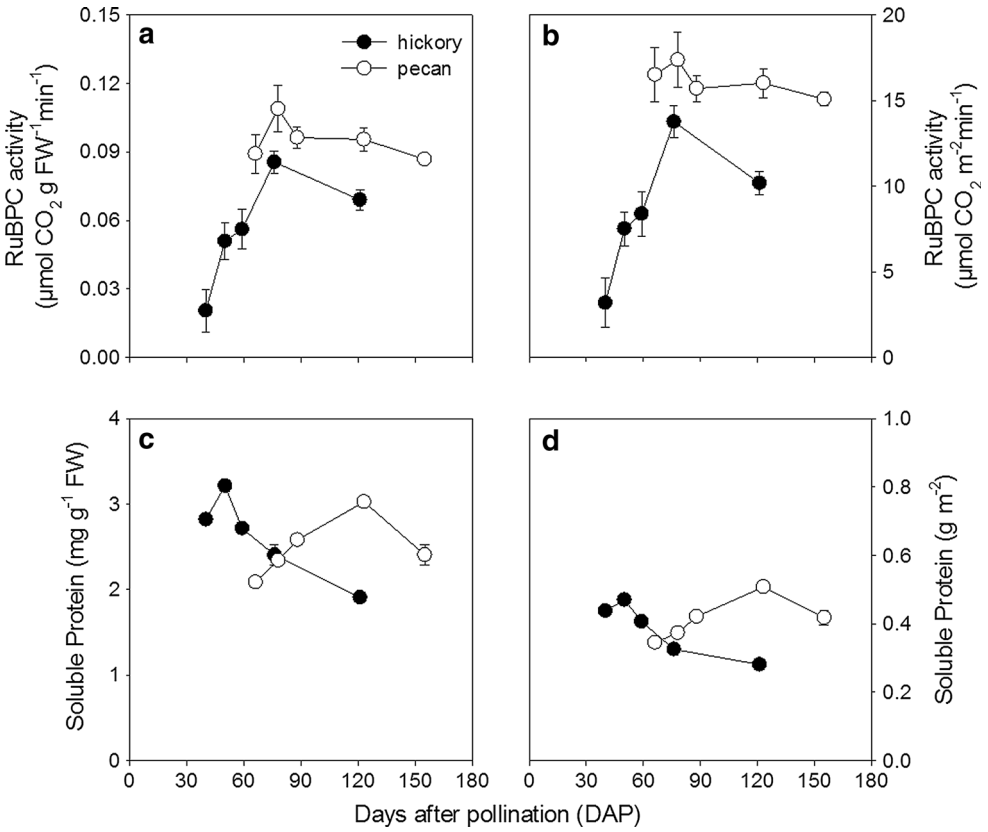

Fig. 7 Activities of Ribulose-1, 5-biphosphate carboxylase (RuBPC) activity and soluble protein content in leaves of hickory and pecan expressed on fresh weight (**a**, **c**) and area basis (**b**, **d**) during the fruit development. Hickory, *filled circles*; pecan, *open circles*. Data are mean ± SE, $n = 6$

at that time (Fig. 7d). RuBPC is usually the major soluble protein of photosynthetic tissue, comprising about 50 % (Makino et al. 1983). Possibly, the hickory leaves had an abundance of other soluble proteins (including RuBPC), that might have served to store nitrogen in the form of proteins, without being fully active (Warren and Adams 2004). An over-investment in RuBPC by *Pinus pinaster* is an adaptation to temporal variation in the nitrogen supply. The RuBPC may aid growth and photosynthesis during periods of nitrogen deficiency (Warren and Adams 2002). Similarly, we suggest that the significantly higher soluble protein per unit area in pecan at 76 DAP might serve as a useful nutrient supply reserve. That may be related to wide adaptability of pecan trees. To test this hypothesis, further studies are necessary to determine the susceptibility of pecan to nitrogen deficiency.

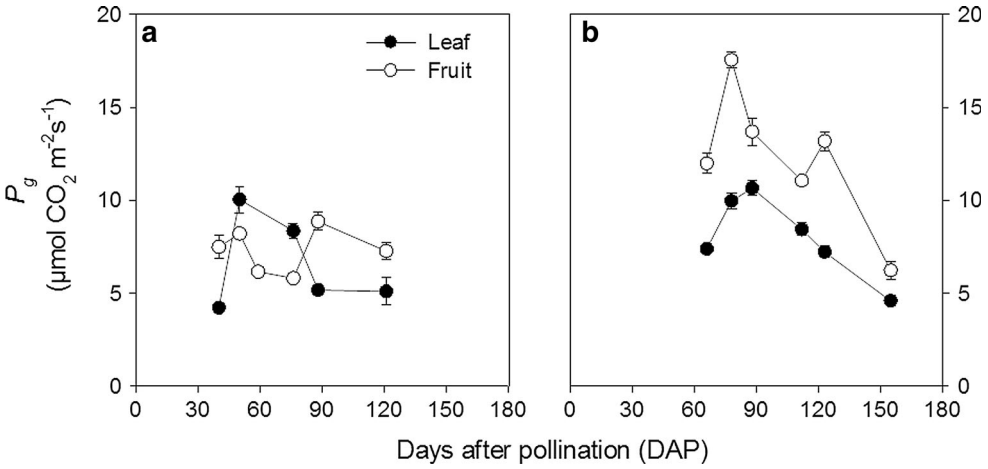

Fig. 8 Changes in the gross photosynthetic rate of leaf and fruit expressed on area basis in hickory (**a**) and pecan (**b**) during fruit development. Leaf, *filled circles*; fruit, *open circles*. Data are mean ± SE, measurements were made at 1300 μmol photons $m^{-2}s^{-1}$. $n = 6$

Table 1 The contribution of fruit photosynthesis to fruit carbon requirement

Hickory		Pecan	
DAP	%	DAP	%
40–50	4.92	66–78	12.6
50–59	6.73	78–88	12.3
59–76	9.61	88–110	14.4
76–88	6.85	110–123	33.8
88–121	13.3	123–155	19.4

The contribution of fruit photosynthesis to fruit carbon requirement was calculated as the total gross fruit photosynthesis (Σ_{gp}, expressed in mg carbon) divided by the sum of the cumulative total respiration (Σ_r, expressed in mg carbon) and the carbon equivalent of the increment in dry matter during an interval of time. We assumed that the daily Σ_{gp} or Σ_r is constant and the value can be determined from the mean of the values on the first day and on the last day during an interval of time. Data are mean ± SE. $n = 6$

Photosynthesis in fruit of hickory and pecan during the fruit development

In this study, we noted that the light saturation points (LSP) of fruits in both hickory and pecan attained peaks of 1996 and 1802 μmol photons m^{-2} s^{-1} at 50 and 88 DAP, respectively. These values are high in comparison to the LSP in leaves of most C_3 plants (Koyama and Kikuzawa 2010). The high LSP is likely related to the high CO_2 availability for photosynthesis produced due to the high respiration in hickory and pecan fruits. Similar high photosynthesis at high light intensity has been observed in *jatropha a*nd *lycopersicum* (Lytovchenko et al. 2011; Ranjan et al. 2012).

The highest values of $P_{(g,\ max)}$ per area in hickory and pecan fruits were 13.53 and 17.10 μmol m^{-2} s^{-1} at 76 and 88 DAP, respectively (Fig. 5c). After this period, $P_{(g,\ max)}$ decreased with fruit age. However, the $P_{(g,\ max)}$ per fruit of hickory and pecan had a continuous increasing trend with fruit development (Fig. 5d). The increase in $P_{(g,\ max)}$ per fruit is related to the increasing fruit surface area (Fig. 2a). The dark respiration rate (R_d) on a fruit basis of hickory significantly increased with the fruit development stage, and peaked at 88 DAP (Fig. 5f). Similarly, the R_d on a fruit basis of pecan significantly increased during fruit development (Fig. 5f). Fruit respiration can be separated into two components: maintenance respiration, which supplies the energy necessary for maintaining a healthy existing phytomass and growth respiration, which is required for the synthesis of new phytomass (Amthor 1989, 2000). Imai and Ogawa (2009) suggested that increased dark respiration on a fruit basis is related to the fast dry mass accumulation for the fruit and seed development. Thus, we suggest that the increase of R_d on a fruit basis is due to the growth respiration by fruit and seed development.

The maximum photosynthetic CO_2 re-fixation in fruits of hickory and pecan initially increased but this was followed by a rapid decrease with age (from 19 to 85 %) (Fig. 5b), indicating varying efficiency of the respired CO_2 re-fixation in fruit during the fruit development. Phosphoenolpyruvate carboxylase (PEPC) has been reported to re-fix the respired CO_2 in fruit (Blanke et al. 1987), which was detected in many fruits (Blanke et al. 1986; Blanke and Notton 1991). The PEPC activity per fruit in apple fruit increased during the fruit development (Blanke et al. 1987). Possibly, changes in the efficiency of the respired

CO_2 re-fixation related to the different PEPC activity during the fruit development. To test this hypothesis, further studies are necessary to explore the physical and kinetic properties of PEPC in hickory and pecan fruits during the fruit development.

The maximum Chl content per area in fruits of hickory and pecan was observed at 50 and 78 DAP, respectively, followed by a rapid decline with fruit age (Fig. 6a, b). This may be due to dilution caused by increasing fruit size.

Contribution of fruit photosynthesis to fruit development

The fruit as a sink acquries carbon originating from photosynthesis, both in the leaf and fruit (Blanke and Lenz 1989). P_g in hickory leaves rapidly decreased after 50 DAP (Fig. 8), and was significantly lower than the rate in fruits from 88 to 121 DAP (corresponding to seed development stage). For pecan, the fruits maintained a significantly higher P_g than leaves during the period of fruit growth and seed development (Fig. 8). Fruit photosynthesis in tomato plays an important role in seed development (Lytovchenko et al. 2011). Similarly, the photosynthesis in hickory and pecan fruits was very important for seed development.

In both hickory and pecan, the contribution of fruit photosynthesis to fruit carbon requirement during the later fruit growth stages was significantly higher than that at the early fruit growth stages, suggesting that fruit photosynthesis plays an important role in the fruit development during the late fruit growth stage (Table 1). Similar results were obtained in studies on peach fruits and cotton fruits (Pavel and DeJong 1993; Hu et al. 2013). The maximum value of the contribution of hickory fruit photosynthesis to the fruit carbon requirement occurred at the end fruit growth stage (Table 1). This increased value may have been due to relatively low fruit respiration. Our results show that the contributions of fruit photosynthesis to fruit carbon requirement in pecan were significantly higher than those in hickory. This might be related to a higher photosynthetic capacity in pecan. Assimilates in leaves are usually used for current-year reproduction, maintenance and growth of woody parts or energy storage for reproduction and new shoot growth during the next growth season, while the photosynthesis by fruits is important to compensate for reproductive cost (Imai and Ogawa 2009). Thus, we suggest that fruit photosynthesis of hickory and pecan also play an important role in offsetting the costs of reproduction.

Conclusion

In conclusion, fruit photosynthesis made a significant contribution to the fruit carbon requirement, especially for seeds development. The fruit development of hickory and pecan was divided into two phases: enlargement of fruit size stage (from fruit setting to 59 DAP for hickory; from fruit setting to 112 DAP for pecan) and the seed development stage (from 59 to 121 DAP for hickory; from 112 to 155 DAP for pecan). However, the photosynthetic rate in leaves of hickory and pecan began to significantly decrease after 50 and 88 DAP, respectively. The P_g per area of hickory fruit was significant higher than that of leaves at late fruit development, while the P_g per area of pecan fruits were always significantly higher than that of leaves during the entire fruit development period. In addition, the surface area of fruits significantly increased with fruit growth.

Author contribution statement Designing the work: Y.Y.H., J.Q.H.; running the experiments: Q.Y.X., J.F.W., Y.R.C., X.Y.Y.; Data analysis and statistics: Q.Y. X., Y.Y. H.; article writing and revising: Q.Y. X., G.H.X., Z.J.W., J.Q.H., Q.X.Z., Y.Y. H.

Acknowledgments This work was supported by a Grant from the Zhejiang Provincial Natural Science Foundation of China (LY15C160003), the National High Technology Research and Development Program of China (863 Program) (2013AA102605), the Zhejiang Provincial Science, Technology Innovation Activity for College Students (2015R412044), and National Natural Science Foundation of China (31100229, 31470682).

Compliance with ethical standards

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

References

Amthor JS (1989) Respiration and crop productivity. Plant Growth Regul 10:271–273

Amthor JS (2000) The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Ann Bot 86:1–20

Aschan G, Pfanz H (2003) Non-foliar photosynthesis—a stratey of additional carbon acquisition. Flora 198:81–97

Baryeh EA (2001) Physical properties of bambara groundnuts. J Food Eng 47:321–326

Bazzaz F, Carlson R (1979) Photosynthetic contribution of flowers and seeds to reproductive effort of an annual colonizer. New Phytol 82:223–232

Biscoe P, Scott R, Monteith J (1975) Barley and its environment. III. Carbon budget of the stand. J Appl Ecol, pp 269–293

Blanke MM, Lenz F (1989) Fruit photosynthesis. Plant Cell Environ 12:31–46

Blanke MM, Notton BA (1991) Kinetics and physiological significance of photosynthetic phosphoenolpyruvate carboxylase in avocado fruit. J Plant Physiol 137(5):553–558

Blanke MM, Whiley AW (1995) Bioenergetics, respiration cost and water relations of developing avocado fruit. J Plant Physiol 145(s1–2):87–92

Blanke MM, Notton BA, Hucklesby DP (1986) Physical and kinetic properties of photosynthetic phosphoenolpyruvate carboxylase in developing apple fruit. Phytochemistry 25(3):601–606

Blanke MM, Hucklesby DP, Notton BA (1987) Distribution and physiological significance of photosynthetic phosphoenolpyruvate carboxylase in developing apple fruit. J Plant Physiol 129(s3–4):319–325

Charles-Edwards DA (1981) The mathematics of photosynthesis and productivity. Academic Press, London

Crews C, Worley R, Syvertsen J, Bausher M (1980) Carboxylase activity and seasonal changes in CO_2 assimilation rates in three cultivars of pecan. J Am Soc Hortic Sci 105:798–801

Diver SG, Smith MW, McNew RW (1984) Influence of fruit development on seasonal elemental concentrations and distribution in fruit and leaves of pecan. Commun Soil Sci Plant 15:619–637

Hieke S, Menzel C, Lüdders P (2002) Effects of leaf, shoot and fruit development on photosynthesis of lychee trees (*Litchi chinensis*). Tree Physiol 22:955–961

Hu YY, Oguchi R, Yamori W, von Caemmerer S, Chow WS, Zhang WF (2013) Cotton bracts are adapted to a microenvironment of concentrated CO_2 produced by rapid fruit respiration. Ann Bot-London 112:31–40

Huang J, Lv FD, He XH (2011) Preliminary study of photosynthesis in *Carya illinoensis*. J Cent South Univ For Technol 31:174–177

Imai S, Ogawa K (2009) Quantitative analysis of carbon balance in the reproductive organs and leaves of *Cinnamomum camphora* (L.) Presl. J Plant Res 122:429–437

Jindal VK, Mohsenin NN (1978) Dynamic hardness determination of corn kernels from impact tests. J Agr Eng Res 23(1):77–84

Kocurek M, Kornas A, Pilarski J, Tokarz K (2015) Photosynthetic activity of stems in two *Clusia* species. Trees 29:1029–1040

Koyama K, Kikuzawa K (2010) Geometrical similarity analysis of photosynthetic light response curves, light saturation and light use efficiency. Oecologia 164:53–63

Kura-Hotta M, Satoh K, Katoh S (1987) Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. Plant Cell Physiol 28:1321–1329

Lawlor DW, Kontturi M, Young AT (1989) Photosynthesis by flag leaves of wheat in relation to protein, ribulose bisphosphate carboxylase activity and nitrogen supply. J Exp Bot 40:43–52

Li BH, Chi DB, Cheng HH, Li ZL (2007) Relationship between photosynthetic characteris and yield of the *Carya cathyensis* Sarg. China For Sci Technol 21:34–37

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol, pp 350–382

Linder S, Troeng E (1981) The seasonal course of respiration and photosynthesis in strobili of scots pine. For Sci 27:267–276

Ling H et al (2014) Comparison of leaf photosynthetic physiological characteristics in five *Carya illinoensis* varieties. Scientia Silvae Sinicae 50:174–178

Lloyd J et al (1995) Measuring and modelling whole-tree gas exchange. Funct Plant Biol 22:987–1000

Lombardini L, Restrepo-Diaz H, Volder A (2009) Photosynthetic light response and epidermal characteristics of sun and shade pecan leaves. J Am Soc Hortic Sci 134:372–378

Lytovchenko A, Eickmeier I, Pons C, Osorio S, Szecowka M, Lihmberg K, Lu YH, Fisahn J, Bock R, Stitt M, Grimm B, Granell A, Fernie AR (2011) Tomato fruit photosynthesis is seemingly unimportant in primary metabolism and ripening but plays a considerable role in seed development. Plant Physiol 157:1650–1663

Makino A, Mae T, Ohira K (1983) Photosynthesis and ribulose-1,5-bisphosphate in rice leaves: changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. Plant Physiol 73(4):1002–1007

Marcelis LFM, Hofman-Eijer LRB (1995) The contribution of fruit photosynthesis to the carbon requirement of cucumber fruits as affected by irradiance, temperature and ontogeny. Physiol Plant 93:476–483

Ogawa K, Furukawa A, Hagihara A, Abdullah AM, Awang M (1995) In situ CO_2 gas-exchange in fruits of a tropical tree *Durio zibethinus* Murray. Trees 9:241–246

Pavel E, DeJong TM (1993) Estimating the photosynthetic contribution of developing peach (*Prunus persica*) fruits to their growth and maintenance carbohydrate requirements. Physiol Plant 88:331–338

Racker E (1962) Ribulose diphosphate carboxylase from spinach leaves: ribulose diphosphate + CO_2 + H_2O → 2 3-P-Glycerate. Method Enzymol 5:266–270

Ranjan S, Singh R, Soni DK, Pathre UV, Shirke PA (2012) Photosynthetic performance of *Jotropha curcas* fruits. Plant Physiol Biochem 52:66–76

Read SM, Northcote DH (1981) Minimization of variation in the response to different protein of the Coomassic Blue G dye binding assay for protein. Anal Biochem 116:53–64

Sayre RT, Kennedy RA, Pringnitz DJ (1979) Photosynthetic enzyme activities and localization in Mollugo verticillata populations differing in the levels of C_3 and C_4 cycle operation. Plant Physiol 64:293–299

Warren CR, Adams MA (2002) Phosphorus affects growth and partitioning of nitrogen to Rubisco in *Pinus pinaster*. Tree Physiol 22:11–19

Warren CR, Adams MA (2004) Evergreen trees do not maximize instantaneous photosynthesis. Trends Plant Sci 9:270–274

Weiss D, Schönfeld M, Halevy AH (1988) Photosynthetic activities in the *Petunia corolla*. Plant Physiol 87:666–670

Xie HE, Huang YJ, Xue XM, Xu CS, Liu L (2008) Growth and development of the *Carya cathayensis* nut. J Zhejiang A & F Univ 4:527–531