

Limitations to photosynthesis of leaves of apple (*Malus domestica*) trees across the growing season prior to and after harvest

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Abstract

Photosynthetic responses to CO₂ were followed on leaves of *Malus domestica* cv. Cox's Orange trees growing in orchard conditions, before and after harvest. Rates of photosynthesis, at ambient and CO₂ saturated concentrations, were stable across the growing season, except for minor decreases at harvest although rates recovered and remained high thereafter. Stomatal conductance increased over the growing season but declined transiently just before harvest and recovered for most of the season. Rates of ribulose-1,5-bisphosphate (RuBP) carboxylation declined before harvest, however, rates of RuBP regeneration mirrored assimilation. After harvest, rates of RuBP regeneration increased whereas rates of carboxylation increased but remained low, limiting photosynthesis. Lack of change in photosynthesis after harvest was at odds with the literature where photosynthesis of high crop load trees declined soon after, suggesting crop load was not as demanding on the Cox's Orange trees as in other studies.

Additional key words: CO₂ response; gas exchange; nonstomatal limitation; post-harvest; pre-harvest; stomatal limitation.

Introduction

It has become well established that fruiting trees have relatively high rates of photosynthesis (Avery 1977). For example, Wünsche *et al.* (2000) showed for cv. Braeburn apple trees that those without fruit ranged in photosynthesis from 3–7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ over selected days. By contrast, trees with a full crop load had photosynthetic rates ranging from 11–14 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the sample period. Similar results were observed with cv. Golden Delicious (Gucci *et al.* 1995, Wibbe and Blanke 1995, 1997) and sweet cherry (Quentin *et al.* 2013). More specifically, Palmer *et al.* (1997) demonstrated that photosynthesis increased in a curvilinear pattern with increasing crop load, thus confirming the strong conclusion that a high sink demand enhanced the photosynthetic capacity of the fruit trees. As a corollary of this response, harvest of the fruit generally results in crop load-induced differences in photosynthesis dissipating within 10–20 days (Palmer *et al.* 1997, Wünsche *et al.* 2000). Furthermore, the decreases in photosynthesis were most marked in the trees with the higher crop loads and least marked on those trees without fruit. However, the effect of the fruit harvest on photosynthesis through

the remainder of the growing season has not been well investigated.

The general assumption for the decrease in photosynthesis after a large crop load has been removed is a consequence of a loss of sink capacity (Herold 1980). This is despite the fact that deciduous horticultural species rely heavily on storing carbohydrates in root reserves for the subsequent bud-break processes in the following spring (Kandiah 1979, Greer *et al.* 2002, Holzapfel *et al.* 2010) and would expect to be a large sink (Palmer *et al.* 1997). Consistent with this hypothesis, for evergreen sweet orange trees (Nebauer *et al.* 2013), crop load had no marked effect on photosynthesis and there were also no seasonal changes in photosynthesis. Similarly, a comparison of summer and winter photosynthetic rates of sweet orange trees indicated reduced winter rates were a consequence of cold nights (Ribeiro *et al.* 2009) and not consequent upon the harvest. For deciduous fruit trees, therefore, it may take some time for the trees to establish the increased demand by the roots and thus measurements up to leaf fall may be required.

Another possibility for the decrease in photosynthesis after harvest may relate to either increased stomatal or nonstomatal, that is biochemical, limitations. The stomatal

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Abbreviations: A_c – modelled rates of carboxylation; A_j – modelled rates of electron transport; C_a – ambient CO₂ concentration; C_i – internal CO₂ concentration; DAB – days after bud break; E – transpiration; g_s – stomatal conductance; J_{max} – maximum rate of RuBP regeneration; P_N – net photosynthetic rate; P_{Nmax} – light- and CO₂-saturated photosynthesis; R_d – day respiration rate; RuBP – ribulose-1, 5-bisphosphate; V_{cmax} – maximum rate of RuBP carboxylation.

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limitation of photosynthesis, originally proposed by Farquhar and Sharkey (1982; see also Jones 1985), varied progressively over the growing season for cv. White Riesling grapevines from about 60% at the start to about 25% at harvest time (Schultz *et al.* 1996). For two apple cultivars, including cv. Golden Delicious and cv. Granny Smith, the stomatal limitation from early in the growing season (33 d after bud break, DAB) ranged from 16 to 18% and peaked at 25 and 19%, respectively, in midsummer (100 DAB) before declining to 22 and 8% in autumn (145 DAB) (Mierowska *et al.* 2002). Other evidence has shown the stomatal limitation can vary with temperature, varying from 13 to 34% between 20 and 40°C for Semillon grapevines (Greer and Weedon 2012) and from 25 to 63% between 15 and 40°C for cv. Red Gala apple trees (Greer 2015). For two oak species (*Quercus ilex* and *Q. faginea*) grown in a Mediterranean climate with mean summer temperatures of 21–22.5°C, the stomatal limitation ranged from 25 to 35% (Juárez-López *et al.* 2008). For the grapevine cultivar cv. Aragonez, grown in conditions where air temperatures reached 33°C during the day, the stomatal limitation averaged 22%. There appears to be few determinations of the stomatal limitation in the post-harvest period of fruiting trees. However, for two sweet cherry cultivars, cv. Kordia and cv. Sylvia, grown in a cool climate (summer maximum 22.5°C), the stomatal limitation, six weeks after harvest, ranged from 13–16% (Quentin *et al.* 2013) but it remains uncertain if this was a post-harvest effect or a cool climate influence. In contrast, for the evergreen sweet orange trees grown in Brazil with warm summers (30–33°C) and winters (25–29°C), the stomatal limitation varied from 40% in winter to 60% in summer, in keeping with other studies on trees grown in warm to hot climates.

There have also been seasonal determinations of the changes in the nonstomatal limitations, including the maximum rates of carboxylation (V_{cmax}) of ribulose 1, 5-bisphosphate (RuBP) and the maximum rates of RuBP regeneration (J_{max}). For example, Greer (2015) followed both limitations on leaves of apple trees grown in a hot climate and demonstrated that V_{cmax} and J_{max} were both high in spring and declined through summer with high temperatures although did recover late in the season, when air temperatures were reduced. A similar pattern in V_{cmax} and J_{max} occurred with grapevines cv. Chardonnay and cv. Merlot, in that both declined progressively through the growing season (Greer 2017), see also Schultz (2003) for similar effects on Riesling vines. A similar pattern was also shown with *Quercus douglasii* trees grown in a similarly hot climate, although there was an initial increase in both V_{cmax} and J_{max} after bud break (Xu and Baldocchi 2003). In contrast, the maritime pine, *Pinus pinaster*, had the highest V_{cmax} and J_{max} in mid-winter and lowest rates in summer (Medlyn *et al.* 2002). For *Pinus ponderosa* growing in the Sierra Nevada, both V_{cmax} and J_{max} increased from spring to peak in summer as did an understory evergreen shrub *Ceanothus cordulatus*, although V_{cmax} and J_{max} peaked later in early autumn (Misson *et al.* 2006). Similar patterns were also noted for several different *Eucalyptus* species (Lin *et al.* 2012). Although V_{cmax} and J_{max} have been measured in

several fruit tree species, including *Juglans regia* (Le Roux *et al.* 1999), *Prunus persica* cv. Fantasia and peach (Rosati *et al.* 1999, Walcroft *et al.* 2002), and *Olea europaea* (Díaz-Espejo *et al.* 2006), there have been few other seasonal evaluations other than those listed above. However, V_{cmax} and J_{max} were measured at a set time after the harvest of sweet cherries but only once (Quentin *et al.* 2013). Thus, there is a poor understanding of the changes that might occur in V_{cmax} and J_{max} limitations of CO₂ assimilation after the removal of the fruit sink.

Accordingly, the hypothesis that apple leaf photosynthesis would decline after the fruit harvest through a reduction in sink capacity was assessed. This was evaluated by measuring the seasonal changes in gas exchange and the response to internal CO₂ of leaves of Cox's Orange apple trees growing in orchard conditions, both prior to and after harvest. A second objective was to assess the stomatal and nonstomatal limitations to assimilation as the season progressed.

Materials and methods

Growth conditions: This study was conducted at the Research Orchard of the Universität Hohenheim, Stuttgart, Germany, located at latitude of 48°42', longitude of 9°12', and an altitude of 390 m a.s.l. The 10-year-old apple trees (*Malus domestica* cv. Cox's Orange) were grafted onto M9 rootstocks and planted in 3-m rows at a spacing of 2 m between trees. The trees were not irrigated but regular rainfall occurred throughout the growing season. Bud break and flowering typically occurred about mid-April and the trees were harvested in mid-September. Six trees were randomly selected and all trees held a moderate crop load (Wünsche, personal communication). All measurements were undertaken on selected fruiting shoots on each tree and all leaves were fully expanded and remained visually green throughout the measurement period, without any signs of senescence.

The site microclimate was monitored nearby within the orchard and air temperature and light intensity were monitored at hourly intervals throughout each day of the growing season.

Leaf gas exchange was monitored with an open gas-exchange system and PLC5 automatic leaf cuvette (*Cirras-1*, PP Systems, Hitchin, UK) and included use of the LED-based lighting system (*CRS131*, PP Systems, Hitchin, UK). Leaf temperature and photon flux density were measured concurrently for each leaf with the system. Photosynthesis (P_N), stomatal conductance (g_s), and transpiration (E) were monitored on representative leaves of bourse shoots. Photosynthetic measurements were conducted from late July until late October.

P_N/C_i responses: Measurements of the photosynthetic response to internal CO₂ concentrations (C_i) were undertaken on selected days prior to harvest using the open gas-exchange system. In each case, fully expanded leaves of each of selected shoots were at a constant saturating PFD of 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperatures were not controlled

and generally increased through the day in keeping with the ambient condition but ranged between 20 and 30°C. After harvest, the ambient temperatures were too low to allow a range of temperatures to be measured, therefore, all gas-exchange measurements were monitored at a constant 20°C. The measurement protocol throughout included measuring the rate of photosynthesis at an ambient CO₂ concentration (400 μmol mol⁻¹) and then the intercellular CO₂ concentration was reduced to about 30–50 μmol mol⁻¹ and the rates measured and then the CO₂ concentrations were progressively increased to about 1,200–1,400 μmol mol⁻¹ and rates measured in 100–200 μmol mol⁻¹ steps. For each day, the P_N/C_i responses were repeated 10–12 times on a new leaf on each occasion.

Data analysis: All P_N/C_i data were analysed statistically using a general linear modelling (GLM) approach with *SAS ver. 9.1.3* (*SAS Institute, Cary, NC, USA*), assuming a completely randomised design. Least squares means and standard errors were determined. The data were analysed in *SAS* (*SAS Institute*) using nonlinear regression according to the Farquhar *et al.* (1980) C₃ model of photosynthesis, following the equations described by Medlyn *et al.* (2002) and adopted by Greer and Weedon (2012), however, using the kinetic constants of Sharkey *et al.* (2007). Firstly, the maximum rates of ribulose-1,5-bisphosphate carboxylation (RuBP, V_{cmax}) and the day respiration rates (R_d) were determined with *SAS* for $C_i < 200$ μmol mol⁻¹. The second step involved using the previously established estimates for day respiration and the maximum rates of RuBP regeneration were determined with *SAS* for $C_i > 250$ μmol mol⁻¹, following the procedure of Silim *et al.* (2010). For each P_N/C_i curve, the stomatal limitation was also determined according to the method of Farquhar and Sharkey (1982) and described by Greer and Weedon (2012). To evaluate the seasonal changes in all parameters, a GLM approach was used to assess the main effect of day across the whole season on photosynthesis, stomatal conductance, transpiration as well as V_{cmax} and J_{max} . In addition, the model also assessed if these parameters were correlated with the mean daily maximum and minimum air temperatures and photon flux densities.

Results

Climate across the growing season: At the start of measurements in early summer (Fig. 1A), the maximum daily PFDs generally averaged 1,200–1,400 μmol m⁻² s⁻¹ although there were cloudy days when the PFDs were below about 800 μmol m⁻² s⁻¹. By late summer, the maximum daily PFDs were declining progressively, such that by the end of measurements, the PFDs were below about 400 μmol m⁻² s⁻¹.

The mean daily maximum and minimum air temperatures (Fig. 1B) followed a similar pattern to the PFDs, with the highest mean daily temperatures over summer averaging between 20 and 25°C and the mean minimum temperatures averaging between 10 and 15°C. The decrease in temperatures also occurred coincident with the decrease in PFD and the maximum temperatures declined

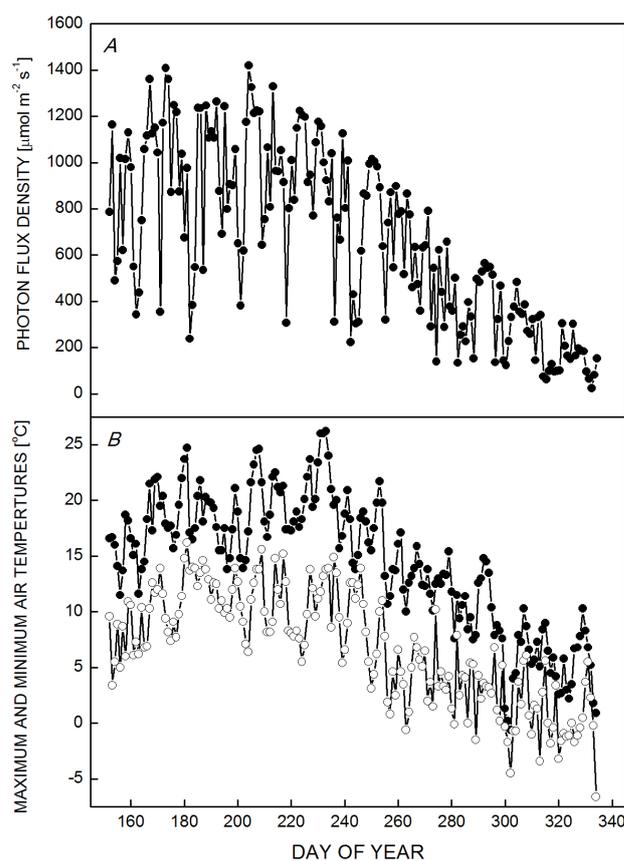


Fig. 1. Seasonal changes in mean daily photon flux density (A) and the mean daily maximum (closed symbols) and minimum (open symbols) air temperatures (B) measured at the Universität Hohenheim orchard nearby to where the trees were growing. Harvest occurred on day 265 (22 September).

from about 20 to 10°C when measurements finished. At the same time, the daily minimum temperatures declined from about 8 to 2°C and close to 0°C on occasions.

Seasonal changes in gas exchange pre- and post-harvest: Mean light-saturated rates of photosynthesis at ambient CO₂ concentrations across the season prior to harvest were relatively constant at about 14–16 μmol m⁻² s⁻¹ although a few days before harvest, the rates had declined to about 10–12 μmol m⁻² s⁻¹ (Fig. 2A). After harvest, there was a recovery of the rates to again average 14–16 μmol m⁻² s⁻¹ although there was a subsequent slight decrease over the season, such that by the last measurements, the rates averaged 12 μmol m⁻² s⁻¹ (Fig. 2B). These results might have occurred from low PFDs about the time of harvest which caused the short-term downregulation of photosynthesis as the PFDs increased and the rates had recovered a few days after harvest. Across the season, there was a significant ($P < 0.001$, $n = 26$) effect of day on photosynthesis, but was relatively weak, only accounting for 15% of the variance. There was, however, no correlation between light-saturated photosynthesis and the seasonal climate.

By contrast, the mean stomatal conductance prior to

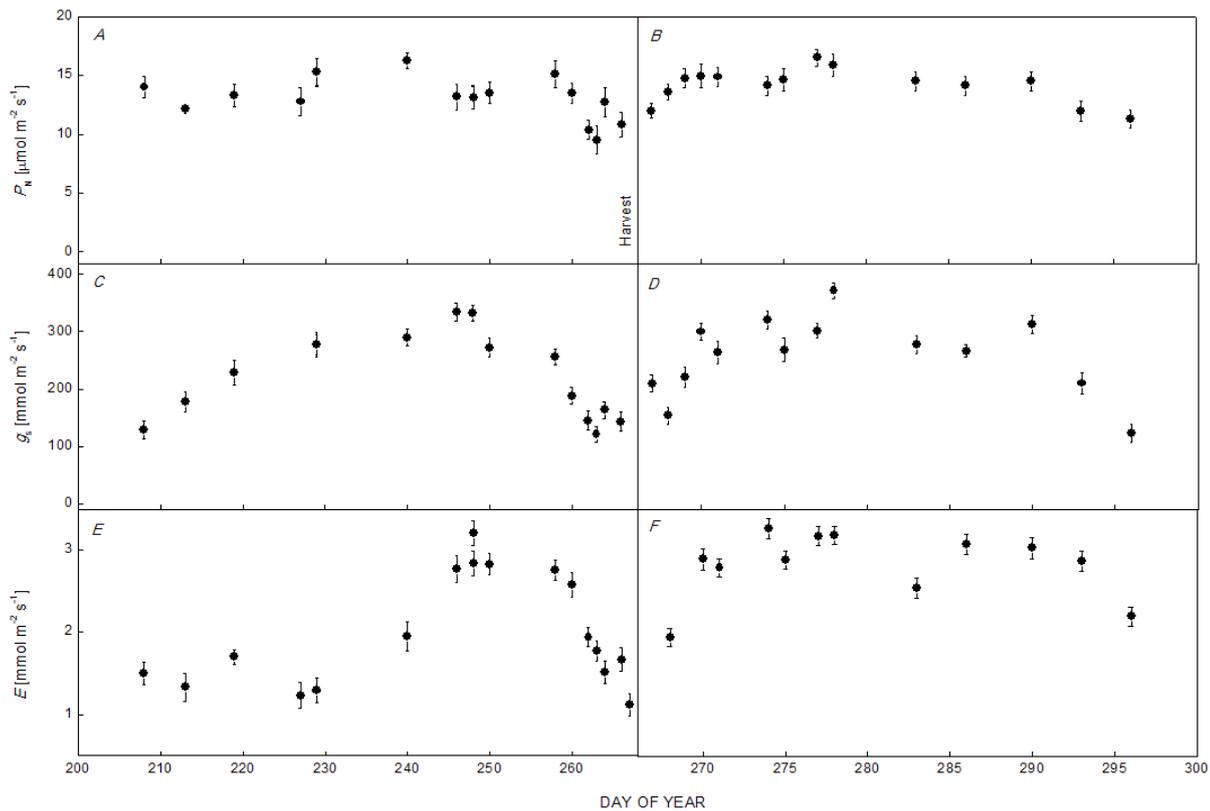


Fig. 2. Seasonal changes in light-saturated net photosynthesis (mean \pm SE, $n = 9-12$) before (A) and after (B) harvest, stomatal conductance before (C) and after (D) harvest, and transpiration before (E) and after (F) harvest for leaves of Cox's Orange apple trees growing in orchard conditions. Harvest occurred on Day 265 as indicated. The average leaf temperature during the pre-harvest period was 26.8°C and the average ambient CO₂ concentration was 409 $\mu\text{mol mol}^{-1}$ and during the post-harvest period, the average leaf temperature was 20.9°C and the average ambient CO₂ concentration was 400 $\mu\text{mol mol}^{-1}$. A snow storm in late October curtailed any more measurements.

harvest increased across the growing season to peak at 330 $\text{mmol m}^{-2} \text{s}^{-1}$ at about the end of summer (Fig. 2C). Thereafter, the conductance declined, such that just at harvest, averaged 150 $\text{mmol m}^{-2} \text{s}^{-1}$. After harvest, the stomatal conductance increased again to about 300 $\text{mmol m}^{-2} \text{s}^{-1}$ and remained more or less constant until the end when there was an indication of some stomatal closure (Fig. 2D). There was a significant effect of day on the stomatal conductance and this accounted for nearly 50% of the variance but there was no correlation with the seasonal climate.

Transpiration followed yet a different pattern prior to harvest, with rates remaining low and relatively constant at 1.5 $\text{mmol m}^{-2} \text{s}^{-1}$ over the early and midsummer but the rates increased markedly at the end of summer to about 3 $\text{mmol m}^{-2} \text{s}^{-1}$ (Fig. 2E). Thereafter, there was a decrease in rates of transpiration that conformed closely to the decrease in stomatal conductance. During the season after harvest, the rates of transpiration initially increased but, for the most part, remained about constant at 2.5–3 $\text{mmol m}^{-2} \text{s}^{-1}$ (Fig. 2F). There was a significant effect of day on the transpiration and this accounted for nearly 40% of the variance but again there was no correlation with the seasonal climate.

P_N/C_i responses pre- and post-harvest: Typical P_N/C_i responses for leaves measured prior to harvest indicated maximum light- and CO₂-saturated photosynthetic rates were about 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with the estimated maximum rate of RuBP carboxylation (V_{cmax}) averaging 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the estimated maximum rate of RuBP regeneration (J_{max}) averaging 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3A). For leaves measured after the fruit were harvested, the typical P_N/C_i response indicated a maximum photosynthetic rate of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and V_{cmax} averaged 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and J_{max} averaged 180 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3B).

Changes in $P_{N\text{max}}$, V_{cmax} , and J_{max} over the growing season:

The maximum CO₂- and light-saturated photosynthetic rates ($P_{N\text{max}}$) remained relatively high and more or less constant over the growing season, averaging 29.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4A). However, just before harvest, the rates decreased to 23.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After harvest, $P_{N\text{max}}$ initially remained low but within a few days recovered, with rates averaging 26.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ until early autumn (Day 290) when the rates decreased again, probably in concert with decreased PFDs and temperatures (Fig. 4B). There was a significant effect of day on the $P_{N\text{max}}$ but this accounted for less than 16% of the variance and there was no correlation with the seasonal climate.

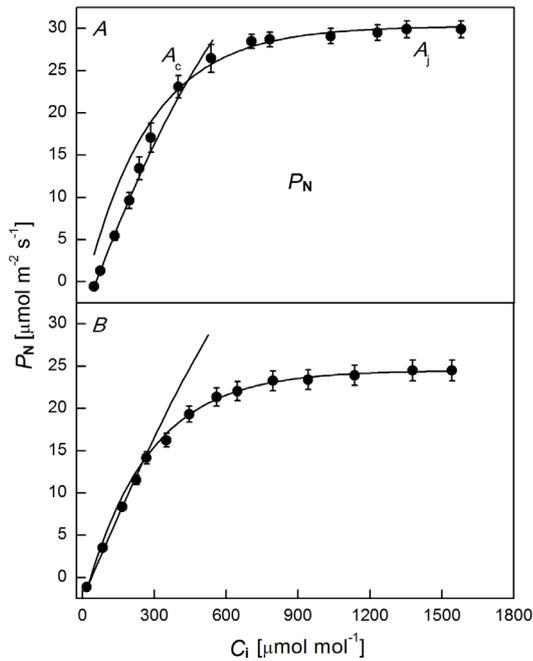


Fig. 3. Typical P_N/C_i responses (mean \pm SE, $n = 3-4$) for Cox's Orange apple leaves before (A) and after harvest (B). Also shown are the modelled rates of RuBP carboxylation (A_c) and RuBP regeneration (A_r) as indicated and determined from fitting the C_3 photosynthesis model of Farquhar *et al.* (1980) to these data.

At the start of measurements in late spring, the apparent V_{cmax} was initially high at $84 \mu\text{mol m}^{-2} \text{s}^{-1}$ but declined slowly and then more rapidly by DAB 240, such that at harvest, the apparent rates were almost 80% lower at $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4C). After harvest, there was some initial recovery in V_{cmax} to about half the spring rates but then a slow progressive decline in V_{cmax} occurred for the remainder of the growing season (Fig. 4D). There was a significant effect of day on seasonal changes in V_{cmax} and this accounted for about 60% of the variance, but there also some correlation with the seasonal climate, accounting for 35% of the variance.

By contrast, the apparent J_{max} initially increased slightly but then remained more or less constant at an average of $165 \mu\text{mol m}^{-2} \text{s}^{-1}$ until just before harvest, where the rates had declined by about 40% to $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 4E). After harvest, there was also some recovery but, unlike V_{cmax} , J_{max} increased to rates higher than occurred in spring and peaked briefly at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ before slowly declining over the rest of the growing season, in keeping with V_{cmax} (Fig. 4F). There was a significant effect of day on seasonal changes in J_{max} and this accounted for about 30% of the variance, but there was no correlation with the seasonal climate.

Stomatal limitation across the growing season: In late spring and early summer, there was a relatively high stomatal limitation at 45–60% but by mid to late summer,

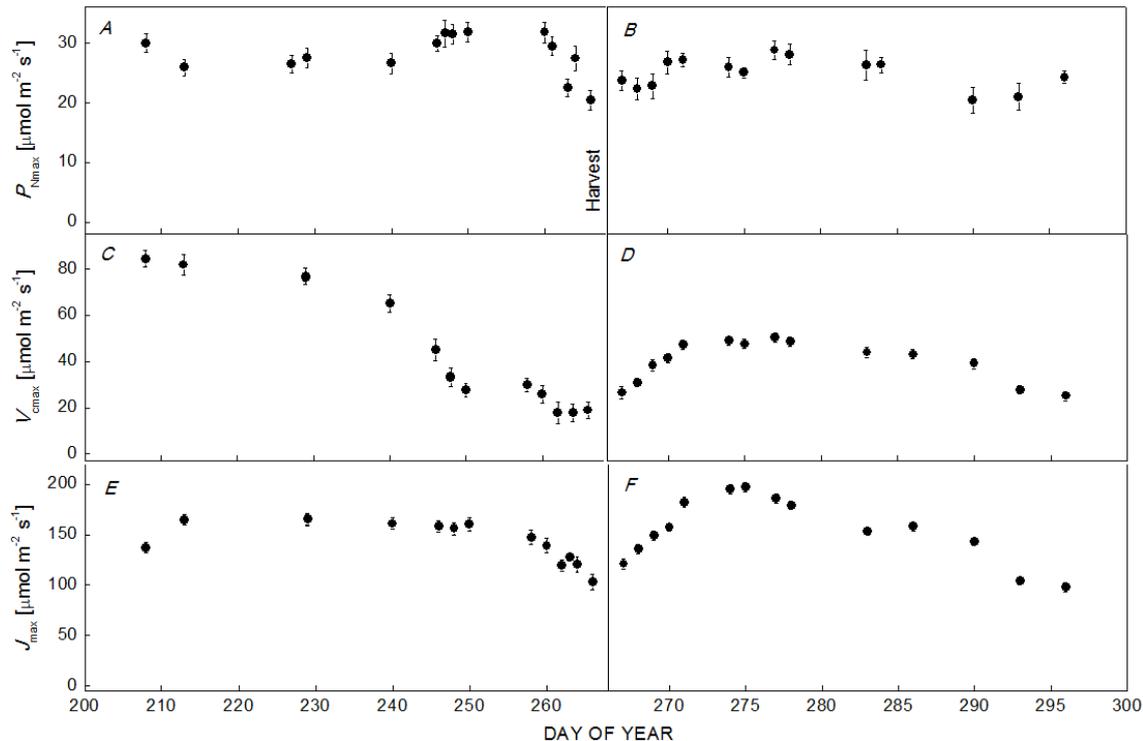


Fig. 4. Light and CO_2 -saturated maximum rates of photosynthesis (P_{Nmax}) of apple leaves of Cox's Orange apple trees growing in orchard conditions before (A) and after (B) harvest, estimated maximum rates of RuBP carboxylation (V_{cmax}) before (C) and after harvest (D) and the estimated maximum rates of RuBP regeneration (J_{max}) before (E) and after (F) harvest. In all cases the values are means \pm SE ($n = 9-12$). Harvest occurred on Day 265 as indicated.

the stomatal limitation had declined to between 30 and 45%, consistent with the decrease in stomatal conductance (Fig. 5A,B). By contrast, after harvest the stomatal limitation decreased further to about 25% but thereafter, remained more or less constant.

Discussion

Across the growing season, rates of photosynthesis of the leaves of the cv. Cox's Orange apple trees at ambient conditions were relatively stable. This conforms to the PFDs remaining above about 900 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$ (cf. Fig. 1) and seemingly high enough to maintain maximal rates (Greer 2018). Similarly, the maximum air temperatures remained mostly above about 20°C which might also explain the stability of the photosynthetic rates. However, rates of photosynthesis of leaves of cv. Braeburn apple trees were also relatively stable, apart from an early peak, from 25 to 158 d after flowering, the rates ranged from 9–12 $\mu\text{mol m}^{-2}\text{ s}^{-1}$ (Palmer *et al.* 1997) as was also shown for this and two other cultivars (Palmer *et al.* 2002). Similarly, rates of photosynthesis of cv. Red Gala apple trees at a comparable temperature (25°C) also indicated an initial early peak and thereafter ranged between 10.2 and 13.3 $\mu\text{mol m}^{-2}\text{ s}^{-1}$ from 53 to 160 DAB (Greer 2015). Likewise, photosynthetic rates of cv. Starkrimson apple trees were also relatively stable across about 150 d of the growing season (Fujii and Kennedy 1985), similarly for cv. Golden Delicious and cv. Granny Smith (Mierowska *et al.* 2002). Thus, despite changes in the seasonal climate, most apple cultivars with a moderate crop load apparently maintained relatively stable rates of photosynthesis over the growing season.

As noted in this study, however, ambient rates of photosynthesis declined at or just before harvest and this was coincident with a marked reduction in stomatal conductance. However, the decrease was also coincident with PFDs declining to around 200 $\mu\text{mol m}^{-2}\text{ s}^{-1}$ and day temperatures declining to about 12°C and a night frost the day before harvest occurred (cf. Fig. 1B). Thus, it seemed most likely that the low PFDs and temperatures were an explanation for the decrease in photosynthesis and perhaps also the decrease in stomatal conductance. Because the study was conducted in a commercial orchard, no fruit could be removed earlier than harvest to assess if these changes over harvest were more directly related to the

fruit removal. Although the harvest date was not provided, Fujii and Kennedy (1985) also noted a marked decrease in photosynthesis occurred on cv. Starkrimson trees from about 164 DAB and this was coincident with fruit dry matter stopping accumulation. However, this was not likely to be related to fruit growth because photosynthesis of fruiting and nonfruiting spurs declined in concert, suggesting leaf senescence and/or climate may have driven the decline. Few studies have determined photosynthesis as close to harvest as the present study, thus, it remains uncertain if rates of photosynthesis decline at this stage more generally.

It was, however, notable that the rates of photosynthesis of the Cox's Orange trees recovered relatively quickly after the fruit harvest, within 3 d rates were comparable with the pre-harvest rates and again remained reasonably steady thereafter. This increase in photosynthesis occurred despite the daily maximum PFDs declining markedly throughout this period and the maximum temperatures were falling as well, although no statistical correlations between photosynthesis and climate were observed. These data might suggest the current measurement conditions, saturating PFDs (1,500 $\mu\text{mol m}^{-2}\text{ s}^{-1}$) and leaf temperatures maintained around 20°C, had a greater effect on rates of photosynthesis than the ambient conditions. However, while stomatal conductance declined prior to harvest, the conductance increased in the post-harvest period and remained reasonably high throughout and this probably accounted for the concurrent increase in transpiration. Thus, the stomatal conductance was unlikely to represent a major limitation to photosynthesis in the post-harvest period. Elsewhere, rates of light saturated photosynthesis measured 20 d after harvest of cv. Braeburn apple trees were also comparable with rates measured 30 d before harvest (Wünsche *et al.* 2000). However, in the same study, 48 d after harvest, the rates of photosynthesis had declined about 40% whereas in the present study 30 d after harvest, rates had declined about 25%. In contrast, also for Braeburn apple trees 11 d after harvest, rates of photosynthesis had declined about 45% for high crop load trees but for trees with a low or no crop load, there was no change in the rates of photosynthesis (Palmer *et al.* 1997). In the Wünsche *et al.* (2000) study, 30 d after harvest, transpiration followed a similar trend to assimilation, declining by 40% in high cropped trees and no change in zero cropped trees compared with a 30% reduction in the

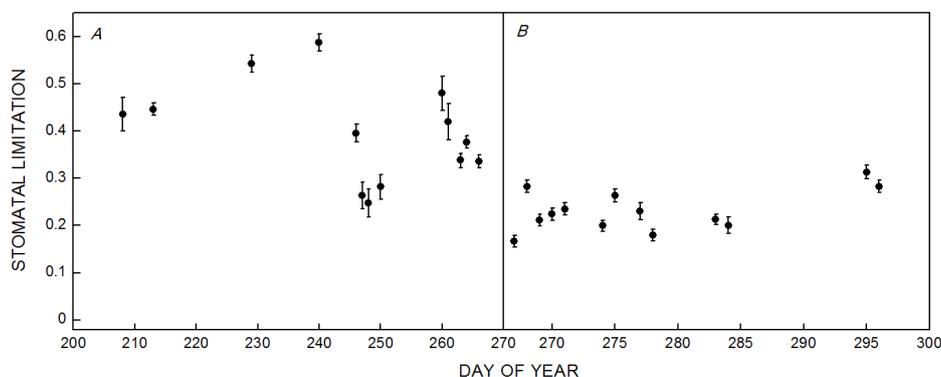


Fig. 5. Stomatal limitation of photosynthesis (mean \pm SE, $n = 9-12$) measured on leaves of Cox's Orange apple trees growing in orchard conditions before (A) and after (B) harvest. In each case, the values were determined from the P_N/C_i curves according to the procedure of Farquhar and Sharkey (1982) and adopted by Greer and Weedon (2012).

present study.

Saturating the photosynthetic process with substrate CO₂ had no marked effect on the seasonal progression of P_{Nmax} which essentially paralleled the rates at ambient conditions across the growing season (*cf.* Figs. 2, 4). This maintenance of the CO₂-saturated rates occurred despite a marked decrease in the apparent maximum rates of RuBP carboxylation and this suggested V_{cmax} was not limiting photosynthesis at ambient and, therefore, also at saturating CO₂ concentrations. By contrast, apparent maximum rates of RuBP regeneration across the growing season were in close accord with the rates of photosynthesis and hence were more likely to be limiting photosynthesis. Certainly, the decrease in J_{max} just before harvest was consistent with the decrease in photosynthesis then. By contrast after harvest, apparent rates of RuBP regeneration increased markedly and were highest across the whole growing season just 8 d after harvest. A concomitant increase in CO₂-saturated rates of photosynthesis did not occur at this time which might, therefore, suggest that J_{max} was no longer limiting and perhaps the low rates of RuBP carboxylation might have become more limiting. However, as it were the rates of photosynthesis at the ambient CO₂ concentration that increased most apparently immediately after harvest and both V_{cmax} and J_{max} also increased in concert, therefore, any discrimination of the dominant limitation was not possible in that short time. Consistent with this, the seasonal changes in P_{Nmax} , V_{cmax} , and J_{max} for *Quercus douglasii* leaves also followed such similar patterns across the growing season although which factor that was most limiting to photosynthesis was not discernible (Xu and Baldocchi 2003).

Although the stomatal limitation of assimilation in the pre-harvest period was generally high early in the growing season and might have limited photosynthesis, the decrease later about 20 d before harvest did not comply with any change in photosynthesis so probably did not limit the process. A stronger but similar conclusion was reached in the post-harvest period when the stomatal limitation was low and relatively constant and, therefore, unlikely to limit photosynthesis much, in keeping with the conclusion about stomatal conductance.

Conclusions: Cox's Orange apple trees had relatively stable rates of photosynthesis across the growing season and appeared to be mostly limited by nonstomatal processes. Prior to harvest, photosynthesis appeared to be more limited by the regeneration of RuBP, as changes in V_{cmax} had no apparent effect on the rates, whereas J_{max} appeared to mirror assimilation. But after harvest, apparent rates of carboxylation of RuBP were relatively low and appeared to be more limiting as J_{max} increased markedly and this change also had no apparent effect on photosynthesis. Perturbations in rates of photosynthesis in the few days before and after the harvest time also occurred in concert with changes in the stomatal conductance and V_{cmax} and J_{max} and may have been as consequence of the removed crop load not demanding much assimilation from the trees but also this change could have been the result of mechanical effects on the trees in the process of harvesting

the fruit. However, it was not possible to remove fruit in the trial so this hypothesis could not be sustained. In addition, the hypothesis that the loss of the fruit sink would cause a decrease in photosynthesis was also not sustained in this study.

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