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RESEARCH PAPER

Effect of fruit load and girdling on leaf photosynthesis in *Mangifera indica* L.

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Abstract

Leaf nitrogen concentration (N_m), mass-to-area ratio, amount of nitrogen per unit leaf area (N_a), non-structural carbohydrate concentration (TNC_a), maximal rate of carboxylation (V_{cmax}), light-saturated rate of photosynthetic electron transport (J_{max}), dark respiration (R_d), net photosynthetic assimilation (A_{net}), quantum yield of photosystem II (Φ_{PSII}), and intercellular CO_2 concentration (C_i) were measured in *Mangifera indica* L. leaves on three types of fruit-bearing branches (non-girdled, NG; girdled with high (HFL) and low (LFL) fruit load), experiencing similar light exposure. TNC_a, V_{cmax}/N_a, J_{max}/N_a, R_d/N_a, C_i, and the initial quantum yield of photosynthetic electron transport (α) were similar in both HFL and NG treatments, but N_m, N_a, and photosynthetic capacity parameters (V_{cmax} and J_{max}) were lower in the HFL than in the NG treatment. The strong depressing effect of girdling on leaf nitrogen concentration cannot therefore be attributed to a change in TNC_a. By contrast, N_a and TNC_a were lower and higher, respectively, in the LFL than in the HFL treatment, suggesting that carbohydrate content may become the driving force behind photosynthetic acclimation to changing source–sink relationships, like the ones resulting from the presence of developing fruits. V_{cmax}/N_a and J_{max}/N_a were lower in the LFL than in the HFL treatment, while R_d/N_a, C_i, and α were not affected by fruit load. It is concluded that girdling and high fruit load affect photosynthesis permanently by decreasing and increasing, respectively, leaf nitrogen concentration. Fruit load, moreover, may have an additional effect on photosynthetic capacity by affecting the relationship between V_{cmax} and J_{max}, and N_a.

Key words: Fruit load, girdling, leaf nitrogen, *Mangifera indica* L., non-structural carbohydrates, photosynthesis, photosynthetic capacity, starch, stomatal conductance.

Introduction

Modelling the distribution of photosynthetic photoassimilates within individual fruit trees has been proposed as a tool to predict the intra-crown heterogeneity of fruit growth (Grossman and DeJong, 1994; Lescourret *et al.*, 1998; Le Roux *et al.*, 2001), which plays a crucial role in fruit quality (Génard *et al.*, 1998). While the effect of light exposure and leaf age on leaf nitrogen concentration and photosynthetic capacity have been well described, little is known about the effects of source/sink balance, and the associated changes in carbon export rate from leaves and leaf carbohydrate concentration, such as the ones resulting from the presence of developing fruits, on leaf nitrogen and photosynthetic capacity within the crown of field-growing trees. This can restrict the ability to predict the spatial distribution of carbon gains and fruit growth within the canopy of fruit trees accurately. Girdled branches with different leaf-to-fruit ratios may provide an excellent system to study the effects of changes in source/sink balances on leaf photosynthesis (Ben Mimoun *et al.*, 1996; Génard *et al.*, 1998).

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Abbreviations: α, initial quantum yield of photosynthetic electron transport; A_{net}, net photosynthesis; C_i, partial pressure of CO_2 intercellular; g_s, stomatal conductance; HFL, high fruit load; J_{max}, light-saturated rate of photosynthetic electron transport; J_t, total light-driven electron flow; LFL, low fruit load; M_a, mass-to-area ratio; N_a, amount of leaf nitrogen per unit leaf area; NG, non-girdled; N_m, leaf nitrogen concentration expressed on a dry matter basis; *NPQ*, non-photochemical quenching; Φ_{PSII}, quantum yield of photosystem II; *Q*, photosynthetic photon flux density; q_P, photochemical quenching; R_d, dark respiration; θ, leaf absorbance; T_l, leaf temperature; TNC_a, total non-structural carbohydrates concentration; V_{cmax}, maximal rate of carboxylation.

Girdling (the removal of a ring of phloem) is a common horticultural practice used to manipulate tree growth and development, and fruit growth, in a variety of fruit species. Its most immediate effect is to stop the basipetal movement of assimilates through the phloem, which results in an accumulation of carbohydrates above the girdle (Roper and Williams, 1989; Schaper and Chacko, 1993; Di Vaio *et al.*, 2001). Girdling has been shown to decrease net photosynthesis (A_{net}) in *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) (Zhou and Quebedeaux, 2003), *Anacardium occidentale* L. (Schaper and Chacko, 1993), *Vitis vinifera* L. (Harrell and Williams, 1987; Roper and Williams, 1989), *Prunus persica* var. nucipersica (Suckow) C.K. Schneid (Di Vaio *et al.*, 2001), and *Mangifera indica* L. (Lu and Chacko, 1998). However, in the presence of a strong sink activity, such as the one provided by the presence of growing fruits, A_{net} may remain high, as shown in *Olea europaea* L. (Proietti and Tombesi, 1990) or *Anacardium occidentale* L. (Schaper and Chacko, 1993).

Several studies have emphasized that leaf photosynthesis can be influenced by the presence of developing fruits. A positive effect of crop load on A_{net} has been reported in many species, including *Citrus unshiu (*Mak.) Marc. (Iglesias *et al.*, 2002), *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) (Hansen, 1970; Palmer, 1992; Palmer *et al.*, 1997; Wünsche *et al.*, 2000), *Prunus cerasus* L. (Layne and Flore, 1995), *Prunus domestica* (Gucci *et al.*, 1991), *Prunus persica* (L.) Batsch. (Ben Mimoun *et al.*, 1996), *Prunus persica* var. nucipersica (Suckow) C.K. Schneid (Di Vaio *et al.*, 2001), and *Vitis vinifera* L. (Loveys and Kriedemann, 1974; Kaps and Cahoon, 1989; Edson *et al.*, 1995; Naor *et al.*, 1997). Carbohydrate accumulation has been shown to have an inhibitory effect on photosynthesis (Azcon-Bieto, 1983; Foyer, 1988; Goldschmidt and Huber, 1992; Martinez-Carrasco *et al.*, 1993), leading to the assumption that a lack of sink activity, resulting from a high leaf-to-fruit ratio, leads to carbohydrate accumulation and feedback inhibition of A_{net}, and vice-versa (Ben Mimoun *et al.*, 1996; Wünsche *et al.*, 2000; Iglesias *et al.*, 2002). However, the mechanisms whereby A_{net} is decreased following carbohydrate accumulation are not well understood. *In vitro* experiments have demonstrated that mesophyll carbohydrate concentration, which depends on the local balance between assimilation and export, can modify the expression of photosynthetic gene promoters (Koch *et al.*, 1992; Sheen, 1994; Jang and Sheen, 1994). Although several carbohydrate-based regulatory mechanisms of photosynthesis may coexist (Quereix *et al.*, 2001), it is reasonable to hypothesize that the long-term consequence of carbohydrate accumulation is a decrease in photosynthetic capacity. Because proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen, photosynthetic capacity is generally strongly related to the amount of leaf nitrogen per unit leaf area, N_a (Field and Mooney, 1986; Evans, 1989; Kellomäki and Wang, 1997; Walcroft *et al.*, 1997, 2002; Urban *et al.*, 2003). A decrease in photosynthetic capacity resulting from reduced sink activity and carbohydrate accumulation would thus be expected to be associated with a decrease in N_a, either the concentration of nitrogen per dry mass unit or the leaf-to-mass ratio, or both. Leaf nitrogen concentration per dry mass has been reported to be higher in fruiting apple trees (Thiebus-Kaesberg and Lenz, 1994), while leaf mass-to-area ratio and chlorophyll concentrations have been found to be higher in leaves of fruiting shoots of *Olea europaea* L. than in non-fruiting shoots (Proietti, 2000). By contrast, lower leaf nitrogen concentrations per dry mass have been observed in fruiting trees of *Prunus persica* (L.) Batsch. (Taylor and May, 1967; Taylor and Van den Ende, 1969), and *Citrus madurensis* Lour. (Lenz, 2000). Decreases in leaf nitrogen concentration throughout the fruit growth period could be attributed to nitrogen dilution in growing leaves or to the remobilization of nitrogen from leaves to fruits (Rufat and DeJong, 2001).

The objective of this study is to improve the understanding of the effect of source/sink relationships on leaf photosynthesis by assessing the effects of fruit load and girdling on leaf nitrogen concentration and the key parameters of photosynthetic capacity of leaves from fruiting branches of *Mangifera indica* L. As A_{net} is potentially affected by intercellular CO_2 concentration (C_i) and the down-regulation of electron flow is usually associated with carbohydrate accumulation, stomatal conductance (g_s) and quantum efficiency of light-use of light-adapted leaves (Φ_{PSII}) were also measured in leaves that were either non-girdled or girdled with either low and high fruit loads.

Materials and methods

Experimental site and plant material

Measurements were performed on leaves from ten 12-year-old trees of *Mangifera indica* L., cv. Lirfa, grafted on 'Maison rouge', randomly selected, although avoiding border trees, within an homogeneous experimental orchard near Saint-Pierre, Réunion island (20°52′48″ S, 55°31′48″ E). Trees were spaced at 5 m (within rows) ×7 m (between rows) and were about 3 m high, with a north-east to south-west row orientation. Trees were irrigated every 2 d on the basis of 100% replacement of evapotranspiration estimated from the equation of Penman–Monteith (Monteith, 1965). Fertilizers were supplied and insects and diseases controlled according to the recommendations of the local agricultural department.

Six weeks after flowering, 60 fruiting branches composed of shoots of the current year and of the previous year were selected among the experimental trees, which represented six branches per tree. Leaf age was found to be unrelated to the observed changes in leaf nitrogen content (Urban *et al.*, 2003). All branches had the same north-west to south-east orientation and a similar height (about 1.5 m). Fish-eye pictures were taken and gap fractions calculated, following the method of Baret *et al.* (1993) and Génard and Baret (1994), to ensure that light exposure did not differ among treatments (HemiView 3.1 SR1, Delta-T devices, UK). Originally, four treatments were considered: non-girdled (NG), girdled with high fruit load (HFL), girdled with low fruit load (LFL) with leaves close to developing fruits, and LFL with leaves far from developing fruits. Fifteen branches were non-girdled and

served as control units. Forty-five branches were girdled to control their leaf-to-fruit ratio precisely (Lescourret *et al.*, 1998; Génard *et al.*, 1998). Because of the potential influence of girdled branches on non-girdled branches, all treatments were represented at least once in each tree. Treatments were randomly allocated to each of the six branches of each tree. Consequently, each treatment was represented one, two or three times in each tree. Girdling was carried out on 9 and 10 October 2002, about 50 d after full bloom, when fruits were 5 cm long, by removing a 10–15 mm wide band of bark in the middle of the main stem of each selected branch. At the time of girdling, leaves were all fully developed. Vegetative flushing after girdling was exceptional, and all new buds were removed. Moreover, no fruit drop was observed during the course of the trial. Fruits were removed to obtain branches with *c*. 10 and 100 leaves per fruit (HFL and LFL; n=17 and n=28, respectively). Then five leaves per branch at the most were removed, if needed, to obtain branches with exactly 10 and 100 leaves per fruit. Originally, a distinction was made between leaves close to and leaves far from the fruits in the LFL treatment, but in the absence of any difference it was decided to pool all the data together. The HFL and LFL treatments (both girdled) were compared to evaluate the effect of fruit load and the associated carbohydrate accumulation on photosynthesis. It was observed that the leaf-to-fruit ratio in the NG treatment was about 100 at the branch level, while reaching 1000 at the whole tree level. Moreover, preliminary observations had shown that accumulation of soluble sugars and starch was of the same magnitude in branches with 10 leaves per fruit than in non-girdled branches. While the LFL and NG treatments (both with 100 leaves per fruit) can be compared to evaluate the effect of girdling on carbohydrate accumulation and photosynthesis, the HFL and NG treatments can thus be compared to evaluate the effect of girdling on photosynthesis, independently of the effect of this practice on carbohydrate accumulation. Measurements were performed from 9 to 29 November 2002, during the period when fruit growth was linear.

Leaf gas exchanges and chlorophyll fluorescence

Net CO_2 assimilation rate (A_{net}) leaf stomatal conductance to water vapour (g_s) and quantum yield of photosystem II (Φ_{PSII}) were measured on 27 November 2002, with an infrared CO_2/H_2O gas analyser and LED-based leaf chamber fluorometer system (LI 6400 and LI 6400–40 LCF, Li-Cor Inc., Lincoln, USA). Φ_{PSII} was calculated according to Genty *et al.* (1989). Measurements were performed around midday, on well-lit, selected leaves from NG, HFL, and LFL treatments (n=9, 9, and 20, respectively). Measurements were performed at partial pressure of ambient CO_2=36 Pa, photosynthetic photon flux density, Q=*c*. 1800 μmol m^{-2} s^{-1}, leaf temperature, T_l=*c*. 30 °C, and water vapour pressure deficit at leaf surface=*c*. 3 kPa. After the end of gas exchange measurements, leaves were harvested and leaf areas measured, then leaves were frozen in liquid nitrogen for further analysis of carbohydrate and nitrogen contents.

Additional leaf gas exchange measurements were performed on 20 November 2002 to establish the relationship between A_{net} and g_s over a larger range of climatic conditions than the one imposed on 27 November 2002. Measurements were performed in the tracking mode to minimize light fluctuations (target value coming from the external sensor, potentially changing every 3 s), at a partial pressure of ambient CO_2=36 Pa, every 2 h from 08.00–16.00 h (n=6–12), with an infrared CO_2/H_2O gas analyser and leaf chamber system with a red+blue light source (LI 6400 and LI 6400–02B, Li-Cor Inc., Lincoln, USA). Water vapour pressure deficit at the leaf surface ranged from 1.2 to 3.1 kPa. It was found that g_s was poorly correlated to water vapour pressure deficit at the leaf surface in 'Lirfa' in this range, provided that the water supply was non-limiting. Comparisons of A_{net} and g_s among treatments have not been presented for this set of data, because they led to the same conclusions as the measurements performed on 27 November 2002.

Φ_{PSII}, photochemical quenching (q_P), and non-photochemical quenching (*NPQ*) coefficients were measured on 29 November 2002 (n=4 for each of the three treatments). Measurements were performed with a LED-based leaf chamber fluorometer system (LI 6400+LI 6400-40 LCF, Li-Cor Inc., Lincoln, USA). after exposing leaves to steady illuminations of 400, 1200, and 2000 μmol m^{-2} s^{-1}. q_P, an estimate of the proportion of open reaction centres, was calculated according to (Schreiber *et al.*, 1986). The minimal chlorophyll fluorescence level when photosystem II centres are open, was measured after applying a far-red pulse of 6 s. *NPQ* was calculated according to Bilger and Björkman (1990). The maximal fluorescence before dawn or after about 30 min of dark adaptation, was measured after applying a saturating flash for 0.8 s.

F_v/F_m, an indicator of photoinhibition (Butler, 1978; Krause, 1988), was measured before dawn or after about 30 min dark adaptation, provided by opaque bags. Measurements were performed before dawn and around 14.00 h on 8, 15, 22, and 29 November 2002 (n=6).

Photosynthetic capacity

The key parameters of photosynthetic capacity, the maximal rate of carboxylation (V_{cmax}, μmol CO_2 m^{-2} s^{-1}), the light-saturated rate of electron transport (J_{max}, μmol m^{-2} s^{-1}), and the rate of CO_2 evolution in the light resulting from processes other than photorespiration (R_d, μmol m^{-2} s^{-1}) were derived from A–C_i curves (Farquhar *et al.*, 1980; Harley *et al.*, 1992) performed on leaves of the NG treatment (n=6), and leaves of the HFL and LFL treatments (n=8), during the period between 9 and 19 November 2002. Leaves were placed in the cuvette to be exposed to high irradiance and ambient CO_2 for at least 15 min before starting A–C_i curves. Eleven measurements were taken (at partial pressure of ambient CO_2=200, 170, 140, 110, 80, 40, 30, 20, 15, 10, and 5 Pa CO_2) for each A–C_i curve. Conditions in the leaf chamber were controlled (T_l=30 °C). Q was set at 1500 μmol m^{-2} s^{-1} (saturating light). The best fit V_{cmax}, J_{max}, and R_d values were inferred by non-linear least square regressions (S-Plus 2000, MathSoft International, Bagshot, UK). R_d was estimated by measurements of the CO_2 evolution rate after 5 min in the dark. At the end of the measurements on each sampling day, leaves were harvested and leaf areas measured, then leaves were frozen in liquid nitrogen for nitrogen and carbohydrate measurements.

Leaf nitrogen and non-structural carbohydrates

Nitrogen and carbohydrate concentrations were assessed on frozen leaf samples taken between 9 and 19 November 2002 at the end of A–C_i curves, and on 27 November 2002 (gas exchange and chlorophyll fluorescence measurements). The total nitrogen concentration (N_m in g nitrogen g^{-1} dry weight), of each sample was measured on 5 mg of plant material powder with an elemental analyser (Carlo Erba Instruments, Milano, Italy), after the method of Colombo *et al.* (1988). Glucose, fructose, and sucrose in the leaves were measured by an enzyme-based analyser (YSI 2007, Yellow Springs Instrument Co., USA). Starch was determined by enzymatic hydrolysis to glucose (Thievend *et al.*, 1972).

Dry mass was assessed by freeze-drying. The masses of starch and soluble sugars were deducted from the dry mass to obtain the structural dry mass, which was used for calculation of N_m and the mass-to-area ratio (M_a in g dry matter m^{-2}). The amount of leaf nitrogen per unit leaf area (N_a in g nitrogen m^{-2}) was calculated as N_a=$N_m M_a$.

Initial quantum yield of photosynthetic electron transport

The initial quantum yield of photosynthetic electron transport for an incident radiation (α) was calculated to evaluate whether differences in Φ_{PSII} between treatments were due to J_{max} or α.

Assuming that photosystems I and II absorb equal amounts of light, the total light-driven electron flow, J_t, may be calculated as (Genty *et al.*, 1989):

$$J_t = 0.5\Phi_{PSII}\theta Q \quad (1)$$

where θ is leaf absorbance.

The effects of Q on Φ_{PSII} may be described by Smith's equation (Smith, 1937), which has often been employed to approximate the light responses of photosynthetic electron transport derived from leaf gas exchange measurements (Tenhunen *et al.*, 1976; Harley *et al.*, 1992; Falge *et al.*, 1996):

$$J_t = \alpha Q[1 + (\alpha Q/J_{max})^2]^{-0.5} \quad (2)$$

Combining equations 1 and 2,

$$\Phi_{PSII} = 2\alpha/\theta[1 + (\alpha Q/J_{max})^2]^{-0.5} \quad (3)$$

Equation 3 shows that at a given Q, differences in Φ_{PSII} among treatments may be due to differences in either α, θ, or J_{max}. It also shows that it is possible to derive α from Φ_{PSII}–Q curves, provided that θ or J_{max} are known. Assuming that θ=0.85, obtained on *Mangifera indica* L. leaves from measurements performed with an integrating sphere (H. Sinoquet, personal communication), is valid for all leaves in this study, and using the J_{max} data obtained on the same leaves from A–C_i curves, α was derived from Φ_{PSII} measurements performed at Q=10, 400, 1200, and 2000 μmol m^{-2} s^{-1} (n=4). α data obtained were confirmed by measurements performed at Q=20, 40, 60, and 80 μmol m^{-2} s^{-1} (Niinemets and Kull, 2001) on additional leaves (n=5) (data not shown).

Statistical analysis

Values were analysed by ANOVA followed by Multiple Comparison of Means (S-Plus 4, Mathsoft, Bagshot, UK). Results are expressed as means ±standard errors (SE). Treatment and time differences were assessed as significant at P<0.05. Comparisons of slopes of the best fit lines for Φ_{PSII} simulated as a function of Φ_{PSII} measured, and g_s as a function of A_{net}, were performed by covariance analysis (Scherrer, 1984).

Results

Effect of fruit load and girdling on nitrogen and non-structural carbohydrate concentrations of leaves

There were significant differences in N_a among treatments. N_a was 8% lower in the LFL than in the HFL treatment, 20% lower in the LFL than in the NG treatment, and 14% lower in the HFL than in the NG treatment (Table 1). Differences in N_a therefore appear more pronounced as a result of girdling than of fruit load. These changes in N_a resulted exclusively from changes in N_m, since M_a remained almost constant regardless of fruit load and girdling (Table 1). Gap fractions did not differ among the three treatments. The values of M_a calculated from gap fractions, using the relationship M_a=83.1 gap fraction+90.0 (L Urban, unpublished results), were close to 132 g dry matter m^{-2}, which is about 7% lower than measured M_a values (Table 1). This is consistent with the previous finding that M_a in leaves close to developing fruits is higher than in leaves on vegetative terminals experiencing similar gap fractions (Urban *et al.*, 2003).

Table 1. *Nitrogen and carbohydrate contents of leaves of non-girdled and girdled fruiting branches with 10 and 100 leaves per fruit (high and low fruit load, respectively): gap fractions, concentration of nitrogen per dry mass unit (N_m), leaf mass-to-area ratio (M_a), amount of nitrogen per unit leaf area (N_a), amounts of soluble sugars, starch, and total non-structural carbohydrates (TNC_a) per unit leaf area*

All measurements were performed on leaves of 12-year-old trees. Means are presented ±SE. Within each row, values followed by different letters differ significantly (P<0.05).

	Non-girdled branches	Girdled branches	
		High fruit load	Low fruit load
Leaf-to-fruit ratio	100	10	100
n	15	17	28
Gap fraction	0.51±0.05.a	0.50±0.07.a	0.51±0.07.a
N_m (g nitrogen g^{-1} dry matter)	1.70±0.05.a	1.42±0.04.b	1.31±0.02.c
M_a (g dry matter m^{-2})	140.2±2.7.a	143.7±2.9.a	142.4±2.6.a
N_a (g nitrogen m^{-2})	2.36±0.07.a	2.04±0.07.b	1.87±0.05.c
Soluble sugars (g m^{-2})	13.0±1.3.a	11.6±0.9.a	13.6±0.8.a
Starch (g m^{-2})	5.4±1.0.a	4.4±0.8.a	19.3±2.1.b
TNC_a (g m^{-2})	18.5±2.0.a	16.0±1.3.a	32.9±2.7.b

Starch and TNC_a were 257% and 78% higher, respectively, in the LFL than in the NG treatment (Table 1). Starch and soluble sugars were not significantly different between the NG and the HFL treatments (Table 1), which supports the idea that the NG is a valid control for the HFL treatment. Starch and TNC_a were 423% and 116% higher, respectively, in the LFL treatment than in the HFL treatment, confirming previous observations about the effect of fruit load on carbohydrate accumulation in leaves of girdled branches (Lescourret *et al.*, 1998; Génard *et al.*, 1998).

Effect of fruit load and girdling on photosynthetic capacity

J_{max} and R_d, were significantly lower in the HFL treatment than in the NG treatment (Table 2). J_{max} was significantly lower in the LFL treatment than in the HFL treatment. There were no significant differences in V_{cmax}/N_a, or J_{max}/N_a between the leaves of the NG and HFL treatments (Table 2). By contrast, V_{cmax}/N_a, or J_{max}/N_a were significantly lower in the LFL treatment than in the HFL treatment. There were no significant differences in R_d/N_a, J_{max}/V_{cmax}, and R_d/V_{cmax} among treatments (Table 2).

Effect of fruit load and girdling on leaf gas exchanges, photochemical quantum efficiency and non-photochemical quenching

At Q=c. 1800 μmol m^{-2} s^{-1} and T_l=30 °C, A_{net}, A_{net}/N_a, and g_s were not significantly different between the NG and HFL treatments, whereas they were substantially higher in the HFL treatment than in the LFL treatment (Table 3).

Table 2. *Characteristics of photosynthetic capacity of leaves from non-girdled and girdled fruiting branches with 10 and 100 leaves per fruit (high and low fruit load, respectively):* V_{cmax}, J_{max}, R_d, V_{cmax}/N_a, J_{max}/N_a, R_d/N_a, J_{max}/V_{cmax}, R_d/V_{cmax}

Measured parameters were derived from A–C_i curves performed on leaves of 12-year-old trees at saturating light (Q=1500 μmol m^{-2} s^{-1}) and leaf temperature=30 °C. Means are presented ±SE. For each row, values with different letters differ significantly (P<0.05).

	Non-girdled branches	Girdled branches	
		High fruit load	Low fruit load
Leaf-to-fruit ratio	100	10	100
n	6	8	8
V_{cmax} (μmol CO_2 electrons m^{-2} s^{-1})	78.6±3.5 a	65.0±6.4 ab	49.6±4.7 b
J_{max} (μmol electrons m^{-2} s^{-1})	115.6±2.1 a	96.1±7.6 b	78.3±6.6 c
R_d (μmol CO_2 m^{-2} s^{-1})	1.76±0.10 a	1.45±0.11 b	1.40±0.15 b
V_{cmax}/N_a	34.4±1.9 a	32.3±2.4 a	25.4±2.1 b
J_{max}/N_a	50.7±2.3 a	48.0±3.1 a	40.1±2.8 b
R_d/N_a	0.76±0.02 a	0.73±0.06 a	0.73±0.08 a
J_{max}/V_{cmax}	1.48±0.06 a	1.50±0.08 a	1.61±0.13 a
R_d/V_{cmax}	0.022±0.002 a	0.023±0.002 a	0.029±0.005 a

Table 3. *Photosynthesis characteristics of leaves from non-girdled and girdled fruiting branches with 10 and 100 leaves per fruit (high and low fruit load, respectively): net photosynthetic assimilation (A_{net}), nitrogen use efficiency of A_{net} (A_{net}/N_a), leaf diffusive conductance to water vapour (g_s), intercellular CO_2 concentration (C_i) and quantum efficiency of radiation use under actinic light (Φ_{PSII})*

Measurements were performed at photosynthetic photon flux density=*c*. 1800 μmol m^{-2} s^{-1} and leaf temperature=*c*. 30 °C on leaves of 12-year-old trees. Means are presented ±SE. For each row, values with different letters differ significantly (P<0.05).

	Non-girdled branches	Girdled branches	
		High fruit load	Low fruit load
Leaf-to-fruit ratio	100	10	100
n	9	9	20
A_{net} (μmol CO_2 m^{-2} s^{-1})	9.04±1.13.a	8.17±0.50.a	5.33±0.56.b
A_{net}/N_a (μmol CO_2 g N^{-1} s^{-1})	3.87±0.65.a	3.86±0.34.a	2.73±0.33.b
g_s (mol H_2O m^{-2} s^{-1})	0.12±0.02 a	0.13±0.01 a	0.07±0.01 b
C_i (μmol CO_2 mol air^{-1})	222±8 a	234±8 a	226±8 a
Φ_{PSII} (mol electrons mol^{-1} photons)	0.15±0.01 a	0.13±0.01 b	0.07±0.01 c

C_i was not significantly affected by any of the treatments. g_s was found to be closely correlated to A_{net} (Fig. 1), and the relationships between g_s and A_{net} were statistically similar in all three treatments.

At Q=1800 μmol m^{-2} s^{-1}, Φ_{PSII} was significantly different among treatments, in a decreasing order for the NG, HFL, and LFL treatments (Table 3). This trend was also found at Q=400, 1200, and 2000 μmol m^{-2} s^{-1}, in another series of measurements on 29 November 2002 (Fig. 2A). However, differences in Φ_{PSII} between treatments tended to decrease with increasing Q. At Q=2000 μmol m^{-2} s^{-1}, there was no significant difference in Φ_{PSII} between the HFL and NG treatments (Fig. 2A).

Q-related decreases in Φ_{PSII} were largely attributable to decreases in q_P (Fig. 2B). q_P was significantly lower in the HFL treatment than in the NG treatment only at Q=2000 μmol m^{-2} s^{-1} (Fig. 2B), and q_P was significantly lower in the LFL treatment than in the HFL treatment at all levels of Q. *NPQ* was significantly lower in the HFL and NG treatments than in the LFL treatment at all levels of Q (Fig. 2C).

There were no significant differences in predawn F_v/F_m among the three treatments on all sampling days (Fig. 3). Moreover, there was no significant decrease in F_v/F_m between predawn and afternoon measurements, in any treatment.

There were no differences in α among treatments (Table 4). Provided that there was no effect of either girdling or fruit load on leaf absorbance, this strongly suggests that differences in Φ_{PSII} among treatments were solely due to differences in J_{max} (equation 3).

Discussion

Effect of fruit load and girdling on leaf nitrogen and carbohydrate contents

The influence of factors other than light and age on the intra-canopy distribution of N_a under a given climate has not been much investigated until now. These results show that low fruit load and girdling have a negative effect on N_a (Table 1). Previously, it was found that photosynthetic light acclimation of *Mangifera indica* L. leaves during the vegetative growth period was driven mainly by changes in M_a (Urban *et al.*, 2003). By contrast, in the current study, differences in N_a associated with fruit load and girdling resulted exclusively from differences in N_m (Table 1). M_a remained almost constant in all three treatments, probably as the result of identical exposures to light, as shown by similar gap fractions values (Table 1). These results show that the response of photosynthetic capacity to fruit load and girdling is different from the pattern of photosynthetic acclimation to light.

While leaf non-structural carbohydrate concentration (TNC_a) decreased with fruit load, N_a, on the contrary, increased with it (Table 1). This observation apparently contradicts previous observations, performed on leaves from non-girdled branches, suggesting that TNC_a may not be the major driving force behind photosynthetic acclimation in *Mangifera indica* L. (Urban *et al.*, 2003). However, considering that in Urban *et al.* (2003) TNC_a only reached a maximum of 12 g m^{-2}, which is substantially lower than

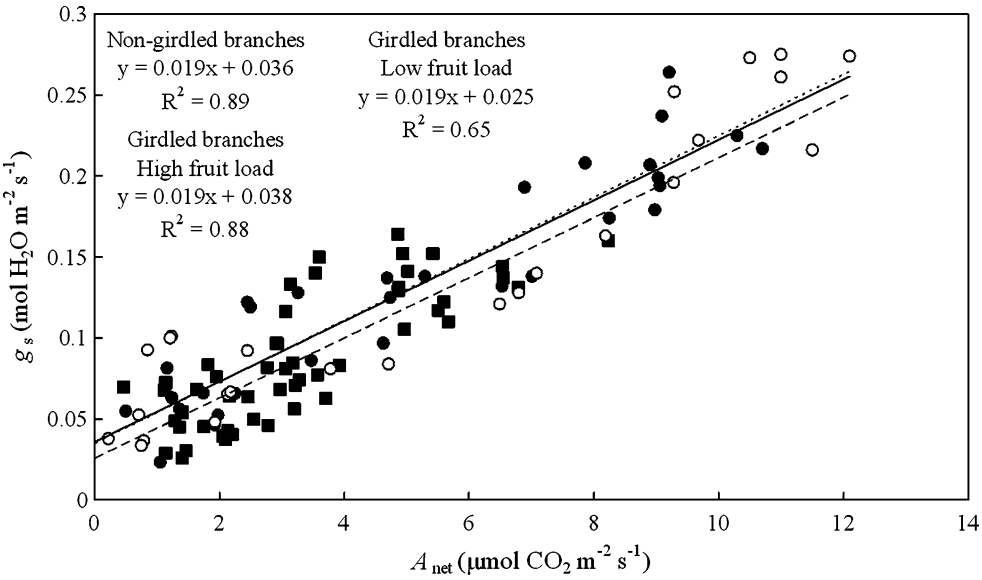

Fig. 1. Leaf diffusive conductance (g_s), as a function of net photosynthetic assimilation rate (A_{net}). Measurements were performed on leaves from non-girdled fruiting branches (open circles) and leaves from girdled branches with different 10 (filled circles) and 100 (filled squares) leaves per fruit. Linear regressions were not significantly different between treatments. The best fit line corresponds to all leaves.

the levels observed in the leaves of the HFL and LFL treatments of the present study, this study's results suggest that leaf carbohydrate accumulation may indeed become the driving force behind decreases in N_a, when TNC_a is above a certain threshold. Below this threshold mechanisms other than sugar-sensing may play a role.

Substantially higher values of N_m and N_a in both the HFL and LFL treatments than in the NG treatment show clearly that girdling had a strong negative effect on leaf nitrogen concentration (Table 1). Considering that the negative effect of girdling on N_m was much more marked than the positive effect of high fruit load, it is improbable that a further increase in fruit load (i.e. fewer than 10 leaves per fruit, as obtained in the HFL treatment) would fully overcome this effect. While the effect of fruit load is more pronounced on leaf carbohydrate concentration than on leaf nitrogen concentration, the major effect of girdling appears to be a strong decrease in leaf nitrogen concentration, which cannot be interpreted in terms of source–sink relationships, since differences in leaf nitrogen concentration between NG and HFL treatments were not associated with differences in leaf carbohydrate status (Table 1).

It has been shown that girdling may reduce g_s, and thus transpiration in leaves of *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) (Zhou and Quebedeaux, 2003), *Vitis vinifera* L. (Harrell and Williams, 1987; Roper and Williams, 1989; Williams *et al.*, 2000), *Mangifera indica* L. (Lu and Chacko, 1998), and *Prunus persica* var. nucipersica (Suckow) C.K. Schneid (Di Vaio *et al.*, 2001). Cytokinin level has been advocated as a potential candidate for controlling photosynthetic capacity (Pons and Bergkotte, 1996; Jordi *et al.*, 2000; Sakakibara, 2000; Ono *et al.*, 2001). No evidence was found that lower N_m and N_a in girdled branches of *Mangifera indica* L. resulted from the negative effect of girdling on g_s and transpiration rate, since g_s was not lower in the HFL treatment than in the NG treatment (Table 3). However, it may be hypothesized that the negative effect of girdling on N_m and thus N_a was the result of a decrease in the concentration in cytokinins in shoot xylem sap, as observed in girdled branches of *Prunus persica* (L.) Batsch. (Cutting and Lyne, 1993). Alternatively, it may be hypothesized that girdling reduced nitrogen transport to the leaves, either directly, by preventing nitrogen, mainly under organic form, from being transported through the phloem, or indirectly, by influencing nitrogen transport through the xylem, as the existence of interactions between xylem and phloem for the transport of nitrogenous compounds would suggest (Weber *et al.*, 1998; Gessler *et al.*, 2003).

Interpreting the effect of fruit load on net photosynthetic assimilation rate

The negative effect of low fruit load on A_{net} is consistent with observations on *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) (Hansen, 1970; Palmer, 1992; Palmer *et al.*, 1997; Wünsche *et al.*, 2000), *Vitis vinifera* L. (Loveys and Kriedemann, 1974; Kaps and Cahoon, 1989; Edson *et al.*, 1995; Naor *et al.*, 1997), *Prunus persica* var. nucipersica (Suckow) C.K. Schneid (Di Vaio *et al.*, 2001), *Prunus domestica* L. (Gucci *et al.*, 1991), and *Prunus persica* (L.) Batsch. (Ben Mimoun *et al.*, 1996). This negative effect may result either (i) from decreases in g_s and the associated changes in intercellular CO_2 concentration, C_i; (ii) from the decrease in electron transport rate resulting from the accumulation of photoassimilates; (iii) from a

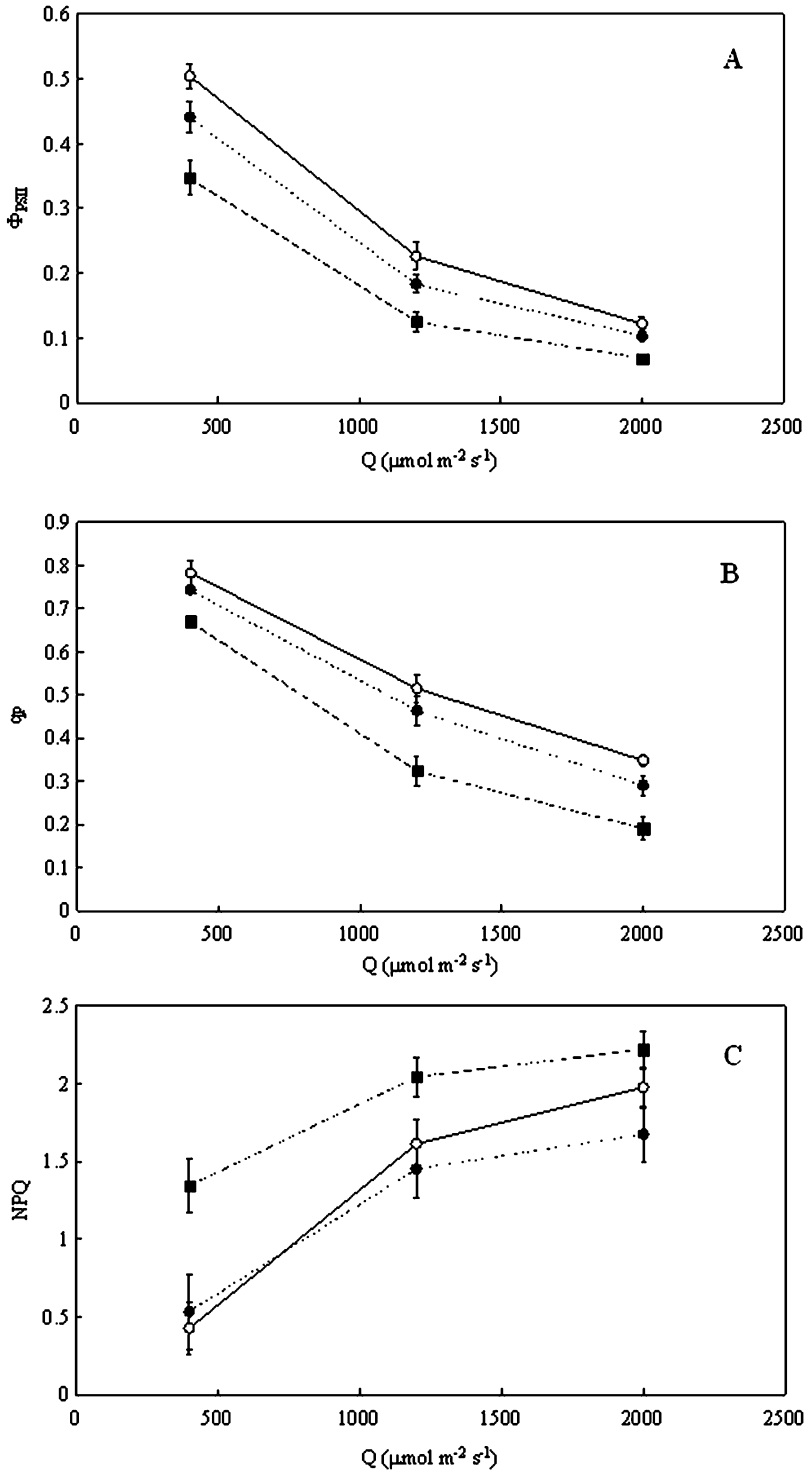

Fig. 2. The quantum efficiency of photosystem II under actinic light (Φ_{PSII}) (A), photochemical quenching (q_P) (B), and non-photochemical quenching (*NPQ*) (C) of leaves from non-girdled fruiting branches (open circles) and leaves from girdled branches with 10 (filled circles) and 100 (filled squares) leaves per fruit. Measurements were performed at Q=400, 1200, and 2000 μmol m^{-2} s^{-1}, on 29 November 2002. Means ±standard errors are given (n=4).

decrease in photosynthetic capacity caused by a decrease in N_a; or (iv) from a change in one or more of the key components of photosynthetic capacity (a decrease in V_{cmax} or J_{max}, or an increase in R_d).

Lower g_s of leaves from the LFL treatment than g_s of leaves from the HFL was not associated with lower C_i (Table 2), demonstrating that the depressing effect on A_{net} of lower fruit load is not attributable to a g_s-associated decrease in C_i.

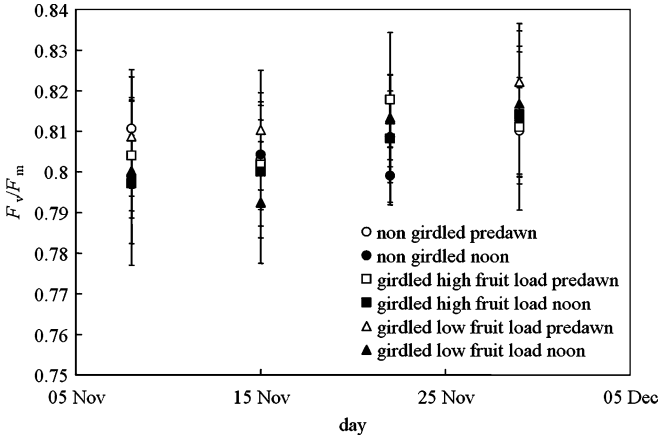

Fig. 3. Predawn F_v/F_m and F_v/F_m measured around noon of leaves from non-girdled fruiting branches (open circles, filled circles) and leaves from girdled branches with 10 (open squares, filled squares) and 100 (open triangles, filled triangles) leaves per fruit. Measurements were performed on 8, 15, 22, and 29 November 2002. Means ±standard errors are given (n=6).

Table 4. *The initial quantum efficiency of radiation use (α) of leaves from non-girdled and girdled fruiting branches with 10 and 100 leaves per fruit (high and low fruit load, respectively)*

Data were derived from Φ_{PSII} measurements performed at Q=10, 400, 1200, and 2000 μmol m^{-2} s^{-1} and leaf temperature=c. 30 °C, on leaves of 12-year-old trees (n=4), assuming θ=0.85 and using J_{max} data obtained from A–C_i curves (equation 6). Means are presented ±SE. Values with different letters differ significantly (P<0.05).

	Non-girdled branches	Girdled branches	
		High fruit load	Low fruit load
Leaf-to-fruit ratio	100	10	100
α (mol electrons mol^{-1} photons)	0.32+0.01 a	0.30+0.03 a	0.33+0.02 a

According to equation 1, and assuming that leaf absorbance was not affected by fruit load, higher Φ_{PSII} values in the HFL treatment than in the LFL treatment (Fig. 2A), demonstrate that photosynthetic electron flow (J_t) is positively correlated with fruit load. This is in agreement with Φ_{PSII} data obtained on *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) at different levels of fruit load (Wünsche *et al.*, 2000), and with numerous observations about the negative feedback effect of starch accumulation on leaf photosynthesis (Paul and Foyer, 2001). The reasons for Φ_{PSII} being positively correlated with low fruit load have not been understood so far. Assuming θ=0.85 in all types of leaves, differences in Φ_{PSII} may be due to differences in either α or J_{max} (equation 3). α was apparently not significantly affected by fruit load (Table 4). Changes in α have been associated with changes in F_v/F_m in a similar magnitude (Niinemets and Kull, 2001; Werner *et al.*, 2001). No difference was found in F_v/F_m between the HFL and LFL treatments. Moreover, there were no significant differences between predawn and afternoon measurements in relation to fruit load (Fig. 3), confirming that the high carbohydrate content associated with low fruit load does not increase either chronic or dynamic photoinhibition (Osmond, 1994), during the period of linear fruit growth in *Mangifera indica* L. This observation is somewhat contradicted by the lower values of q_P found in leaves of the LFL treatment than in leaves of the HFL treatment (Fig. 2B). High q_P is generally considered to be essential in protecting leaves from photoinhibition (Ögren, 1991; Ögren and Rosenqvist, 1992; Maxwell *et al.*, 1995; Baroli and Melis, 1998; Niinemets and Kull, 2001). However, higher levels of dissipation of excitation energy as heat, as estimated from *NPQ* measurements (Fig. 2C), in the LFL treatment than in the HFL treatment, may well have provided leaves with increased protection against photodamage (Osmond, 1994).

Considering that low A_{net} in the LFL treatment was neither due to low values of α nor to high values of R_d (Table 3), the next issue to address is to examine whether it is attributable to low N_a, or to low J_{max}/N_a or V_{max}/N_a values in the LFL treatment. The reduction in leaf nitrogen content in the LFL treatment does not fully account for the observed reduction in A_{net} as a function of fruit load. Compared with the HFL there was a 35% reduction in A_{net} in the LFL treatment, but there was only a 29% reduction in photosynthetic nitrogen use efficiency (A_{net}/N_a in Table 3). This suggests that factors other than leaf nitrogen content, like a shift in the fundamental relationships between the parameters of photosynthetic capacity and N_a may have played a role in the observed reduction in A_{net}. V_{cmax}/N_a and J_{max}/N_a were indeed lower in the LFL treatment than in the HFL treatment (Table 3), demonstrating that the lower photosynthetic capacity associated with low fruit load is not only attributable to lower leaf nitrogen content, but also to a decrease in the two major components of photosynthetic capacity, i.e. V_{cmax} and J_{max}, at a given leaf nitrogen concentration. Spatial and temporal variability in leaf photosynthetic capacity has been documented in a range of deciduous forest tree species (Wilson *et al.*, 2000) and *Prunus persica* (L.) Batsch. (Walcroft *et al.*, 2002). It has been hypothesized that this variability could be caused by temperature or light acclimation processes (Walcroft *et al.*, 2002). This study's results show that fruit load does not affect the allocation of nitrogen between the different components of the photosynthetic machinery, but that less leaf nitrogen is allocated to the photosynthetic machinery, at low fruit load, possibly as the consequence of reduced sink activity.

Effect of fruit load and girdling on the relationship between g_s *and* A_{net}

Cowan and Farquhar (1977) have hypothesized that stomata were regulated in such a way as to maximize A_{net} while minimizing water losses by transpiration. This

hypothesis underlies the models of Ball *et al.* (1987) and Leuning (1995), where g_s is linearly correlated to A_{net}. The existence of an unique relationship between g_s and A_{net}, regardless of fruit load (Fig. 3), confirms observations made on *Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) (Wünsche *et al.*, 2000), and suggests that the co-regulation of A_{net} and transpiration was not affected by fruit load. However, the slope of the relationship between g_s and A_{net} was lower during the phase of active fruit growth than during the phase of vegetative growth (Urban *et al.*, 2003), 0.019 versus 0.025, which questions the robustness of the model of g_s established for *Mangifera indica* L. during the period of vegetative growth (Urban *et al.*, 2003).

Conclusion

This study's results provide new insight in the effects of girdling and fruit load on leaf photosynthesis. The existence of a substantial difference in N_m, and thus N_a and photosynthetic capacity parameters, between NG and HFL treatments, indicates that high fruit load does not totally counterbalance the negative effect of girdling on photosynthesis. Moreover, models of fruit growth derived from studies made on girdled fruit-bearing branches (Génard *et al.*, 1998; Lescourret *et al.*, 1998) must be used with care when considering their integration in models at tree level. Low fruit load exercises a negative effect on A_{net} by decreasing N_m and N_a, V_{cmax}/N_a, and J_{max}/N_a, but not C_i. Moreover, no evidence was found for any down-regulation of photosynthetic electron flow rates due to differences in parameters other than J_{max}, as expected from the accumulation of non-structural carbohydrate in leaves resulting from low fruit load in girdled branches. These results clearly demonstrate that the decrease in A_{net} associated with low fruit load cannot simply be associated with the straightforward inhibiting effect of carbohydrate accumulation on electron flow and that sub-models of leaf photosynthesis used in models of intra-crown heterogeneity of fruit growth must be reassessed to take into account the effects of phenology and the associated changes in source–sink relationships on photosynthetic capacity.

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