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Stomatal Regulation of Photosynthesis in Apple Leaves: Evidence for Different Water-use Strategies between Two Cultivars

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• *Background and Aims* Leaf responses to environmental conditions have been frequently described in fruit trees, but differences among cultivars have received little attention. This study shows that parameters of Farquhar's photosynthesis and Jarvis' stomatal conductance models differed between two apple cultivars, and examines the consequences of these differences for leaf water use efficiency.

• *Methods* Leaf stomatal conductance (g_{sw}), net CO_2 assimilation rate (A_n), respiration (R_d) and transpiration (E) were measured during summer in 8-year-old 'Braeburn' and 'Fuji' apple trees under well-watered field conditions. Parameters of Farquhar's and Jarvis' models were estimated, evaluated and then compared between cultivars. Leaf carbon isotope discrimination ($\Delta^{13}C$) was measured at the end of the growing season.

• *Key Results* A single positive relationship was established between V_{Cmax} (maximum carboxylation rate) and N_a (leaf nitrogen concentration per unit area), and between J_{max} (maximum light-driven electron transport rate) and N_a. A higher leaf R_d was observed in 'Fuji'. The g_{sw} responded similarly to increasing irradiance and leaf temperature in both cultivars. g_{sw} responded to lower vapour pressure deficit in 'Fuji' than in 'Braeburn'. Maximal conductance (g_{swmax}) was significantly smaller and A_n was more limited by g_{sw} in 'Braeburn' than 'Fuji'. Lower g_{sw}, E and higher intrinsic water use efficiency were shown in 'Braeburn' and confirmed by smaller leaf $\Delta^{13}C$ compared with 'Fuji' leaves.

• *Conclusions* The use of functional model parameters allowed comparison of the two cultivars and provided evidence of different water use 'strategies': 'Braeburn' was more conservative in water use than 'Fuji', due to stomatal limitation of A_n, higher intrinsic water use efficiency and lower $\Delta^{13}C$. These physiological traits need to be considered in relation to climate adaptation, breeding of new cultivars and horticultural practice.

Key words: Apple, carbon isotope discrimination, leaf nitrogen, leaf temperature, irradiance, *Malus × domestica*, modelling, photosynthesis, stomata, transpiration, vapour pressure deficit, water use efficiency.

INTRODUCTION

The responses of fruit tree crops to fluctuating or changing climatic conditions may help in understanding current practical problems in fruit tree management, such as yield variability. The vegetative and reproductive growth of trees depends on assimilate production which is controlled by tree architecture and leaf functions, both modulated by environmental interactions (Flore and Lakso, 1989; Lakso, 1994). At branch scale, Massonnet *et al.* (2004) showed that two apple cultivars differed in transpiration rate, suggesting that this may result either from variability in branch structure, which affects light interception within the tree crown, or from differences in leaf physiological functions, or from both. Architectural diversity has been characterized among apple cultivars: Lespinasse (1992) and Costes *et al.* (2003) classified apple cultivars into four groups (types I to IV) based on branching and fruiting patterns. Massonnet (2004) showed that two group IV apple cultivars ('Fuji' and a new hybrid 'X3305') have a spatial leaf distribution conferring greater light interception by the canopy than two group III cultivars ('Braeburn' and

'Ariane'). The present study addresses the question of leaf functional differences between two of these cultivars, 'Fuji' and 'Braeburn'.

Stomatal conductance (g_{sw}) and net CO_2 assimilation rate (A_n) in C_3 fruit species depend upon conditions such as solar irradiance (Marini and Sowers, 1990; Francesconi *et al.*, 1997), leaf temperature (Seeley and Kammereck, 1977; Berry and Bjorkman, 1980), vapour pressure deficit (Watson *et al.*, 1978; Fanjul and Jones, 1982), soil and plant water status (Schulze, 1986) and mineral nutrition. The capacity of cultivars to adjust to environmental variations, and their water use strategy in particular, is affected by differences within a species in leaf biochemistry (e.g. carboxylation rate of Rubisco, electron transport rate) and stomatal responses. Among apple cultivars, stomatal regulation has been related to tree vigour; rapid growth was generally linked to high g_{sw} (Atkinson *et al.*, 2000; Li *et al.*, 2002). Oren *et al.* (1999) showed that stomatal sensitivity to leaf-to-air vapour pressure deficit (VPD) varies both within and between species. In the short term and at leaf scale, stomatal movements control the trade-off between A_n and transpiration (E), and hence water use efficiency (WUE) which is the ratio between carbon gain and

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water loss (Farquhar *et al.*, 1989). The intrinsic water use efficiency (IWUE), which was defined by Comstock and Ehleringer (1992) as the ratio of A_n rate to g_{sw}, is less dependent upon instantaneous environmental conditions (air temperature and relative humidity) than WUE, and is under tight genetic control in many tree species (Brendel *et al.*, 2002, 2007; Casasoli *et al.*, 2006). WUE can be estimated by carbon isotope discrimination ($\Delta^{13}C$), an integrative variable, which is linearly and negatively correlated with WUE in many C_3 species (Farquhar and Richards, 1984; Guehl *et al.*, 1995).

Quilot *et al.* (2002) and Tardieu (2003) suggested that ecophysiological models could be valuable tools for studying complex processes, i.e. interrelated physiological functions, particularly for comparing cultivars. Each genotype can be represented by a set of response parameters for a given range of environmental conditions (Tardieu, 2003). Thus, we hypothesized that parameters from ecophysiological models could be applied to apple cultivars and used to characterize the differences in leaf function between them.

The objective of this study was to identify the physiological traits that are cultivar-dependent in apple trees. To this end stomatal conductance, photosynthesis and dark respiration were compared in 'Fuji' and 'Braeburn'. The comparison was based on (*a*) parameters of the empirical stomatal conductance model of Jarvis (1976) and maximal stomatal conductance; and (*b*) parameters of the biochemical photosynthesis model of Farquhar *et al.* (1980). The relationship between leaf A_n and g_{sw}, E and $\Delta^{13}C$ was also examined to assess water use strategies of these apple cultivars.

MATERIALS AND METHODS

Plant material

Two apple cultivars belonging to two different architectural types of the Lespinasse's classification (Lespinasse, 1992) were studied: 'Braeburn' (type III), and 'Fuji' (type IV), grafted on dwarfing M9 rootstock. Three representative 8-year-old trees of 'Braeburn' and of 'Fuji' planted in December 1994, 6 m × 1·8 m apart, in a north–south orientation and trained using the Solaxe system (Lauri and Lespinasse, 2000) were used. The experimental plot was carefully irrigated using a microjet system monitored by tensiometers to avoid soil water deficits. All trees were unpruned, and the crop loads adjusted by chemical and manual thinning. The annual fruit yields were between 25 kg and 35 kg per individual tree. All experiments were carried out at INRA experimental station (Mauguio, located near Montpellier, South of France) during the 2002 growing season.

Leaf physiological traits

For all the measurements described, leaves were sampled at random within tree crowns at an external position for sunlit leaves and an internal position for shaded leaves. The sample details of each experiment are specified below.

Stomatal conductance. Stomatal responses to PPF (photosynthetic photon flux, $\mu mol\ m^{-2}\ s^{-1}$), T_l (leaf temperature, °C) and VPD (leaf to air water vapour pressure deficit, kPa) were measured using a portable infrared photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA). Stomatal responses were measured between early July and early August on different samples of four sunlit leaves, simultaneously in the two cultivars. Each set of measurements was obtained by varying only one environmental parameter, the others being set at standard conditions, i.e. PPF = 1500 $\mu mol\ m^{-2}\ s^{-1}$, T_l = 25 °C, VPD ≤ 1·5 kPa. CO_2 concentration of the air at the leaf surface (C_a) was maintained at 35 Pa during these measurements. Each g_{sw} value was recorded after equilibration for at least 20 min (steady-state condition) as described by Le Roux *et al.* (1999). Leaves used for the measurements were located in comparable positions for all cultivars, thus minimizing uncontrolled effects resulting from the presence of fruits near the measured leaf, the length of the bearing shoot or the leaf position on that shoot.

Stomatal responses to PPF (1500, 1000, 600, 400, 200, 100, 50, 0 $\mu mol\ m^{-2}\ s^{-1}$) were obtained by varying the intensity of a red/blue LED light source (LI-6400-02B; Li-Cor, Inc.). Stomatal responses to T_l [20 °C, 25 °C, 30 °C (± 1 °C)] were measured early in the morning with leaf chamber temperature regulated by integrated Peltier coolers that allowed the temperature to be controlled to within ± 6 °C of the air temperature. Stomatal responses to increasing VPD (1·0, 1·5, 2·0, 2·5, 3·0 and 3·5 kPa) were obtained by regulating the relative humidity of the air in the LI 6400 cuvette chamber either by scrubbing a fraction of the inlet air through a desiccating column or by humidifying it through a series of 25-L cans containing water.

The stomatal response to the different environmental factors was expressed as a fraction of the highest g_{sw} value reached for each leaf (Le Roux *et al.*, 1999).

Maximal stomatal conductance (g_{swmax}, mol $m^{-2}\ s^{-1}$) was estimated on an independent sample of 12–17 sunlit or shaded leaves of each cultivar under the standardized environmental conditions previously described, simultaneously with the measurements of stomatal response to the environment.

Leaf photosynthesis and respiration. Net CO_2 assimilation rate (A_n) in relation to internal concentration of CO_2 (C_i) was measured *in situ* during the same period (early July) with the LI-6400 system, by changing C_a, in the following order: 0, 180, 2·5, 150, 5, 100, 7·5, 60, 10, 40, 15, 20 and 30 Pa. The alternating high and low C_a values avoided leaf saturation by assimilates resulting from persistently high CO_2. All measurements were made under standard environmental conditions (T_l = 25 °C, PPF = 1500 $\mu mol\ m^{-2}\ s^{-1}$, VPD ≤ 1·5 kPa). Eight sunlit and shaded leaves were measured in both cultivars.

Respiration (R_d) was estimated simultaneously over the same period (early July) by measuring CO_2 production rate at the end of the night, before sunrise (0600–0800 h in solar time), for eight sunlit and shaded leaves.

Leaf nitrogen concentration. All leaves measured for stomatal conductance or photosynthesis were immediately collected

and placed in a cool box. Leaf area (A_l, m^2) was recorded with a flatbed scanner (CanoScan 4400F, Canon) coupled to the Optimas software (V6·5; Media Cybernetics, Silver Spring, MD, USA). Leaves were then rapidly frozen in liquid nitrogen and stored in a freezer at $-26\,^{\circ}$C before freeze drying (Heto VR1 CT110, Denmark) for dry mass (W, g) determination. Leaf nitrogen content (N) was measured by catharometry after dry ignition (Agronomy Laboratory Cirad Montpellier, France), and leaf nitrogen concentration expressed per unit area (N_a, g m^{-2}).

Leaf carbon isotope discrimination. The primary growth of 73–81 long shoots per cultivar was monitored at weekly intervals from April to June. Coloured labels were positioned below the apex of each growing shoot, indicating the leaf positioned below the label, the period of its expansion during the 2002 growing season. In October, three different sets of ten sunlit leaves per cultivar were collected on the basis of leaf expansion date (3 April, 17 April and 3 May) from three trees per cultivar. These samples were placed immediately on ice, frozen in liquid nitrogen and lyophilized. Leaves were crushed and homogenized using a centrifugal crusher. Leaf discrimination for ^{13}C (Δ) was calculated as proposed in Farquhar *et al.* (1989):

$$\Delta = [(\delta_a - \delta_l)/(\delta_l + 1000)]1000 \tag{1}$$

where δ_a and δ_l are the carbon isotopic compositions of air and leaf, respectively, expressed in δ units (‰) relative to the international Pee Dee Belemnite (PDB) standard. Measurements of δ_l were obtained on a flow isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) and a constant value of -8 ‰ was used for δ_a as described by Farquhar *et al.* (1989).

Estimation of model parameters

Leaf stomatal conductance model. Jarvis' empirical model (Jarvis, 1976), modified by Stewart (1988), describes stomatal conductance as a combination of independent relative functions (varying from 0 to 1) characterizing leaf response to PPF, T_l, VPD, C_a and Ψ (bulk soil water potential):

$$g_{sw} = g_{swmax} f_1(\text{PPF})f_2(T_l)f_3(\text{VPD})f_4(C_a)f_5(\Psi) \tag{2}$$

In this equation, g_{swmax} (mol m^{-2} s^{-1}) is expressed as a linear function of leaf nitrogen concentration per unit area (N_a), as proposed by Le Roux *et al.* (1999), and f_1 to f_5 are empirical functions whose best fit values for these parameters, i.e. those presenting the closest correlation, were estimated by linear or non-linear least squares regression using SigmaPlot software (2001, SPSS Inc., Chicago, IL, USA). Relative g_{sw} (dimensionless) is drawn from eqn (2) as the ratio of g_{sw} to g_{swmax}.

To simplify the Jarvis-Stewart model, the functions f_4 and f_5 which describe the responses of relative g_{sw} to C_a and to Ψ, respectively, were neglected. Indeed, C_a variations in the orchard during the day are negligible in comparison to other sources of variation. Regarding the f_5

function, it was considered that soil water potential showed little variation in the irrigated conditions used.

Leaf photosynthesis model. The parameters of Farquhar's model (Farquhar *et al.*, 1980), modified by Le Roux *et al.* (1999), were estimated in shaded and sunlit leaves of the two cultivars, assuming that mesophyll CO_2 conductance (g_i) was infinite. This assumption has been questioned (Evans and von Caemmerer, 1996; Ethier and Livingston, 2004; Warren and Dreyer, 2006) because g_i generally limits photosynthesis and leads to the underestimation of V_{Cmax} (maximum carboxylation rate). Estimating g_i is still a matter of controversy, and it was decided not to take this parameter into account. All values of V_{Cmax} presented are therefore 'apparent' values that underestimate the real values.

Apparent V_{Cmax} and maximum electron transport rate (J_{max}) were estimated from A_n–C_i curves, and dark respiration rate (R_d) from direct field measurements (see above). The best fit by non-linear least squares regression (SAS macro; P. Montpied, EEF, INRA Champenoux, pers. comm.) resulted from measurements made with C_a at <30 Pa for V_{Cmax} and >50 Pa for J_{max}. Four to seven leaf data sets were retained for the determination of V_{Cmax} and J_{max}, and six or seven for R_d. Farquhar's parameters were expressed in relation to the individual leaf N_a value, this variable being closely correlated to intercepted irradiance (DeJong and Doyle, 1985).

Comparison of model parameters between the cultivars

All statistical analyses were performed using Statistica 6 software (StatSoft, Inc., Tulsa, OK, USA). The cultivar effect was computed by a one-way ANOVA after ensuring that the data were normally distributed and that the variances were homogeneous. When the parameters also depended on another variable, the cultivar effect was determined by a covariance analysis (ANCOVA), using the continuous variable as covariate. Mean values for both cultivars were compared by the Newman–Keuls test for parametric analyses (more than ten replicates) or the Mann–Whitney test for non-parametric analyses (less than ten replicates).

Evaluation of model predictions

According to the similarity or dissimilarity of cultivar response to environmental variables, single or cultivar-specific sets of parameters were adopted for each model. Given that independent measurements were used to parameterize Jarvis' and Farquhar's models, the quality of model outputs was tested by cross-validation between the two data sets. Leaf gas exchange measurements made to parameterize Farquhar's model were used to evaluate the quality of Jarvis' model. For this purpose, the environmental conditions prevailing during these measurements (PPF, T_l and VPD) were used as inputs into the Jarvis equation to calculate g_{sw} values. Reciprocally, the independent measurements made to parameterize Jarvis' model and the resulting g_{sw} estimations were used to evaluate the

quality of Farquhar's model, inferring C_i values from the equation drawn from Harley *et al.* (1992):

$$C_i = C_a - (A_n \times 1{\cdot}6 \times 1000/g_{sw}) \qquad (3)$$

The quality of these estimations, by comparison with measured values, was determined by two parameters: root mean square error (RMSE) and bias (*b*). These were calculated as follows:

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n}(X_{s,i} - X_{m,i})^2/n} \qquad (4)$$

$$b = \sum_{i=1}^{n}(X_{s,i} - X_{m,i})/n \qquad (5)$$

where $X_{s,i}$ are the estimated values, $X_{m,i}$ the measured values and *n* the number of observations.

RESULTS

Stomatal responses to environmental variables

'Braeburn' and 'Fuji' cultivars showed a similar stomatal response to PPF when fitted by an equilateral hyperbola (Fig. 1A). Non-parametric statistical analysis detected a significant difference ($P = 0{\cdot}034$) between the two cultivars only at 100 μmol m^{-2} s^{-1}. Relative g_{sw} was at a maximum when PPF exceeded 1000 μmol m^{-2} s^{-1}.

The response of relative g_{sw} to leaf temperature (T_l) was represented by a second order polynomial in 'Fuji' and 'Braeburn' (Fig. 1B). For both cultivars, the optimal temperature for g_{sw} was close to 29 °C, without significant differences ($P > 0{\cdot}05$) between the two cultivars whatever T_l.

The response of g_{sw} to VPD displayed two stages in both cultivars (Fig. 1C): at low VPD, relative g_{sw} was around 1,

but it decreased linearly as VPD increased beyond a given threshold. The VPD threshold was significantly higher in 'Braeburn' (1·84 kPa) than in 'Fuji' (1·38 kPa) according to the Mann–Whitney test ($P = 0{\cdot}028$). The rate of g_{sw} decrease that resulted from VPD increase was slightly faster in 'Braeburn' than in 'Fuji', although the slopes were not significantly different ($P > 0{\cdot}10$; Fig. 1C).

Maximal stomatal conductance

A positive linear relationship was fitted between maximal stomatal conductance (g_{swmax}) and leaf nitrogen concentration per leaf area (N_a) for each cultivar (Fig. 2). The covariance analysis with N_a as the covariate showed a significant difference ($P = 0{\cdot}005$) in mean g_{swmax} with
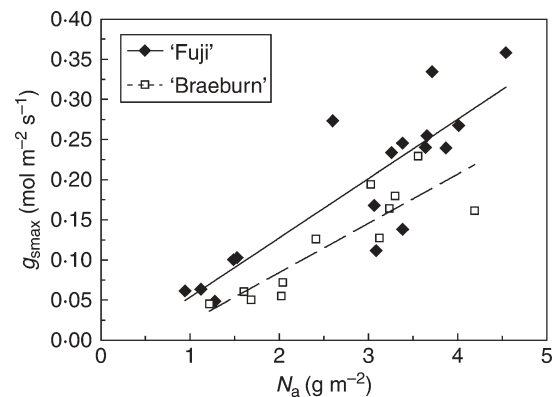
FIG. 2. Relationships between maximum stomatal conductance, g_{swmax}, and leaf nitrogen per unit area, N_a, determined in 'Fuji' and 'Braeburn' apple cultivars. In the linear equation ($g_{swmax} = a_N N_a + b_N$) the fitting parameters are: 'Fuji': $a_N = 0{\cdot}074$, $b_N = -0{\cdot}020$ ($R^2 = 0{\cdot}74$); 'Braeburn': $a_N = 0{\cdot}062$, $b_N = -0{\cdot}038$ ($R^2 = 0{\cdot}77$). The ANCOVA with N_a as the covariate detected significant differences ($P = 0{\cdot}005$) between the cultivars. Mean g_{swmax} values followed by different letters are significantly different: 'Fuji', 0·19[a], 'Braeburn', 0·12[b].
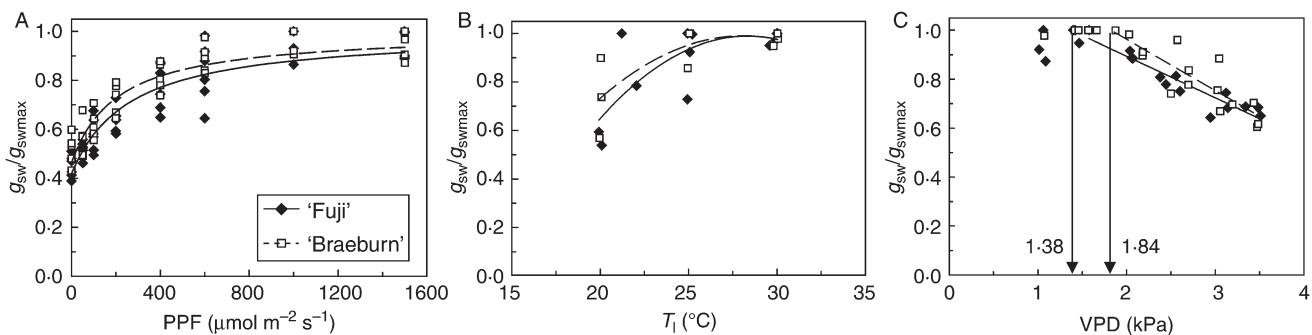
FIG. 1. Response of stomatal conductance, g_{sw}, normalized by the maximum stomatal conductance value, g_{swmax}, to (A) irradiance (PPF), (B) leaf temperature (T_l) and (C) leaf-to-air water vapour pressure deficit (VPD) in 'Fuji' and 'Braeburn' apple cultivars. In (A), [$g_{sw}/g_{swmax} = (a_P \text{ PPF} + b_P)/(c_P \text{ PPF} + d_P)$] fitting parameters: 'Fuji': $a_P = 66{\cdot}10^{-4}$, $b_P = 0{\cdot}716$, $c_P = 66{\cdot}10^{-4}$, $d_P = 1{\cdot}716$ ($R^2 = 0{\cdot}93$); 'Braeburn': $a_P = 90{\cdot}10^{-4}$, $b_P = 0{\cdot}856$, $c_P = 90{\cdot}10^{-4}$, $d_P = 1{\cdot}856$ ($R^2 = 0{\cdot}94$). In (B), ($g_{sw}/g_{swmax} = a_T T_l^2 + b_T T_l + c_T$) fitting parameters: 'Fuji': $a_T = -49{\cdot}10^{-4}$, $b_T = 0{\cdot}28$, $c_T = -2{\cdot}94$ ($R^2 = 0{\cdot}57$); 'Braeburn': $a_T = -39{\cdot}10^{-4}$, $b_T = 0{\cdot}22$, $c_T = -2{\cdot}12$ ($R^2 = 0{\cdot}61$). In (C), the threshold VPD value ensuring $g_{sw} = g_{swmax}$ is noted. Equation beyond this value ($g_{sw}/g_{swmax} = a_D \text{ VPD} + b_D$) uses fitting parameters: 'Fuji': $a_D = -0{\cdot}17$, $b_D = 1{\cdot}23$ ($R^2 = 0{\cdot}89$); 'Braeburn': $a_D = -0{\cdot}21$, $b_D = 1{\cdot}39$ ($R^2 = 0{\cdot}75$). Results of the Mann–Whitney test: in (A) $P = 0{\cdot}034$ at PPF = 100 μmol m^{-2} s^{-1} but $P > 0{\cdot}05$ at the other PPF levels; in (B) $P > 0{\cdot}05$ at three temperatures; in (C) $P = 0{\cdot}029$ between the threshold values. The ANCOVA procedure using VPD as the covariate detected no significant differences ($P > 0{\cdot}05$) between the cultivars. Responses to PPF, T_l and VPD were recorded under standard conditions (see Materials and Methods).
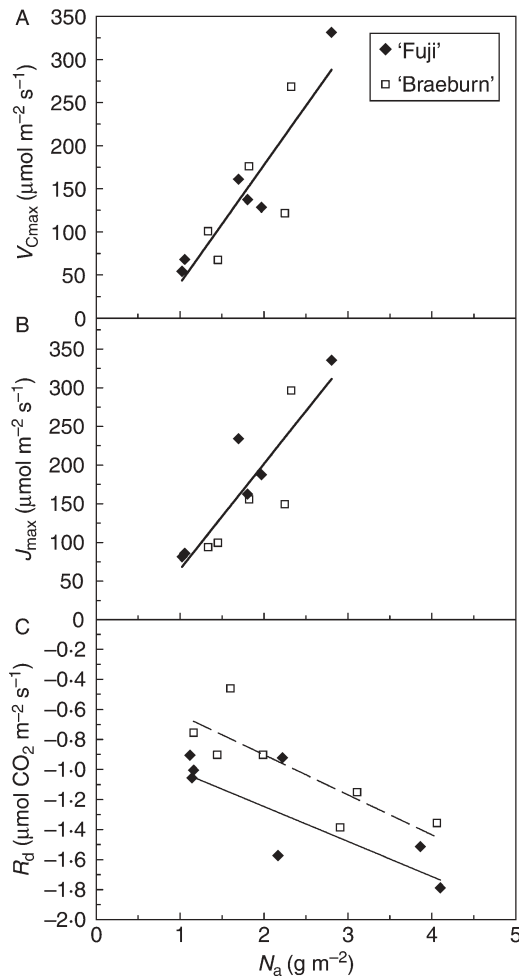
FIG. 3. Relationships between (A) maximum carboxylation rate, V_{Cmax}, (B) maximum electron transport rate, J_{max}, and (C) dark respiration rate, R_d, and leaf nitrogen per unit area, N_a, in 'Fuji' and 'Braeburn' apple cultivars. In (A), linear equation ($V_{Cmax} = a_V N_a + b_V$) and fitting parameters $a_V = 137.3$, $b_V = -96.94$ ($R^2 = 0.77$). In (B), linear equation ($J_{max} = a_J N_a + b_J$) and fitting parameters: $a_J = 136.4$, $b_J = -70.98$ ($R^2 = 0.76$). In (C), linear equation ($R_d = a_R N_a + b_R$) and fitting parameters: 'Fuji': $a_R = -0.23$, $b_R = -0.78$ ($R^2 = 0.79$); 'Braeburn': $a_R = -0.27$, $b_R = -0.37$ ($R^2 = 0.67$). The covariance analysis using N_a as covariate detected no significant differences between the cultivars for V_{Cmax} and J_{max}, but significant differences ($P = 0.007$) for R_d. The R_d means followed by different letters are significantly different: 'Fuji', -1.31^a, 'Braeburn', -0.96^b. Conditions for the estimation of V_{Cmax}, J_{max} and R_d are given in Materials and Methods.
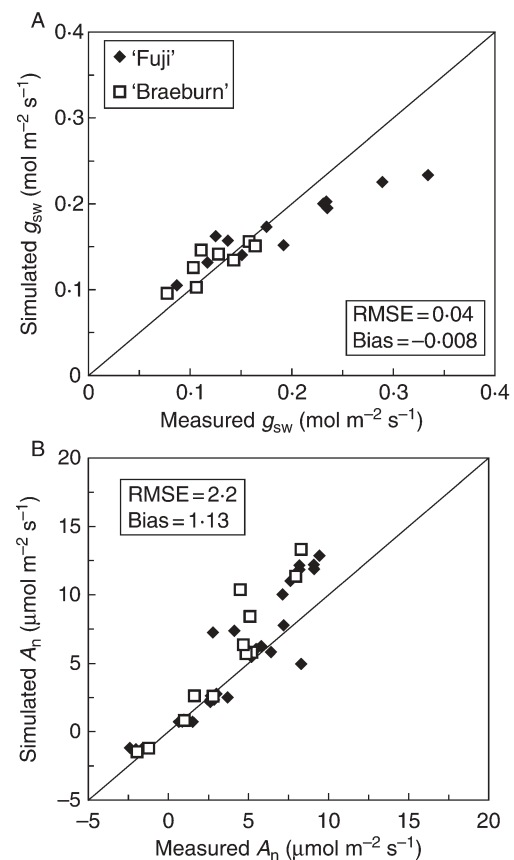

FIG. 4. Comparison of (A) stomatal conductance and (B) photosynthetic rate measured *in situ* on leaves of 'Fuji' and 'Braeburn' apple cultivars, and simulated by the Jarvis and Farquhar sub-models, respectively. The root mean square error (RMSE), the bias and the 1:1 line are indicated.

larger values in 'Fuji' than in 'Braeburn', although the slopes were similar (0.074 and 0.062, respectively). Moreover, the range of g_{swmax} variations was different between cultivars: maximal g_{swmax} was significantly larger in 'Fuji' (0.36 mol m^{-2} s^{-1}) than in 'Braeburn' (0.23 mol m^{-2} s^{-1}; Fig. 2).

Leaf photosynthesis and respiration

A unique positive and linear relationship between V_{Cmax} and N_a, and between J_{max} and N_a, was observed in both cultivars, as shown by the ANCOVA analyses (Fig. 3A, B).

Of the two variables combined in N_a, i.e. nitrogen concentration on a mass basis (N_w) and leaf mass to area ratio (W_a), W_a played the most important role in J_{max} and V_{Cmax} variations. Indeed, the correlation was higher between V_{Cmax}, J_{max} and W_a ($R^2 = 0.56$ and 0.54, respectively) than between V_{Cmax}, J_{max} and N_w ($R^2 = 0.23$ and 0.29, respectively; data not shown). The mean $J_{max} : V_{Cmax}$ ratio was 1.2 at 25 °C.

The relationship between leaf respiration (R_d) and leaf nitrogen per mass area (N_a) differed significantly, with 'Fuji' greater R_d than 'Braeburn' (ANCOVA analysis, $P = 0.009$; Fig. 3C).

Evaluation of model predictions

The g_{sw} values predicted by Jarvis' model satisfactorily matched the measured values in both cultivars, as shown by the RMSE (0.04) and bias (-0.008; Fig. 4A). The different points were close to the 1:1 line, particularly for 'Braeburn'. Some of the highest g_{sw} values were underestimated by the model for 'Fuji'.

Predictions of leaf photosynthesis using Farquhar's model resulted in a fairly good match between estimated and measured values for all cultivars as shown by the RMSE
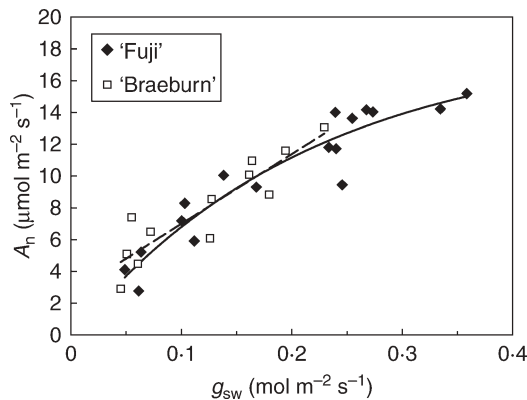
FIG. 5. Relationships between net assimilation, A_n, and stomatal conductance, g_{sw}, in 'Fuji' and 'Braeburn' apple cultivars. Equation for 'Fuji': $[A_n = (a_A \ b_A \ g_{sw})/(a_A + b_A \ g_{sw}) + c_A]$; equation for 'Braeburn': $(A_n = a_A \ g_{sw} + b_A)$. Fitting parameters: 'Fuji': $a_A = 18.685$, $b_A = 4.671$, $c_A = -0.183$ $(R^2 = 0.90)$; 'Braeburn': $a_A = 43.835$, $b_A = 2.611$ $(R^2 = 0.82)$. The covariance analysis, using g_{sw} as the covariate, revealed significant differences $(P < 0.05)$ between the cultivars for A_n. The mean A_n values followed by different letters were significantly different: 'Fuji', 10.06^a, 'Braeburn', 8.84^b. A_n and g_{sw} were measured in sunlit and shaded leaves $(n = 12$ and 17 in 'Braeburn' and 'Fuji', respectively) under standardized environmental conditions: $PPF = 1500 \ \mu mol \ m^{-2} \ s^{-1}$, $T_l = 25 \ °C$ and $VPD < 1.5 \ kPa$.

(2.2) and bias (1.13; Fig. 4B). However, the model over-estimated (2-fold) some of the highest values for leaf photosynthesis, particularly at high PPF (>600 $\mu mol \ m^{-2} \ s^{-1}$, data not shown).

Relationship between stomatal conductance and photosynthesis

The range of g_{sw} variations for sunlit or shaded leaves was narrower in 'Braeburn' (between 0.05 and 0.23 mol $m^{-2} \ s^{-1}$) than in 'Fuji' (between 0.05 and 0.36 mol $m^{-2} \ s^{-1}$; Fig. 5), in accordance with the lower g_{swmax} found in this cultivar. 'Braeburn' also had lower maximal leaf A_n values (13.1 $\mu mol \ CO_2 \ m^{-2} \ s^{-1}$) than 'Fuji' (15.2 $\mu mol \ m^{-2} \ s^{-1}$, Fig. 5). Differences between cultivars were confirmed by independent measurements made during the same growing season using a Licor LI-6200 system (data not shown). Specific relationships between A_n and g_{sw} were adjusted by a non-linear fit in 'Fuji' and by a linear fit in 'Braeburn' (Fig. 5). Analysis with g_{sw} as the covariate yielded significant differences in

A_n between the cultivars $(P = 0.007)$, with lower CO_2 net assimilation rates in 'Braeburn'.

Leaf ecophysiological traits under optimized conditions

The data sets stemming from leaf responses to environmental variables were pooled together, restricting the range of irradiance and air humidity variations to conditions which determine optimal (or sub-optimal) g_{sw}: $600 \leq PPF \leq 1500 \ \mu mol \ m^{-2} \ s^{-1}$ and $0.90 \ kPa \leq VPD \leq 2.1 \ kPa$. On this basis, the average g_{sw} value of sunlit leaves was 25 % smaller in 'Braeburn' than in 'Fuji' $(P = 0.0036$; Table 1), and the average A_n value 8 % smaller in the first cultivar (n.s.). Averaged E was 17 % lower, and WUE 5 % higher, when 'Braeburn' sunlit leaves were compared with equivalent 'Fuji' leaves, but these differences were not significant $(P > 0.10)$. In contrast, the IWUE of 'Braeburn' leaves was 15 % greater than in 'Fuji' $(P < 0.001)$.

Carbon isotope discrimination of sunlit leaves

Smaller carbon isotope discrimination ($\Delta^{13}C$) occurred in leaves that expanded in May rather than in April for both cultivars (Fig. 6). 'Fuji' leaves displayed a higher $\Delta^{13}C$ than 'Braeburn' leaves irrespective of when they expanded.
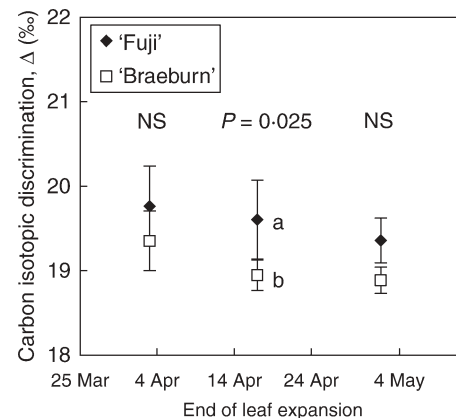

FIG. 6. Carbon isotope discrimination, $\Delta^{13}C$, for sunlit apple leaves which expanded at start, middle and end of extension growth period in 'Fuji' and 'Braeburn' apple cultivars. All leaves were sampled in October for isotopic analysis. $\Delta^{13}C$ of the two cultivars were statistically compared using the Mann–Whitney test. *P*-values are indicated where the differences are significant and NS where they are not. Values followed by different letters are significantly different.

TABLE 1. *Averaged ($\pm$ s.d.; n = 24) ecophysiological traits of sunlit leaves in two apple cultivars*

	A_n ($\mu mol \ CO_2 \ m^{-2} \ s^{-1}$)	g_{sw} ($mol \ H_2O \ m^{-2} \ s^{-1}$)	E ($mmol \ H_2O \ m^{-2} \ s^{-1}$)	WUE ($mmol \ CO_2 \ mol^{-1} \ H_2O$)	IWUE ($\mu mol \ CO_2 \ mol^{-1} \ H_2O$)
Braeburn	10.895 ± 1.775	0.166 ± 0.051^b	2.034 ± 0.989	6.132 ± 2.001	68.941 ± 14.210^a
Fuji	11.784 ± 2.874	0.223 ± 0.074^a	2.458 ± 1.126	5.325 ± 1.492	55.328 ± 9.281^b
Cultivar effect	n.s.	**	n.s.	n.s.	***

Measurements were performed during July and August 2002 with the following conditions: $CO_2 = 35 \ Pa$; $600 \leq PPF \leq 1500 \ \mu mol \ m^{-2} \ s^{-1}$; $20 \ °C \leq T_l \leq 30 \ °C$; $0.90 \ kPa \leq VPD \leq 2.1 \ kPa$.

Values compared by one-way ANOVA procedure for determination of the cultivar effect. **$P < 0.01$; ***$P < 0.001$; n.s., not significant. Values followed by different letters are significantly different according to Mann-Whitney test.

Non-parametric statistics detected a significant difference between 'Fuji' and 'Braeburn' during April ($P = 0.025$; Fig. 6).

DISCUSSION

Quality of model parameter estimations

This study describes the parameterization of Jarvis' stomatal conductance model and of Farquhar's photosynthesis model for leaves of two apple cultivars, and compares the estimated parameters. The stomatal behaviour in response to changing PPF and T_1 was similar to that commonly found in other species (Leuning *et al.*, 1995; Le Roux *et al.*, 1999), and previously observed in apple (Warrit *et al.*, 1980; Pretorius and Wand, 2003). The response of g_{sw} to VPD consisted of a two-step process: g_{sw} was close to maximum at low or moderate VPD and showed a severe decrease after a VPD threshold. This response was similar to that observed by Watson *et al.* (1978) in apples, and by Mediavilla and Escudero (2004) in different oak species. Dragoni *et al.* (2004) obtained the same result in 'Royal Empire' apple trees, and concluded that at low VPD, stomatal conductance was limited by the feedback control exerted by photosynthetic products, while the stomatal response to VPD became a limiting factor at higher VPDs. In other studies (e.g. Winkel and Rambal, 1990; Jones, 1998; Le Roux *et al.*, 1999), the initial plateau was not observed, but this may be due to measurements being started at VPD values close to the threshold (around 1·5 kPa). The g_{swmax} values observed in the present study were smaller than those observed in earlier studies with apple trees (Wünsche *et al.*, 2000; Glenn *et al.*, 2001) but comparable with those described under South African climate (Pretorius and Wand, 2003; Gindaba and Wand, 2007). Indeed, the estimation of g_{swmax} remains difficult. The environmental conditions *a priori* supposed to maximize g_{sw} were drawn from the literature (e.g. optimal temperature commonly 25 °C; Pretorius and Wand, 2003), but it was observed *a posteriori* that some of these values were not fully adequate in the present conditions: for the two cultivars analysed here, optimal temperature was near 29 °C. However, as measurements were performed in similar conditions for both cultivars, the comparison discussed below remains valid.

Parameters of leaf photosynthetic capacity were linearly related to nitrogen concentration per unit leaf area (N_a), as shown in other deciduous tree species, such as walnut (Le Roux *et al.*, 1999) and peach (Le Roux *et al.*, 2001; Walcroft *et al.*, 2002). V_{Cmax} at 25 °C was larger than in a series of forest tree species (Dreyer *et al.*, 2001). In addition, the $J_{max} : V_{Cmax}$ ratio (1·2) was lower than usually reported even if it is subject to marked variability (Leuning, 1997). Reviewing the literature, Wullschleger (1993) calculated a mean $J_{max} : V_{Cmax}$ ratio of 1·64 for 109 species including *Malus* sp. The high nitrogen status of the experimental trees in the present study, indicated by the large N_a values (maximum up to 4 g m^{-2}, i.e. N_w of 3·4 %; Fig. 2) of sunlit leaves, could account for the high V_{Cmax} values observed. Indeed, Grassi *et al.* (2002)

showed that V_{Cmax} values increase and the $J_{max} : V_{Cmax}$ ratio decreases with high mineral nutrient supply in *Eucalyptus*. The excess N is preferentially allocated to Rubisco rather than to the proteins that regulate the rate of electron transport. The consequence is therefore an increase in V_{Cmax} but not in J_{max}, thus decreasing the J_{max}/V_{Cmax} ratio. The high V_{Cmax} values estimated here also suggest a large mesophyll conductance to CO_2 in apple leaves; this requires further examination and explicit estimates of mesophyll conductance. Indeed, different studies demonstrated that mesophyll resistance to CO_2 transfer imposes limitation to photosynthesis in many species (Warren and Adams, 2006).

Quality of model predictions

In the present study, Jarvis' model produced a fair prediction of leaf stomatal conductance over the range of environmental conditions which are commonly encountered in a Mediterranean climate. As the model underestimated some g_{sw} values, this can result either from the simplification of the Jarvis' model considered here (i.e. C_a and Ψ variations neglected) or from underestimation of g_{swmax}.

Leaf photosynthesis estimated by Farquhar's model was slightly more problematic. Measured values matched predicted values when PPF was below 600 μmol m^{-2} s^{-1}, but the model overestimated A_n values at higher irradiance. This was explained by the J_{max} module in Farquhar's model which was identified as insufficiently limiting at high PPF. This said, the overestimated photosynthesis rate for strongly sunlit leaves could be considered as having little impact on estimates of photosynthesis at larger scales (e.g. the branch) since most of the leaves on a branch do not receive high irradiance because of within-tree shade and leaf blade orientation (Massonnet, 2004).

Cultivar-dependent leaf functions: differences in water use strategy

The experimental plan used in this study, which included two apple cultivars, allowed the cultivar effect to be explored on leaf photosynthetic and stomatal properties, and also on transpiration rate and water use, in the middle of the growing season.

Based on these instantaneous measurements, the two apple cultivars displayed a similar photosynthetic capacity (V_{Cmax} and J_{max}) on the basis of normalized leaf N_a values. In both cultivars, V_{Cmax}, J_{max} and R_d increased with N_a, as commonly observed in numerous other species (Reich *et al.*, 1998), including walnut (Le Roux *et al.*, 1999), peach (Le Roux *et al.*, 2001) and mango (Urban *et al.*, 2003). The relationship between R_d and N_a is interpreted to be a consequence of changed leaf structure resulting from different positions within the canopy, and higher maintenance costs in sunlit leaves considering their carbon budget (Mitchell *et al.*, 1999). This is supported by structural modifications between sunlit and shaded leaves observed in these two cultivars (Massonnet, 2004). The thinner palisade tissue in shaded leaves and lower leaf mass to area ratio could account for the diminished

respiration rate. The higher leaf R_d in 'Fuji' suggests that the potentially larger photosynthetic rate in this cultivar is partly counterbalanced by greater respiratory losses.

The comparison of the two cultivars revealed different stomatal responses. 'Braeburn' and 'Fuji' differed with respect to maximal stomatal conductance, 'Fuji' having significantly higher g_{swmax} than 'Braeburn'. The two cultivars showed similar responses to PPF and T_l. The stomatal response to VPD showed some differences between cultivars: a significantly lower VPD threshold value was observed in 'Fuji', whereas the rate of relative g_{sw} decrease beyond this threshold was slightly steeper in 'Braeburn'.

'Braeburn' showed a linear relationship between A_n and g_{sw}, i.e. no apparent saturation of the photosynthesis process even at the highest observed g_{sw}. In this case, carbon gain is likely to be limited more by stomatal conductance than by the photosynthetic apparatus itself. Such a linear relation between A_n and g_{sw} has already been reported in apple (Lakso, 1979) and mango (Urban *et al.*, 2004), but it was shown here that this cannot be generalized. Indeed, 'Fuji' showed some non-stomatal limitation of leaf photosynthesis (rectangular hyperbola fit; Fig. 5). With this curvilinear relationship, A_n reaches a plateau at the highest observed g_{sw} values, suggesting that A_n is limited either by the amount of Rubisco or its activity, or by the rate of electron transport. In this case, the trade-off between CO_2 assimilation and E is modified, lowering the WUE value. Cultivars with a prevailing stomatal limitation generally appear more water-conserving and consequently exhibit a higher WUE than cultivars with limitation by the photosynthetic machinery (Jones, 1985). The present results are in accordance with this interpretation since instantaneous gas exchange measurements on sunlit leaves indicated slightly higher WUE, and significantly higher IWUE, in 'Braeburn' than in 'Fuji'. The higher water use efficiency in 'Braeburn' was confirmed when integrated over a much longer time scale (i.e. the whole growing season), by the smaller values of carbon isotope discrimination ($\Delta^{13}C$) in its leaves. Farquhar and Richards (1984) pointed out that the isotopic composition reflects the effect of plant water status on photosynthesis, with a linear negative relationship between $\Delta^{13}C$ values and WUE in various species. In the present study, values of leaf $\Delta^{13}C$ were very informative in terms of the physiological properties of a cultivar and complemented instantaneous stomatal conductance measurements, because $\Delta^{13}C$ integrated environmental conditions which varied throughout the growing season. The g_{sw} values, predicted by the Jarvis model, result from the response to several environmental variables, without consideration of their interaction. This possible imbalance is overcome by using the more integrative variable, $\Delta^{13}C$, which was therefore useful for comparing functional capacities between cultivars, in addition to the parameters of the ecophysiological models. As the present comparison considered two apple cultivars, it would be worthwhile to examine leaf ecophysiological traits in more depth, among a larger cultivar range. This was performed in a recent study showing a strong variability of leaf ecophysiological traits (e.g. g_{sw}, IWUE, A_{nmax} ...) among a recombinant apple F_1 population

(Regnard *et al.*, 2007), opening new perspectives for breeding.

CONCLUSIONS

In the present study, the results of both $\Delta^{13}C$ analysis and instantaneous responses of g_{sw} to environmental variables indicated different water use strategies in the two apple cultivars analysed. Based on the results, 'Braeburn' is more water-conserving than 'Fuji'. Stomatal responses to environment, and leaf respiration rate, differed between the two cultivars; they also had contrasted crown architectures. As a consequence, combined functional and architectural traits probably play complementary roles in determining the physiological functions of these apple cultivars at the integrated scales of fruiting branches or whole trees. Further examination of a larger range of cultivars is required. The present study also shows that the description of the environmental plasticity of different cultivars is of interest for horticulture in order to determine the potential adaptation of a cultivar to a given climate and consequently the need of finely tuning cultural practices (e.g. irrigation) when the environmental conditions are limiting for growth and productivity.

LITERATURE CITED

Atkinson CJ, Policarpo M, Webster AD, Kingswell G. 2000. Drought tolerance of clonal *Malus* determined from measurements of stomatal conductance and leaf water potential. *Tree Physiology* **20**: 557–563.

Berry J, Bjorkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* **31**: 491–543.

Brendel O, Pot D, Plomion C, Rozenberg P, Guehl JM. 2002. Genetic parameters and QTL analysis of $\delta^{13}C$ and ring width in maritime pine. *Plant, Cell and Environment* **25**: 945–953.

Brendel O, Le Thiec D, Scotti-Saintagne C, Bodénès C, Kremer A, Guehl JM. 2007. Quantitative trait loci controlling water use efficiency and related traits in *Quercus robur* L. *Tree Genetics & Genomes.* doi: 10.1007/s11295-007-0107-z.

Casasoli M, Derory J, Morera-Dutrey C, Brendel O, Porth I, Guehl JM, *et al.* **2006.** Comparison of quantitative trait loci for adaptive traits between oak and chestnut based on an expressed sequence tag consensus map. *Genetics* **172**: 533–546.

Comstock JP, Ehleringer JR. 1992. Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proceedings of the National Academy of Sciences of the USA* **89**: 7747–7751.

Costes E, Sinoquet H, Kelner JJ, Godin C. 2003. Exploring within-tree architectural development of two apple tree cultivars over 6 years. *Annals of Botany* **91**: 91–104.

DeJong TM, Doyle JF. 1985. Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). *Plant, Cell and Environment* **8**: 701–706.

Dragoni D, Lakso AN, Piccioni RM. 2004. Transpiration of an apple orchard in a cool humid climate: measurement and modeling. *Acta Horticulturae* **664**: 175–180.

Dreyer E, Le Roux X, Montpied P, Daudet FA, Masson FA. 2001. Temperature response of leaf photosynthetic capacity in seedlings from seven temperate tree species. *Tree Physiology* **21**: 223–232.

Ethier GJ, Livingston NJ. 2004. On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant, Cell and Environment* **27**: 137–153.

Evans JR, von Caemmerer S. 1996. Carbon dioxide diffusion inside leaves. *Plant Physiology* **110**: 339–346.

Fanjul L, Jones HG. 1982. Rapid stomatal responses to humidity. *Planta* **154**: 135–138.

Farquhar GD, Richards RA. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* **11**: 539–552.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* **149**: 78–90.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503–537.

Flore JA, Lakso AN. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *Horticultural Reviews* **11**: 229–287.

Francesconi AHD, Lakso AN, Denning SS. 1997. Light and temperature effects on whole-canopy net carbon dioxide exchange rates of apple trees. *Acta Horticulturae* **451**: 287–294.

Gindaba J, Wand SJE. 2007. Climate-ameliorating measures influence photosynthetic gas exchange of apple tree. *Annals of Applied Biology* **150**: 75–80.

Glenn DM, Puterka GJ, Drake SR, Unruh TR, Knight AL, Baherle P, Prado E, Baugher TA. 2001. Particle film application influences apple leaf physiology, fruit yield, and fruit quality. *Journal of the American Society for Horticultural Science* **126**: 175–181.

Grassi G, Meir P, Cromer R, Tompkins D, Jarvis PG. 2002. Photosynthetic parameters in seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. *Plant, Cell and Environment* **25**: 1677–1688.

Guehl JM, Nguyen-Queyrens A, Loustau D, Ferhi A. 1995. Genetic and environmental determinants of water use efficiency and carbon isotope discrimination in forest trees. In: Sandermann H, Bonnet-Masimbert M, eds. *EUROSILVA Contribution to Forest Tree Physiology*. Paris: INRA, 297–321.

Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992. Modeling photosynthesis of cotton grown in elevated CO_2. *Plant, Cell and Environment* **15**: 271–282.

Jarvis PG. 1976. The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society of London, B* **273**: 593–610.

Jones HG. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell and Environment* **8**: 95–104.

Jones HG. 1998. Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* **49** (special issue): 387–398.

Lakso AN. 1979. Seasonal changes in stomatal response to leaf water potential in apple *Journal of the American Society for Horticultural Science* **104**: 58–60.

Lakso AN. 1994. Apple. In: Schaffer B, Andersen PC, eds. *Handbook of environmental physiology of fruit crops*. Boca Raton, FL: University of Florida/CRC Press, 3–35.

Lauri PE, Lespinasse JM. 2000. The vertical axis and solaxe systems in France. *Acta Horticulturae* **513**: 287–296.

Le Roux X, Grand S, Dreyer E, Daudet FA. 1999. Parameterization and testing of a biochemically based photosynthesis model for walnut (*Juglans regia* L.) trees and seedlings. *Tree Physiology* **19**: 481–492.

Le Roux X, Walcroft AS, Daudet FA, Sinoquet H, Chaves MM, Rodrigues A, Osorio L. 2001. Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. *Tree Physiology* **21**: 377–386.

Lespinasse Y. 1992. Le pommier. In: Gallais A, Bannerot H, eds. *Amélioration des espèces végétales cultivées*. Paris: INRA, 579–594.

Leuning R. 1997. Scaling to a common temperature improves the correlation between the photosynthesis parameters J_{max} and Vc_{max}. *Journal of Experimental Botany* **48**: 345–347.

Leuning R, Kelliher FM, de Pury DGG, Schulze ED. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell and Environment* **18**: 1183–1200.

Li F, Cohen S, Naor A, Shaozong K, Erez A. 2002. Studies of canopy structure and water use of apple trees on three rootstocks. *Agricultural Water Management* **55**: 1–14.

Marini RP, Sowers DL. 1990. Net photosynthesis, specific leaf weight, and flowering of peach as influenced by shade. *HortScience* **25**: 331–334.

Massonnet C. 2004. *Variabilité architecturale et fonctionnelle du système aérien chez le pommier* (Malus × domestica Borkh.): *comparaison de quatre cultivars par une approche de modélisation structure-fonction*. PhD Thesis, Ecole Nationale Supérieure Agronomique, Montpellier, France.

Massonnet C, García-Villanueva E, Costes E, Regnard JL. 2004. Integrating apple tree aerial and root architecture in a structure–function approach. *Acta Horticulturae* **636**: 601–608.

Mediavilla S, Escudero A. 2004. Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. *Forest Ecological Management* **187**: 281–294.

Mitchell KA, Bolstad PV, Vose JM. 1999. Interspecific and environmentally induced variation in foliar dark respiration among eighteen southeastern deciduous tree species. *Tree Physiology* **19**: 861–870.

Oren R, Sperry JS, Katul GG, Pataki DE, Ewers BE, Phillips N, Schafer KVR. 1999. Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell and Environment* **22**: 1515–1526.

Pretorius JJJB, Wand SJE. 2003. Late-season stomatal sensitivity to microclimate is influenced by sink strength and soil moisture stress in 'Braestar' apple trees in South Africa. *Scientia Horticulturae* **98**: 1–15.

Quilot B, Génard M, Kervella J, Lescourret F. 2002. Ecophysiological analysis of genotypic variation in peach fruit growth. *Journal of Experimental Botany* **53**: 1613–1625.

Regnard JL, Ducrey M, Porteix E, Segura V, Costes E. 2007. Phenotyping apple progeny for ecophysiological traits: how and what for? 27th International Horticultural Congress, Séoul, Korea. *Acta Horticulturae*, in press.

Reich PB, Walters MB, Ellsworth DS, Vose JM, Volin JC, Gresham C, Bowman WD. 1998. Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. *Oecologia* **114**: 471–482.

Schulze ED. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Annual Review of Plant Physiology* **37**: 247–274.

Seeley EJ, Kammereck R. 1977. Carbon flux in apple trees: the effects of temperature and light intensity on photosynthetic rates. *Journal of the American Society for Horticultural Science* **102**: 731–733.

Stewart JB. 1988. Modelling surface conductance of pine forest. *Agricultural and Forest Meteorology* **43**, 19–35.

Tardieu F. 2003. Virtual plants: modelling as a tool for the genomics of tolerance to water deficit. *Trends in Plant Science* **8**: 9–14.

Urban L, Le Roux X, Sinoquet H, Jaffuel S, Jannoyer M. 2003. A biochemical model of photosynthesis for mango leaves: evidence for the effect of fruit on photosynthetic capacity of nearby leaves. *Tree Physiology* **23**: 289–300.

Urban L, Lu P, Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. *Tree Physiology* **24**: 387–399.

Walcroft A, Le Roux X, Diaz-Espejo A, Dones N, Sinoquet H. 2002. Effects of crown development on leaf irradiance, leaf morphology and photosynthetic capacity in a peach tree. *Tree Physiology* **22**: 929–938.

Warren CR, Adams MA. 2006. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant, Cell and Environment* **29**: 192–201.

Warren CR, Dreyer E. 2006. Temperature response of photosynthesis and internal conductance to CO_2: results from two independent approaches. *Journal of Experimental Botany* **57**: 3057–3067.

Warrit B, Landsberg JJ, Thorpe MR. 1980. Responses of apple leaf stomata to environmental factors. *Plant, Cell and Environment* **3**: 13–22.

Watson RL, Landsberg JJ, Thorpe MR. 1978. Photosynthetic characteristics of the leaves of Golden delicious apple trees. *Plant, Cell and Environment* **1**: 51–58.

Winkel T, Rambal S. 1990. Stomatal conductance of some grapevines growing in the field under a Mediterranean environment. *Agricultural & Forest Meteorology* **51**: 107–121.

Wullschleger SD. 1993. Biochemical limitations to carbon assimilation in C_3 plants – a retrospective analysis of the A/C_i curves from 109 species. *Journal of Experimental Botany* **44**: 907–920.

Wünsche JN, Palmer JW, Greer DH. 2000. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy *Journal of the American Society for Horticultural Science* **125**: 93–99.