Changes in vascular and transpiration flows affect the seasonal and daily growth of kiwifruit (*Actinidia delicosa*) berry

Brunella Morandi,* Luigi Manfrini, Pasquale Losciale, Marco Zibordi and Luca Corelli Grappadelli

Dipartimento Cultive Arboree, University of Bologna, V.le Fanin 46 40127, Bologna, Italy

*For correspondence. E-mail bmorandi@agrsci.unibo.it

Received: 30 November 2009 Returned for revision: 13 January 2010 Accepted: 17 February 2010 Published electronically: 9 April 2010

---

**Background and Aims** The kiwifruit berry is characterized by an early stage of rapid growth, followed by a relatively long stage of slow increase in size. Vascular and transpiration flows are the main processes through which water and carbon enter/exit the fruit, determining the daily and seasonal changes in fruit size. This work investigates the biophysical mechanisms underpinning the change in fruit growth rate during the season.

**Methods** The daily patterns of phloem, xylem and transpiration in/outflows have been determined at several stages of kiwifruit development, during two seasons. The different flows were quantified by comparing the diurnal patterns of diameter change of fruit, which were then girdled and subsequently detached while measurements continued. The diurnal courses of leaf and stem water potential and of fruit pressure potential were also monitored at different times during the season.

**Key Results** Xylem and transpiration flows were high during the first period of rapid volume growth and sharply decreased with fruit development. Specific phloem import was lower and gradually decreased during the season, whereas it remained constant at whole-fruit level, in accordance with fruit dry matter gain. On a daily basis, transpiration always responded to vapour pressure deficit and contributed to the daily reduction of fruit hydrostatic pressure. Xylem flow was positively related to stem-to-fruit pressure potential gradient during the first but not the last part of the season, when xylem conductivity appeared to be reduced.

**Conclusions** The fruit growth model adopted by this species changes during the season due to anatomical modifications in the fruit features.

**Key words:** *Actinidia delicosa*, fruit growth, xylem, phloem, fruit transpiration, water relationships, vapour pressure deficit.

---

**INTRODUCTION**

From a biophysical point of view, fruit growth may be defined as a balance between incoming and outgoing fluxes: when this balance is positive fruit increase in weight; when it is negative, they shrink (Fishman and Génard, 1998). Some fruits, such as tomato (Guichard et al., 2005) and peach (Huguet et al., 1998; Morandi et al., 2007a), alternate periods of expansion and shrinkage during the day, indicating that strong variations may occur in this balance daily. Apples in their early and mid-season developmental stages show a somewhat comparable behaviour (Higgs and Jones, 1984; Lang, 1990; Berger and Selles, 1993). Kiwifruit berries approaching maturity show only slight changes in daily fruit diameter (Morandi et al., 2007b).

Carbon and water enter the fruit via phloem and xylem streams, whereas water is lost to the atmosphere via transpiration. The effects of dry matter gain and loss from fruit photosynthesis and respiration on fruit diameter variation can be considered to be negligible on a daily scale (Blanke and Lenz, 1989). Fruits, for example apple at mid-season (Lang, 1990), grape (Bondada et al., 2005; Keller et al., 2006) and citrus (Huang et al., 2000), may lose water also via xylem backflow from fruit to leaves that generally occurs during the warmest hours of the day.

Xylem and phloem flows are driven by hydrostatic pressure gradients along the vascular path (Münch, 1930; Minchin and Thorpe, 1987; Minchin et al., 1996) and assimilates can be passively unloaded to sink cells either by diffusion or by bulk flow, depending on whether their transport responds to concentration or turgor pressure gradients, respectively (Patrick, 1990, 1997; Lalonde et al., 2003). When no, or too low, water potential gradients exist between phloem and sink cells, apoplasmic phloem unloading may become a necessary tool to actively transport carbohydrates into the fruit (Patrick, 1990, 1997; Lalonde et al., 2003).

Fruit transpiration depends on fruit surface area, surface conductance and environmental conditions, and may be partly regulated by stomatal conductance (Jones and Higgs, 1982; Lescourret et al., 2001; Li et al., 2002; Gibert et al., 2005). Many fruit species exhibit a strong seasonal relationship between fruit transpiration and xylem flow: in peach, daily xylem inflow is proportional to transpiration and increases with fruit development (Fishman and Génard, 1998; Morandi et al., 2007a); in apple (Lang, 1990; Drazeta et al., 2001, 2004), grape (Düring et al., 1987; Findlay et al., 1987; Creasy et al., 1993) and kiwifruit (Dichio et al., 2003; Montanaro et al., 2006), xylem flux decreases during the season along with fruit surface conductance. Although this relationship is strong and occurs in many fruits, the reasons for it have not yet been fully documented.

Kiwifruit ‘Hayward’ berry development is characterized by a first period of rapid diameter expansion, followed by a period.
of slow increase in fresh weight that lasts until harvest (Beever and Hopkirk, 1990; Gallego et al., 1997; Ferrandino and Guidoni, 1998). During the first period, fruit transpiration is very high due to the thin cell walls of the epidermis and the large amounts of live tricomes, which increase the fruit surface exposed to the atmosphere (Hallett and Sutherland, 2005). By contrast, kiwifruit berry epidermis is characterized by very few stomata, so that stomatal conductance is responsible for a very low proportion of kiwifruit water loss (Hallett and Sutherland, 2005). Later in the season, important anatomical changes, such as cell wall suberization and loss of tricome functionality, occur in the fruit epidermis, producing a dramatic reduction in surface conductance (Hallett and Sutherland, 2005). Similarly to transpiration, xylem flux is very high at the beginning of the season, progressively decreasing with fruit development. It is not clear, however, whether this reduction is due to anatomical changes in the xylem vessels, such as disruptions or occlusions localized either in the pedicel (as occurs in tomato; Van Ieperen et al., 2003) or in the fruit tissue (as occurs in apple; Drazeta et al., 2001, 2004), or simply to diminished hydrostatic pressure gradients due to low transpiration rates and high phloem flows at the end of the season, as occurs in grape (Bondada et al., 2005; Keller et al., 2006; Choat et al., 2009).

Large amounts of dry matter accumulate in kiwifruit berry during the season (Okuse and Ryugo, 1981; Given, 1993). Richardson et al. (1997) report an almost linear increase in total dry weight content of kiwifruit berry from fruit set to harvest. As carbohydrates are translocated in the phloem, fruit dry matter accumulation should be related to the amount of phloem sap flowing into the fruit tissue during the season, as well as to the carbohydrate concentration of the phloem sap, which may change during fruit development; however, no information is available on the behaviour of kiwifruit phloem flow, either on a daily or on a seasonal scale.

Vascular and transpiration in/outflows represent the main biophysical determinants of fruit fresh and dry matter gains. Therefore, separate analyses of these flows and of their relationships with the environment and tree water status are of basic importance to explain and understand the process underpinning fruit growth.

Lang and Thorpe (1989) and Lang (1990) have developed a methodology to separate and assess phloem, xylem and transpiration flows to and from a fruit. Although other experimental methods have been developed (Ho et al., 1987; Windt et al., 2009), the one proposed by Lang (1990) is readily adoptable under field conditions. However, this method is based on several assumptions and can lead to systematic errors, as reported by Fishman et al. (2001).

Using this and other techniques, the contribution of phloem, xylem and transpiration to fruit growth have been determined in grape (Lang and Thorpe, 1989; Greenspan et al., 1994), apple (Lang, 1990), tomato (Ho et al., 1987; Guichard et al., 2005) and peach (Morandi et al., 2007a). In most of these fruits the model of growth changes during the season, whereas in others, such as peach, the contribution of vascular and transpiration flow to fruit growth remains stable during the entire period of fruit development.

In kiwifruit, phloem, xylem and transpiration flows have not yet been quantified, either on a daily or on a seasonal scale. The present study investigates the biophysical mechanisms leading to fresh and dry matter accumulation in the fruit, and describes how changes in berry anatomical features which occur during fruit development affect the seasonal and daily behaviour of fruit growth.

MATERIALS AND METHODS

Plant material and environmental data

This study was conducted during 2006 and 2007 on ten kiwifruit, Actinidia deliciosa (A.Chev.) C.F.Liang & A.R.Ferguson, vines of the cultivar ‘Summerkiwi 4605’, located at the experimental farm of the University of Bologna (Cadriano, Bologna, Italy). This is an early maturing cultivar, usually harvested 3–4 weeks before the widely planted variety ‘Hayward’. The orchard, trained as T-bar at a density of 1112 vines ha⁻¹, was managed according to standard cultural practices.

In 2006, full bloom occurred on 23 May and fruit were harvested on 10 October, 20 weeks after full bloom (WAFB); in 2007 the date of full bloom was 11 May and harvest occurred on 25 September, 19 WAFB.

Temperature, relative humidity and rainfall data were available from a weather station (A840 Base Station, Adcon Telemetry GmbH, Klosterneuburg, Austria) placed in an adjacent orchard in the farm. Data were collected at 15-min intervals during the whole season. From these data vapour pressure deficit (VPD) was calculated in both years.

Seasonal patterns of fresh and dry weight

In both years, the seasonal patterns of fruit diameter, fresh and dry weight were monitored from 3 WAFB until harvest. The maximum and minimum transversal diameters of 20 tagged fruit, randomly chosen, were measured by calliper, at time intervals ranging from 5 to 12 d, depending on the fruit phenological stage. At each recording time (t), diameter data from each fruit were averaged (D, mm) and converted to fresh weight (Wfresh, g) using the following conversion equation:

\[ W_{\text{fresh}} = aD^b \]

where \( a \) and \( b \) were 0.0013 (± 0.0002 s.e) and 2.8640 (± 0.0529 s.e). This equation was obtained in 2006 by regressing diameter and weight data of 138 fruit picked during the whole season from the orchard where the experiments were set. The coefficient of determination (\( R^2 \)) of the relationship was >0.98.

Daily relative growth rates (RGR, g g⁻¹ day⁻¹) at each recording time (t) (i.e every 5–12 d) were calculated using the following equation:

\[ \text{RGR}_{t1} = \frac{(W_{\text{fresh},t1} - W_{\text{fresh},t0})}{(t_1 - t_0)W_{\text{fresh},t0}} \]

where \( W_{\text{fresh},t1} \) and \( W_{\text{fresh},t0} \) are the fruit fresh weights (g) calculated at any given recording date (\( t_1 \)) and on the date before (\( t_0 \)), respectively.

In both years, fruit dry matter percentage (DM %) was determined destructively at time intervals of about 10 d, during the season. On each measurement date, ten fruit from
the same orchard were harvested and from each fruit, a transverse slice of about 4 g was cut and dried at 60°C. Using these data, fruit dry matter content (g per fruit) and the specific rates of fruit dry matter accumulation (gDM gFW⁻¹ d⁻¹) were calculated for each sampling date.

**Xylem, phloem and transpiration flows**

Daily patterns of fruit growth, phloem inflow, xylem in/outflow and transpiration outflow were determined at different stages during fruit development, following Lang (1990). This method assumes fruit diameter variation in a finite time interval as the result of the algebraic sum among phloem, xylem and transpiration flows. Vascular and transpiration flows are then calculated as the difference between the diameter variations of intact, girdled and detached fruit. This calculation is based on the further assumptions that: (1) xylem flow is not affected by girdling and (2) transpiration rate is not affected by detachment. Fishman *et al.* (2001) report how assumption (1) can lead to some systematic errors, causing under- and over-estimation of phloem and xylem flows, respectively. However, these errors seem to be limited to specific times during the day and, to date, this is the only method which allows estimation of vascular and transpiration flows in the field, at short times scales and on a statistically sound number of samples.

Fruit diameter variations over time were monitored at 15-min intervals by using custom-built gauges interfaced to a CR1000 data-logger (Campbell Scientific Ltd, Shepshed, UK). The gauges consisted of a light, stainless steel frame supporting a variable linear resistance transducer (Megatron Elektronik AG & Co., Munich, Germany). Temperature effects on the frame and the sensor were tested and showed negligible errors under normal field conditions (Morandi *et al.*, 2007c).

In 2006, vascular and transpiration flows were determined in the following periods: 12–19 June (3 WAFB), 30 June – 5 July (5 WAFB), 4–10 August (10 WAFB), 11–16 August (11 WAFB) and 11–18 September (16 WAFB). In each period diameter variations over time were simultaneously monitored on 12 representative, well-exposed fruit placed on the east side of the row. Nine of these fruit were subjected to the following sequence of conditions: ‘intact’ (with normal vascular connections), ‘girdled’ (with the phloem connection severed) and ‘detached’ (with all vascular connections severed). Phloem connections were severed by girdling the shoot directly above and below the pedicel insertion. In ‘detached’ fruit the peduncle surface was covered with glue to avoid any water loss, and fruit were hung in their original position using thin wire. Fruit were monitored for 2 or 3 d on each of the ‘intact’ and ‘girdled’ conditions and only for 1 or 2 d on the ‘detached’ condition; for each fruit, data collected on days with the same condition were then averaged. Detached fruit were monitored for a shorter period of time to avoid excessive dehydration of the tissue, which could lead to an underestimate fruit transpiration. Depending on the weather conditions, the whole experiment lasted from 5 to 8 d, as only data collected on clear and sunny days were used for calculations. During this period, three of the 12 fruit were continuously monitored in intact conditions and served as controls to verify that fruit daily growth rate and pattern did not change significantly during the period of measurement.

For each fruit, diameter data were converted to weights by the specific conversion equation reported above (eqn 1). The relative changes of fruit fresh weight (g g⁻¹) in a given time interval (t) were then calculated on each of the three conditions: normal (N), girdled (G) and detached (D), and phloem (P), xylem (X) and transpiration (T) flows were computed using the following equations:

\[
P_t = N_t - G_t
\]

\[
X_t = G_t - D_t
\]

\[
T_t = D_t
\]

Fruit growth rate, and phloem, xylem and transpiration flows were expressed both as weight changes per whole fruit (g fruit⁻¹) and per unit fruit weight (g g⁻¹) to allow comparisons between years, stages and cultivars. At each recording time, data from the nine fruits measured were averaged and standard errors were computed for all the parameters considered.

As day to day variability in the weather conditions might have affected the results obtained in 2006, during the following season we chose to set up the experiments slightly differently in order to double-check the results obtained the previous year. The three treatments (‘intact’, ‘girdled’, ‘detached’) were applied simultaneously, each to four fruit, monitored for 24 h. Every day the condition of each fruit was changed: ‘intact’ fruit were girdled, ‘girdled’ fruit were detached and ‘detached’ fruit were substituted with new ‘intact’ fruit. Three more fruit were left intact for the whole period and served as controls so that a total of 15 fruit were monitored simultaneously. In this second year, each experiment lasted from 8 to 15 d and, on each day, diameter data from fruit in the same condition (‘intact’, ‘girdled’, ‘detached’) were averaged and converted to weights by the specific conversion equation reported above. Experiments were performed in 11–20 June (4 WAFB), 22–29 June (6 WAFB), 27 July – 6 August (11 WAFB), 16–30 August (14 WAFB) and 10–17 September (17 WAFB).

Whole fruit (g fruit⁻¹) and specific (g g⁻¹) changes of mean fresh weights in a given time interval (t) were then calculated on each of the three conditions: normal (N), girdled (G) and detached (D), and phloem (P), xylem (X) and transpiration (T) flows were computed using the equations reported above. At each recording time, data from each day were averaged and standard errors were computed for all the parameters considered so that replicates were fruit in 2006 and days in 2007.

For each measurement period, relative water losses and phloem relative contribution to fruit growth were also calculated as the ratio between daily transpiration and daily total inflows (phloem + xylem) and between daily phloem flow and daily total inflows, respectively.

Parallel, independent measurements of daily fruit water losses via cuticle transpiration were carried out in the second year at 6 WAFB to double-check the reliability of measurements carried out with the gauges. Ten fruit comparable
with those monitored by the gauges were detached and their fresh weight and diameter were immediately measured. Peduncles were then covered with glue and fruit were hung with thin wire as close as possible to their original position. Twenty-four hours later the weight and diameter measurements were repeated and daily transpiration was estimated as the average weight difference between the two measurements. Measurements were repeated on four clear, sunny days.

Xylem functionality

In the second season, at 5 and 14 WAFB, a complementary trial was conducted to test the fruit xylem functionality.

The daily patterns of xylem and transpiration flows were determined on control fruit and on fruit whose epidermis was partially removed to enhance water losses (treated) using the method described above (Lang, 1990). The growth of eight fruit under normal conditions was monitored for 2 d by the fruit gauges; on day 3 all fruit were girdled and four of them were treated, immediately after girdling, by removing the epidermis on about half the fruit surface with a knife. The epidermis was removed on the two sides of the fruit perpendicular to those in contact with the gauge. The following day at the same hour, all fruit were detached and monitored for 24 h. Fruit were hung in the same position in the canopy and the cut surface of the peduncles was covered with glue to avoid water losses. Immediately after detachment, a further, thin slice of fruit flesh was removed in the ‘treated’ fruit to maintain the same open pathway for water loss. As fruit epidermis removal caused fast dehydration of the ‘opened’ surface, in this experiment xylem and transpiration flows were calculated only for the 3-h period from 0900 to 1200 h following the treatment application. Control and treated fruit were compared for xylem and transpiration flows by a t-test.

Water relationships

The daily patterns of leaf, stem and fruit pressure potentials in the xylem were monitored on 3 July, 9 August and 15 September in 2006 (5, 11 and 16 WAFB, respectively) and on 27 June and 29 August in 2007 (6 and 14 WAFB, respectively). On each day, measurements were taken around 0400, 0700, 0900, 1100, 1300, 1500, 1700, 1900 and 2400 h, using a Scholander pressure chamber.

With this technique, the pressure potential recorded on leaf and stem can be assumed to be equal to the water potential as the concentration of the xylem sap is almost null. By contrast, fruit pressure potential may not coincide with its water potential, as the osmotic concentration in the fruit apoplast may be significantly high (Matthews and Shackel, 2005). For these reasons, here ‘water potential’ is the parameter measured on leaf and stem and ‘pressure potential’ the parameter measured on fruit.

Leaf water potential was measured on four well-exposed shoot leaves covered by aluminum foil just before excision (Turner and Long, 1980), using a Scholander (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) pressure chamber. The water potential of the stem was measured very close to a fruit: four leaves placed on fruit-bearing shoots were chosen. Each leaf was covered with aluminium foil at least 90 min prior to measurement to allow equilibration with the stem. This is different from the ‘standard’ stem water potential, which is normally measured in the inner part of the canopy (McCutchan and Shackel, 1992; Naor et al., 1995; Naor, 1998). This is therefore termed stemf here.

Fruit pressure potential was measured on four fruit close to the leaves used for stemf determinations. For every measurement time, stemf-to-fruit pressure potential gradient was calculated as the difference between stemf water potential and fruit pressure potential. Means (±s.e.) were computed for leaf and stemf water potentials and for fruit pressure potential at each recording time. For all measurement days, fruit pressure potential was correlated to transpiration, xylem and phloem flows recorded on that day. Similarly, stemf-to-fruit pressure potential gradient was correlated to xylem flow. As flow data were collected more often during the day than hydrostatic pressures, on each day only the values corresponding to the time when water potentials were measured were used for the correlations. For each correlation, slope (±s.e.), r and P were calculated.

RESULTS

Seasonal patterns of fruit growth

In both years, kiwifruit berries initially showed a rapid volume increase followed by a period of slow diameter growth (Fig. 1A). Highest RGRs were recorded during the first part of the season, with fruit growing at 0.06–0.07 g g−1 d−1 between 4 and 5 WAFB. A progressive decrease in fruit RGR occurred until about 10 WAFB; thereafter, it maintained steady or slightly decreasing values, in the range 0.005–0.01 g g−1 d−1, until harvest (Fig. 1B).

Fruit dry matter content and percentage increased almost linearly from the earliest stages until harvest during both seasons (Fig. 1C). The rates of specific dry matter gain were high at the beginning of the season and progressively decreased until about 10–12 WAFB. Thereafter, average values of approx. 0.002 g DM gFW−1 d−1 were maintained until harvest (Fig. 1D).

Seasonal patterns of phloem, xylem and transpiration flows to/from the fruit.

Kiwifruit-specific transpiration decreased with fruit development: small fruit at 3–4 WAFB transpired on average 0.4–0.5 g water gFW−1 d−1, corresponding to 7–8 g water per fruit d−1. As they increased in size, transpiration decreased, levelling at 12–14 WAFB until harvest, when a fruit weighing 60–70 g lost around 0.03–0.05 g water gFW−1 d−1 (1–2 g water per fruit d−1; Fig. 2A, B). Fruit relative water losses (transpiration vs. total inflows) ranged from 85 to 110 % at the beginning and at the end of the season, respectively; however variability was high for this parameter and the trend was not significant (r = 0.54; P = 0.12) (Fig. 2B). The parallel measurement of fruit transpiration performed by weighing detached fruit showed similar water losses to those measured, at the same time, using the fruit gauges (data not shown).
Xylem flow was also very high early in fruit development, reaching its maximum at 3–4 WAFB, when it accounted for 92/93% of the daily total inflows. Subsequently, xylem flow decreased sharply until 12–14 WAFB, when it accounted for 82% of the total inflows during the first season and only for 50% in the second season. Thereafter, xylem flow remained stable or decreased slowly, accounting for 50–60% of daily total inflows (Fig. 2C, D).

Phloem inflow was smaller than xylem inflow during the whole season. At fruit-specific level (g g⁻¹) it decreased during the season, similarly to xylem and transpiration inflows (Fig. 2E) but showed constant rates at the whole-fruit level (g fruit⁻¹) (Fig. 2F).

The relative contribution of phloem to fruit growth increased with fruit development (r = 0.79; P = 0.005), accounting for 10 and 40–50% of daily total inflow during the early and late stages, respectively (Fig. 2F). A positive, linear correlation was found between specific phloem flow and daily dry matter gain (Fig. 3).

Daily patterns of phloem, xylem and transpiration flows to/from the fruit

3–4 WAFB (early stage). Young kiwifruit berries grew at the highest rates during late afternoon and night, reaching maxima after 1800 h. Immediately after dawn, fruit growth rate progressively slowed and became negative, leading to a shrinkage that stopped between midday and 1500 h (Fig. 4A, B). At the daily scale, specific transpiration rate was positively related to VPD, with R² values of 0.64 and 0.75 in the two years (data not shown), and it increased from dawn to maxima around noon. It then decreased in the afternoon and maintained low values during the night (Fig. 4A, B). The daily pattern of xylem flow mirrored transpiration: it increased after dawn, although it did not balance fruit water losses until 1200 and 1600 h in 2006 and 2007, respectively. Thereafter, it decreased until the next morning (Fig. 4A, B). Phloem inflow maintained lower values than xylem, but with several fluctuations during the day. In the first year, it showed inflows mostly during the morning and the night (Fig. 4A), whereas in the second year it appeared quite constant from dawn to dusk (Fig. 4B).

5–7 WAFB (mid stage). At this time of season, as in the earlier stage, fruit grew from late afternoon to dawn and shrank during the morning and mid-day hours. In the two years, minima and maxima in RGR occurred around 0900 and 1900 h, respectively, with higher absolute values in the first than in the second season (Fig. 4C, D). At an hourly scale specific transpiration rate followed VPD (R² = 0.88 and 0.94 for 2006 and 2007, respectively – data not shown), and increased from dawn to mid-afternoon, again with higher values in 2006 than in 2007 (Fig. 4C, D). Xylem flow was low at dawn and became negative in the early morning. In addition to transpiration, this slight backflow contributed to fruit shrinkage at this time of the day. From mid-morning, although a positive xylem flow was recovered, it was not sufficient to balance transpiration water losses until mid to late afternoon in both years. When xylem flow exceeded transpiration losses, fruit started to increase their volume. Maxima in xylem inflow were reached in the late afternoon for both seasons, although differences of up to 30–40% were recorded between the two years (Fig. 4C, D).

Phloem flow contributed to fruit growth mainly during the morning, providing about 60 and 70% of its total daily contribution between 0900 and 1200 h (Fig. 4C, D).

10–12 WAFB (mid to late stage). In both seasons, fruit RGR, specific vascular flows and transpiration at 10–12 WAFB were similar in patterns but lower in absolute values than those shown in the earlier periods. At this time, fruit shrinkage
occurred later during the day and lasted until mid-afternoon so that maxima in RGR were reached in the late evening for both seasons. Transpiration rate was linearly related to VPD ($R^2 = 0.6$ and $0.8$ in the two years). Despite similar environmental conditions between this and the earlier two periods, maxima in fruit transpiration rate were about $30\%$ lower than those at 5–7 WAFB, with maximum values ranging from $0.05$ to $0.08$ mg g$^{-1}$ min$^{-1}$ (Fig. 4E, F). Similarly to transpiration, xylem flow showed $30\%$ lower values than the previous period, reaching maxima around 2000 h. As at 5–7 WAFB, phloem contribution was highest around mid-day: $80\%$ of phloem unloading occurred between 0900 and 1600 h at 10 WAFB in 2006 and between 0900 and 1800 h at 11 WAFB in 2007 (Fig. 4E, F).

14–18 WAFB (late stage). At 14 WAFB, in 2007, fruit daily RGR showed slightly positive values (on average $0.015$ mg g$^{-1}$ min$^{-1}$) during the evening and the night, whereas fruit shrank (minima in RGR were recorded at 1330 h) around mid-day (data not shown). Later in the season, at 16 WAFB in 2006 and at 17 WAFB in 2007, fruit RGR maintained values close to zero during the entire 24 h with no or negative daily net growth (Fig. 4G, H).

In the 2007 experiments, transpiration followed VPD, with $R^2$ of $0.65$ and $0.89$ at 14 and 17 WAFB, respectively.
Fig. 4. Diurnal courses of fruit mean relative growth rate (RGR) and specific phloem, xylem and transpiration flow rates and vapour pressure deficit (VPD) at (A) 3, (C) 5, (E) 11 and (G) 16 WAFB in 2006 and at (B) 4, (D) 6, (F) 11 and (H) 17 WAFB in 2007. Maximum s.e. values for RGR were 0.046, 0.015, 0.007 and 0.015 for (A), (C), (E) and (G), respectively, in 2006 and 0.035, 0.023, 0.020 and 0.024 for (B), (D), (F) and (H), respectively, in 2007. Maximum s.e. values for transpiration were 0.319, 0.029, 0.013 and 0.005 for (A), (C), (E) and (G), respectively, in 2006 and 0.129, 0.044, 0.020 and 0.018 for (B), (D), (F) and (H), respectively, in 2007. Maximum s.e. values for phloem were 0.161, 0.034, 0.011 and 0.004 for (A), (C), (E) and (G), respectively, in 2006 and 0.025, 0.036, 0.020 and 0.028 for (B), (D), (F) and (H), respectively, in 2007. Maximum s.e. values for xylem were 0.332, 0.029, 0.015 and 0.007 for (A), (C), (E) and (G), respectively, in 2006 and 0.106, 0.044, 0.027 and 0.020 for (B), (D), (F) and (H), respectively, in 2007. Data were recorded at 15-min intervals, during all experiments. For each parameter, data are the means of 6–9 replicates.
Xylem functionality

Removing kiwifruit berry epidermis at 5 WAFB did not affect fruit transpiration. During the first 3 h after treatment, hourly water losses were on average 36.8 and 24.1 mg g⁻¹ h⁻¹ for control and treated fruit, respectively. Similarly, no differences between treatments were found for xylem flow, with hourly rates of 36.5 and 21.9 mg g⁻¹ h⁻¹ for control and treated fruit, respectively.

At 14 WAFB, fruit epidermis removal resulted in a rise of fruit hourly water loss that reached on average 7.0 mg g⁻¹ h⁻¹ during the first 3 h after treatment. This value was significantly higher than for control fruit (1.1 mg g⁻¹ h⁻¹) (P = 0.005) during the same time interval. Despite such large differences in water loss, control and treated fruit did not show differences (P = 0.86) in the hourly amount of xylem flow, which was 1.3 and 0.6 mg g⁻¹ h⁻¹ for peeled and control fruit, respectively.

Water relationships

Throughout the season, leaf and stem water potentials and fruit pressure potential decreased after dawn, showed the most negative values around mid-day, increased during the afternoon and reached the highest daily values during the evening, which were then maintained during the night. Leaves always reached the most negative water potentials between 1000 and 1200 h (Fig. 5). Fruit pressure potential decreased in the morning, reaching minima at about 1500 h in all measurements. An exception to this was the measurement carried out at 6 WAFB in 2007, when fruit pressure potential showed the lowest value at noon (Fig. 5).

Fruit pressure potential was negatively related to fruit transpiration rate whereas no significant correlations were found between fruit pressure and phloem flow at any time during the season (Table 1). Stem₃-to-fruit pressure potential gradient was positively affected by fruit transpiration rate at 6 but not at 14 WAFB. Similarly, fruit xylem inflow responded to stem₃-to-fruit pressure potential gradient during the first stages of fruit development (6 WAFB), whereas no correlation was found between these parameters later in the season (Fig. 6).

DISCUSSION

Seasonal growth in fresh and dry weight shown in both years by ‘Summerkiwi’ fruit is 2–3 weeks shorter than ‘Hayward’, but with a pattern similar to that already reported for this species (Gallego et al., 1997; Ferrandino and Guidoni, 1998), which is characterized by a first period of high RGR, decreasing sharply until reaching very low values during the second half of the growth season (Fig. 1A, B).

On the other hand, seasonal dry matter accumulation is constant at the whole-fruit level (Fig. 1C), but decreases on a specific basis (Fig. 1D), although less sharply than RGR (Fig. 1D).

Different methodologies were used to assess phloem, xylem and transpiration flows in the two years; however, similar results were found both on a seasonal and on a daily scale (Figs 2 and 4). Specific vascular flows and transpiration to/from the kiwifruit berry were very high in the early stage and progressively decreased with fruit development. Initially, xylem and phloem imports largely exceeded transpiration losses, producing the high volume increase typical of the first rapid growth stage. Later, the volumes exchanged diminished and the balance between incoming and outgoing fluxes was progressively reduced, mainly due to a sharp decrease in xylem flow, resulting in a decrease of fruit growth rate. Specific phloem flow was lower and decreased less sharply than xylem flow during the season (Fig. 2C), showing a correspondence with the rates of fruit dry matter gain (Fig. 1D). A similar correspondence was found between absolute phloem flows (Fig. 2F) and the rates of dry matter accumulation per whole fruit, as both these parameters maintained constant values during the season.

The linear relationship between daily phloem flow and dry matter gain (Fig. 3) suggests that phloem sap may maintain a fairly constant concentration (around 10–12 %) during fruit development. The concentration of kiwifruit phloem sap has not been directly determined; the value estimated here is in the range of, or slightly lower than, those adopted for medium-cropped peach trees (9–17 %) in the model of Fishman and Génard (1998), and for tomato (12 % at 25 °C) in the model of Liu et al. (2007). Phloem sap concentrations of 17 and 16 % were independently reported for tomato (Ruan and Patrick, 1995) and Ricinus communis (Peuke, 2010), respectively. In view of the above, the results reported in this paper appear quite realistic; therefore, the potential underestimation of daily phloem flow due to possible flaws in the methodology should be quite low.

Despite some seasonal variability, phloem flow was one-tenth that of xylem flow in the early stage but, as the latter decreased, the former reached similar values at the end of fruit development. The increase in fruit dry matter percentage (Fig. 1B) that characterizes kiwifruit berry development (Okuse and Ryugo, 1981; Given, 1993) must be attributed largely to a seasonal increase in the relative contribution of phloem to fruit growth (Fig. 2F). In addition, the slight seasonal increase in the relative amount of water losses (transpiration vs. total inflows) could have partly contributed to dehydrate the fruit tissue during the last stages of fruit development. However, this trend was not significant during the season (Fig. 2B).

The seasonal decrease in transpiration rate (Fig. 2A) is due to anatomical modifications of the fruit epidermis (Schmid, 1978; Hallett and Sutherland, 2005). Such changes affect fruit water status as water losses by transpiration reduce fruit pressure potential (Table 1) and increase the force driving xylem water into the fruit, provided that xylem vessels are functional and water is available. Therefore, the high transpiration rates recorded at the beginning of the season are a key feature for the fruit to import large amounts of xylem sap by bulk flow, as shown by the linear relationship between the
stem-to-fruit pressure potential gradient and xylem flow (Fig. 6). Later in the season, this relationship disappears (Fig. 6). This lack of relationship could be attributed in part to changing mechanical properties within the fruit, the effect of which might not have been detected using the method adopted here. However, as no changes in xylem flow were found between intact fruit and fruit whose water loss was enhanced by peeling off part of the epidermis, the progressive seasonal decrease in xylem flow cannot be simply attributed to a reduction in the pressure potential gradient in the xylem path to the fruit, as occurs in grape (Bondada et al., 2005; Keller et al., 2006), but to a progressive decrease in vessel hydraulic conductivity, occurring either in the pedicel or in the fruit.

At the daily scale, minute variations in fruit diameter showed how kiwifruit berry grows during the late afternoon and night and shrinks around midday, regardless of fruit developmental stage (Fig. 4). However, daily variations in RGR are higher during the first period of fruit development, when xylem and transpiration in/outflows are highest (Figs 2 and 4A–D).

During the day, transpiration rate always responds to changes in environmental conditions: as VPD increased after sunrise, transpiration increased (Fig. 4) and fruit pressure potential decreased. Despite the physiological relationship between water losses by transpiration and water gains by xylem flow, these parameters are not always related over 24 h. In fact, depending on the time of day when water is available for fruit expansion, xylem flow and transpiration losses may or may not coincide. For example, between 3 and 4 WAFB they showed a symmetrical daily pattern ($R^2 > 0.99$; Fig. 4A, B). This may be due to the low daily VPDs recorded in June (Fig. 4A, B). The ensuing lower environmental demand for water may result in a higher stem water potential, with the possibility for the fruit to promptly recover the high amount of water transpired, at any time during the day.
Along with stem-to-fruit pressure potential gradients (Fig. 5C), as these may even become negative (as after 0900 h), negative xylem flows can be observed in response to VPD. The dependence on VPD of these backflows was also visible during later stages (10–11 WAFB), especially in 2007, in response to the particularly high VPD recorded during that period (Fig. 4F). When xylem vessels are functional, as in the early to mid stage of fruit development, water backflow from fruit to leaves may occur in kiwifruit, due to competition between the two organs. In fact, in the morning, most of the water available at vine level appears to be directed to transpiring leaves, which reduce their water potential more quickly than fruit (Fig. 5). In the afternoon, when leaf stomatal conductance decreases (data not shown) and leaf water potential increases rapidly, more water becomes available for the fruit. Phloem flow always reached lower values than xylem flow during the day, although it showed higher variability over the day. The hourly fluctuations and negative values occasionally recorded for phloem flow can partly be attributed to the errors arising from the methodology applied (Fishman et al., 2001). In several species, stem phloem flow determinations based on magnetic resonance techniques showed more constant rates over the 24 h (Peuke et al., 2001; Windt et al., 2006).

However, in most instances, the highest rates of phloem import occurred during the morning (5 – 7 WAFB) and at the beginning of the afternoon (10–11 WAFB), when fruit transpiration is high, xylem flow is low or negative, and fruit shrink at their highest daily rates (Fig. 4). At this time fruit pressure potential decreases rapidly due to transpiration water losses and the molecules entering the fruit via phloem flow may further lower fruit osmotic potential, thus enhancing the fruit capacity to subsequently import xylem water at high flow rates. This occurs generally in the afternoon, when xylem import exceeds transpiration losses, allowing the fruit first to recover their initial volume, then to increase their size. The daily mechanism described is similar to that reported for peach at stage III (Morandi et al., 2007a), although kiwifruit xylem flow and transpiration are higher when the fruit is young, and reach values lower than peach around 10 WAFB (i.e. when peaches grow fastest). This comparison suggests that fruits that transpire large amounts of water, for example kiwifruit berry in the early stage, and peach during the whole season, may have adopted a similar mechanism of daily growth, based on high daily fluctuations in diameter and hydrostatic pressure.

In conclusion, extremely large amounts of water are exchanged by kiwifruit during the period of fast volume increase, as a result of fruit surface conductance and high xylem functionality. These features appear to be important for fruit cell expansion, which is maximum at this time of the season: high turgor pressure at certain times during the day are needed for cell walls to extend; as cells expand, this further reduces fruit pressure potential and allows new fresh mass to enter the fruit (Schmalstig and Cosgrove, 1990; Cosgrove, 1993a, b). This delicate balance between incoming and outgoing mass, based on high water fluxes, enables early kiwifruit berries to grow at the highest daily RGRs; but it also makes the fruit extremely susceptible to water stress, as reported by Miller et al. (1998). Later in the season, this water flux from the tree to the atmosphere is progressively reduced due to anatomical changes occurring both on the fruit epidermis and in the xylem vessels, leading to a decrease in fruit growth rate.

In kiwifruit, two physiologically distinct models of fruit growth seem to be adopted during the season. The present study provides one of the most complete descriptions of the biophysical aspects of such mechanisms, the outcome of which is the fresh and dry matter accumulation in kiwifruit berries. The changes in fruit epidermis anatomy and xylem functionality provide an explanation of the shift between the two models. Knowledge of the seasonal and daily behaviour of fruit vascular and transpiration flows may provide an important background for improvements to kiwifruit horticultural management.

LITERATURE CITED


