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An external heat pulse method for measurement of sap flow through fruit pedicels, leaf petioles and other small-diameter stems

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ABSTRACT

The external heat ratio method is described for measurement of low rates of sap flow in both directions through stems and other plant organs, including fruit pedicels, with diameters up to 5 mm and flows less than 2 g h^{-1}. Calibration was empirical, with heat pulse velocity (v_h) compared to gravimetric measurements of sap flow. In the four stem types tested (*Actinidia* sp. fruit pedicels, *Schefflera arboricola* petioles, *Pittosporum crassifolium* stems and *Fagus sylvatica* stems), v_h was linearly correlated with sap velocity (v_s) up to a v_s of approximately 0.007 cm s^{-1}, equivalent to a flow of 1.8 g h^{-1} through a 3-mm-diameter stem. Minimum detectable v_s was approximately 0.0001 cm s^{-1}, equivalent to 0.025 g h^{-1} through a 3-mm-diameter stem. Sensitivity increased with bark removal. Girdling had no effect on short-term measurements of *in vivo* sap flow, suggesting that phloem flows were too low to be separated from xylem flows. Fluctuating ambient temperatures increased variability in outdoor sap flow measurements. However, a consistent diurnal time-course of fruit pedicel sap flow was obtained, with flows towards 75-day-old kiwifruit lagging behind evaporative demand and peaking at 0.3 g h^{-1} in the late afternoon.

Key-words: *Actinidia chinensis*; *Actinidia deliciosa*; fruit; heat pulse velocity; phloem; transpiration; xylem.

INTRODUCTION

Heat-based sap flow techniques are widely used to measure transpiration and study plant–water relations. All of these techniques estimate sap flow by measuring either the effect of the moving sap on the heat balance of a heated portion of a stem ('heat balance' methods) or the effect of the moving sap on the propagation of a discrete pulse of heat released into the stem ('heat pulse' methods). All have advantages and disadvantages depending on the application, and their theory and use have been reviewed elsewhere (Swanson 1994; Smith & Allen 1996; Burgess, Adams & Bleby 2000).

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Most techniques involve insertion of heaters and detectors into sapwood, and are therefore suited to stems of diameters larger than 10 mm. Only a few are suitable for plant organs between 5 and 10 mm in diameter, and below this range the thermal instability and delicacy of stems, petioles, pedicels or roots 1–4 mm in diameter make non-destructive measurement of sap flow very difficult. Current limitations include poor detection of low rates of sap flow, instrument complexity and cost. This study describes a new type of heat pulse sap flow sensor capable of non-destructively measuring low rates of sap flow in stems down to 2 mm diameter or less. Our primary goal was to develop a simple, low-cost sensor for measurement of sap flow in both directions through the pedicels of developing kiwifruit berries.

Measurement of sap flow provides a number of benefits for studies of plant–water relations. Sap flow sensors can be easily replicated and automated, and installed without disrupting the microclimate around the plant (Smith & Allen 1996). When installed at different positions within the same plant, they can provide information on the dynamics of water flow around the plant. Recent examples include the role of tissue capacitance in buffering transpirational water loss (James *et al.* 2003; Burgess & Dawson 2008); documenting reversal in sap flow direction, particularly in large-diameter roots growing in drying soil (Burgess *et al.* 1998; Smith *et al.* 1999); and the coupling of stomatal physiology to plant hydraulic capacity and root functioning (Clearwater *et al.* 2004). In the case of rapidly growing fleshy organs such as fruit, comparison of sap flow with measurements of water potential gradients, transpiration and growth can provide useful information concerning factors that influence the accumulation of water and solutes (carbohydrates, minerals, etc.) in the organ of interest. There are many aspects of fruit biology, production and quality that require an improved understanding of the balance between water supply and loss from the developing fruit. These include changes in phloem and xylem functionality during fruit development (Bussieres 2002; Dichio, Remorini & Lang 2003); fruit softening and shrivel disorders in grapes and kiwifruit (Tyerman *et al.* 2004; Thorp *et al.* 2007); cracking and splitting of tomatoes, bell peppers and many other fruits (Aloni *et al.* 1999; Bertin *et al.* 2000); and the balance

between fresh weight growth and non-structural dry matter accumulation in fruits such as tomatoes, apples, kiwifruit and prunes (Richardson, McAneney & Dawson 1997; Guichard *et al.* 2001). This latter example is a major quality factor influencing fruit storability, processing, nutritional value and consumer perception of fruit flavour and texture. However, despite their relevance, published accounts of direct *in vivo* measurements of sap flow to or from developing fruit are rare (Higuchi & Sakuratani 2006; Windt 2007). The reason is probably that there are currently no methods suitable for routine measurement of the small flows involved.

The sap flow techniques most commonly applied to small-diameter stems have been heat pulse probes and constant power heat balance gauges. Cohen and co-authors have successfully used heater and thermocouple probes, inserted into the herbaceous stems of plants such as cotton, soy bean and maize, to measure sap flow in one direction using a heat pulse technique (Cohen *et al.* 1988, 1990, 1993; Cohen & Li 1996). The insertion of a probe can damage the vascular tissue, and, therefore, requires a stem of large-enough diameter that probe insertion is not overly disruptive of normal sap flows. The diameters of stems reported by Cohen *et al.* were typically in the range from 5 to 20 mm, and the sap velocities measured in these fast-growing annuals were relatively high. The diameters of many fruit pedicels and leaf petioles are in the range of 1–4 mm, and, depending on stem anatomy, the vascular cylinder within these may be less than 1 mm in diameter. We therefore chose an approach that did not require insertion of a probe into the stem.

Constant power heat balance gauges, effectively a heated insulated collar that is wrapped around the stem, can be custom made and are commercially available for measuring sap flow in stems and roots of diameters from 2 to 125 mm (Sakuratani 1984; Baker & van Bavel 1987; Smith & Allen 1996; Senock & Leuschner 1999). With minor modification, this technique can be used to measure sap flow in both directions (Burgess *et al.* 2000). However, the calculation of flow from this method includes a measurement of the temperature differential between the two ends of the gauge. Large errors occur when sap flows are low and the differential approaches zero. Flows through *Grevillea* roots less than approximately 10 g h^{-1} were excluded from analysis by Smith *et al.* (1999), and Sakuratani, Aoe & Higuchi (1999) excluded flows less than approximately 2 g h^{-1}. Coners & Leuschner (2002) successfully measured flows through tree fine roots 3–4 mm in diameter, but flows less than 2 g h^{-1} required an empirical correction. Higuchi & Sakuratani (2005, 2006) reduced the size of their gauge, and successfully measured minimum sap flows of approximately 1 g h^{-1} through mango inflorescences and fruit pedicels. However, based on transpiration measurements, maximum sap flows through developing kiwifruit pedicels and many other small-diameter stems are often less than 0.5 g h^{-1}, and if sap flow reversal occurs, the transition through zero is often the period of most interest. A variation of the heat balance technique is currently available commercially (Phytech Ltd, Rehovot, Israel) for measurement of low rates of sap flow in leaf petioles and small stems. However, these are described as 'relative' sensors by the manufacturers, for semi-quantitative measurement of flow rate and direction. We therefore chose not to use a heat balance technique, and instead developed an external version of a heat pulse method that is particularly sensitive to low rates of sap flow.

Marshall (1958) suggested that a sensitive heat pulse configuration would be obtained by recording the ratio of the temperature deflections measured at equal distances above and below the heating element following the release of a heat pulse. Burgess *et al.* (1998) named this approach the 'heat ratio' method, and demonstrated its utility for measuring bidirectional sap flow with probes inserted into roots. In this study, we used the heat ratio method with a small external heater (an electronic chip resister) and temperature sensors (fine gauge thermocouples) glued to a cork block and pressed against the surface of the stem. Externally applied heat pulses were used in early sap flow studies (Bloodworth, Page & Cowley 1955; Closs 1958), and two recent reports also used an external heat pulse method to measure sap flow in stems down to 3 mm in diameter (Bauerle *et al.* 2002; Helfter *et al.* 2007). Both of these two recent examples used a non-contact laser as the heat source, and infrared thermometers or a thermal-imaging camera for detecting heat pulse propagation. In all of these external heat pulse examples, the sensor configurations and associated mathematical derivations used were less suitable for low flow rates (<2 g h^{-1}), and cannot be used for detecting sap flow in both directions. The use of lasers and thermal detectors also requires relatively bulky and expensive equipment. A disadvantage of the method described in this study is that the gauge is in contact with the stem, and therefore contributes to conduction of the heat pulse. The application of an external heat pulse to a small-diameter stem and gauge also clearly violates the assumption of thermal homogeneity underlying the numerical descriptions of heat pulse propagation (Marshall 1958). We therefore paid particular attention to the relationship between measured heat pulse velocity and actual sap flux density, and the effect that different species and types of stem had on this relationship. Helfter *et al.* (2007) also reported the detection of phloem flows using their external heat pulse method, an advance that will be of great value if it can be routinely repeated. We therefore tested the effect of girdling and bark removal on sap flow measured with our external heat ratio sensors.

METHODS

Gauge design

Sap flow gauges were made from a rectangular block of cork aggregate 18 mm long, 7 mm wide and 3.5 mm high, cut from a self-adhesive granulated cork flooring tile. The heating element was a 47 Ω chip resistor, 3.2 mm long, 1.6 mm wide and 0.55 mm high (Kamaya RMC1/8K470FTP, Part no. 421-9034, RS Components, Auckland, New Zealand), with 0.2-mm-diameter enameled copper

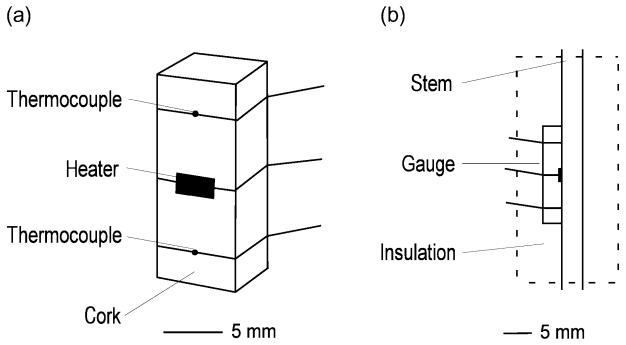

Figure 1. Diagram of the external heat ratio gauge, showing (a) arrangement of the heating element and thermocouples on a cork block, and (b) positioning of the gauge and insulation on a stem.

wire soldered to each end. The heating element and lead wires were glued using cyanoacrylate glue to the centre of the cork block, with the long axis of the resistor perpendicular to the long axis of the cork block (Fig. 1). Two copper–constantan thermocouples, made from 0.13-mm-diameter single-stranded Teflon-insulated thermocouple wire (TFCC-005 and TFCP-005, Omega Engineering, Manchester, UK) were similarly glued to the cork block 6 mm above and below the centre of the heating element (Fig. 1). The heating element was pressed into the cork block to bring its outer surface level with the thermocouple junctions, and a layer of clear nail varnish was spread over the entire face of the gauge to provide electrical insulation between the gauge and the stem it was applied to. The gauge was 'taped' to the stem of interest using strips of laboratory film (Parafilm M, Alcan Packaging, Neenah, WI, USA), taking care that the heater and thermocouples were firmly held against the surface of the stem, and that the axis formed by the heater and thermocouple junctions on the face of the gauge was centred on, and parallel to, the long axis of the stem (Fig. 1). Multiple turns of film were wound around the gauge and stem to achieve firm contact along the full length of the gauge. The gauge and stem were then tightly enclosed in closed-cell foam pipe insulation secured with tape and extending at least 20 mm above and below the position of the gauge on the stem.

The heating element was connected in series with a 120 Ω current-limiting resistor to a 12 V battery via a relay controlled by a datalogger (CR10X; Campbell Scientific, Logan, UT, USA). The two thermocouples were connected individually to the datalogger via thermocouple extension wire (Omega Engineering, part no. PP-T-24). The datalogger was used to fire the heat pulse every 10 min, and record the thermocouple temperatures for 200 s after the heat pulse was released. A 6 s heat pulse, dissipating 0.24 W from the heating element, was chosen to give an appropriate balance between avoiding excessive heating of the stem tissue next to the heater and an adequate temperature inflection at the position of the thermocouples. An extra thermocouple embedded in kiwifruit pedicels indicated that the 6 s heat pulse elevated temperatures within the stem adjacent to the heating element by a maximum of 15 °C during application of the pulse. Shorter-, higher-wattage heat pulses resulted in higher temperatures and caused death of the adjacent tissue. Prior to each heat pulse, the absolute temperature of each thermocouple was measured, and for 200 s after the heat pulse the change in temperature from the starting value and the ratio of the two temperature differentials was calculated. A period when the ratio of the two temperature differentials was most stable was chosen (55–74 s after heat pulse release), and the average ratio used to calculate the heat pulse velocity (Burgess *et al.* 1998).

Sap flow theory

Most previous applications of heat pulse sap flow measurements have involved the release of a discrete pulse of heat from a linear heater inserted radially into the sapwood of woody plant stems. Temperature sensors inserted into the sapwood at known positions upstream and downstream of the heater are used to record the temperature change follow the release of the heat pulse. If sap flow is occurring, convection of heat by moving sap causes the centre of the heat pulse to move with a velocity, v_h, that is proportional to the rate of sap flow. A variety of analytical solutions, first developed by Marshall (1958), have been used to estimate v_h from the measured temperature profiles. Marshall (1958) showed that for low rates of sap flow, a particularly sensitive measurement is obtained by recording the temperature rises δT_1 and δT_2 (°C) at equal distances x (cm) above and below the heater (respectively) along the same axis as the flow of sap:

$$v_h = \frac{k}{x}\ln\left(\frac{\delta T_1}{\delta T_2}\right) \quad \text{cm s}^{-1}, \tag{1}$$

where k is the thermal diffusivity (cm^2 s^{-1}). In most applications, 'above' the heater refers to the acropetal direction and the direction of the predominant transpirational flow of xylem sap. When there is no sap flow, the logarithm of the ratio $\delta T_1/\delta T_2$ is zero. When sap flow occurs, the ratio is greater than or less than 1, and the sign of the measurement indicates the direction of sap flow (Burgess *et al.* 2000). The thermal diffusivity was estimated from

$$k = \frac{x^2}{4t_m} \quad \text{cm}^2\ \text{s}^{-1}, \tag{2}$$

where t_m is the time (s) between the heat pulse and the maximum temperature rise recorded x cm above or below the heater, under conditions of zero sap flow (Green, Clothier & Jardine 2003). In probe-based heat pulse applications, the value of k should be between the value for sap and dry sap wood (Marshall 1958). In this study, the effective k is a property of the gauge material (cork) and the stem that it is in contact with. We measured t_m by installing the gauge on excised stems, and recording heat pulses with no imposed xylem flow, and calculated k from Eqn 2. Measured t_m

(55–65 s) varied little between individual stems, and similar values were obtained when a layer of cork was pressed against the gauge instead of a plant stem. This suggests that a significant proportion of the heat is conducted through the cork, and that the value of k is dominated by the thermal properties of the cork. We therefore assumed k was constant for each stem type (for kiwifruit pedicels plus the cork gauge assembly, k was 0.0013 $cm^2\ s^{-1}$).

When probes are used, perturbation of heat transfer by the probes and by wounded sapwood around the probes causes a reduction in measured v_h (Swanson & Whitfield 1981). Measured v_h is therefore usually corrected using non-linear correction factors derived from two-dimensional numerical models that predict the effects of probe materials and wound size on v_h (Swanson & Whitfield 1981; Green *et al.* 2003). In this study, the geometric complexity of an external gauge combined with various arrangements of both hydroactive (functional xylem) and non-hydroactive (bark, cortex, pith, etc.) tissues within the stems of interest meant that these models were not directly applicable. However, because the gauge and much of the tissue within the stems were not conducting sap, it was expected that v_h would be less than that predicted by heat pulse theory alone. For simplicity, it was assumed that

$$v_c = v_h m_{bark}, \quad (3)$$

where v_c is heat pulse velocity corrected for the effect of non-hydroactive components within the system (gauge materials, bark, etc.), and m_{bark} is an empirical multiplier that accounts for this effect (Edwards, Becker & Cermak 1996). Sap velocity expressed on a total sap wood area basis (v_s) is then calculated as

$$v_s = v_c (k_w V_{wood} + V_{water}), \quad (4)$$

where k_w is a coefficient related to the thermal properties of the woody matrix (0.441 at 20 °C), and V_{wood} and V_{water} are the volume fractions of the woody matrix and water, respectively, in the xylem (Becker & Edwards 1999). The multiplier ($k_w V_{wood} + V_{water}$) in Eqn 4 is based on the assumption that sapwood is a thermally homogenous matrix of moving sap and stationary wood. Its value is less than 1, thus accounting for the fact that rapid convection of heat within the lumens means that v_c should be faster than v_s. In conventional sap flow theory, v_s is equivalent to the product of average sap velocity in the lumens of the xylem and ratio of lumen area to total sapwood area (Edwards *et al.* 1996). This correction was also difficult to apply to the current study because the combination of external gauge and stem was probably not thermally homogenous, and only a proportion of the cross section of the stem was hydroactive xylem. For calibration purposes, we therefore took the simplest approach of finding the slope of the relationship between measured heat pulse velocity v_h and measured sap velocity:

$$v_s = v_h m_{sap}, \quad (5)$$

where m_{sap} is a coefficient that we assumed incorporates some of the effects [m_{bark} and ($k_w V_{wood} + V_{water}$)] described by Eqns 3 and 4. For most experiments, sap flow ($g\ h^{-1}$) was measured gravimetrically or by gas exchange, and v_s was expressed as sap flow per unit total cross-sectional area of the stem at the point of gauge attachment, including all hydroactive and non-hydroactive tissues. Ideally, v_s should be expressed as flow per unit sapwood area, but in reality the xylem anatomy of small stems and roots is often more complex than the homogenous cylinder of sapwood found in larger woody stems. This makes it difficult to determine the effective 'sapwood' area, and v_s is sensitive to errors in determination of xylem cross-sectional area. Rapid heat transfer between the hydroactive and non-hydroactive tissues within a small stem means that inclusion of total cross-sectional area may also help to reduce variability in m_{sap}. The same approach was adopted by Cohen & Fuchs (1989) using heat pulse probes in herbaceous stems with complex anatomies. To investigate potential sources of variation in m_{sap} (Eqns 3 and 4), samples of all stems used for calibration experiments were kept for measurement of anatomical properties (e.g. bark thickness, proportion of xylem area to total cross-sectional area) and volume fractions of wood and water, based on Archimedes's principle and oven dry weight (Edwards & Warwick 1984).

Plant material

The sap flow gauges were tested with stems from a range of plant species, including pedicels of green kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* 'Hayward'] and yellow kiwifruit (*Actinidia chinensis* Planch. var. *chinensis* 'Hort16A'), petioles of the 'dwarf umbrella tree' [*Schefflera arboricola* (Hayata) Kanehira, a common ornamental plant with compound leaves and long slender petioles], distal stems ('twigs') cut from a mature copper beech tree (*Fagus sylvatica* L.), and the main stem of seedlings of karo (*Pittosporum crassifolium* Banks & Sol. ex A.Cunn.), a fast-growing small tree native to New Zealand (Table 1). For kiwifruit, the pedicels were from fruit that varied in age from approximately 50 d (growing rapidly) to more than 200 d (mature fruit) after anthesis. The green and yellow kiwifruit species are taxonomically closely related, and their fruit pedicels were similar in anatomy and morphology, but the two cultivars differ in their seasonal timing of anthesis and fruit growth. For practical reasons, green kiwifruit were used for the majority of tests with excised pedicels and detached fruiting shoots, and gold kiwifruit were used for testing measurements of sap flow into growing fruit in the orchard.

Comparison between gauge output and gravimetric measurements of flow

Controlled testing of the sap flow gauges in both flow directions was achieved by excising stems and connecting them to a supply of filtered (2 μm), pressurized tap water using

Table 1. Summary of the species and types of stem used for testing of the external heat ratio sap flow method

Species	Organ	*n*	Diameter range (mm)	m_{sap}	$(k_w V_{wood} + V_{water})$
Actinidia deliciosa	Fruit pedicel	16	2.6–3.8	2.5 ± 0.1	0.83 ± 0.01
Schefflera arboricola	Leaf petiole	7	2.0–3.1	2.6 ± 0.1	0.87 ± 0.01
Fagus sylvatica	Distal stem	6	2.5–3.5	3.3 ± 0.1	0.63 ± 0.01
Pittosporum crassifolium	Seedling stem	3	4.8–5.5	2.6 ± 0.1	0.72 ± 0.01

m_{sap} is the average slope of the relationship between v_s (sap velocity or sap flux density) and v_h (measured heat pulse velocity; Eqn 5). $(k_w V_{wood} + V_{water})$ is the theoretical multiplier used to estimate v_s from v_h, based on sapwood density and water content (Eqn 4).

PTFE compression fittings (catalog # A-06473-00; Cole-Parmer, Vernon Hills, IL, USA) and 2.3-mm-OD diameter PTFE tubing (Fig. 2). After collection from the plant, the stem ends were recut under water and inserted into the compression fittings while allowing a low-pressure flow of water to flush air bubbles from the fittings. In the case of kiwifruit pedicels, several millimetres of bark was also stripped from each end of the pedicel, and the ends thoroughly washed in water and recut to remove mucilage before insertion into the compression fittings. A sap flow gauge was installed on the stem and insulated in the normal way. Three-way plastic luer stop-cock valves were used to create a manifold for directing the flow of water in either direction through the excised stem and onto a reservoir on a three-decimal place electronic balance (PB303S; Mettler Toledo, Greifensee, Switzerland) (Fig. 2). After installing the stem and gauge, the water supply was switched to a high-pressure water–air accumulator tank, with compressed air supplied from a tank and pressure regulator. A solenoid valve was used to briefly vent the air side of the accumulator tank, allowing the pressure to be dropped in controlled increments (Fig. 2). The datalogger was used to trigger the heat pulse and measure the signal from the sap flow gauge, control the solenoid valve and record water flows from the balance via an RS-232 serial connection. A typical test was started by supplying water at a pressure of up to 300 kPa, waiting for flows to stabilize, then leaving the datalogger programmed to record the gauge signal and gravimetric flow every 10 min, and incrementally reduce the pressure every 30 min until the applied pressure and flow had reduced to zero. The flow direction was then reversed by adjusting the three-way valves, and the calibration sequence restarted in the opposite direction without disturbing the stem or sap flow gauge.

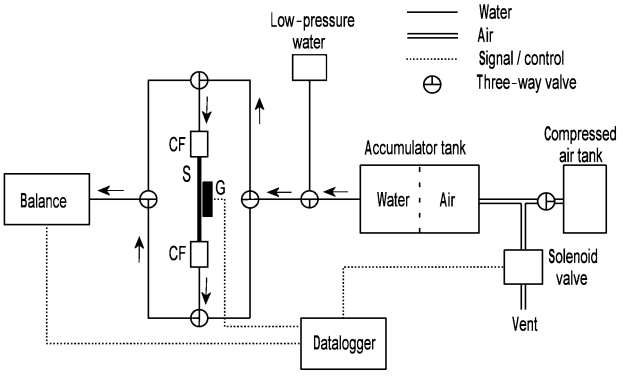

Figure 2. Schematic diagram of the apparatus used to impose measured flows in both directions through the xylem of an excised stem, while also recording the output from an external heat ratio sap flow gauge installed on the stem. Arrows show the direction of flow for the indicated alignment of the three-way valves. CF, compression fitting; S, stem; G, gauge.

Effect of bark tissue and detection of *in vivo* flows

Tests were conducted with excised stems prepared as described earlier. In one series of tests, the effect of bark tissue on gauge measurements was assessed by repeating the calibration exercise using the same excised green kiwifruit pedicel and gauge, after a window of bark had been sliced from the pedicel to expose the xylem and the gauge re-installed in the same position. In other tests, intact potted plants were allowed to transpire normally, and flow was measured by placing the plant on a balance (also connected to the datalogger) with the soil surface covered (*P. crassifolium* seedlings), or by enclosing a leaf in the chamber of a plant gas exchange system (LI6400; Li-Cor, Lincoln, NE, USA) and measuring transpiration of the whole leaf (one *S. arboricola* petiole). In these examples, the sap flow gauge was installed on the external surface of the stem, without disturbing the bark or petiole cortex. Attempts were made to detect phloem flows, usually by installing the gauge on a leaf petiole or seedling stem (on the bark or on the xylem after cutting a window in the bark) and supplying leaves distal to the gauge with light, while darkening the rest of the plant. Flows were recorded for several days, then the stem was steam or mechanically girdled close to the gauge, and the sap flow signal was monitored for any obvious changes caused by girdling.

Measurement of flow through fruit pedicels

The sensitivity of the gauge to sap flow in intact kiwifruit pedicels was tested using both excised shoots and fruit growing on normal vines in an orchard. Current-year lateral shoots with a fruit were cut from a green kiwifruit vine 60 d after anthesis, recut immediately under water, brought into

the laboratory and supplied with light from a desk lamp. A sap flow gauge was installed on the fruit pedicel on top of the bark, and the fruit and shoot were subjected to treatments intended to induce changes in pedicel sap flow, for example, cutting of the pedicel distal or proximal of the gauge, or removing the water supply from the cut base of the shoot and allowing the entire shoot to dehydrate. Sap flow gauges were also installed on top of the bark of intact yellow kiwifruit pedicels in the orchard 75 d after anthesis, and the recorded sap flow signal was compared with estimated fruit transpiration for the same period. Fruit transpiration was estimated by multiplying the hourly vapour pressure deficit, recorded by an automated weather station 100 m from the orchard, by fruit surface conductance to water vapour. Fruit surface conductance of yellow kiwifruit was measured by taking a sample of detached fruit from the same vines, and monitoring their weight loss while they were exposed to a wind speed of approximately 1 ms^{-1}, and constant temperature and humidity conditions in the laboratory (Maguire, Banks & Lang 1999). Kiwifruit berry surface conductance can be assumed constant for a given stage of development, regardless of ambient conditions, because the exocarp lacks functional stomata. By 75 d after anthesis, fruit transpiration is also relatively insensitive to wind speed, because resistance to vapour transfer between the fruit and atmosphere is dominated by skin properties, rather than boundary layer conductance. Growth in fruit fresh weight and dry matter content was also monitored over the same period by destructive harvesting at 2–3 week intervals.

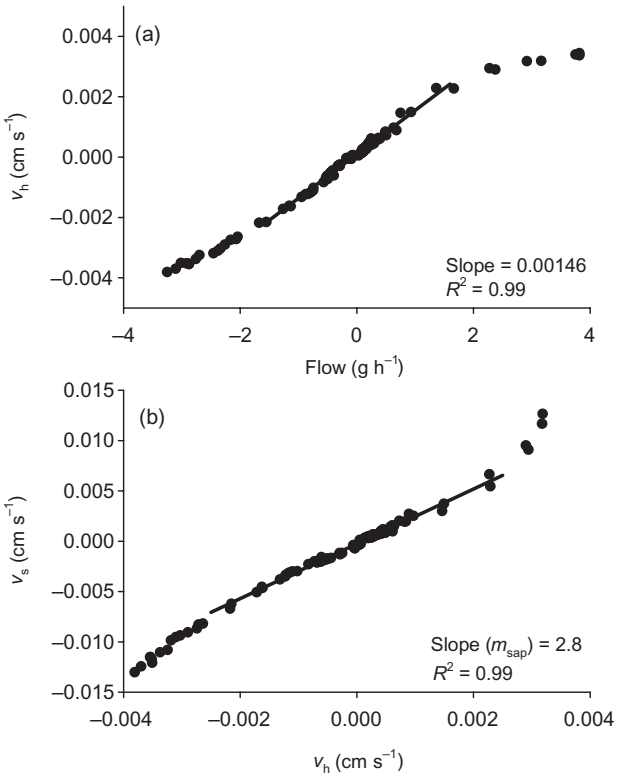

Figure 3. (a) Example of the relationship between heat pulse velocity (v_h) measured with an external heat ratio sap flow gauge and imposed flow through an excised *Actinidia deliciosa* fruit pedicel 2.9 mm in diameter. (b) The relationship between gravimetrically measured sap velocity and gauge measured v_h for the same stem as in (a). Lines indicate linear regressions for flows ± 1.7 g h^{-1}, equivalent to v_h ± 0.0025 cm s^{-1}.

RESULTS

Gauge calibration

Heat pulse velocity (v_h) measured using the external gauges was linearly related to the flow rate of xylem sap in both directions through all of the stems tested, at least up to a flow rate of approximately 1.5 g h^{-1} (Fig. 3a). The response was curvilinear at higher flow rates that were outside the normal physiological range for the organs of interest. When the flow rate was expressed as a sap flux per unit total stem cross-sectional area (v_s), v_h was consistently one-half or less than v_s (Fig. 3b). The average multiplier for the linear portion of the curve relating v_s to v_h (m_{sap}, Eqn 5) was 2.5 or 2.6 for stems of three of the species studied (*A. deliciosa* pedicels, *S. arboricola* petioles and *P. crassifolium* stems), but was higher (3.3) for *F. sylvatica* stems (Fig. 3, Table 1). The flow rates through excised *F. sylvatica* stems for a given applied pressure were between 10 and 100 times the flows through stems from the other three species, and examination of transverse freehand sections from each stem revealed that the xylem of *F. sylvatica* stems contained a higher frequency of larger-diameter vessels. The intercept of the relationship between v_s and v_h was not always zero (not shown). When non-zero intercepts were found, closer examination usually revealed an error in positioning of one or both of the thermocouples on the gauge ($x \neq 6$ mm), or poor alignment or poor contact between the gauge and the stem. Poor results (variable m_{sap}, non-zero intercepts) were obtained if the gauge was not firmly held against the stem (e.g. the gauge could be 'wiggled' after it had been taped in place).

To understand the sources of variation in m_{sap} between individual stems, the relationship between various measures of stem morphology, anatomy and the value of m_{sap} was investigated. No consistent relationship was found between stem diameter, bark thickness (defined as the depth of tissue between the stem surface and xylem) or the ratio of xylem to non-xylem tissue cross-sectional area and m_{sap}. Variation between species in the density and water content of the stems [the ($k_w V_{wood} + V_{water}$) coefficient from Eqn 4] was also not related to the average value of m_{sap} for each species and organ type (Table 1). Beech stems had the lowest value for this coefficient, but the highest value of m_{sap}, the opposite of the expected pattern (Table 1, Eqn 5).

However, the presence of bark between the gauge and the xylem did influence the value of m_{sap} in both excised and intact stems. When the bark was removed and the gauge re-installed on the xylem in the same position, the measured value of v_h for a given flow rate was increased by an average

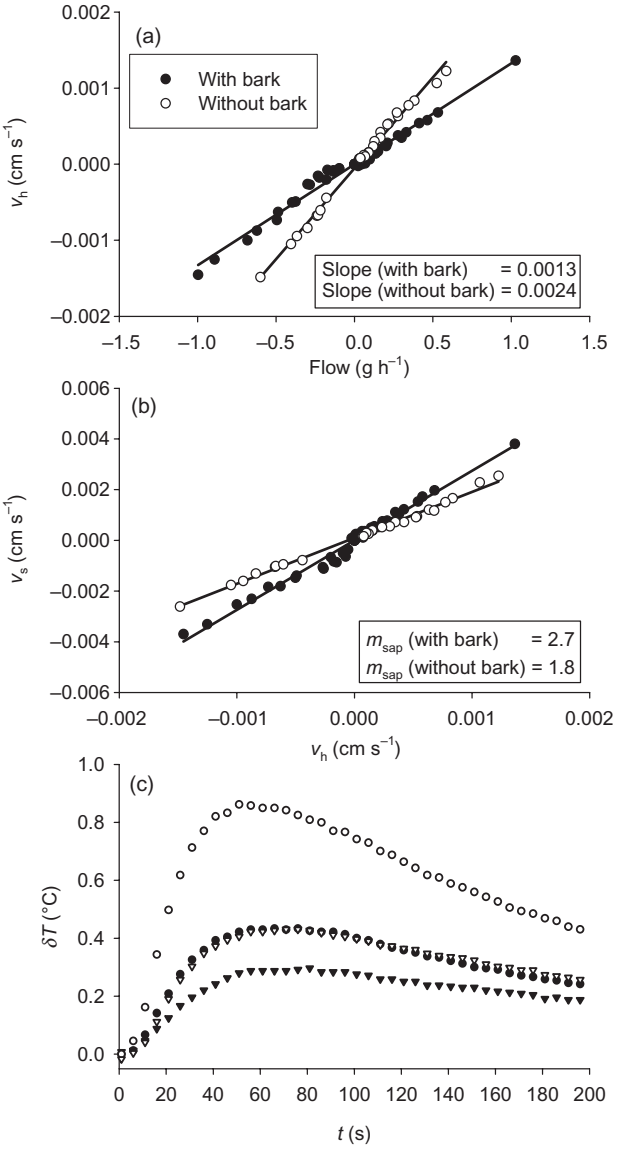

Figure 4. Example of the external heat ratio gauge response to imposed flow in an excised *Actinidia deliciosa* fruit pedicel 3.1 mm in diameter, before (closed symbols) and after (open symbols) bark removal. (a) Heat pulse velocity (v_h) as a function of flow rate. (b) Sap velocity (v_s) as a function of v_h. (c) The heat pulse as recorded by the downstream (circles) and upstream (triangles) temperature sensors, for an imposed flow of –0.6 g h^{-1}.

of 47% (paired *t*-test, $P = 0.001$) when this exercise was repeated on six excised kiwifruit (*A. deliciosa*) pedicels (Fig. 4a). Gauge sensitivity was increased disproportionately compared with the reduction in stem cross-sectional area (calculated v_s is also increased by bark removal, because cross-sectional area is reduced), resulting in a decrease in the average value of m_{sap} from 2.5 to 2.2 (*t*-test, $P = 0.03$) (Fig. 4b). The increased sensitivity with bark removal was caused by an increase in heat transferred to the two thermocouples, particularly by convection to the down-stream thermocouple (Fig. 4c).

Measurement of *in vivo* sap flow

The external gauges provided sensitive and repeatable measurements of sap flow when installed on living stems in the laboratory. Heat pulse velocity (v_h) measured on a leaf petiole was highly correlated with transpiration from the lamina of the same leaf, measured with a gas exchange system (Fig. 5a). Similar results were obtained when sap flow gauges were installed on the main stem of potted tree seedlings, and v_h compared with whole plant transpiration measured by placing the plant on a balance (Fig. 5b). In this second example, the gauge was sensitive enough to resolve sap velocities of less than 0.0001 cm s^{-1}, equivalent to flows of less than 0.08 g h^{-1} through a stem 5 mm in diameter. In all experiments, variation in measured sap flow was

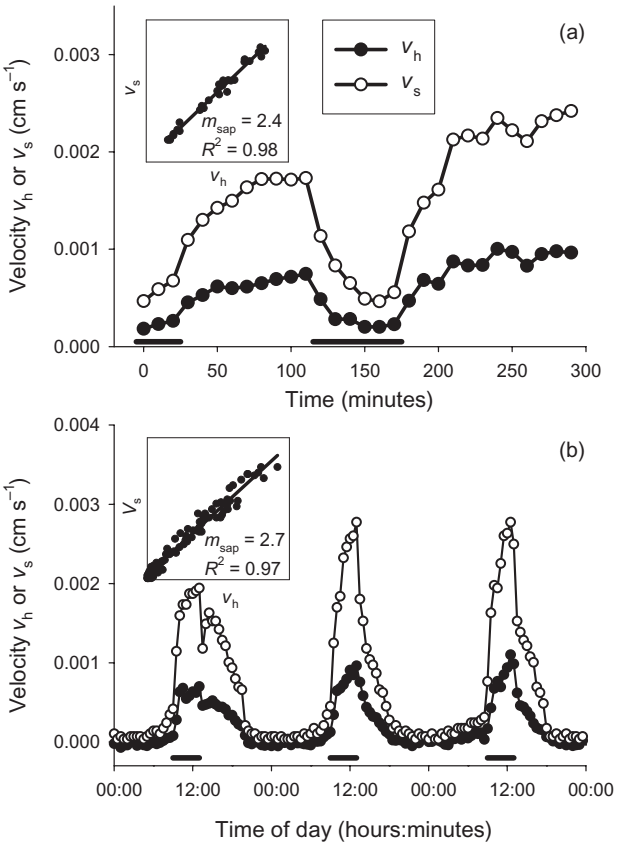

Figure 5. Comparison between *in vivo* measurements of heat pulse velocity (v_h, closed symbols), measured using an external heat ratio sap flow sensor, and actual sap velocity (v_s, open symbols) through (a) a *Schefflera arboricola* leaf petiole (diameter 2.6 mm), and (b) a *Pittosporum crassifolium* seedling stem (diameter 5.0 mm). In (a), v_s was calculated from gas exchange measurements of transpiration from the leaf lamina, and horizontal bars indicate periods when the leaf lamina was darkened by covering the gas exchange cuvette. In (b), v_s was calculated from measurements of weight loss from the potted seedling placed on a balance, and horizontal bars indicate a 4 h period each day when a desk lamp was turned on above the potted seedling (which was otherwise lit by normal laboratory lighting). Insets show the relationship between v_s and v_h, and the resulting estimates of m_{sap}.

dominated by variation in xylem sap flow. No effect on measured sap flow was detected when leaf petioles or stems were mechanically or steam girdled above or below the gauge (not shown).

When installed on the pedicel of a developing green kiwifruit growing on a shoot removed from a vine, gauge measurements of v_h indicated a small flux of sap towards the fruit which rapidly reversed when the shoot was removed from its water reservoir and allowed to dehydrate (Fig. 6). This response indicates that shoot dehydration reversed the apoplastic pressure gradients driving flows in the xylem, and caused the dehydrating shoot to withdraw water from the fruit. All flow ceased when the pedicel was then cut from the shoot (Fig. 6).

While stable measurements of sap flow were easily obtained in a laboratory, measurements were more variable when gauges were installed on the pedicels of growing yellow kiwifruit berries in an outdoor environment. Measured sap flow was particularly noisy during day-light hours when ambient temperatures were more variable (Fig. 7). In some examples, the variation was large enough to obscure any diurnal trends (not shown), and was clearly not related to any underlying variation in actual sap flow. To investigate the source of this variability, the datalogger was reprogrammed to save 1 min of temperature measurements before the heat pulse was initiated, and the 20 s period of temperature differentials normally used to estimate v_h. Examination of these data revealed that day-time variability in measured sap flow was associated with periods of increased variation in the rate and direction of temperature change prior to initiation of the heat pulse (Fig. 7a,b). The

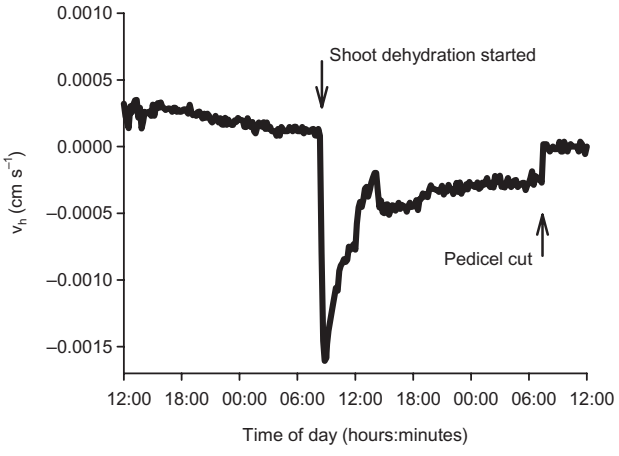

Figure 6. Changes in heat pulse velocity (v_h) measured using an external heat ratio gauge installed on the pedicel of an *Actinidia deliciosa* fruit growing on detached shoot. At the beginning of the experiment, the shoot was cut from the vine and allowed to continue transpiring with the cut end immersed in a reservoir of water. Dehydration of the shoot was started at the time indicated by removing the shoot from the reservoir, and allowing transpiration to continue. Positive values for v_h indicate sap flow towards the fruit; negative values indicate sap flow from the fruit to the shoot. Sap flow was stopped by cutting the pedicel above and below the gauge.

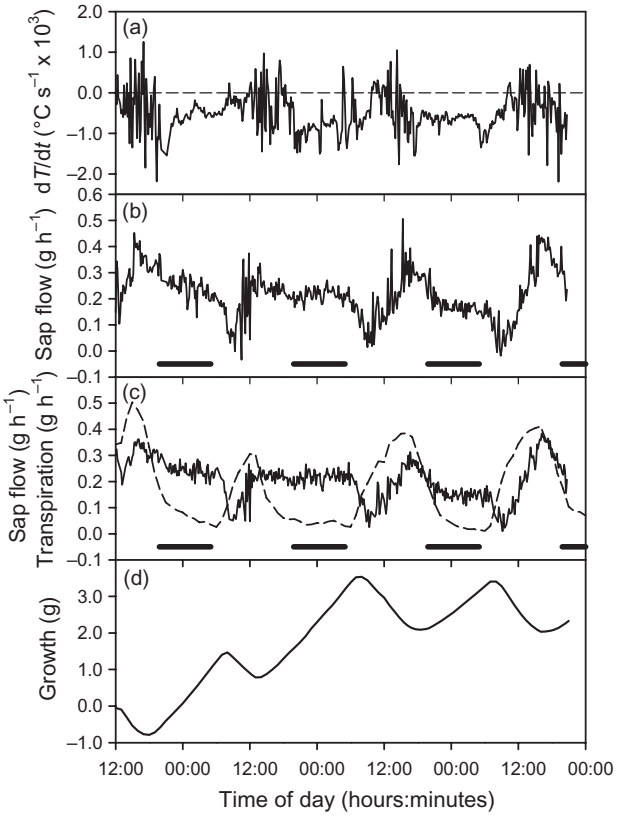

Figure 7. Example of sap flow measurements on the pedicel of an intact *Actinidia chinensis* fruit growing in an orchard. (a) The rate of change in temperature measured by the proximal thermocouple over the 60 s period prior to intiation of the heat pulse. (b) Sap flow to a single fruit over a 4 d period, calculated from measurements of heat pulse velocity by assuming $m_{sap} = 2.5$. (c) Sap flow recalculated after correcting measurements of the heat pulse at each thermocouple for the rate of change in temperature prior to the heat pulse. The dashed line shows fruit transpiration estimated as the product of fruit surface conductance to water vapour and the vapour pressure deficit. (d) Predicted fruit growth, calculated as the cumulative sum of the difference between pedicel sap flow and fruit transpiration.

overall median value for the rate of change in temperature prior to the heat pulse was negative, suggesting that the gauge and stem were usually still cooling slightly from the previous heat pulse 10 min earlier (Fig. 7a). Variability in measured sap flow was reduced when the measurements of temperature differentials during the heat pulse were corrected by linear extrapolation for the rate of change in temperature immediately prior to the heat pulse (Fig. 7c). Extra attention paid to insulation of the gauge and stem also reduced variability in measured v_h.

The diurnal pattern of sap flow through the living kiwifruit pedicels suggested relatively constant, positive sap flow towards the fruit during the night, a sudden decrease in sap flow to near zero after dawn, followed by a steady increase to peak sap flow towards the fruit near the end of the day (Fig. 7c). Changes in fruit sap flow lagged behind the diurnal trend in fruit transpiration estimated from fruit

surface conductance, and ambient air temperature and humidity. Subtraction of estimated fruit transpiration from measured sap flow resulted in an estimated pattern of volume growth (ignoring solutes in the phloem or xylem sap) that is typical of many fleshy fruits, including kiwifruit – expansion during the night, decreasing volume during the morning and a resumption of growth around the middle of the afternoon (Fig. 7d). The estimated net accumulation of water by the fruit over the 3 d period was 0.9 g d^{-1}, a value close to the measured average 1.0 g d^{-1} of fresh weight growth obtained from repeated destructive harvesting of fruit from the same vines at the same time during the season.

DISCUSSION

The external heat ratio method was effective for measuring bidirectional sap flow in stems between 2 and 5 mm in diameter. With appropriate calibration, the method was sensitive enough to provide an absolute measure of sap flux densities from less than 0.0001 up to 0.0050 cm s^{-1} in seedling stems, fruit pedicels and leaf petioles. We are not aware of any other sap flow method that can measure such low sap velocities, in small stems, and in both directions. The gauges are also relatively simple and inexpensive (materials costing a few cents per gauge) to make, and are therefore amenable to replication if suitable datalogging equipment is available.

Heat pulse velocity was linearly related to sap velocity, except when high velocities were imposed using excised stems. In his original tests, Marshall (1958) also found that the heat ratio method was limited by higher sap velocities. While these higher velocities were above the normal *in vivo* range for the stems we examined, if high sap velocities are expected, an alternative heat pulse or heat balance configuration can be adopted (Cohen *et al.* 1988; Burgess *et al.* 2000). Miniature heat balance gauges have been used to measure high flow rates through fine roots and stems with similar diameters to the stems examined in this study, but heat balance gauges cannot measure accurately when flows are less than 1–2 g h^{-1} (Senock & Ham 1993; Senock & Leuschner 1999; Coners & Leuschner 2002). The current method is therefore complimentary to the heat balance method, in that it is more suitable for flows less than 2 g h^{-1}.

Within the flow range where measured heat pulse velocity was linearly related to sap velocity, v_s was consistently between 2.5 and 3.3 times measured v_h. The actual value for the slope of the relationship is dependent on what assumptions are made about the effective 'sapwood' area of the stem, and how the gauge itself contributes to the measurement (the heat pulse travels through the cork backing of the gauge, as well as the stem). Idealized theory for heat pulse probes predicts that v_h should be similar or higher than v_s (Marshall 1958; Becker & Edwards 1999), but in reality the effects of the probes and wounding caused by probe installation on heat transfer to the moving sap usually cause v_h to be underestimated by between 50 and 90% (Swanson & Whitfield 1981; Green & Clothier 1988; Cohen & Fuchs 1989; Green *et al.* 2003). It is likely that underestimation of v_h with the present method is also caused by the slowing of heat pulse propagation caused by gauge materials and bark or cortical tissue between the gauge and the xylem.

For practical purposes, we suggest that an empirical calibration, obtained for each species and organ type, can be used to estimate v_s from v_h. We usually installed and calibrated gauges without removing the bark or cortical tissue, because this process is relatively invasive with small-diameter organs. In the case of kiwifruit berry pedicels, partial bark removal can result in death of the pedicel and abscission of the fruit. Where accurate absolute measures of sap flow are required, we have found that stems can be removed from the plant at the end of an experiment, without disturbing the gauge installation, and a unique calibration obtained for each stem by imposing measured xylem flows in the laboratory. Variation in gauge sensitivity between stems of the same type is significant, but can also be reduced with consistent gauge assembly and installation. It is also advisable to check for a zero offset at the end of each experiment by imposing zero sap flow conditions, either by cutting the stem or preventing transpiration. Under zero flow conditions, the time between heat pulse release and the maximum temperature rise can also be determined, and thermal diffusivity (k) calculated for each gauge installation (Eqn 2). These properties are likely to vary with the species and organs used, and the materials used to make the gauge. During our tests, zero offsets were usually caused by small errors in positioning of one or both of the thermocouples on the gauge. If a significant offset is detected, the results can be corrected by measuring or estimating the actual distances between the heater and thermocouple, and v_h recalculated using the new values (Burgess *et al.* 1998).

Our calibrations suggest that the average gauge response was similar for all but one of the four stem types tested (Table 1). The beech stems were more porous than the other stem types, with a higher number of large vessels visible in the sapwood. Heat pulse measurements rely on thermal equilibrium between the stationary sapwood and the moving sap, a condition that may not be met when a proportion of the sap is moving in large vessels with particularly high lumen sap velocities (Marshall 1958). The overall effect is a reduction in measured v_h compared with actual sap velocity expressed on an area basis (v_s), as observed with the beech stems (Table 1). Higher maximum sap velocity can be expected in species and organs with high axial porosity. In these instances, a miniature heat balance method may be more appropriate (discussed earlier). The similarity in gauge response between the less porous *Actinidia*, *Pittosporum* and *Schefflera* stems suggests that thermal homogeneity can be assumed for these stems. In these examples, an alternative to empirical calibration that may be appropriate in the future is to use numerical models of heat transfer to analytically derive a relationship between v_h and v_s, a technique that has already been successfully applied to probe-based heat pulse methods (Swanson 1994; Green *et al.* 2003). A similar approach could be used to investigate the contribution of gauge design and

materials (e.g. cork or other materials) to heat transfer and estimates of thermal diffusivity.

The gauge response appeared insensitive to stem diameter, suggesting that a single calibration factor can be assumed for stems within the diameter range tested (2–5.5 mm). While there is no reason why the method cannot be used with larger stems, it is more likely that the assumption of thermal homogeneity between the moving sap and all parts of the stem cross section and gauge will not be met (Swanson 1994). This may be particularly true for relatively large herbaceous stems with complex stem anatomies and large internal voids of non-hydroactive tissue. In these cases, the best approach may be to develop an empirical calibration for that type of stem over the range of stem diameters of interest (Cohen & Fuchs 1989; Cohen & Li 1996).

No phloem flow was detected by using the external heat ratio sensors to record flow dynamics before and after girdling treatments on leaf petioles or stems. Estimating mass flows in the phloem is notoriously difficult (Canny 1971; Van Bel 2003), but they are thought to be significantly smaller than those in the xylem. In a recent study, Windt *et al.* (2006) used magnetic resonance imaging to provide a comprehensive comparison of the dynamics of phloem and xylem flows in the stems of tomato, poplar, tobacco and castor bean plants. Phloem flows were relatively constant, without the large diurnal fluctuations of xylem flows. During the day, the ratio of phloem to xylem flows was 0.1 or less, while at night the ratio varied between 0.04 and 0.55, depending on the species. Direct comparisons with area-based sap flux densities used in this study are difficult, but if the phloem mass flows measured by Windt *et al.* (2006) are converted to sap flux densities by dividing by an approximate stem cross-sectional area, values of 0.0001–0.0003 cm s^{-1} are obtained. Such flows are at the limit of detection using the sap flow gauges described in this study, and will be difficult to separate from the larger and more variable fluxes occurring close by in the xylem.

In contrast, Helfter *et al.* (2007) reported successful detection of phloem flows in small stems using a similar external heat pulse method. However, in that study, phloem flows in sapling stems were interrupted by removing the bark and exposing the xylem, a procedure that not only prevents phloem transport, but that also changes the balance between conduction and convection of the heat pulse. In our tests with excised stems (no phloem flows before or after bark removal), application of the heat pulse directly to the xylem also resulted in a larger proportion of the applied heat entering the xylem sap and being convected to the downstream temperature sensor (Fig. 4). In intact seedlings, the effect is an increase in measured upward heat pulse velocity (even though xylem sap velocity has not changed), which Helfter *et al.* (2007) attributed to the loss of downward phloem flows. Their reported heat pulse velocities for downward phloem flow in transpiring seedlings were also of a similar magnitude to xylem heat pulse velocity in the same stems, an unusual result given the lower rates of mass flow expected in the phloem. We therefore conclude that phloem flows have yet to be successfully measured or separated from xylem flows using non-destructive, heat-based sap flow methodologies.

Our original goal was to develop a sap flow method suitable for *in vivo* measurement of sap flow in developing kiwifruit pedicels. Measurements of fruit sap flow on mature vines were found to be more challenging than working with excised shoots in the laboratory. Even with insulation and shielding, the measurements were affected by transient fluctuations in temperature of a similar magnitude and rate of change to the applied heat pulse. Similar problems have been reported by other groups attempting to measure sap flow through small stems in the field (Cohen *et al.* 1988; Groot & King 1992; Gutierrez *et al.* 1994). The resulting noise in the measured sap flow signal was increased in environments where rapid diurnal temperature fluctuations occurred (e.g. a poorly ventilated glasshouse), and on partially cloudy days or windy days. Some of the noisy data could be excluded or corrected on the basis of temperature measurements prior to initiation of the heat pulse. We are also investigating the potential for improving the signal by enclosing the gauge and stem in a thermal heat sink to dampen externally imposed temperature gradients.

Acceptable measurements of normal fruit pedicel sap flow were obtained despite the increased variability in sensor output experienced outdoors. These data are among the first direct measurements of sap flow into normal developing fruit (Higuchi & Sakuratani 2006). The results show a relatively constant background flux of sap towards the fruit of approximately 0.2 g h^{-1}. Presumably, flow is occurring in both the xylem and phloem. This flux is interrupted each morning by a sudden drop towards zero flow, then a recovery to higher levels in the afternoon. Pedicel sap flux lags behind predicted fruit transpiration, an effect that we attribute to the capacitance of fruit pericarp tissues and a related delay in the propagation of apoplastic pressure gradients from the transpiring fruit surface through the flesh to the receptacle. The reduction in sap flow in the morning suggests that as leaf transpiration begins, there is a more rapid drop in the apoplastic pressure potential within the shoot xylem compared with the fruit apoplast, to an extent that net sap flow almost reverses in direction.

Reversal or 'back flow' of xylem sap flow from the fruit to the plant has been indirectly inferred in kiwifruit and other developing fruits, but has only been directly measured once, in mango (Higuchi & Sakuratani 2006). Back flow has been implicated in a range of fruit quality issues, including accumulation of minerals (Lang 1990; Lang & Volz 1998) and reductions in fruit weight prior to harvest (shrivel disorders; Tyerman *et al.* 2004; Keller, Smith & Bondada 2006). Understanding some of these problems has been hampered by the inability to measure pedicel sap flow and distinguish between water loss by transpiration and by back flow (Greenspan, Schultz & Matthews 1996). Experiments with these sap flow gauges are continuing as we examine the driving forces for water and dry matter accumulation in developing fruit, including the occurrence of phenomena such as xylem flow reversal and loss of xylem functionality as fruits mature.

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