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Hydraulic resistance of developing *Actinidia* fruit

Mariarosaria Mazzeo[1,*], Bartolomeo Dichio[1], Michael J. Clearwater[2,3], Giuseppe Montanaro[1] and Cristos Xiloyannis[1]

[1]*Dipartimento delle Culture Europee e del Mediterraneo: Architettura, Ambiente, Patrimoni Culturali (DiCEM), Università degli Studi della Basilicata, Italy,* [2]*Department of Biological Sciences, University of Waikato, Hamilton 3240, New Zealand and* [3]*Plant and Food Research Institute of New Zealand, Te Puke Research Center, RD 2 Te Puke, New Zealand*
** For correspondence. E-mail mmazzeo@inwind.it*

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• *Background and Aims* Xylem flows into most fruits decline as the fruit develop, with important effects on mineral and carbohydrate accumulation. It has been hypothesized that an increase in xylem hydraulic resistance (R_T) contributes to this process. This study examined changes in R_T that occur during development of the berry of kiwifruit (*Actinidia deliciosa*), identified the region within the fruit where changes were occurring, and tested whether a decrease in irradiance during fruit development caused an increase in R_T, potentially contributing to decreased mineral accumulation in shaded fruit.
• *Methods* R_T was measured using pressure chamber and flow meter methods, the two methods were compared, and the flow meter was also used to partition R_T between the pedicel, receptacle and proximal and distal portions of the berry. Dye was used as a tracer for xylem function. Artificial shading was used to test the effect of light on R_T, dye entry and mineral accumulation.
• *Key Results* R_T decreased during the early phase of rapid fruit growth, but increased again as the fruit transitioned to a final period of slower growth. The most significant changes in resistance occurred in the receptacle, which initially contributed 20 % to R_T, increasing to 90 % later in development. Dye also ceased moving beyond the receptacle from 70 d after anthesis. The two methods for measuring R_T agreed in terms of the direction and timing of developmental changes in R_T, but pressure chamber measurements were consistently higher than flow meter estimates of R_T, prompting questions regarding which method is most appropriate for measuring fruit R_T. Shading had no effect on berry growth but increased R_T and decreased dye movement and calcium concentration.
• *Conclusions* Increased R_T in the receptacle zone coincides with slowing fresh weight growth, reduced transpiration and rapid starch accumulation by the fruit. Developmental changes in R_T may be connected to changes in phloem functioning and the maintenance of water potential gradients between the stem and the fruit. The effect of shade on R_T extends earlier reports that shading can affect fruit vascular differentiation, xylem flows and mineral accumulation independently of effects on transpiration.

Key words: Calcium concentration, dye tracer, hydraulic resistance, partitioning of fruit resistance, shade, *Actinidia deliciosa*, kiwifruit.

INTRODUCTION

The relative contributions of xylem, phloem and transpiration fluxes to the water balance of fleshy berry fruits change as the fruit develop. In many fruits, such as tomato, grape and kiwifruit, inward xylem flow decreases as the fruit mature (Ho *et al.*, 1987; Greenspan *et al.*, 1994; Morandi *et al.*, 2010). The role of the xylem in late-season fruit development is of particular interest because of its involvement in mineral transport and quality disorders correlated with fruit mineral content (Montanaro *et al.*, 2006; Rogiers *et al.*, 2006; Tonetto de Freitas *et al.*, 2011). Ontogenetic changes in xylem flows have also been associated with 'shrivel' disorders, caused either by a reversal in pressure gradients and loss of water to the shoot (Tilbrook and Tyerman, 2009), or by an imbalance between inward vascular flows and transpiration (Clearwater *et al.*, 2012). In grapes and kiwifruit the decrease in xylem flow into the fruit has been attributed to a reduction in the hydrostatic pressure gradients driving flow, and a higher relative contribution of phloem water to the end of season water balance (Bondada *et al.*, 2005; Keller *et al.*, 2006; Choat *et al.*, 2009; Morandi *et al.*, 2010). Apart from the 'buffering' of water supplies by the phloem (Choat *et al.*, 2009), the gradient in pressure from stem to fruit pericarp is primarily a function of the transpiration rate, determined by skin water vapour conductance and the vapour pressure deficit, and the hydraulic resistance of the flow pathway (Tyerman *et al.*, 2004; Montanaro *et al.*, 2012).

The role of fruit hydraulic resistance (R_T) in xylem flow variation during fruit development has been examined in some detail in tomato (Malone and Andrews, 2001; Van Ieperen *et al.*, 2003) and grapes (Tyerman *et al.*, 2004; Choat *et al.*, 2009). In both cases a large increase in R_T during fruit development has been documented, but the exact timing, location and significance of variation in R_T for the regulation of flow in both the xylem and the phloem remains of debate (Choat *et al.*, 2009; Windt *et al.*, 2009). In kiwifruit, a progressive increase in R_T within the fruit was hypothesized by Morandi *et al.* (2010) to support the conclusion that an ontogenetic decrease in inward xylem flow could not be attributed to a reduction in the pressure potential gradient alone. Dye inflow has been used as a tracer to infer a decrease

in xylem 'functionality' during fruit development in kiwifruit (Dichio *et al.*, 2003), but there have been no direct measurements of whole-fruit R_T, or attempts to partition R_T within the pathway from pedicel to receptacle and into the pericarp.

For xylem-borne mineral nutrients, particularly Ca, variation in the xylem functionality or R_T is often invoked to explain seasonal accumulation patterns (Saure, 2005; Tonetto de Freitas *et al.*, 2011). The xylem represents the main route for Ca delivery to fruit because the phloem transports negligible amounts (Bukovac and Wittwer, 1957). However, the involvement of R_T as a factor affecting fruit Ca nutrition has only been examined indirectly, using dyes as a tracer for xylem functioning, and by correlating anatomical changes in xylem structure with fruit calcium disorders (Malone and Andrews, 2001; Dichio *et al.*, 2003; Dražeta *et al.*, 2004). Direct assessment of fruit hydraulic resistance is needed to understand fruit Ca accumulation patterns and explain the causes of fruit developmental disorders related to low Ca content that are a significant cause of loss for the kiwifruit and other major fruit industries (Poovaiah, *et al.*, 1988; Ferguson *et al.*, 2003; Thorp *et al.*, 2003).

In addition to developmental changes, fruit R_T may also be affected by environmental conditions during fruit growth. In particular, the level of irradiance on the fruit during development is a strong determinant of flesh quality and mineral content in many fleshy fruits (Biasi and Altamura, 1996). In kiwifruit shading negatively affects vascular differentiation and fruit transpiration, and is correlated with decreased final fruit Ca concentration (Biasi and Altamura, 1996; Montanaro *et al.*, 2006). Whether irradiance directly affects R_T in kiwifruit or any other fruit is unknown. Stem xylem R_T is lower in sun-exposed shoots of woody plants (Schultz and Matthews, 1993), and leaf R_T decreases with increasing growth irradiance, photosynthetic capacity and short-term increases in irradiance during transpiration (Carins Murphy *et al.*, 2012; Aasamaa and Sõber, 2012). However, fruit differ from leaves in that there may be little or no active stomatal control of transpiration, specific transpiration rates in most fruits are usually much lower than leaves, particularly later in fruit development (Clearwater *et al.*, 2012), and it can be argued that the primary function of the xylem in developing fruit is the facilitation of growth, rather than gas exchange.

The goal of this study was to investigate how R_T changes during the development of the kiwifruit berry, and to identify where within the berry the ontogentic changes in R_T are most significant. It was expected that R_T would increase during fruit development, and that the timing and location of the increase in R_T would coincide with the previously identified cessation of dye entry into the fruit in the receptacle zone that occurs approximately half way through development (Dichio *et al.*, 2003). During preliminary measurements it was also noted that the absolute magnitude of fruit R_T depended on the method used to measure it, potentially affecting predictions of inward and outward xylem fluxes and general understanding of vascular functioning in developing fruit. Therefore, two alternative methods for measuring fruit R_T were compared. A flow meter was used to record the flow of pressurized water into the fruit, via the pedicel. This technique allows R_T to be partitioned between different parts of the fruit and pedicel by sequential excision of the distal end of the flow pathway, and has been used most recently to document the hydraulic architecture of developing grape berries (Tyerman *et al.*, 2004; Choat *et al.*, 2009). Results from the flow meter were compared with measures of R_T obtained for the same population of fruit using a pressure chamber to force water out of the fruit via the pedicel. The pressure chamber provided a reference for comparison of R_T with the flow meter results, but cannot be used to partition R_T within the fruit. A second experiment investigated, for the first time in any fruit, whether R_T and dye entry were affected by irradiance during development. It was hypothesized that shading would increase R_T and decrease dye entry, because of the previous finding that shading reduced vascular differentiation in kiwifruit berries.

MATERIALS AND METHODS

Plant material and experimental sites

All experiments used the standard commercial green kiwifruit cultivar *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* 'Hayward'.

The study of developmental changes in R_T, the partitioning of R_T within the fruit, and the comparison between flow meter and pressure chamber methods was conducted in New Zealand at the Plant and Food Research Te Puke research orchard (37°49′S, 176°19′E) using fruit sampled from non-irrigated horizontal pergola-trained vines (415 plants ha^{-1}) grafted onto open pollinated seedling rootstocks of *A. deliciosa* 'Bruno', and managed following local commercial practices. The study of shading effects on R_T and dye entry was conducted on fruit from own rooted vines in Italy at the Pantanello Experimental Farm, Basilicata Region (40°23′N, 16°45′E). The shading treatments were applied to vines in an orchard located close to the experimental farm, with vines trained on a pergola system (625 plants ha^{-1}) and regularly microjet-irrigated (120 L h^{-1}) to wet the entire soil surface according to local commercial practices.

Pressure chamber measurements of R

The developmental time course of whole-fruit R_T was recorded in New Zealand using the pressure chamber method (Lang and Ryan, 1994). At the midpoint of flowering (50 % anthesis, 21 November) 200 self-terminated fruiting shoots with open flowers were selected and flowers pruned to leave one flower per shoot. During early fruit growth, defined as up to 50 d after anthesis (DAA), hydraulic measurements were completed approximately every 10 d on a minimum of 12 fruits, collected pre-dawn. For the remainder of fruit development when fruits were growing more slowly, measurements were completed at 70, 93, 123 and 163 DAA. On each occasion, the fruiting shoots were immediately defoliated, excised and sealed in a plastic bag and transferred promptly to the laboratory. Six duplicate custom-made pressure chambers were used, with measurements completed simultaneously on six fruit at a time.

For each measurement of R_T the fruit pedicel was cut at the proximal end under distilled water, inserted into an 8-mm-long silicone tube connected to the hub of a hypodermic needle, and arranged in the pressure chamber with the needle passing through the rubber septum of the chamber, with the exit point sealed with putty (Lang and Ryan, 1994). Outside the chamber

expressed sap was passed from the needle via a plastic capillary tube (2 mm internal diameter) into a pre-weighed 1·5-mL microcentrifuge tube. With this arrangement it was assumed that the sap at the cut end of the pedicel was at atmospheric pressure, and the pressure difference driving the flow of sap from the fruit during pressurization (ΔP) was equivalent to the chamber pressure measured relative to atmospheric. R_T (MPa s g^{-1}) was calculated according to the Ohm's law analogy of Tyree and Ewers (1991) as:

$$R_T = \Delta P / F \quad (1)$$

where F (g s^{-1}) was the flow rate of sap exuded from pressurized fruit over a 10-min period, after a constant flow was achieved. In a preliminary experiment the maximum flow rate of exuded sap was reached in the range 1·0–1·6 MPa applied chamber pressure, and therefore 1·3 MPa was chosen as the standard pressure for all measurements.

Flow-meter measurements of R *and partitioning of* R *within the fruit*

R_T was also measured in New Zealand on an identical sample of 12 fruit using a high-pressure flow meter. After cutting the fruit pedicel a 10-mm length of bark was removed and the cut xylem face cleaned and recut with a razor blade to prevent mucilage released from the bark from entering the xylem during measurements. The pedicel was attached to the flow meter using a compression fitting (EW06473-00, Cole Parmer, Vernon Hills, IL, USA), water supplied at a constant pressure of 0·55 MPa and flow measured as the distance moved by a bubble along a transparent capillary tube of 1·5 mm internal diameter, timed over 30-s intervals. Three-way luer stop-cocks, additional tubing and syringes were used to provide a reservoir of low-pressure water for initial connection of the pedicel to the flow meter, and for the introduction and purging of the bubble from the measurement tube. Before each measurement flow was recorded with no applied pressure and the result subtracted from flow recorded at high pressure before calculation of hydraulic resistance using eqn (1). For estimates of fruit growth rate, berry length and total weight were recorded before and after flow meter measurements, respectively.

To partition hydraulic resistance within the fruit, after R_T was recorded for the intact fruit (berry + pedicel), each fruit was progressively cut back from the stigmatic end into four portions with transverse cuts using a sharp disposable blade (Fig. 1), following an approach similar to that of Tyerman *et al.* (2004). The cut locations were at 50 % berry length (removing the distal half of the fruit), at 20 % of berry length, measured from the calyx end (removing the proximal portion of the fruit but leaving the receptacle zone attached), and at the distal end of the pedicel, immediately proximal to the fruit. After each cut, flow was measured at least three times until constant. Ambient temperature was recorded and used to correct for any viscosity effects (Tyree *et al.*, 1995).

R_T was partitioned assuming four resistances arranged in series:

$$R_T = R_{pe} + R_r + R_p + R_d \quad (2)$$

where R_{pe}, R_r, R_p and R_d are the resistances of the pedicel, receptacle, proximal and distal portions of the fruit, respectively

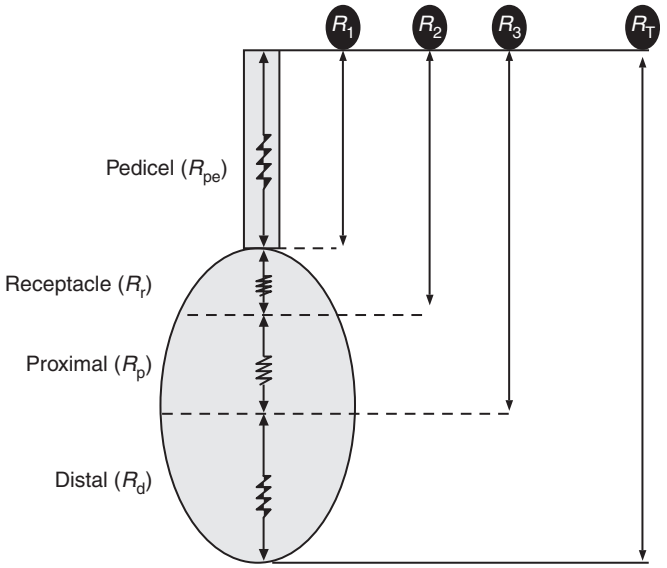

FIG. 1. Schematic representation of the Ohm's law analogy used to partition total hydraulic resistance (R_T) into four component resistances (R_{pe}, R_r, R_p, R_d), arranged in series. The fruit pedicel was connected to the flow meter, R_T measured, and the fruit then cut back sequentially at the positions indicated by the transverse dotted lines to obtain the partial resistances R_3, R_2 and R_1, from which the component resistances were calculated by subtraction (eqns 2–6).

(Fig. 1). Each component resistance was calculated as:

$$R_d = R_T - R_3 \quad (3)$$

$$R_p = R_3 - R_2 \quad (4)$$

$$R_r = R_2 - R_1 \quad (5)$$

$$R_{pe} = R_1 \quad (6)$$

where R_T, R_1, R_2 and R_3 are the measured resistances of the fruit before and after each cut (Fig. 1).

On the same date of fruit sampling for the hydraulic resistance determinations, a group of ten fruit were collected and promptly transferred to the laboratory for length (mm) and fresh mass (g) determination. These measurements were used to calculate the length and mass relative growth rate (RGR) (Fisher, 1921) over the period of two consecutive observations, and the average RGR values were referred to the mid-point of that period.

Effect of shading on R_T*, dye entry and mineral accumulation*

The effect of reduced irradiance on R_T, dye uptake and mineral accumulation of fruit was measured in Italy. A neutral, porous shade cloth (2591WO Ombraverde 70, Arrigoni SpA, Uggiate-Trevano, Italy) causing a 30 % reduction in incident light (measured using a spectroradiometer, LI-1800; Li-Cor Inc., Lincoln, NE, USA) was positioned on wooden frames approx. 0·3–0·4 m above one half of the canopy of ten randomly chosen vines, 10 d after full bloom. The unshaded half of each vine served as the control treatment. During the growing season, pruning was conducted to maintain a constant canopy density by removing vigorous shoots from both the shaded and the control halves of the canopy.

Around mid-bloom (15 May) 520 newly open flowers per treatment were selected on approx. 130 terminate fruiting shoots randomly selected from within the ten vines and labelled. Natural bee pollination ensured roughly simultaneously fruit-set (by 20 May). Thirty fruits were sampled pre-dawn from each treatment at 3- to 4-d intervals up to 42 DAA, then weekly until 80 DAA, and then on 101, 114, 132 DAA. On each occasion, the fruiting shoots were immediately defoliated, excised and sealed in a plastic bag and transferred promptly to the laboratory where the fruit were screened for defects and 12 used for measurement of R_T and 12 for dye uptake. R_T was measured using the pressure chamber method with similar equipment and procedures to those described for measurements in New Zealand.

Dye transport into the fruit was assessed following the methods of Dichio *et al.* (2003). Fruit were detached from their shoot by cutting the pedicel at the proximal end under water, the cut end cleaned in distilled water, and then re-cut under water with a sharp blade. The distal end of the pedicel was smeared with a thin band of melted paraffin wax to prevent capillary movement of dye solution over the surface of the fruit, and the pedicel end was inserted into a vial containing 1 mL dye solution (0·5 % filtered aqueous toluidine blue). The fruit were then left to transpire for 75 min on a woody frame under uniform air flow of 0·8 m s^{-1} provided by a fan under constant laboratory conditions of 25 °C and 55 % relative humidity.

At the end of transpiration period the fruit were removed from the vial and immediately sliced transversely at 25, 50 and 75 % of berry length, measured from the pedicel–berry junction. The number of stained median–dorsal carpellary bundles (Clearwater *et al.*, 2012) on the distal cut face at each position was counted using a stereomicroscope (SMZ100, Nikon) and expressed as a proportion of the total number of bundles (stained and unstained). For estimates of growth rate, fruit length and total weight were recorded before and after cutting of the fruit, respectively.

On 160 DAA ten fruit per treatment were randomly sampled for N, P, K, Ca and Mg determinations. Fruit were sliced, dried (60 °C, to constant weight) using a ventilated drying cabinet (FED 400, WTB-Binder, Tuttlingen, Germany), then ground in a ball mill to a fine powder. A 0·5-g aliquot of powder was ashed (24 h, 580 °C), digested in 2 M HCl and brought to a final volume of 100 mL with distilled water. A 0·2-mL aliquot was then diluted to a final volume of 10 mL with distilled water and used for Ca, Mg and K determination (ICP/OES, Varian 710-ES). Phosphorus was determined using the Olsen method and N by Kjeldhal's method.

Statistical analysis

Comparisons between treatments were carried out using Student's *t*-test at the 0·05 or 0·01 probability levels using Microsoft Office Excel 2003.

RESULTS

Changes in hydraulic resistance during development

Fruit size exhibited the pattern of growth that is typical of kiwifruit, with a rapid increase in weight and size up to 60 DAA,

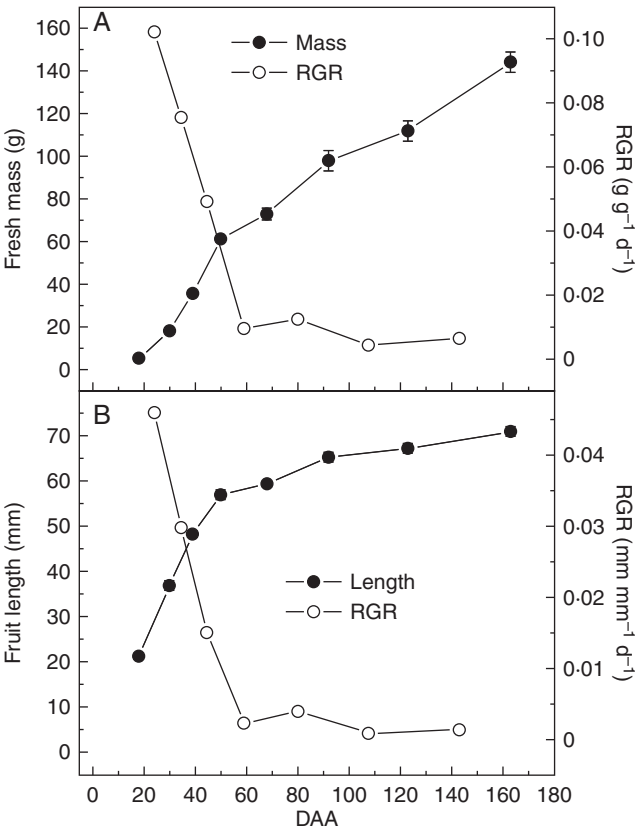

FIG. 2. Time-course of absolute and relative (RGR) growth in (A) berry fresh weight and (B) length of *Actinidia deliciosa* cv 'Hayward' kiwifruit grown in New Zealand and used for measurements of seasonal changes in fruit hydraulic resistance. DAA = days after anthesis. Points are the mean of 12 fruit ± s.e. Note that values of RGR were plotted at the middle of each observation period.

followed by a brief reduction in relative growth rate around 70 DAA (Fig. 2). A less pronounced second peak in growth occurred around 90 DAA and was followed by gradual slowing of growth until the final harvest (Fig. 2).

The patterns of change in total fruit hydraulic resistance (R_T) during development in New Zealand were similar for the two methods of measurement, with R_T declining quickly during early fruit growth, then increasing again after 68 DAA (Fig. 3). However, flow meter-measured R_T was consistently 2–10 times lower than pressure chamber-measured R_T, and there was a less pronounced increase in flow meter R_T during the final phase of fruit development (Fig. 3).

Developmental changes in resistance of all three measured fruit portions (R_3, R_2, R_1) were similar to the changes in R_T, with decreasing resistance early in fruit development during the period of most rapid fruit growth, followed by an increase later in development (Fig. 3B). Up to 40 DAA there were detectable changes in resistance with sequential excision of the distal, proximal and receptacle portions of the fruit. On the first date of measurement (18 DAA) resistance decreased by more than half when the distal half of the fruit was excised. After 40 DAA, excision of the distal and proximal portions of the berry had little effect on resistance (Fig 3B). Total resistance for the remainder of fruit development was therefore dominated by the receptacle (Fig. 4). Partitioning of R_T between the pedicel, receptacle, and

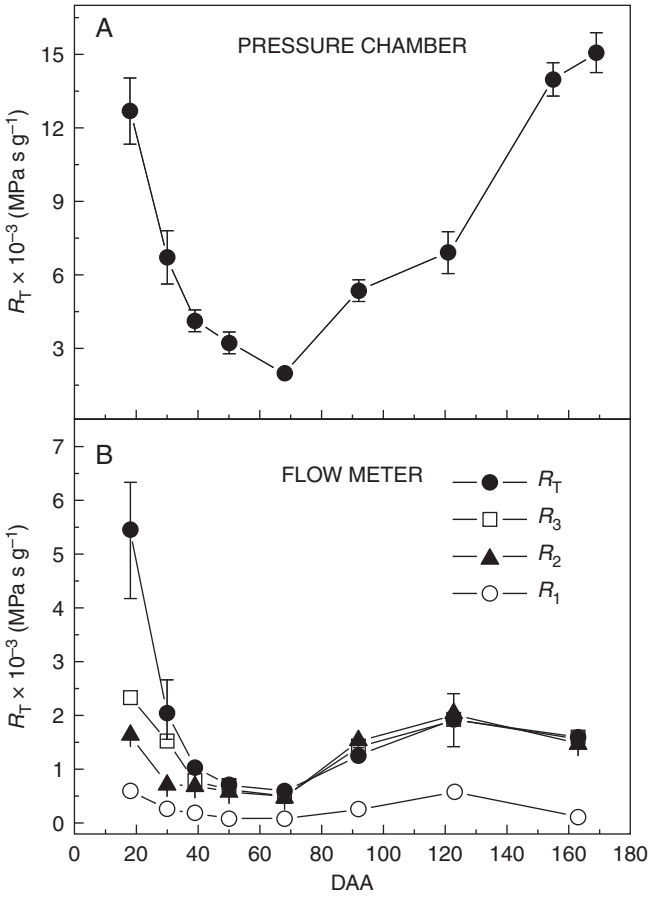

FIG. 3. Developmental time-course of total hydraulic resistance (R_T) measured using (A) the pressure chamber and (B) the flow meter method on whole-fruit and on fruit portions (R_3, R_2, R_1) obtained by cutting-back the fruit at three positions (see Fig. 1), of *A. deliciosa* cv. 'Hayward' kiwifruit grown in New Zealand. Values are the mean ± s.e. of 12 fruit. Note the differences in *y*-axis scale between the two figures.

distal and proximal portions of the fruit confirmed that the distal 80 % of the fruit (most of the berry volume) initially contributed 70 % to R_T (Fig. 4, 18 DAA, R_d and R_p combined). However, from 98 DAA onwards the receptacle contributed more than 80 % and the remainder of the berry less than 10 %. Compared with changes in the berry, the contribution of the pedicel to R_T was low and relatively constant, between 7 and 20 % (Fig. 4), a reflection of the way R_{pe} varied in concert with R_T throughout fruit development (Fig. 3B).

Effect of shade on hydraulic functioning

In a separate experiment in Italy the effect of shade on R_T was investigated using the pressure chamber method and the same fruit cultivar. The pattern of growth of the fruit in Italy was similar to that observed in New Zealand, with rapid growth up to 60 DAA. There was no difference in the pattern of growth or rate of growth between control and shaded fruit (data not shown). The magnitude and ontogenetic pattern of declining then increasing R_T (Fig. 5) was similar to that observed in New Zealand (Fig. 2A). Shade increased R_T by approx. 30 % during

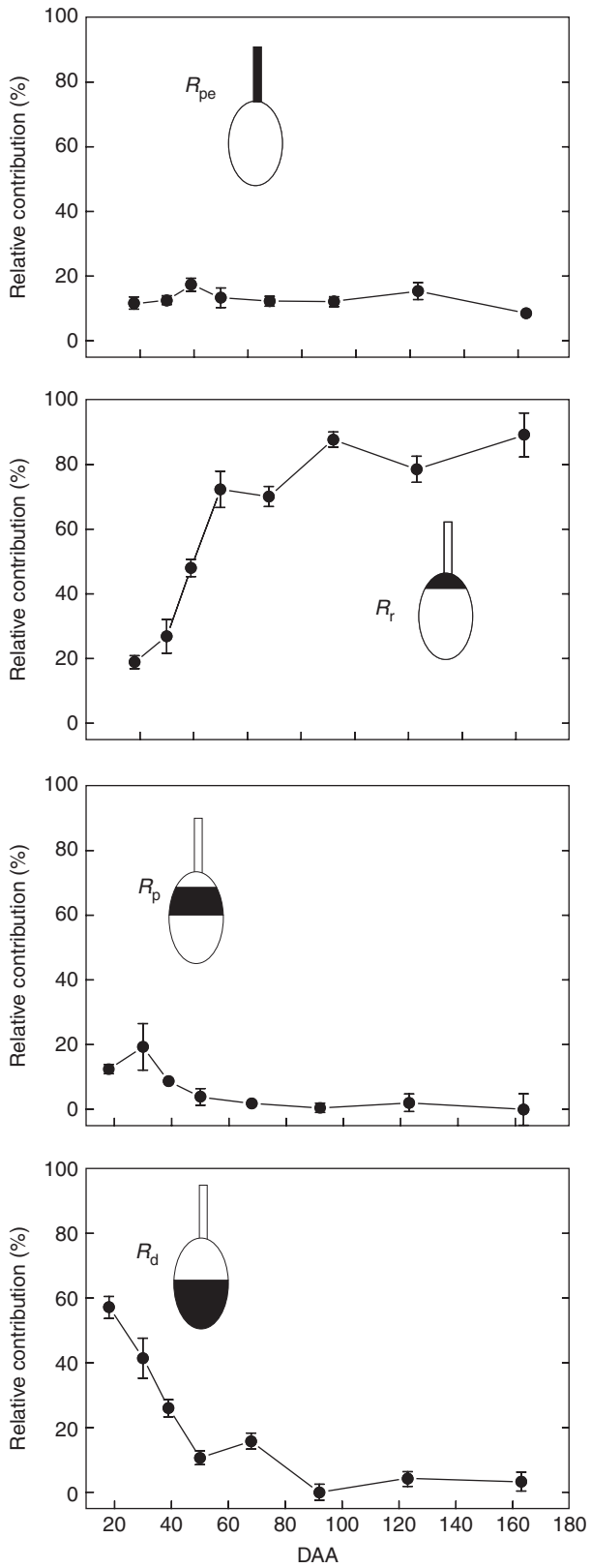

FIG. 4. Developmental changes in the relative contribution of each component of hydraulic resistance within the kiwifruit berry (pedicel, R_{pe}; receptacle, R_r; proximal, R_p; distal, R_d) to R_T. Values are the mean ± s.e. of 12 fruit.

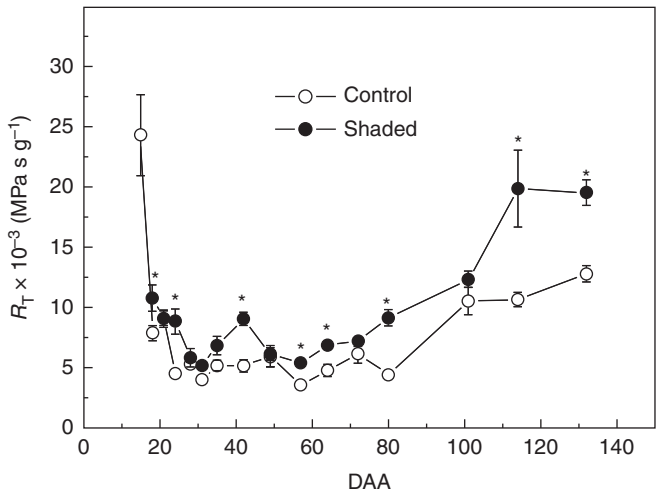

FIG. 5. Developmental time-course of berry hydraulic resistance (R_T) measured using the pressure chamber method in control and shaded fruit of *A. deliciosa* cv. 'Hayward'. Each point represents the mean ± s.e. of 12 fruit. Comparing treatments at the same time, asterisks indicate a significant difference at $P = 0{\cdot}05$ (Student's *t*-test). Note that the shade treatment began at 10 DAA.

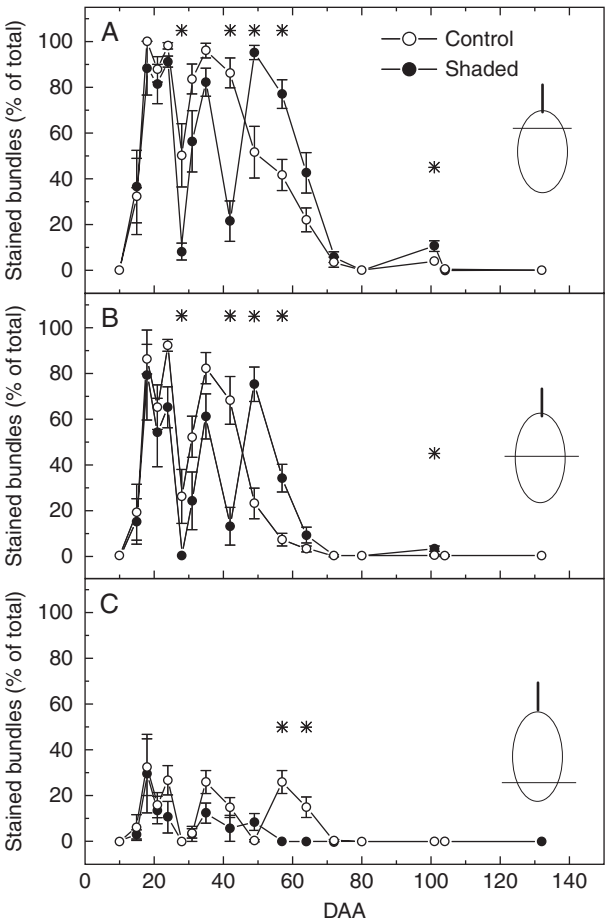

FIG. 6. Developmental time-course of xylem functionality (measured as the percentage of dye-stained bundles) of median–dorsal carpellary bundles in control and shaded berries of *A. deliciosa* cv. 'Hayward' kiwifruit in Italy. Functionality was assessed at proximal (A), median (B) and distal (C) positions within the fruit. Values are the mean ± s.e. of 12 fruit; asterisks indicate a significant difference at $P = 0{\cdot}05$ (Student's *t*-test).

the middle period of fruit development and by as much 70 % during the final phase of fruit development, and caused the final rise in R_T to occur approx. 20 days earlier (Fig. 5).

Dye infusion in the shading experiment also revealed declining hydraulic functioning within the proximal regions of the fruit during development (Fig. 6). Shading caused more pronounced oscillations in functionality and an earlier reduction in dye transport to distal portions of the fruit (Fig. 6). An average of 35 median dorsal carpellary bundles (stained and unstained) were counted for each position (proximal, median and distal) within the fruit, and the number of bundles was constant during development (data not shown). The proportion of stained bundles oscillated in both shaded and control fruit during the first 60 DAA, before falling gradually to zero at all positions by 80 DAA. A slight recovery at more proximal positions within the fruit was observed around 100 DAA, but thereafter no dye penetrated beyond the receptacle into the fruit. During the first 60 DAA, when dye did enter a bundle within the fruit, it was most likely to reach between the median and distal positions before further movement ceased (Fig. 6B, C). Shading delayed the loss of dye transport to proximal and median positions, but caused an earlier permanent loss of dye transport to the distal position (Fig. 6).

The increased hydraulic resistance and reduced dye penetration caused by shading were also accompanied by a decrease in final fruit Ca concentration (Table 1). Shading had no effect on the concentrations of the four other measured macronutrients (N, P, K and Mg; Table 1).

DISCUSSION

These results show that ontogenetic changes in xylem hydraulic resistance do occur and are likely to contribute to seasonal changes in xylem in-flows into developing kiwifruit berries. They also show that, unlike the grape berry, the receptacle zone is an important contributor to increasing xylem hydraulic resistance during the second half of berry development. These findings provide an explanation for previous observations of reductions in fruit accumulation of xylem-mobile Ca during development. Support is also found for the hypothesis that the effect of shade on Ca accumulation is connected to a shade-induced increase in xylem hydraulic resistance. While the two methods of measuring fruit hydraulic resistance agreed in terms of the developmental time course of changes in resistance, there were large differences in the absolute magnitude of measured R_T. This finding has implications for the interpretation of previous measurements of fruit hydraulic resistance.

The estimates of R_T obtained using the pressure chamber were approximately three times higher than those recorded using a flow meter. Which estimate is closer to the actual value of fruit hydraulic resistance? The two methods differ in the driving force used to induce flow and the direction of water movement, and may also differ in the pathway through which flow occurs during measurement. The pressure chamber causes flow from the fruit apoplast, which must then be replaced by dehydration

TABLE 1. *Concentration (% dry weight) (± s.e.) of macronutrients in fruit grown with and without artificial shading*

	N	P	K	Ca	Mg
Control	1·01 ± 0·06	0·19 ± 0·02	2·16 ± 0·20	0·27 ± 0·02	0·09 ± 0·005
Shaded	0·96 ± 0·07	0·18 ± 0·01	2·21 ± 0·13	0·19 ± 0·02*	0·09 ± 0·005

* Significant difference between means in the same column ($P < 0·01$, Student's *t*-test, $n = 10$).

of the symplast (Wei *et al.*, 2000). Higher estimates of R_T obtained using this method may therefore reflect the additional pressure gradients required to cause movement of water across cell membranes of the fruit pericarp. However, both Tyerman *et al.* (2004) and Choat *et al.* (2009) assumed that inward flow meter measurements on grape berries also included the resistance of a composite osmotic barrier (pericarp cell membranes and other structures) that was in series with the xylem. The flow meter pushes water into the fruit in the direction of normal transpiration flow, but is also likely to cause the flooding of embolized conduits (Trifilò *et al.*, 2010) and airspaces (Sack *et al.*, 2002) inside the fruit. Flooding of air spaces during flow meter measurements of leaves had little effect on estimates of hydraulic resistance, when compared with methods that do not cause flooding, suggesting that the pathways circumvented by flooding contribute little to *in-vivo* resistance in leaves (Sack *et al.*, 2002). Whether this finding applies to developing fruit is unknown.

The evaporative flux method is a third option for measuring R_T that has also been used frequently with leaves. The technique relies on allowing transpiration from the detached organ and measuring the flows and pressure gradients that result; it is therefore the method that should most closely approximate natural flows also in whole vine (Dichio *et al.*, 2013). While not yet widely used with any fruit, a preliminary estimate of R_T for late-season kiwifruit obtained using the evaporative flux method (25×10^3 MPa s g^{-1}; Clearwater *et al.*, 2012) was similar to, or higher, than that obtained using the pressure chamber (15×10^3 MPa s g^{-1}; Fig. 3). The flow meter, however, gave a significantly lower estimate of R_T than the two other techniques at the same developmental stage ($1·5 \times 10^3$ MPa s g^{-1}; Fig. 3), and it is this technique that has been used to investigate the concept of hydraulic isolation in grape (Tyerman *et al.*, 2004; Choat *et al.*, 2009). If the flow meter underestimates resistance, then the degree of hydraulic isolation will also be underestimated and the magnitude of potential xylem 'back-flow' to the shoot overestimated. Therefore, there is a need to further investigate the effect of methodology on estimates of berry hydraulic conductance and overall vascular functioning for kiwifruit and other species. Differences in climatic conditions between Italy and New Zealand may have contributed to differences in the absolute value of the fruit hydraulic resistance between the two study sites, but will not have affected the comparison between flow meter and pressure chamber methods in New Zealand. Summer maximum air temperatures reach 18–25 °C in New Zealand, but can be up to 35 °C in Italy, resulting in differing physiological responses in field-grown kiwifruit (e.g. the propensity for photoinhibition) (Montanaro *et al.*, 2007). Recently, Way *et al.* (2013) demonstrated that growth at elevated temperatures (ambient +5 °C) induces higher hydraulic resistance in stems of *Populus tremuloides*, so the absolute value of R_T may be temperature-dependent.

The general pattern of increasing R_T during the latter half of berry development is consistent with previous reports (Lang and Ryan, 1994; Tyerman *et al.*, 2004; Choat *et al.*, 2009). Comprehensive measurements of berry hydraulic resistance throughout development are rare, with previous studies measuring R_T only during the later stages of fruit development (Tyerman *et al.*, 2004; Trifilò *et al.*, 2010), or comparing a limited number of time-points immediately before and after the onset of ripening (Choat *et al.*, 2009). This study documents for the first time a steep decrease in R_T coincident with the most rapid phase of fruit growth during the first 60 d after pollination. Hydraulic capacity expanded in coordination with the increase in fruit volume and length, consistent with recent findings that xylem vascular differentiation continues as grape berry volume increases (Chatelet *et al.*, 2008). Temporal oscillation in dye uptake during this period (Fig. 6; Dichio *et al.*, 2003; Dražeta *et al.*, 2004) suggests that some variation may occur in xylem continuity within individual vascular bundles that is not reflected in measurements of R_T. A decline in dye uptake at veraison in grape has been attributed to a change in the pressure gradients that contribute to passive dye movement (Bondada *et al.*, 2005; Keller *et al.*, 2006). Changes in these gradients are probably the result of decreasing berry transpiration, growth or solute partitioning; while the rates of transpiration and growth do decrease during the first 60 d of berry growth in kiwifruit (Smith *et al.*, 1995; Dichio *et al.*, 2003) and grape (Greer and Rogiers, 2009), there is no evidence that they oscillate. From 70 d onward R_T increased, regardless of the hydraulic method used, consistent with dye infusion results and previous reports for other fruits. This period includes the second phase of fruit expansion at 90 DAA (veraison in grapes) and the onset of ripening in kiwifruit, which occurs at approx. 150 DAA. Choat *et al.* (2009) detected no change in R_T until after veraison in grapes and concluded that declines in xylem inflow during this period could not be attributed to increased hydraulic resistance. The higher frequency measurements presented here show that in the kiwifruit berry the rise in R_T begins before the second phase of fruit growth and long before fruit ripening, suggesting that declining xylem inflows (Morandi *et al.*, 2010) are correlated with increasing hydraulic resistance.

Increasing R_T after the first phase of rapid berry growth was strongly associated with an increase in receptacle hydraulic resistance. The sequential excision of distal fruit portions combined with flow meter measurements showed that the resistance of the receptacle zone decreased the least during the first phase of berry growth, and a rise in receptacle resistance accounted for almost all of the increase in R_T from 70 DAA onwards. The relative contribution of receptacle resistance to

R_T therefore increased throughout fruit development. The results of dye infusion were consistent with this pattern, with almost complete cessation of dye entry beyond the receptacle from 70 DAA onwards. The lack of dye in distal portions of the fruit during this period is not indicative of a loss of xylem functionality beyond the receptacle, because if dye cannot pass beyond the receptacle then the functionality of more distal vascular bundles remains untested. Dye has been observed to move readily within the xylem of ripening kiwifruit berries when it was introduced directly to the pericarp of fruit that were still attached to the vine (Clearwater *et al.*, 2012). Receptacle resistance dominates during later fruit growth to the point that the hydraulic properties of the more distal portions of the fruit are difficult to measure via the pedicel.

The cause of increases in receptacle resistance in kiwifruit warrants further investigation. Choat *et al.* (2009) associated post-veraison increases in resistance of grapes with the deposition of gels or solutes within receptacle xylem conduits. In kiwifruit the increase in resistance occurs continuously from 70 DAA. While there is no sharp transition in phloem unloading mechanism or pericarp solute content, like that observed at veraison in grapes, in kiwifruit the period from 70 DAA does correspond with the onset of starch accumulation and a gradual restriction in symplasmic phloem unloading (Richardson *et al.*, 1997; N. Gould and M. J.Clearwater, unpubl. data). It is therefore possible that there is a functional connection between changes in receptacle hydraulic resistance, phloem unloading and berry carbohydrate accumulation. In addition, the kiwifruit receptacle zone incorporates a complex junction between median dorsal and ventro-median carpellary vascular bundles, adjacent to a central woody 'mucro' that becomes progressively more massive and lignified during fruit development (Sharrock and Hallett, 1992; Mazzeo, 2008). The physical cause of increased receptacle resistance may be related to the pattern of expansion, differentiation and lignification of vascular bundles adjacent to the sclerified mucro (Mazzeo, 2008).

Levels of irradiance are known to affect xylem differentiation and mineral accumulation in kiwifruit berries, and reduced irradiance causes decreased solute content and quality in many fruits (Biasi and Altamura, 1996). Here these findings are extended to show that reduced irradiance causes a measurable increase in berry xylem hydraulic resistance. In the earlier study, shade reduced the number and total cross-sectional area of xylem elements in the pedicel and carpellary bundles, and the density of lateral branch bundles in the outer pericarp, independently of any effect on fruit growth or photosynthesis (Biasi and Altamura, 1996). The present study also found no effect of shade on growth, but a pronounced effect on xylem hydraulic functioning, in terms of both increased R_T and reduced dye entry into the fruit. In both experiments mineral accumulation was also reduced by shading, confirming a correlation between xylem differentiation, hydraulic functioning and reduced xylem inflows into developing fruit (Montanaro *et al.*, 2006). The location within the fruit of increased hydraulic resistance caused by shading was not identified, but uniform reduction of dye entry throughout the fruit up to 70 DAA supports the earlier finding by Biasi and Altamura (1996) of a reduction in the quantity of differentiated xylem conduits at all levels in the vascular hierarchy. Beyond 70 DAA, dye entry into proximal and median zones of the fruit was briefly maintained by shade, possibly because shading delayed the onset of changes in the receptacle zone discussed above.

Shading reduced accumulation of the xylem-mobile element Ca in the kiwifruit berry. In contrast, the final concentrations of the phloem-mobile elements (N, P, K; Clark and Smith, 1988; Buxton *et al.*, 2007) were not affected by shade. Mg tends to behave as an intermediate between exclusively xylem- and phloem-mobile elements (Clark and Smith, 1988, Buxton *et al.*, 2007), and in this experiment Mg concentrations were unaffected by shade. Some of the effect of shading on Ca accumulation may have been caused by reductions in vapour pressure deficit and fruit transpiration caused by shading (Montanaro *et al.*, 2010, 2012). However, the general correlations between xylem hydraulic resistance and xylem-mobile mineral accumulation, observed in the shading experiment, and temporal changes in hydraulic resistance and the major phases of fruit growth, observed in the hydraulic architecture measurements, suggest an overall coordination between xylem hydraulic functioning and the balance between phloem and xylem in-flows into the fruit. Xylem hydraulic resistance does not directly influence the rate of transpiration, but does influence the pressure gradients developed for a given level of transpiration. These pressure gradients in turn influence phloem fluxes (Clearwater *et al.*, 2012), the balance between xylem and phloem flows (Choat *et al.*, 2009), differential accumulation of predominantly phloem- or xylem-mobile minerals, and the potential for any xylem back flow later in fruit development when apoplasmic sugar accumulation is significant. A coordinated ontogenetic increase in hydraulic resistance may be a mechanism for maintaining moderate negative (inward) xylem pressure gradients while skin vapour conductance and transpiration rates fall. Changes in fruit xylem hydraulic architecture may also be functionally coordinated with a transition in the mechanism of phloem unloading and type of carbohydrate accumulated by developing kiwifruit berries. These topics require further investigation if we are to understand the genetic and physiological controls on fruit development and the determinants of quality in economically important fruit crops.

This study documents the seasonal pattern of the fruit total hydraulic resistance and its partitioning among various fruit components, and shows that the most significant changes in resistance occurred in the receptacle (rising to 90 % of total resistance late in the season). Increased R_T in the receptacle zone may be connected to changes in phloem functioning and the maintenance of water potential gradients between the stem and the fruit. The effect of shade on R_T (increased) and on fruit Ca accumulation (reduced) reported here extends earlier reports that shading can affect fruit vascular differentiation, xylem flows and mineral accumulation.

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