Fruit Morphological and Physiological Traits Influence Calcium Transport and Accumulation in Kiwifruit

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Keywords: calcium partitioning, transpiration, xylem functionality, light, wind, exposure

Abstract

Effects of exposure to light and air movement on the accumulation of some mineral elements in fruit of kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*) are presented. Exposed fruit (>40% full sunlight) exhibited calcium content twice that of shaded fruit (<20% full sunlight). Trends of Ca accumulation are discussed in relation to various known physiological and morphological fruit traits. Xylem functionality, fruit transpiration, fruit hair viability and fruit hydraulic conductance show significant changes during the first 8-10 weeks after full bloom. Each of these can, in part be held to account for the early cessation of Ca import into the fruit. Future research perspectives and opportunities for field manipulation to increase fruit Ca content are introduced.

INTRODUCTION

In kiwifruit, as in most fruits, fruit quality and shelf life are related to fruit mineral composition, and particularly, to low fruit calcium concentration (Poovaiah et al., 1988; Thorp et al., 2003; Ferguson et al., 2003). It has been widely shown that after an early rise, fruit Ca concentration actually decreases because Ca influx ceases by the mid-growth stage, whereas volume growth continues till harvest (Clark and Smith, 1988; Xiloyannis et al., 2001). Recently, fruit Ca content has been investigated in response to several factors such as light intensity, wind speed, position within the canopy and leaf:fruit ratio (Montanaro et al., 2006; Dichio et al., 2006; Thorp et al., 2003; Ferguson et al., 2003); however, the underlying physiological mechanisms regulating fruit calcium inflow to the fruit are still not at all well understood.

This paper focuses on physiological and morphological traits that, from our own results and those of others, seem to affect the rates of Ca transport and accumulation in the fruit. Opportunities for further research and for commercially practicable field manipulations likely to increase fruit calcium are also introduced.

MATERIALS AND METHODS

Except where otherwise noted, trials were carried out in Southern Italy (40°20'N, 16°48'E) on mature own-rooted kiwifruit vines 'Hayward' (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A.R. Ferguson) trained to pergola with 494 plants ha^{-1}. The vines were managed to local commercial practice. Bloom commenced during the last week of May and supplementary bee pollination ensured good and roughly synchronous fruit-set between the end of May and the beginning of June.

A few days after fruit-set, two light exposure treatments were imposed on single canes. These were: (i) an 'exposed' treatment involving watershoot removal where fruiting canes received >40% of the available Photosynthetic Photon Flux Density (PPFD) at 12:00 h, and (ii) a 'shaded' treatment using shade cloth (Arrigoni, CO – Italy, mod. 2591WO Ombraverde 90, 80%) where canes received <20% of the available PPFD at 12:00 h. For details of light measurement methods see Montanaro et al. (2006).

Fruit transpiration was measured every 7-10 days on attached fruit using a portable gas exchange analyser (ADC-LCA4, ADC BioScientific Ltd, Hoddesdon,

England). Sap flow (heat balance method) was monitored on whole canes using a Dynagage sap-flow device (Dynamax Inc., Houston, USA). Detailed methodological descriptions are reported in Xiloyannis et al. (2003).

Fruit (fruit + fruitstalk comprising both peduncle and pedicel) hydraulic conductance was determined weekly throughout fruit development using home-made equipment based on the Scholander pressure chamber (Model 600, PMS Instruments, Corvallis, OR-USA) and working at a standard pressure of 1.3 MPa obtained using compressed N_2. About 12 fruit per treatment were sampled before dawn and the cut fruitstalk was immediately dipped in distilled water to minimise air embolism. The whole fruit was enclosed in a plastic bag to reduce transpiration, and promptly transferred to the laboratory. Fruitstalks were re-cut under water before beginning the measurements. After discarding the first few drops of sap exuded when fruit were first pressurised, sap was collected for 10 min into pre-weighed 1.5 ml plastic vials and immediately weighed. Fruit hydraulic conductance was calculated according to Reid et al. (2005) and sap density was assumed to be 1 $mg{\cdot}mm^{-3}$.

Concurrent with other measurements, 10 fruit per treatment were sampled and dried (48h in a ventilated oven at 60°C) and Ca, K and Mg concentrations were determined using a spectrophotometer (model AA-40, Varian Inc., Palo Alto, USA) on acid digested samples (H_2SO_4 + HNO_3). Organic N was assayed using the Kjeldhal method. All samplings and measurements were done on fruit from a similar position in the canopy to reduce variability.

Observations of fruit hair viability were made weekly in Palmerston North, New Zealand, during the first 70 days after full bloom (AFB). On each sampling, ~100 hairs per fruit from 10 fruit were carefully removed with a sharp blade and held for 1h in a drop of Alexander's stain solution (Alexander, 1980) under a glass cover slip on a warmed (50°C) microscope slide. This double stain discriminates between live cells (protoplasts stain pink) and dead cells (walls stain blue/green). The proportion of hairs that contained some live (i.e., pink) cells was counted under a light microscope.

RESULTS AND DISCUSSION

Analysis revealed different accumulation patterns between Ca and Mg, and N and K during fruit growth, while fruit fresh weights (110 g ± 3.5 at day 187 AFB) were not significantly different between the two treatments (Student's *t*-test, P=0.05) (Fig. 1). Amounts of N and K (mg per fruit) increased linearly during the early period 17-18 weeks AFB; thereafter their accumulation slowed, reaching a maximum value by about day 156 AFB. At this time, fruit contained approximately 135 mg of N and 285 mg of K. By contrast, the Ca and Mg accumulation patterns exhibited a shorter, roughly linear phase up to 60 and 90 days AFB respectively, when accumulation of Ca reached a plateau and accumulation rate of Mg rates decreased. The accumulations of N and K were not significantly affected by the two exposure treatments whereas those of Ca and Mg were, with the accumulation in exposed fruit both faster and reaching a higher asymptotic value. This result may be explained by taking into account the restricted phloem mobility of both Ca and Mg (White and Broadley, 2003). By the end of the experiment, the mass of Ca in the exposed fruit (up to about 40 mg per fruit) was approximately double that in the shaded ones. The exposure treatments did not significantly influence final fruit dry matter. In exposed fruit, these values were 19.21 ± 0.57g and in shaded fruit, 20.16 ± 0.6g (± S.E. in Student's *t*-test, for P=0.05).

Fig. 1 shows that from 60-70 days AFB onwards, fruit Ca levels (mass per fruit) in both exposed and shaded treatments was relatively constant, although in exposed fruit, Ca accumulation did seem to continue for a few days longer. This cessation of Ca import has already been linked with declines in the functionality of the xylem pathway (Dichio et al., 2003). Interestingly, we report here that the Ca accumulation profile (Fig. 1) is also consistent with that of cumulative fruit transpiration (Fig. 2). This consistency is in regard both to shape and timing. Also note that fruit Ca accumulation and fruit transpiration respond very similarly to exposure treatments, although Ca accumulation is enhanced

roughly 2-fold by increased exposure, whereas the transpiration increase is somewhat lower – about a 1.2-fold increase. Increased exposure seems also to have prolonged the period of rapid fruit transpiration. The closeness of these various correlations (viz. fruit Ca accumulation and fruit transpiration) suggests some causal linkage between the two.

On the other hand, it must be noted that the higher rate of transpiration in exposed (compared with shaded) fruit could also be associated with the higher rate of sap flow found in exposed canes (Fig. 3). However, for this effect to be causative, rather than merely correlative, would imply that it was mediated via a change in the Ca composition of the flowing xylem sap. Such a change in sap composition would more likely be the other way around in that Ca would be more dilute in the xylem stream under rapid-transpiration conditions, so we do not favour this interpretation.

Differences in fruit transpiration under the two exposure treatments only partly explain the difference in fruit Ca accumulation, confirming that correlations between the rate of transpiration and Ca import are not straightforward. This has already been suggested for other species (Banuelos et al., 1987; Koontz and Foote, 1966; Pomper and Grusak, 1977). Our findings seem to agree with Bangerth (1979) that there is likely no simple proportionality relationship between fruit Ca and fruit transpiration. Further investigation of transpiration-independent mechanisms in relation to Ca accumulation in fruit would be well worthwhile if we are to better elucidate this important area of fruit physiology in kiwifruit.

Fruit transpiration will certainly depend upon external factors of the aerial microenvironment but it will also depend on a number of internal or fruit factors, particularly those that affect the rate of diffusion of water from the inside of the fruit through to the surrounding air. These will include the barrier properties of the fruit surface, the fruit surface area, and the driving force for water vapour transfer (Maguire et al., 2003). Interestingly, there are quite a number of morphological and anatomical changes that occur in kiwifruit at around 60 days AFB. Each of these change factors has the potential for involvement in a more complex mechanism responsible for the critical early decline in fruit Ca accumulation.

One of these change factors is the decline in the physical integrity and functionality of the fruit xylem. This structure:function decline occurs in many species of fruit and has been reported also for kiwifruit (Dichio et al., 2003). It has already been remarked that this decline coincides with a decline in fruit transpiration (Montanaro et al., 2006). The primary effect of a progressive dysfunction of the fruit xylem will be a change in the predominant pathway taken by water entering the fruit from the vine, diverting it from a mainly apoplastic one to a mainly symplastic one. This change will tend to reduce the import by the fruit of all symplast-immobile ions such as Ca. Incidentally, this water inflow diversion will also probably increase the import of some important, largely symplastically transported species such as sugar and potassium (Lang et al., 1986).

Another marked change that occurs at about the same time (around 60-70 days AFB) is the collapse of the external tissue layers (Xiloyannis et al., 2001) of the fruit and the formation of a suberized periderm (Schmid, 1978). Smith et al. (1995) reported dramatic declines in skin surface conductance in kiwifruit and attribute these declines to the development of this dermal layer. They report that the surface conductance of very young fruit is extremely high - about 10-times greater than in apple. However, while just after bloom, skin conductance is about 0.8 mmol water m^{-2} s^{-1}, this very high value falls by almost 90% over the next few days declining to only about 0.1 mmol water m^{-2} s^{-1} by day 21 AFB (Smith et al., 1995). The decline continues on, but more gradually through to about day 60 AFB, thereafter then on the surface conductance value remains low and relatively constant right through to harvest at about day 170 AFB. We note that the skin conductance result of Smith et al., (1995) fits well with our own cumulative fruit transpiration data reported in Fig. 3.

Another change that occurs in kiwifruit skin has to do with the loss in vitality of the fruit's dermal hairs (noted in Dichio et al., 2004). Again, this loss occurs with a similar synchrony and so it too probably bears a degree of responsibility for the observed

decline in fruit transpiration, and also in fruit Ca accumulation. From an anatomical point of view the dermal hairs of kiwifruit have been reasonably well characterised (White, 1986) but, as yet no information exists as to their possible influence on the transpiration physiology of the fruit. It is therefore worthwhile at this stage to introduce to the discussion some relevant aspects of their development and physiology.

The juvenile kiwifruit already has a full set of hairs (trichomes) at bloom and no new hairs develop from this time on. The hairs are also fully formed and are typically 2.5 to 3.0 mm long and their bases are about 0.1 mm in diameter giving them an aspect ratio (length/basal diameter) of about 25. In the very young fruit, the hairs are packed together so closely that their bases are almost touching. Subsequent surface expansion of the fruit moves the hairs apart so that they become increasingly isolated from one another during the growing season.

Hairs are of quite simple structure (Fig. 4) and contain no vascular tissue. This requires that any evaporative water loss occurring from the more apical cells must be made good by diffusion of water up from the skin through the more basal cells. This diffusion pathway (it is likely that it will be predominantly through the cell wall matrix, not through the protoplasts) is probably sufficient to maintain hair vitality early on when the hairs' close proximity to one another means that they will offer a degree of mutual protection from water loss. However, as they move apart with fruit growth, this protection will become less and less, and so the acropetal diffusion pathway will probably become limiting at some stage. This understanding fits with our observation that cell death (drying) is first evident in the most apical hair cell and this then progresses downwards (basipetally) through the hair eventually reaching the most basal ones last.

A simple calculation based upon hair dimensions (the area of a hair ≅ π·base radius·hair length) and hair density (hairs mm^{-2} of fruit surface) (data not shown) proves that, in a very young kiwifruit, the still-living hairs are sufficiently numerous as to increase the 'living' surface area of the fruit by a factor of around 40-times, compared with a hairless fruit of the same dimensions. This massive amplification of fruit surface area is almost certainly responsible for the very high surface conductance of a young kiwifruit. The amplification factor steadily decreases as the fruit surface area increases with growth (data not shown), but the hair dimensions and hair numbers per fruit remain the same (i.e., the hair density decreases). The proportion of living hairs (hairs with some live hair cells) falls from close to 100% at the time of fruit set to about 50% by 30 days AFB and by 60 days AFB all the cells of all hairs are dead (Fig. 5). Death of the single long apical hair cell (Fig. 4) occurs much sooner.

We interpret the rather striking skin surface conductance results of Smith et al. (1995) and our own fruit transpiration results (Fig. 2), not in terms of the development of a more complex dermal layer (although this too could be involved), but predominantly in terms of the basipetal progression of cell death in the dermal hairs. During this short period of early fruit growth, as hair cell death progresses, the 'hair effect' will change from one in which they increase water loss from the fruit skin to one in which they *decrease* it. The fully dead (dry) hairs will serve to reduce fruit water loss by entrapping a thick (c. 3 mm) boundary layer of still, moist air around the fruit. This effect is much the same as that of the layer of fur that reduces the rate of heat loss from the skin of mammals. Moreover, this understanding is consistent with the behaviour, elsewhere reported (Dichio et al., 2006) that, under the influence of an increasing forced air stream, the kiwifruit's boundary layer resistance breaks down progressively and fruit transpiration increases very significantly. This effect is again much the same as that in which wind increases superficial heat loss from furry mammals (the chill factor).

Fig. 6 shows preliminary results on seasonal changes in the hydraulic conductance of fruit + fruitstalk grown under different conditions of exposure. Within the first 35 days AFB, hydraulic conductance increased rapidly. This is presumably due to the differentiation of new xylem vessels as a result of a post bloom rise in the activity of the intrafascicular vascular cambium, probably stimulated by seed set (Drazeta et al., 2004). Conductance rose to about 0.22 cm^3 MPa^{-1} $s^{-1}\times10^{-3}$ in both exposed and shaded fruit. For

about 20 days, conductance remained relatively stable but then decreased progressively after day 60 AFB, according to Dichio et al. (2003). In the late season, hydraulic conductance of the fruit + fruitstalk (measured using a pressure bomb) was very low (0.05 - 0.1 cm^3 MPa^{-1} s^{-1} × 10^{-3}) (Fig. 6). We note that this result is in conflict with the data emerging from our dye studies (Dichio et al., 2003), which show a much earlier cessation of flow. This discrepancy will be addressed more thoroughly in a separate study and may have to do with the rather high axial pressure gradient applied to the xylem vessels resulting from the application of a bomb pressure of 1.3 MPa (this high bomb pressure is a requirement of our method in order to obtain measurable volumes of exuded sap). The problem is that the pressure is applied over a relatively short distance of about 70 mm between the fruit flesh and the proximal end of the fruitstalk – this is an unnaturally high gradient for the xylem that would normally experience axial gradients some 3-orders of magnitude less. An alternative explanation of the discrepancy (Bondada et al., 2005) could be a reduction in the available pressure gradients (the driving force of xylem sap flow), but this explanation requires confirmation through further experimentation and analysis.

We note that, on average fruit + fruitstalk conductance in exposed fruit was about 40% higher than in shaded fruit, and this is consistent with their higher Ca accumulation and transpiration rates (Figs. 1 and 2). This difference implied either that xylem vascular development was greater in the more exposed fruit or that the onset and progression of xylem dysfunction was somewhat delayed or reduced. The picture emerging emphasises that physical alterations along the fruit xylem pathway do account for some of the reduction in xylem water inflow to the fruit and consequently the lowered rates of Ca import.

CONCLUSIONS

Results indicate some positive effects on fruit quality (i.e., Ca content) are obtainable associated with characteristics of the micro-environment close to the fruit. Particularly, increase in fruit / cane exposure to light and air movement are favourable to fruit Ca import. Therefore, it may be concluded that careful attention to canopy architecture and management in commercial kiwifruit orchards (e.g., by judicious early summer pruning) could offer useful tools for improving kiwifruit quality.

ACKNOWLEDGEMENTS

The study was financially supported by the Italian Ministry of University and Scientific Research. Special grants PRIN2005 and BRIMET.

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Figures

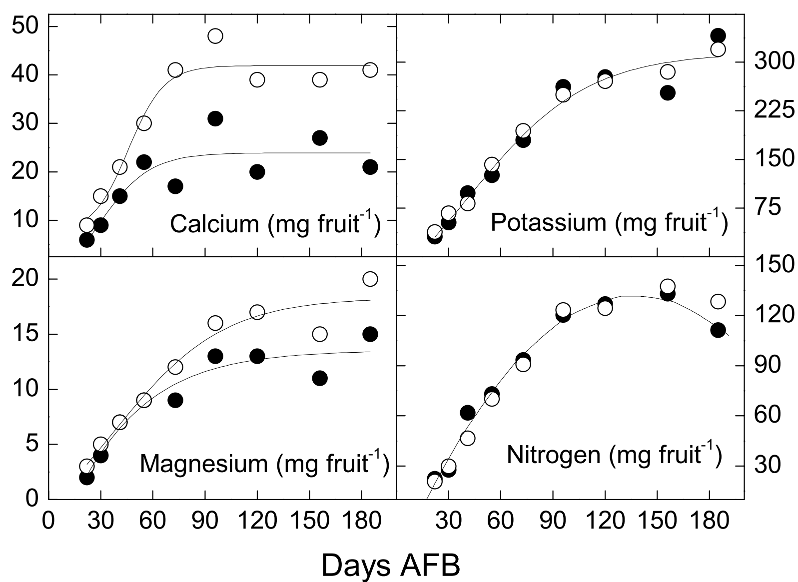

Fig. 1. Seasonal accumulation patterns of Ca, K, Mg and N in exposed (o) and shaded (•) fruit (AFB= after full bloom, where 0= 27 May). Each point is the mean of three analyses.

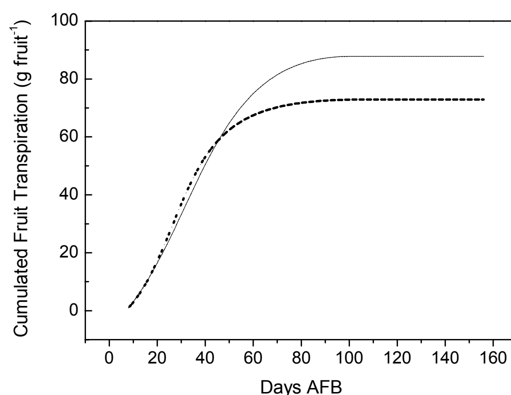

Fig. 2. Cumulative fruit transpiration with time in exposed (continuous line) and shaded (dotted line) fruit. (Re-drawn from Montanaro et al., 2006).

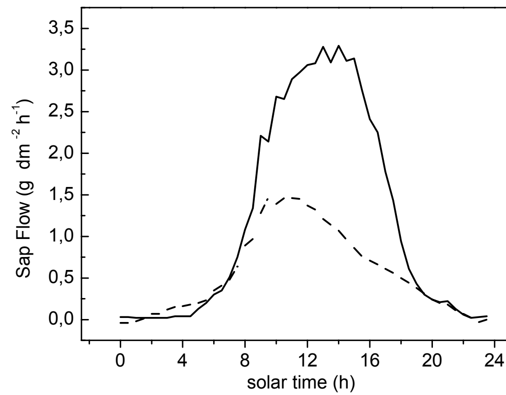

Fig. 3. Typical profile for the diurnal course of sap flow (g dm^{-2} h^{-1}) in exposed (continuous line) and shaded (dotted line) fruit. Each line represents the average result from 3 flow sensors.

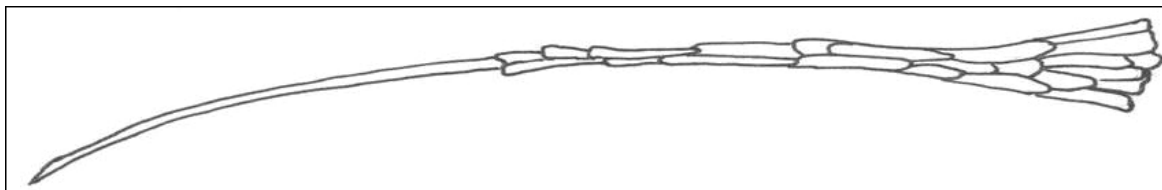

Fig. 4. Drawing of a dermal hair from 'Hayward' kiwifruit. Cell death occurs basipetally, from left to right in this drawing.

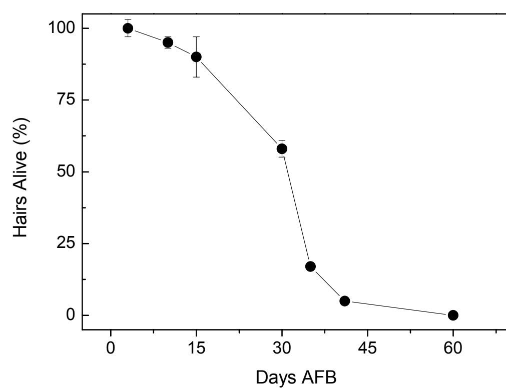

Fig. 5. Hair viability (% of hairs in the sample containing some live cells). Fruit were grown under normal commercial conditions in New Zealand and were not subjected to any special treatment. Each point is the average (±SE) of 100 observations.

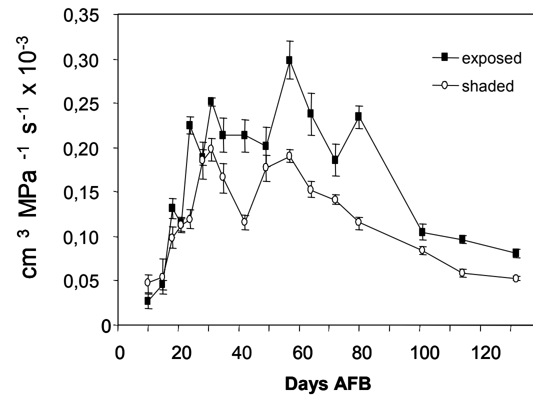

Fig. 6. Hydraulic conductance of fruit + fruitstalk in exposed (■) and shaded (□) fruit measured using a pressure bomb method. Each point is the average of 12 fruit.