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ORIGINAL ARTICLE

Phenolic compounds in young developing kiwifruit in relation to light exposure: Implications for fruit calcium accumulation

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Abstract
The interaction between light availability and the biosynthesis of phenolic compounds in fruit of kiwifruit (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A. R. Ferguson) was investigated. Fruits were exposed either to natural light or were artificially shaded while growing on mature vines and were analysed weekly during the first 11 weeks of development. Phenols were identified and quantified by using High Performance Liquid Chromatography (HPLC). Results showed that the predominant phenolic compounds were hydroxycinnamic acids (HCAs), flavonols and the flavan 3-ol epicatechin. Calcium (Ca^{2+}), the main mineral nutrient involved in fruit quality was also determined. Light significantly increased the accumulation of both phenols and Ca^{2+} into the fruit. This work expands the list of known phenolics in kiwifruit and provides a possible explanation for the seasonal pattern of Ca^{2+} import into the fruit. Results on light–phenol interaction being apparently beneficial for fruit Ca^{2+} accumulation, suggest that accurate canopy management could enhance fruit quality.

Keywords: Actinidia deliciosa *var.* deliciosa, *shading, hydroxycinnamic acids, xylem flow, calcium, canopy management*

Introduction

Plants are static organisms that must necessarily accommodate themselves to their surroundings by adaptation of their development and growth responses. Secondary metabolites are believed to be essential compounds aiding plant fitness in a number of ways, by improving both resistance to pathogens and herbivores and to stress conditions (Feucht & Treutter 1999, Treutter 2006), on the one hand, and enhancing reproduction by providing pollinator attraction (Kliebenstein 2004), on the other.

Throughout evolution, plant–environment interactions have produced a large number of secondary metabolites. These compounds have mostly been synthesised by the modification of the backbone structure of existing ones (Kliebenstein 2004). Despite their huge numbers in some key commercial crops, both the identification of these compounds and their physiological roles are not at all well defined. This is the case with kiwifruit phenolic compounds. Extensive studies have been made on phenolic composition in the leaf and the mature fruit (Ferguson 1984, Ippolito et al. 1997, Greaves et al. 2001, Wurms et al. 2003) as well as on the juice (Dawes & Keen 1999). These studies gave more emphasis to their health protective role against oxidative damage to the human cell (Chun et al. 2005, Park et al. 2006). Hence, it appears that little published information exists on the specific components of the phenolic moiety in kiwifruit during growth and on their possible physiological roles.

Light is an environmental signal that may induce biosynthesis of plant phenolic compounds (Grand et al. 1982, Douglas 1996, Rozema et al. 1997), presumably because of their UV-filtration action (Rozema et al. 1997, Gitz et al. 2004), as well as it is essential for driving photosynthesis and other key metabolic pathways (Thum et al. 2003).

The plant–light interaction also affects some qualitative characteristics of the fruit, particularly its mineral composition. It has been shown that high-light growing conditions improve fruit quality in apples (Jackson et al. 1977, Jackson 1980), grapes (Hopping 1977) and kiwifruit too (Snelgar & Hopkirk 1988, Biasi et al. 1995). The mechanism underlying this phenomenon is not completely understood. In kiwifruit, as in most fruits, fruit quality and fruit shelf life appear to be related to calcium (Ca^{2+}) content (Poovaiah et al. 1988,

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Benge et al. 2000, Ferguson et al. 2003, Thorp et al. 2003) and that Ca^{2+} accumulation is enhanced by increased light intensity (Biasi et al. 1995, Montanaro et al. 2006). Calcium is a nutrient, mainly transported through the xylem (McLaughlin & Wimmer 1999, White & Broadley 2003, Ho & White 2005). Consequently its seasonal pattern necessarily reflects the formation and functionality of the xylem conduits (Dichio et al. 2003). Some matters remain still to be determined. For example, it is not clear whether the dynamics of the import of Ca^{2+} is due to alterations of the xylem pathway in the fruitstalk or in the berry itself. In view of the specific role that some compounds have in providing the building blocks for the xylem conduits (Douglas 1996, Rozema et al. 1997, Boerjan et al. 2003), valuable information may plausibly be drawn from the determination of phenolics in the fruitstalk and in the berry. For these reasons, the present study was designed to determine whether differences in phenolic content between sun-exposed and artificially shaded fruits are present by separately investigating berries and fruitstalks during fruit development. We hypothesized that fruits subjected to low-light conditions would have low phenol content. Testing this hypothesis required assessment not only of the size of the phenolic pool during the season but also of the identification of particular compounds involved. In addition to the identification and the behaviour of phenols in response to light availability, we aimed also to give an interpretation of the results in relation to fruit Ca^{2+} accumulation. Thus, we focused on the early stage of fruit growth (i.e., about 80 days after fruit-set), when total fruit Ca^{2+} already accounts for almost the entire content at harvest (Ferguson 1980, Clark & Smith 1988).

Materials and methods

Experimental site and plant material

Trials were carried out during the 2004 growing season in Southern Italy (Metaponto, N 40°20′ E 16°48′) on mature, own-rooted, kiwifruit plants (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A. R. Ferguson) (cultivar Hayward) trained to a pergola system. Vines were planted on sandy loam soil at a spacing of 4.5 m within rows and 4.5 m between rows, with a North to South row orientation. The pollinator was cv. Tomouri and the proportion of male:female vines was 1:8. Every third plant in every third row was a male.

The vines and the soil were managed following local commercial practice. Soil mineral contents (soluble fraction, 30 April, 10–40 cm depth) were 33.4 ppm K, 2.53 ppm Mg^{2+} and 10.1 ppm Ca^{2+}. Fertilizer was applied by fertigation, at a rate of 10 kg ha^{-1} nitrogen and 20 kg ha^{-1} phosphorous, approximately every 30 days from May to August, while 64 kg ha^{-1} potassium was distributed once only in July. In January, 8 t ha^{-1} of organic fertilizer (22.2 C/N, Eco-Pol SpA, Italy), containing on a dry matter basis 2.02% total N, 1.86% K_2O and 0.68% P_2O_5, were applied directly to the soil. The vines were regularly microjet-irrigated during the season, approximately every 10 days in May and June and every 4–5 days during the periods of highest evapotranspirative demand (July, August and September). Bloom was during the last 10 days of May and natural bee pollination ensured normal and roughly simultaneous fruit-set at 30 May ($\pm$ 1 day).

Shade and light treatments

From 1 June and throughout the growing season, 2 light exposure treatments were imposed on 100 canes selected from 30 plants chosen at random. Each cane had 7–8 terminating shoots, approximately 25 fruits and a similar leaf:fruit ratio.

Before the experiment started, on all 30 chosen vines some watersprouts (3–4 per vine) were removed to minimize their possible shading effects due to the upright and fast growth. Subsequently, the shaded treatment (<20% available Photosynthetic Photon Flux Density [PPFD] at midday) was imposed on half of the selected canes using a neutral shade cloth (Arrigoni, CO, Italy, mod. 2591WO) causing 80% reduction in incident light. The available PPFD at midday within the canopy of the remaining canes was up to 40% of the incident light, hence these were defined as exposed. Radiation measurements were carried out using 18 quantum sensors (Model SKP 215, Skye Instruments LTD, Llandrindod Wells, UK). Sensors were placed in 3 areas per treatment (3 sensors each) at a maximum distance of 10 cm from fruit. Moreover, one sensor was placed above the canopy to measure the incident PPFD. All sensors were connected to a datalogger (CR10, Campbell Scientific), which was programmed to monitor the PPFD at 60 s intervals and to compute the averages at 15 min intervals. For each sensor, daily PPFD was obtained by integrating the data recorded every 15 min.

Fruit samples

From fruit-set till the 11th week, 25 fruits per treatment were sampled each week from 4–5 of the selected canes, always at the same time of day (i.e., about 10:00 am). Each cane supplied fruits just one time throughout the experiment. To minimize variability, fruits were harvested from the two basal and early fruiting shoots of each selected cane, in accordance with the recommendations of Ferguson et al. (2003) and Thorp et al. (2003). Ten of 25 fruits were weighed (fresh weight) immediately, sliced and fruit dry matter (d.wt., i.e., skin plus

flesh) was determined after 48 h drying (at 60°C), and total Ca content (skin plus flesh) was measured after acid digestion ($H_2SO_4+HNO_3$) using an atomic spectrophotometer (Varian, AA-40).

The remaining 15 fruits were immediately sliced, frozen with liquid nitrogen and placed in a refrigerator at −80°C, lyophilized and the skin was thoroughly scraped off using a sharp blade. Skin was removed in order to separately analyse it; unfortunately the amount collected was not sufficient to correctly warrant the extraction procedure. For analysis of the berry, 5 bulk samples (3 fruits each, 15 fruits total) were prepared with slices from the upper, middle and lower zone of fruits. At the first sampling date, the very small berries (1 cm length) were analysed in their entirety. For fruitstalk analysis, a bulk sample was composed of 3 entire fruitstalks. Each bulk sample was ground in a mixer ball mill to a fine powder which was used for the extraction.

Extraction and quantification of phenolic compounds

The dry powder was extracted with methanol (100%), containing Naringenin (0.1 mg ml^{-1}) as internal standard, for 30 min in a water bath at 4°C during sonication. Methanolic aliquots were used directly for High Performance Liquid Chromatography (HPLC) analyses.

HPLC was carried out with a Kontron chromatograph using a HyperClone ODS C18 column (150 × 4.60 mm, 3 μm) (Phenomenex, CA, USA) and 5% aqueous formic acid (solvent A) and methanol (solvent B). The following elution profile was used with a flow rate of 0.6 ml min^{-1}: 0–5 min, 0–2.5% B in A, 10 min, 2.5% B in A, 15 min, 2.5–5% B in A, 10 min, 5% B in A, 25 min, 5–10% B in A, 10 min, 10% B in A, 25 min, 10–20% B in A, 35 min, 20–30% B in A, 15 min, 30–90% B in A, 15 min, 90% B in A. Phenolic compounds were detected with a diode array and their UV absorbance spectra were monitored.

The quantification of epicatechin was carried out by a postcolumn derivatization method using the selective reagent 4-dimethylamino-cinnamaldehyde with detection at 640 nm (Treutter 1989).

Phenolic compounds were identified according to their UV absorbance spectra. Hydroxycinnamic acids were calculated as chlorogenic acid, flavonols as rutin, epicatechin as epicatechin. Standards were purchased from Roth (Karlsruhe, Germany).

Statistical analyses

Mean differences in Ca and phenolic compounds accumulation between fruits differently illuminated were determined by Student's *t*-test method. Curve fitting were by Origin® 6.1 (OriginLab Corporation, USA).

Results

During the experimental period, the incident light per day ranged from 30–40 mol m^{-2} day^{-1} PPFD, except for some overcast days (Figure 1). In the exposed treatment, the daily available PPFD was on average 67% ± 1.51 of the incident above canopy PPFD. In the shaded treatment the available PPFD was only 1.95% ± 0.11 of the above canopy PPFD (Figure 1).

Fruitstalk phenolic compounds

The predominant phenolic compounds of the fruitstalk were hydroxycinnamic acids (HCAs), flavonols and the flavan 3-ol epicatechin. The HCAs and flavonols of both sun and shade fruits had a similar pattern throughout the experiment, exhibiting high concentrations just a few days after fruit-set and a minimum at day 30 for HCAs and 20 for flavonols, thereafter their concentrations increased again (Figures 2a, 3a). The concentration of HCAs of exposed fruitstalks were significantly above (Student's *t*-test, $p = 0.05$) those of shaded ones during the early fruit development stage until day 30 (Figure 2a), levels being approximately 1.5 or twofold those of the shaded ones. Within 30 days after fruit-set, HCAs concentration fell from 3–1.6 mg g^{-1} d.wt. in the exposed situation and from 2.5–1.1 mg g^{-1} d.wt. when shaded.

Only during the first 10 days after fruit-set and later at day 49, the concentrations of flavonols in the fruitstalk were significantly affected by high light exposure (Student's *t*-test, $p = 0.05$), showing 0.71 mg g^{-1} d.wt. in sun fruit 0.46 mg g^{-1} d.wt. in shaded fruits (Figure 3a).

The concentration of epicatechin was stimulated by light during the first 20 days after fruit-set, thereafter, no significant variations were observed between the treatments (Figure 4a, Student's *t*-test, $p = 0.05$). The exposed fruitstalks showed the high-

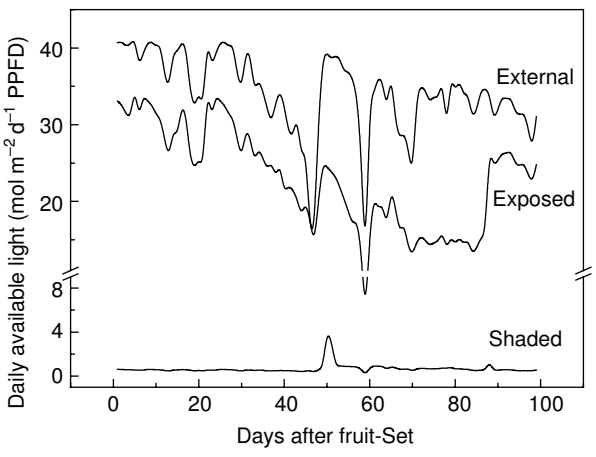

Figure 1. Available light during the experiment. Each line represents the mean of 9 Quantum Sensors (Skye) placed in 3 areas per treatment. (0 = 30 May), the y-axis (radiation) is broken from 9 to 11.

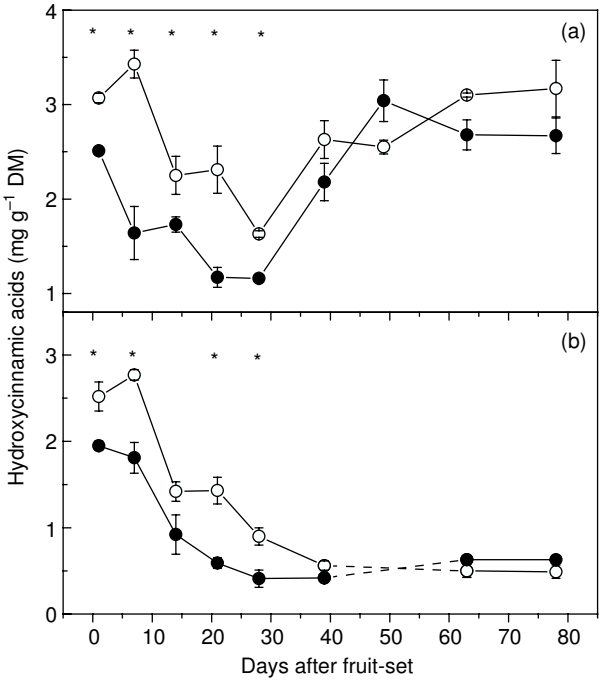

Figure 2. Hydroxycinnamic acids (mg g^{-1} d.wt.) of fruitstalk (a) and berry (b) sampled from exposed (○) and shaded (●) canes during the experiment. Values (means ± SE) were determined from 5 HPLC analyses. (Means separation by Student's *t*-test, *significant at $p = 0.05$). The dotted line between day of experiment 39 and 63 is due to a missing analysis of berry samples.

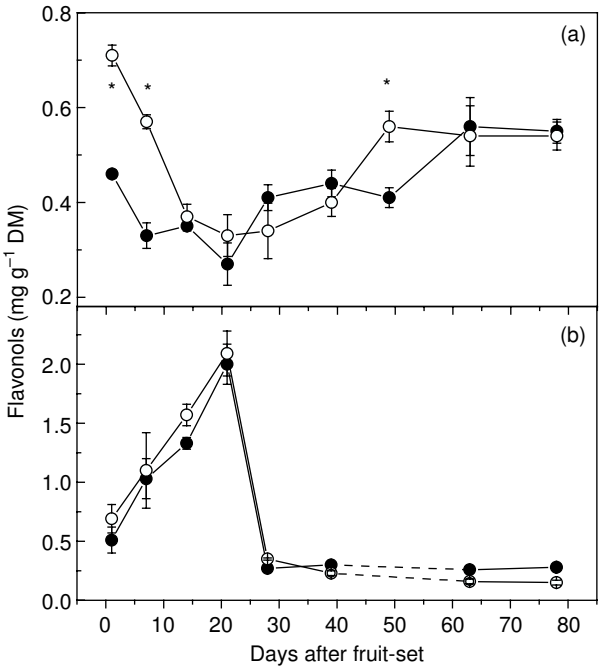

Figure 3. Flavonols (mg g^{-1} d.wt.) of fruitstalk (a) and berry (b) of fruits grown in exposed (○) and shaded (●) canopy position during the experiment. Values (means ± SE) were determined from 5 HPLC analyses. (Means separation by Student's *t*-test, *significant at $p = 0.05$). The dotted line between day of experiment 39 and 63 is due to a missing analysis of berry samples.

est concentrations of epicatechin just 1 week after fruit-set being about 9.5 ± 0.39 mg g^{-1} d.wt. Thereafter, concentration gradually declined reaching the value of 7.78 ± 0.36 mg g^{-1} d.wt. at day 28. In the shaded fruitstalks, epicatechin increased slightly from week 1–4 after fruit-set reaching values similar to that of exposed ones (Figure 4a).

Berry phenolic compounds

As in the fruitstalk, the prevailing phenolic compounds in the berry were HCAs, flavonols and the flavan 3-ol epicatechin. They showed the highest levels in very young fruits, i.e., within the first 3 weeks after fruit-set.

A continuous decline was found for HCAs (Figure 2b). Just after fruit-set, their concentrations in the exposed berries of 2.52 ± 0.17 mg g^{-1} d.wt. were significantly above (Student's *t*-test, $p = 0.05$) those in shaded ones of 1.95 ± 0.05 mg g^{-1} d.wt. This difference remained until day 30. At day 40 after fruit-set the concentration of hydroxycinnamic acids was about 0.5 mg g^{-1} d.wt. in both exposed and shaded berry (Figure 2b).

The flavonols in the berries were not affected by light (Figure 3b). Moreover, their behaviour differed from that of the other compounds identified during fruit development. Just after fruit-set, they started with a low concentration of approximately 0.5 mg g^{-1} and increased to a maximum at day 20 with values close to 2.1 mg g^{-1} d.wt. Concentration decreased sharply thereafter, reaching the lowest values of 0.3 mg g^{-1} d.wt. at day 28 after fruit-set (Figure 3b).

During early stages of fruit development, the concentration of epicatechin progressively decreased reaching negligible values after 6 weeks (Figure 4b). In addition, this compound was not affected by light treatment. The initial values were approximately 10 mg g^{-1} d.wt. and the concentrations of approximately 1 μg g^{-1} d.wt. were reached at day 39 after fruit-set (Figure 4b).

Fruit growth and Ca^{2+} accumulation

Throughout the experiment, fruit dry matter (not shown) was not significantly affected (Student's *t*-test, $p = 0.05$) by the treatments. At day 79 after fruit-set it reached 12.8 ± 0.57 g f^{-1} in the sun and 11.2 ± 0.6 g f^{-1} in shade. In this study, calcium has measured as total (see Methods section) with no attempt to share between different forms. Accumulation of Ca^{2+} into fruit was significantly affected by the treatments, showing lowest values in fruit grown under low light availability (Table I). At approximately week 6 following fruit-set, shaded fruits had accumulated 10.92 ± 0.61 mg f^{-1} while the exposed ones exhibited 13.41 ± 0.75 mg f^{-1}. Further increase of that nutrient was recorded during the subsequent weeks and at day 78 after fruit-set had reached 32.33 ± 1.36 mg f^{-1} in sun fruit and 24.8 ± 1.78 mg f^{-1} in shaded fruit.

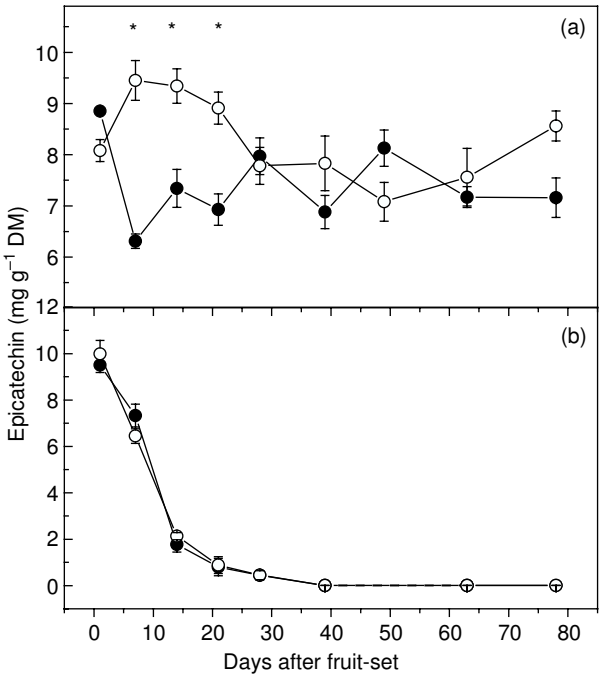

Figure 4. Epicatechin (mg g^{-1} d.wt.) of fruitstalk (a) and berry (b) of fruits sampled from exposed (○) and shaded (●) canes during the experiment. Values (means±SE) were determined from 5 HPLC analyses. (Means separation by Student's *t*-test, *significant at $p=0.05$). The dotted line between day of experiment 39 and 63 is due to a missing analysis of berry samples.

Discussion

This study reports on the natural occurrence of phenolic compounds in developing kiwifruit (berry and fruitstalk) in response to shading. Results on phenols were hard to compare with literature values because of the poor information existing on kiwifruit that mainly reports the leaf or mature fruit phenolic composition and associated with anti-fungal activity (Ippolito et al. 1997, Greaves et al. 2001, Wurms et al. 2003).

In general, excepting for the epicatechin of shaded fruitstalks and flavonols of berry, the pattern of phenolic compound changes showed a decrease within the early weeks after fruit-set. In particular, the concentration of HCAs and epicatechin of the berry rapidly declined reaching their lowest values 40 days after fruit-set. The quick fall in concentration of the phenolic compounds in the early developmental stage has been already reported on grape berries and on apple fruit (Crippen & Morrison 1986, Treutter 2001). Moreover, it occurs at the same time with changes in a number of physiological and structural aspects of the fruit (e.g., fruit transpiration, dysfunction of the fruit vascular system; see Dichio et al. 2003, and Montanaro et al. 2006 for details) confirming the relevance of the early stage of fruit development.

This study shows a light-induced increased concentrations of HCAs in fruits (30–50% higher than shade, on a whole experiment basis), which is similar to the response of anthocyanins in sun grape berries

Table I. Fruit Ca content (mg f^{-1}) of fruit sampled from exposed and shaded canes during the experiment. Nutrient values are the mean (±SE) of ten fruits and include skin and seed tissue. (Means separation by Student's *t*-test, *significant at $p=0.05$).

Days after fruit-set	Exposed	(±SE)	Shaded	(±SE)
1	3.14	0.25	0.11*	0.01
7	3.69	0.18	1.63*	0.10
14	5.06	0.33	3.21*	0.13
21	6.33	0.96	4.93	0.78
28	9.54	0.99	7.65	0.57
39	13.41	0.75	10.92*	0.61
78	32.33	1.36	24.80*	1.78

(Crippen & Morrison 1986). Reduced formation of phenolic compounds in shaded fruits reflects the light-dependency of the enzymes' activity involved in their biosynthetic pathway (i.e., phenylalanine ammonia-lyase and dihydroflavonol-reductase) (Treutter 2001). However, in the berry, except for HCAs, the biosynthesis of the identified compounds was not enhanced by increased radiation, likely due to different mass and optical characteristics between berry and fruitstalk.

A different partitioning of phenylpropanoid and flavonoid metabolism in the fruitstalk, in comparison to the berry, is clearly evident. This is particularly obvious for flavonols (Figure 3), which reached their maximum concentrations in berries at day 20, while at the same time they showed a minimum in the fruitstalk. Additionally, flavonoid showed a different behaviour in berries. While epicatechin is declining (Figure 4b), which is in parallel to the pool of HCAs, flavonols are accumulating (Figure 3b).

Sun fruits concomitantly exhibited increased Ca^{2+} and HCAs concentrations showing a poor correlation ($R^2=0.67$) with the berry (Figure 5). Such a result is in agreement with the findings of Awad and de Jager (2002), who reported a similar correlation between chlorogenic acid versus Ca^{2+} concentration in the skin of apple fruit. In view of the widely documented involvement of HCAs in the xylem formation (Douglas 1996, Rozema et al. 1997, Kliebenstein 2004) and the light-induced increase of that compounds (Figure 2), it may be reasonably hypothesized that light-induced increase in HCAs promotes xylem conductance (more or larger vessels) increasing Ca^{2+} accumulation.

This experiment was also designed to determine whether there were differences in phenolic content between berry and fruitstalk. Concentrations of HCAs in the berry decreased by approximately 80%, up to 40 days after fruit-set, in both treatments (Figure 2b). Afterwards, the amounts of these secondary metabolites remained constantly close to 0.5 mg g^{-1} d.wt. until the end of experiment, whilst, in the fruitstalk it increased toward the initial value (Figure 2a). The lack of such enhanced concentration in the berry might correlate with a failure in the secondary growth of the xylem (Biasi & Altamura

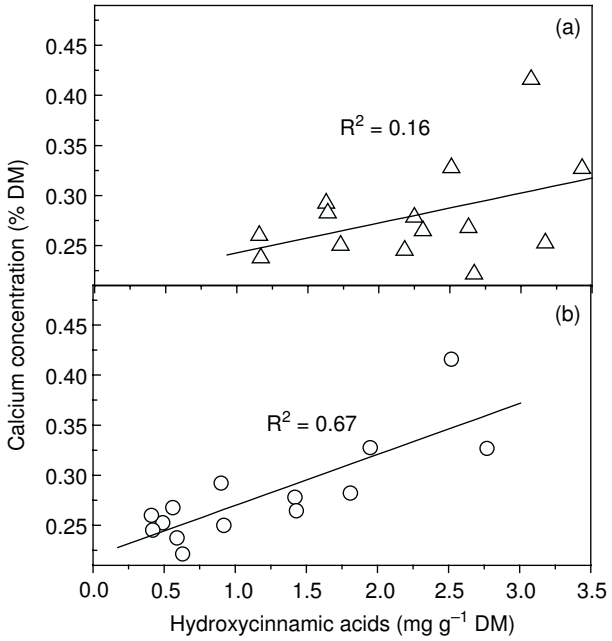

Figure 5. Relationships between hydroxycinnamic acids (mg g^{-1} d.wt.) and fruit Ca concentration (% d.wt.) in fruitstalk (a) and berry (b). Data are from both exposed and shaded fruits.

1996) thus it could be responsible for reduced Ca^{2+} accumulation.

In summary, our results showed which are the predominant phenolic compounds in berry and fruitstalk of young developing kiwifruit; moreover, our findings indicate that the synthesis of phenolic compounds is significantly lowered when the surrounding environment is poorly illuminated. Finally, it is suggested that promoting the interaction between fruits and light during the early 5–6 weeks of fruit growth, for example through canopy management (i.e., by early watersprouts removal) should promote a higher natural occurrence of phenols, especially those (i.e., hydroxycinnamic acids) that are likely chemical prerequisites for fruit quality.

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References

Awad MA, de Jager A. 2002. Relationships between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. Sci Hortic 92:265–276.

Benge JR, Banks NH, Tillman R, Nihal De Silva H. 2000. Pairwise comparison of the storage potential of kiwifruit from organic and conventional production systems. NZJ Crop Hort Sci 28:147–152.

Biasi R, Altamura MM. 1996. Light enhances differentiation of the vascular system in the fruit of *Actinidia deliciosa*. Physiol Plantarum 98:28–35.

Biasi R, Costa G, Manson PJ. 1995. Light influence on kiwifruit (*Actinidia deliciosa*) quality. Acta Hort 379:245–251.

Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. Ann Rev Plant Biol 54:519–546.

Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J Sci Food Agric 85:1715–1724.

Clark CJ, Smith GS. 1988. Seasonal accumulation of mineral nutrients by kiwifruit. II. Fruit. New Phytol 108:399–409.

Crippen DD, Morrison JC. 1986. The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. Am J Enol Vit 37:243–247.

Dawes HM, Keen JB. 1999. Phenolic composition of kiwifruit juice. J Agric Food Chem 47:2398–2403.

Dichio B, Remorini D, Lang A. 2003. Developmental changes in xylem functionality in kiwifruit fruit: Implications for fruit Ca accumulation. Acta Hort 610:191–195.

Douglas CJ. 1996. Phenylpropanoid metabolism and lignin biosynthesis: From weeds to trees. Trends Plant Sci 1:171–178.

Ferguson AR. 1984. Kiwifruit: A botanical review. Hort Rev 6:1–64.

Ferguson IB, Thorp TG, Barnett AM, Boyd LM, Triggs CM. 2003. Inorganic nutrient concentrations and physiological pitting in 'Hayward' kiwifruit. J Hortic Sci Biotechnol 78(4):497–504.

Ferguson IB. 1980. Movement of mineral nutrients into the developing fruit of the kiwifruit (*Actinidia chinensis Planch*.). NZ J Agric Res 23:349–353.

Feucht W, Treutter D. 1999. The role of flavan-3-ols and proanthocyanidins in plant defence. In: Inderjit, Dakshini KMM, Foy CL, Principles and practices in plant ecology. Boca Raton, FL: CRC Press. pp 307–338.

Gitz III DC, Liu-Gitz L, McClure JW, Huerta AJ. 2004. Effects of a PAL inhibitor on phenolic accumulation and UV-B tolerance in *Spirodela intermedia* (Koch.). J Experim Bot 55:912–927.

Grand C, Boudet AM, Ranjeva R. 1982. Natural variations and controlled changes in lignification processes. Holzforschung 36:217–223.

Greaves TJ, McGhie TK, Piller GJ, Meekings JS, Reglinski T, Klages K, Boldingh HL. 2001. Carbon status during leaf development in kiwifruit and subsequent resistance of wounded tissue to *Botrytis cinerea*. Crop Protection 20:553–560.

Ho LC, White PJ. 2005. A cellular hypothesis for the induction of Blossom-End Rot in tomato. Ann Bot 95:571–581.

Hopping ME. 1977. Effect of light intensity during cane development on subsequent bud break and yield of 'palimino' grape vines. NZ J Experim Agric 5:287–290.

Ippolito A, Lattanzio V, Nigro F, Di Venere D, Linsalata V, Lima G, Castellano MA, Salerno M. 1997. Mechanisms of resistance to *Botrytis cinerea* in wounds of cured kiwifruits. Acta Hort 444:719–724.

Jackson JE, Palmer JW, Perring MA, Sharples RO. 1977. Effects of shade on the growth and cropping of apple trees. III. Effects on fruit growth, chemical composition and quality at harvest and after shortage. J Hortic Sci 52:267–282.

Jackson JE. 1980. Light interception and utilization by orchard systems. Hortic Rev 2:208–267.

Kliebenstein DJ. 2004. Secondary metabolites and plant/environment interactions: A view through *Arabidopsis thaliana* tinged glasses. Plant Cell Environ 27:675–684.

McLaughlin SB, Wimmer R. 1999. Ca physiology and terrestrial ecosystem processes. New Phytol 142:373–417.

Montanaro G, Dichio B, Xiloyannis C, Celano G. 2006. Light influences transpiration and Ca accumulation in fruit of kiwifruit plants (*Actinidia deliciosa* var. *deliciosa*). Plant Sci 170:520–527.

Park YS, Jung ST, Kang SG, Drzewiecki J, Namiesnik J, Haruenkit R, Barash D, Trakhtenberg S, Gorintstein S. 2006. *In vitro* studies of polyphenols, antioxidants and other dietary indices in kiwifruit (*Actinidia deliciosa*). Int J Food Sci Nutr 57(1–2):107–122.

Poovaiah BW, Glenn GM, Reddy ASN. 1988. Ca and fruit softening: Physiology and biochemistry. Hortic Rev 10:107–152.

Rozema J, van de Staij J, Bjorn LO, Caldwell M. 1997. UV-B as an environmental factor in plant life: Stress and regulation. Trees 12:22–28.

Snelgar WP, Hopkirk G. 1988. Effect of overhead shading on yield and fruit quality of kiwifruit (*Actinidia deliciosa*). J Hortic Sci 63(4):731–742.

Thorp TG, Ferguson IB, Boyd LM, Barnett AM. 2003. Fruiting position, mineral concentration and incidence of physiological pitting in 'Hayward' kiwifruit. J Hortic Sci Biotechnol 78(4):505–511.

Thum KE, Shasha DE, Lejay LV, Coruzz GM. 2003. Light- and carbon-signaling pathways. Modeling Circuits of Interactions. Plant Physiol 132:440–452.

Treutter D. 1989. Chemical reaction detection of cathechins and proanthocyanidins with 4-dimetylaminocinnamaldehyde. J Chromatogr 467:185–193.

Treutter D. 2001. Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Reg 34:71–89.

Treutter D. 2006. Significance of flavonoids in plant resistance: A review. Environ Chem Lett 4(3):147–157.

White PJ, Broadley MR. 2003. Ca in plants. Ann Bot 92:487–511.

Wurms KV, George MP, Lauren DR. 2003. Involvement of phenolic compounds in host resistance against *Botrytis cinerea* in leaves of the two commercially important kiwifruit (*Actinidia chinensis* and *A. deliciosa*) cultivars. NZ J Crop Hortic Sci 31:221–233.