Fruit Calcium Content in Relation to Phenolic Compounds in Stalk and Berry of Young Developing Fruits of *Actinidia deliciosa* var. *deliciosa*

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Abstract

The pattern of phenolic compounds in both berry and fruit stalk of kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*), in relation to calcium (Ca) accumulation was studied. Fruit were collected weekly during the early stages of fruit growth, Ca was determined by atomic absorption spectroscopy and phenolics using high performance liquid chromatography techniques. Ca concentrations fell to a minimum value of 0.22% DM by 30–40 days after fruit-set. The predominant phenolic compounds were hydroxycinnamic acids, flavonols and the flavan 3-ol, epicatechin. Generally, phenols reached their lowest concentrations correspondingly to the Ca decrease. Thereafter, only hydroxycinnamic acids in the stalk showed an increase, suggesting that in the berry secondary xylem formation did not occur, thereby accounting for the early cessation of Ca imports into the fruit.

INTRODUCTION

The mineral composition of fruit is significant in determining fruit quality. Low concentrations of calcium (Ca) in fruit have been associated with physiological disorders in a number of species including kiwifruit (Ferguson and Watkins, 1989; Saure, 1996; Ho and White, 2005; Thorp et al., 2003; Ferguson et al., 2003).

Certain minerals, notably Ca, are well known to be transported only in the xylem (McLaughlin and Wimmer, 1999); weak transpiration of fruit and restricted mobility of Ca in the phloem sap can explain why Ca concentrations are so low in fruit compared with other organs (Clark and Smith, 1988; Xiloyannis et al., 2001). In addition, it has been shown that the xylem in the fruit of kiwifruit becomes progressively dysfunctional during development (Dichio et al., 2003) at the same time as Ca import into the fruit stops (Montanaro et al., 2006). However, little information is available on xylogenesis processes in fruit in relation to Ca, particularly in the first 5-6 weeks following fruit-set during which approximately 80% percent of total fruit Ca is imported (Xiloyannis et al., 2001; Montanaro et al., 2006).

Lignin is a major structural component of xylem tissues (Douglas, 1996) and it is biosynthesised by the phenylpropanoid pathway via hydroxycinnamic acids. Thus it is reasonable to consider the occurrence of these secondary metabolites which are directly involved in xylem in fruit becoming dysfunctional. This work reports preliminary results on the lignification process in fruit in relation to Ca accumulation. Using a biochemical approach the formation of phenolic compounds which are, at least in part, chemical prerequisites of xylogenesis in kiwifruit stalks and berry were investigated.

MATERIALS AND METHODS

Trials were carried out in Southern Italy (Metaponto, N 40°20' E 16°48') on mature own-rooted kiwifruit plants (*Actinidia deliciosa* var. *deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson) ('Hayward', "Pergola" system, 494 plants ha^{-1}) during the 2004 season. The vines were regularly microjet-irrigated; blooming occurred during the last 10 days of May, and bee pollination ensured normal and simultaneous fruit-set by 30 May.

Twenty-five fruit (including stalk) were collected weekly from 5 selected vines by

week 1 to week 11 after fruit-set. Ten of the 25 fruit were weighed (fresh weight) immediately, and fruit dry matter (DM) (skin plus flesh) was determined after 48 h drying (60°C). Fruit were aggregated into three bulk samples, each of which was analysed for calcium. Ca concentration (skin plus flesh) was measured on acid digested samples (skin plus flesh) (H_2SO_4 + HNO_3) using an atomic absorption spectrophotometer (Varian, AA-40).

The remaining 15 fruit were immediately sliced, frozen (-80°C) lyophilised and the skin was thoroughly scraped off, except for the week 1 samples. For the analysis of both berry and stalk, fruit were grouped and 5 bulk samples. The dry powder was extracted with methanol (100%) containing naringenin (0.1mg ml^{-1}) as internal standard for 30 min in a water bath at 4°C during sonication. Methanolic aliquots were directly used for HPLC analyses (Kontron chromatograph, HyperClone ODS C18 column 150 x 4.60 mm; 3 μm) and phenols were eluted using 5% aqueous formic acid (solvent A) and methanol (solvent B) as solvents. Epicatechin was quantified using the postcolumn derivatisation method described by Treutter (1989).

Phenolic compounds were identified according to their UV absorbance spectra and retention time.

RESULTS AND DISCUSSION

During the first 20 days of fruit growth, fruit Ca concentrations decreased, falling rapidly from 0.33% DM to 0.24% DM (Fig. 1), and then continuing to decline until day 79 after fruit-set reaching 0.22% DM. These results confirm the importance of this stage of fruit growth for Ca accumulation (Xiloyannis et al., 2001).

The predominant phenolic compounds were flavonols, hydroxycinnamic acids and the flavan 3-ol, epicatechin, in both stalk and berry. In this preliminary study fruit stalks contained high concentrations of hydroxycinnamic acids (2.5 mg g^{-1} DM) just few days after fruit-set but these fell to a minimum of 1.1 mg g^{-1} DM at day 25 (Fig. 2). Subsequently, hydroxycinnamic acids concentrations increased to 3.0 mg g^{-1} DM at the end of week 7 after fruit-set, thereafter remaining unchanged at 2.7 mg g^{-1} DM in the next two samples. In the berry, hydroxycinnamic acids concentration was 1.95 mg g^{-1} DM just after fruit-set (Fig. 2), but subsequently declined to 0.5 mg g^{-1} DM by day 40 after fruit-set. In the berry, similarly the concentration of epicatechin fell starting at 9.5 mg g^{-1} DM then declining to a concentration of approximately 1μg g^{-1} DM at day 39 after fruit-set (Fig. 3). In the stalk epicatechin remained unchanged around 7 mg g^{-1} DM in the last samples.

Concentrations of Ca and some phenolic compounds in kiwifruit fruit change during the early stages of development. In general, except for epicatechin in the stalk, the pattern of phenolic compounds decreased within the first 30 days after fruit-set (Fig. 4). In particular, concentrations of hydroxycinnamic acids and epicatechin of the berry rapidly declined reaching their lowest values 40 days after fruit-set (Figs. 2 and 3). These observations fit with other findings concerning physiological and structural aspects of the fruit. The substantial reduction in concentration of phenolics by week 8 occurred at the same time as transpiration is blocked and the fruit vascular system becomes permanently dysfunctional, both of which occur about 8 weeks after fruit-set (Dichio et al., 2003; Smith et al., 1995; Xiloyannis et al., 1999; Montanaro et al., 2006). Furthermore, collapse of the fruit external layer and development of a suberized periderm occur during the same period (Smith et al., 1995; Xiloyannis et al., 2001). Thus, it is confirmed that the early stage of fruit development is crucially important for many physiological and biochemical changes.

This study was designed to investigate the pattern of phenolic compounds in fruit analysing separately the berry and the stalk. Biosynthesis of phenolic compounds may be relevant for xylem formation being beneficial for Ca flow into developing fruits. Ca accumulation appears to be related to the abundance of these phenolics (Fig. 5) in the berry (R^2 = 0.84). For apples, it has been postulated that dysfunction of vascular tissues within the pome is responsible for low Ca import (Lang and Ryan, 1994), while in

kiwifruit the phenomenon is still unclear since observations on the breakdown of the xylem conductivity are for entire fruit (Dichio et al., 2003). Within 40 days of fruit-set in kiwifruit, concentrations of hydroxycinnamic acids, known precursors of lignin (Grand et al., 1982; Douglas, 1996), fell by approximately 80% and 60% in berry and stalk, respectively. Thereafter, hydroxycinnamic acids increased significantly only in the stalk; this may be associated with secondary growth of the xylem (Biasi and Altamura, 1996). However, even though hydroxycinnamic acids concentrations in the stalk increased, Ca concentrations in fruit remained unchanged.

CONCLUSIONS

The results in this paper show that in the kiwifruit berry approximately 30–40 days after fruit-set, there appears to be no further synthesis of the phenolics, such as hydroxycinnamic acids, that are thought to be involved in xylogenesis. It is therefore suggested that the lack of secondary xylem formation in the conducting tissues in the berry may be associated with the permanent dysfunction of xylem in the fruit, thereby accounting for the cessation of calcium influx. Further work is required to investigate the possible antioxidant activity of the phenolics involved in xylogenesis.

ACKNOWLEDGEMENTS

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Figures

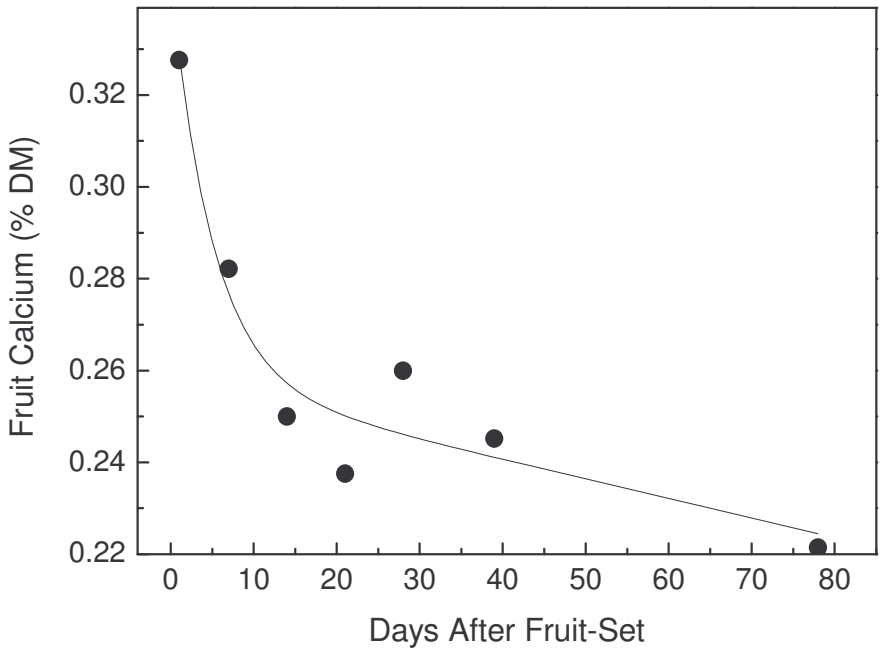

Fig. 1. Fruit calcium concentration (% DM) during the first 11 weeks of fruit growth in kiwifruit. Each point is the mean of three analyses.

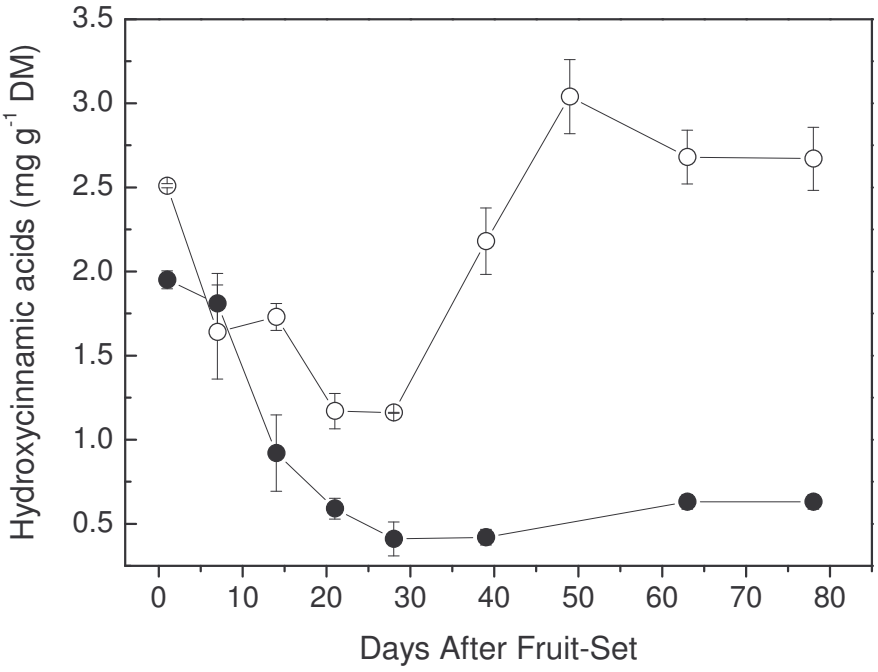

Fig. 2. Hydroxycinnamic acids (mg g^{-1} DM) (± SE) in berry (•) and stalk (o) during the first 11 weeks of fruit growth in kiwifruit. Each point is the mean of five analyses.

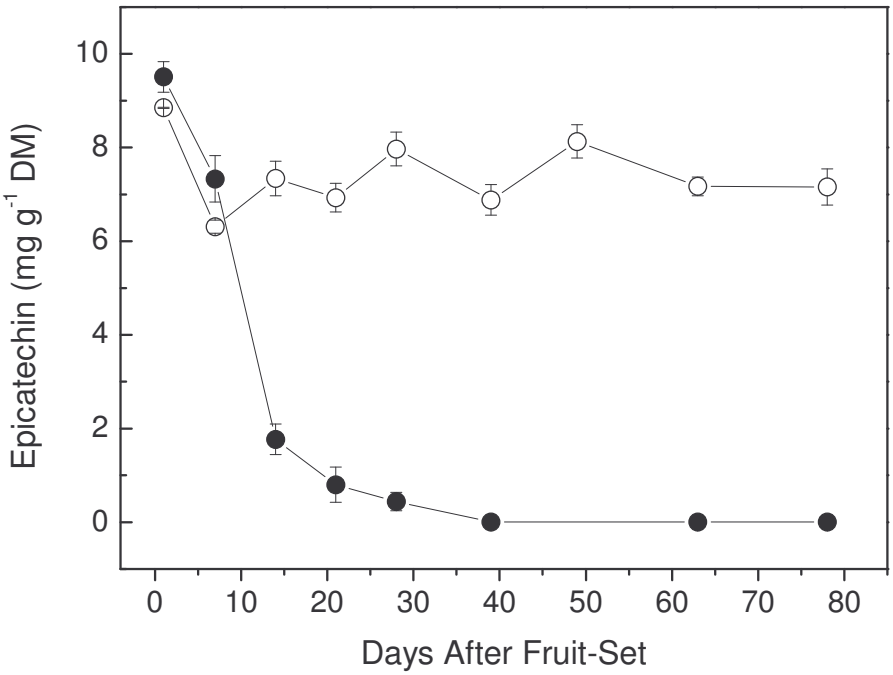

Fig. 3. Epicatechin (mg g^{-1} DM) (± SE) in berry (•) and stalk (o) during the first 11 weeks of fruit growth in kiwifruit. Each point is the mean of five analyses.

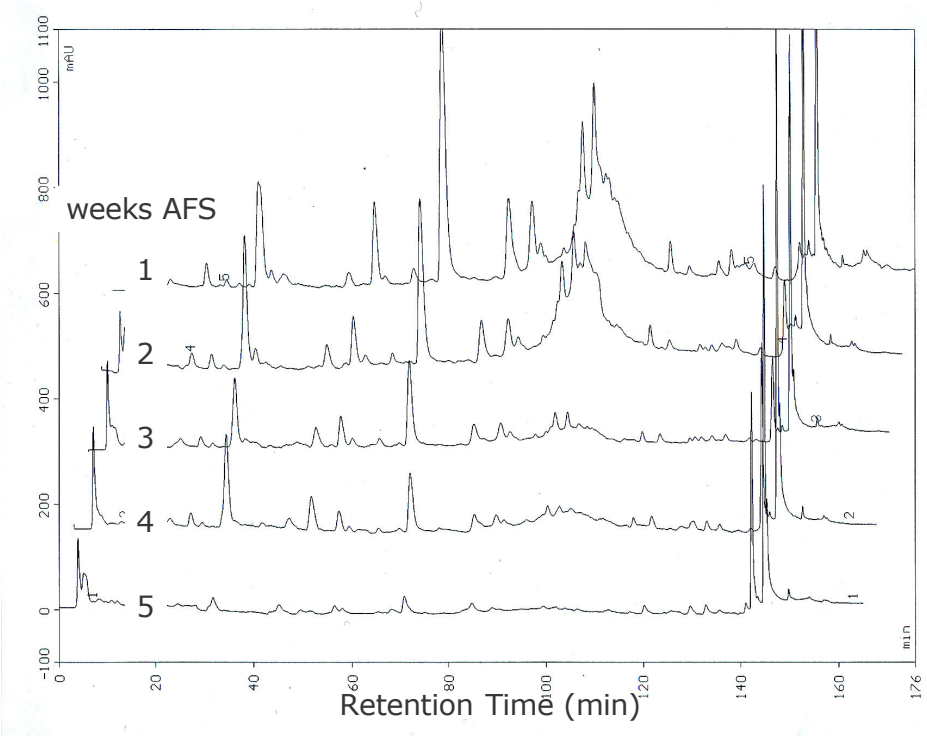

Fig. 4. HPLC elution chromatograms of phenolic compounds in kiwifruit (berry) during the first five weeks after fruit-set (AFS).

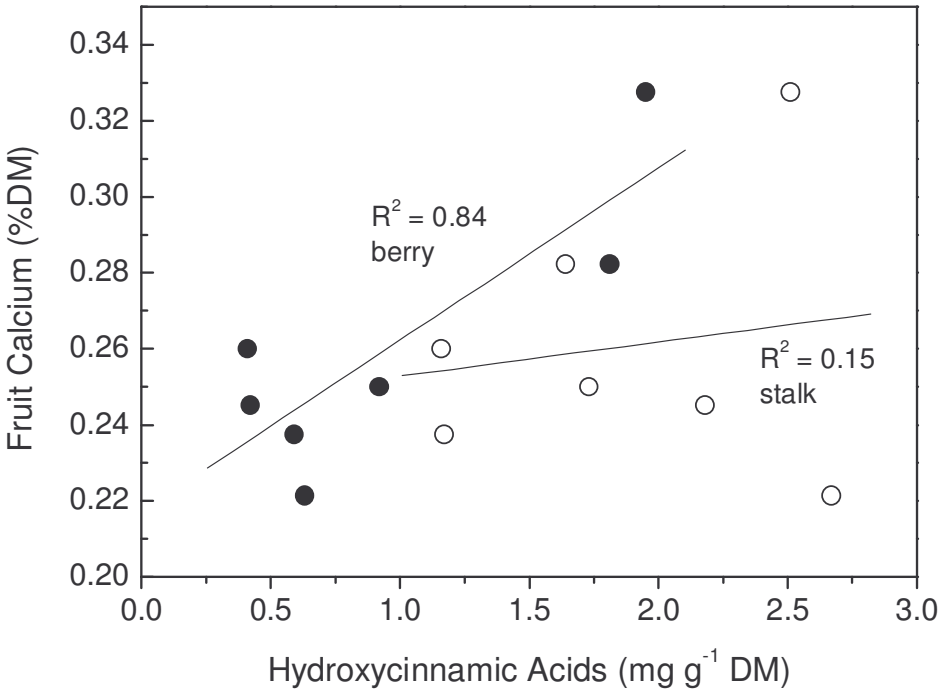

Fig. 5. Relationship between kiwifruit calcium concentration (% DM) and hydroxycinnamic acids (mg g^{-1} DM) in berry (•) and stalk (o) during the first 11 weeks of fruit growth.