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Fruiting position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg and Ca in the flavedo

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ABSTRACT

Adequate K, Mg and Ca supply is important to develop well-structured and functional cell walls and membranes in fruit, and insufficient levels or imbalances of these minerals are known to be involved in various postharvest disorders. Microclimatic variation exists in the 'Nules Clementine' mandarin tree canopy and results in lower photosynthetically active radiation (PAR) levels and temperature as well as a higher humidity inside the tree canopy. The aim of this experiment was to determine the impact of this variation in microclimate on accumulation patterns of K, Ca and Mg in the flavedo of the fruit rind during stages II and III of fruit development. Fruit mass, dimensions, rind colour development and mineral composition of the flavedo were measured to describe the condition of fruit borne on the outside and inside of the tree's canopy. The data revealed that canopy position influenced mineral nutrient accumulation patterns in the flavedo. Outside fruit flavedo accumulated significantly higher concentrations of Ca and Mg in all three seasons (2005–2007). In contrast, inside fruit flavedo (shaded fruit) accumulated significantly higher levels of K compared with outside fruit flavedo. The accumulation of K and Ca differed from that of kiwifruit and apple in that Ca concentration increased and K decreased towards maturity. These results suggest that xylem, as in citrus leaves, is the main vasculature supply conduit to the citrus fruit flavedo for mineral nutrients. The reduction of transpiration potential by lower temperatures and higher humidity inside the canopy could be responsible for the reduced accumulation of Ca and Mg. The high K concentration of inside fruit flavedo is suggested to be a stress response, due to the low light levels, to maintain osmotic potential in the shaded rind tissue, and this imbalance could possibly lead to a reduction in rind condition, which manifests through rind breakdown symptom development.

1. Introduction

A citrus tree develops a dense canopy of leaves that results in microclimatic variation within the canopy, e.g. temperature (Barry et al., 2000), vapour pressure deficit (VPD) (Syvertsen and Albrigo, 1980) and photosynthetically active radiation (PAR) (Greene and Gerber, 1967; Jahn, 1979). These factors are also known to influence water movement and fruit tissue water content (Kaufmann, 1970), as well as photosynthesis (Jahn, 1979) in citrus trees. In addition, a relationship exists between areas in the canopy with high light levels and temperatures and fruit with high juice and soluble solids content (Reitz and Sites, 1948; Barry, 2000; Morales et al., 2000). The higher juice and soluble solids content of exposed fruit are thought to be the result of a more rapid rate of development, and therefore higher sink–source ratio, compared to partially shaded fruit (Barry et al., 2000), which could be seen as. Although higher air temperature in the exposed canopy results in higher sugar content than shaded canopy positions, it is thought that the additional effect of higher PAR, although not quantified (Sites and Reitz, 1949), and water stress (Syvertsen and Albrigo, 1980) of exposed fruit will in particular play an important role in causing differences in juice soluble solids content between canopy positions (Barry et al., 2000).

However, canopy microclimate not only impacts on differences in fruit internal quality, but also on differences in accumulation of mineral nutrients in leaves and fruit (Koo and Sites, 1956; Fallahi and Moon, 1989). It can be concluded from these studies that leaves and fruit positioned inside the dense citrus canopy have higher levels of N, P and K compared with leaves and fruit borne on the outside. In contrast outer, more exposed leaves and fruit have higher Ca and, to a lesser extent, Mg levels. This variation of mineral nutrient (and other solutes) distribution within the citrus tree canopy could be argued to depend on microclimatic factors, e.g. temperature, light and relative humidity within the canopy, as these factors affect water movement – the mobile phase of all solutes – in the xylem and phloem vascular bundles. Water movement in the xylem, and

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the associated mineral nutrient transport, is generally accepted to occur due to evaporative water loss from the leaves, driven by a VPD gradient pulling water and therefore solutes towards the transpiring leaf surface (Fisher, 2000).

Transpiration rate can also be influenced by fruit anatomical changes, e.g. declining conductance in apple fruit stalks (*Malus domestica* Borkh.) (Lang and Ryan, 1994) and xylem discontinuation in some grape cultivars (*Vitis vinifera* L.) (Coombe and McCarthy, 2000). In *Citrus* fruit the possible plugging of stomata by the development of surface wax, as well as the reduction in stomata per unit surface area, are thought to influence fruit transpiration (Albrigo, 1972). However, fluctuations in transpiration rate do not influence all mineral nutrients equally and, whereas Ca movement is significantly reduced, K and Mg movement are not drastically affected by a reduction in transpiration rate (Marschner, 1995).

Phloem-facilitated transport of mineral nutrients follow Munch-pressure flow dynamics between the source (leaf) and sink (fruit) (Patrick, 1997). However, the difference in mobility of mineral nutrients in the phloem results in high concentrations of K and Mg and very low concentrations of Ca and B being transported (Bukovac and Wittwer, 1957). This occurrence is suggested to be due to the variation in element solubility in the phloem sap (Tromp, 2005).

A fruit's mineral concentration can therefore be a function of either xylem or phloem supply, although a shift from xylem towards phloem transport is thought to occur in apple fruit (Lang, 1990). In contrast to most other fruit types, citrus fruit consists of a fleshy pulp and a leathery rind which are hydrolytically separated (Koch and Avigne, 1990). The accumulation of mineral nutrients in citrus fruit pulp follows, to a large extent, the same pattern as apple (Ferguson and Watkins, 1983) and kiwifruit (*Actinidia deliciosa* Chev.) (Clark and Smith, 1988), whereby Mg concentrations increase towards fruit maturity. In contrast, citrus rind has a high Ca concentration and lower K at maturity. This pattern is thought to be due to different contributions by the xylem and phloem towards these fruit parts (Storey and Treeby, 2000).

Mineral nutrient status and accumulation of especially Ca, K and Mg in various fruit types have been closely associated with postharvest disorders, although not necessarily as the underlying mechanism, in citrus [*Citrus sinensis* (L.) Osb] (Storey and Treeby, 1994), avocado (*Persea americana* Mill.) (Van Rooyen and Bower, 2005), apple (Ferguson and Watkins, 1983), tomato (*Solanum lycopersicum* Mill.) (Del Amor and Marcelis, 2006) and kiwifruit (Ferguson et al., 2003). The influence of position within the tree or vine has also been suggested to influence fruit sensitivity to postharvest physiological disorders due to the impact on mineral nutrient allocation and eventual composition in plum (*Prunus salicina* Lindl.) (Taylor et al., 1993) and kiwifruit (Thorpe et al., 2003).

'Nules Clementine' mandarin fruit (*C. reticulata* Blanco.) develops a progressive rind disorder, called rind breakdown (RBD), which is more prevalent in fruit flavedo that develop under low light conditions. It is suspected that a weak rind condition due to lower pigment and carbohydrate content in the flavedo of fruit from the inside part of the tree canopy could result in increased susceptibility to RBD (Cronje et al., 2011). However, no studies have been done on the influence of canopy position on the K, Mg and Ca contents in the flavedo of 'Nules Clementine' mandarin. The aim of this experiment was to determine if K, Mg and Ca accumulation in the flavedo is influenced by canopy position (sun exposed vs. shaded) during the period after physiological fruit drop which coincides with developmental stages II and III (Bain, 1958). It is hypothesised that the citrus flavedo receives most of its mineral nutrients via the xylem and consequently the accumulation rate will be affected by microclimatic factors affecting transpiration rate.

2. Materials and methods

2.1. Sites and plant material

The experiment was conducted in two orchards of 'Nules Clementine' mandarin budded on Carrizo citrange {[*Poncirus trifoliata* (L.) Raf.] × [*Citrus sinensis* (Osb.) L.]} rootstock. In the 2005 and 2007 seasons, fruit were sampled from the orchard at the University of Stellenbosch experimental farm, Western Cape province, South Africa, whereas in 2006, a commercial orchard in the Paarl area, 20 km from the experimental farm was used. This was necessary due to low crop load on the experimental farm orchard in the 2006 season. Both these orchards were planted with a north–south row orientation. The Stellenbosch orchard was planted in 1991 at a spacing of 4.5 m × 2.5 m, and the Paarl orchard in 1993 at a spacing of 5 m × 3 m.

2.2. Treatments, fruit sampling and data collection

Eight adjacent single-tree replicates ($n = 8$) were used from which 25 inside and 25 outside fruit per replication tree of each treatment were sampled monthly, commencing after physiological fruit drop and coinciding with stages II and III of fruit development (January–May) (Bain, 1958). The 25 fruit from a tree made up one replicate. The treatments were two canopy positions, i.e. outside (90–100% of full sunlight) or inside (<80% of full sunlight). The outside fruit were picked from the most sun-exposed side of the canopy (east side) and the inside fruit were picked from the inside of the canopy (out of direct sunlight). In both cases fruit were sampled from a height of 1–2 m.

At commercial harvest, eight replicates of 25 fruit each from the inside and outside canopy position were picked from the same trees and transported to a commercial packhouse where they were drenched (thiabendazole 1000 mg L^{-1}; guazatine 500 mg L^{-1}; 2.4-D sodium salt 250 mg L^{-1}) before receiving all standard commercial packhouse treatments [thiabendazole, 500 mg L^{-1}; imazalil, 500 mg L^{-1}; 2,4-dichlorophenoxyacetic acid, 125 mg L^{-1}, and polyethylene citrus wax application (Citrushine®, Johannesburg, South Africa)]. These fruit were stored at 7.5 °C and scored for rind breakdown incidence after 14 weeks.

To quantify the microclimate in the canopy, light profile measurements were recorded during 2005 in the top, middle and below the leaf canopy of eight trees. This measurement was only done once to determine the different light levels in a canopy between the inside and outside and not to document these change during the day or season. The measurements were done between 10 h and 12 h on a clear day on 21 January 2005 using a light meter (Li-250 light meter with a Li-190SA quantum meter, Li-COR, Lincoln, NE, USA), which took point measurements integrated over 15 s. The 80-cm long probe, consisting of 80 individual light meters, was divided into eight 10-cm zones from which the average values were plotted. Two TempTale4/humidity data loggers (Sensitech Inc., Beverly, MA, USA), measuring temperature and humidity were placed in one tree in outside (eastern side of canopy) and inside (next to trunk) position to quantify the climatic variables in the canopy.

Rind colour of each fruit was determined during the monthly sampling (January, February March, April and May), using a chromameter (Minolta NR 4000, Osaka, Japan). Fruit dimensions (length and diameter) and weight were measured prior to removing the flavedo with a zester. During 2006, the pedicel diameter of each fruit was measured at each monthly sampling (except in March). The flavedo of the 25 inside and 25 outside fruit per replicate tree was pooled for every monthly sampling date to ensure that there was enough material for analysis. The flavedo was frozen in liquid nitrogen whereafter it was freeze-dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and stored at −80 °C.