Journal of the Science of Food and Agriculture, Volume 99, Issue 8, June 2019, pp. 4142-4149
DOI: 10.1002/jsfa.9644

Investigating the involvement of ABA, ABA catabolites and cytokinins in the susceptibility of 'Nules Clementine' mandarin to rind breakdown disorder

Lembe Samukelo Magwaza [a,b,c], Maria del Carmen Alamar [c], Samson Zeray Tesfay [a], Asanda Mditshwa [a], Umezuruike Linus Opara [d, e], Leon Alexander Terry [c*]

[a] Discipline of Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, South Africa

[b] Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, South Africa

[c] Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

[d] Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Science, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa

[e] Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Food Science, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa

* Corresponding author: Leon A. Terry

Email: l.a.terry@cranfield.ac.uk

Published by Wiley. This is the Author Accepted Manuscript issued with: Creative Commons Attribution Non-Commercial License (CC:BY:NC 4.0).
The final published version (version of record) is available online at DOI:10.1002/jsfa.9644. Please refer to any applicable publisher terms of use.

Abstract

BACKGROUND: 'Nules Clementine' mandarin was used to investigate the potential involvement of endogenous plant hormones in mediating citrus fruit susceptibility to rind breakdown disorder (RBD). The effect of light exposure (*viz*. canopy position and bagging treatments) on the endogenous concentration of ABA, 7'hydroxy-abscisic acid (7-OH-ABA), ABA-glucose ester (ABA-GE) and dihydrophaseic acid (DPA), and *t*-zeatin was tested using four preharvest treatments: outside, outside bagged, inside and inside bagged. Phytohormones concentration was evaluated during 9 weeks of postharvest storage at 8 °C. **RESULTS:** The shaded fruit inside the canopy had the highest RBD score (0.88) at the end of postharvest storage, while sun-exposed fruit had the lowest score (0.12). Before storage, ABA concentration was lowest (462.8 μg kg^{-1}) for inside fruit, and highest in outside bagged fruit (680.5 μg kg^{-1}). Although ABA concentration suddenly increased from the third week, reaching a maximum concentration of 580 μg kg^{-1} at week 6 in fruit from inside position, it generally reduced 1.6-fold ranging from 240.52 to 480.65 μg kg^{-1} throughout storage. The increase of 7-OH-ABA was more prominent in fruit from inside canopy. Overall, the concentration of ABA-GE increased 3-fold with storage time. DPA concentration of bagged fruit from inside canopy position was significantly higher compared to outside fruit. The lower ABA-GE and higher DPA concentration in inside bagged fruit throughout storage also coincided with higher RBD. **CONCLUSION:** The strong positive correlations between 7-OH-ABA, DPA and RBD incidence demonstrated that these ABA catabolites could be used as biomarkers for fruit susceptibility to the disorder.

Keyword: Senescence; citrus; rind quality; postharvest stress; chilling injury

INTRODUCTION

The marketability of fresh citrus fruit is highly dependent on external appearance, and rind quality is a decisive factor for consumer acceptance.[1-3] However, citrus fruit are susceptible to several rind physiological disorders during postharvest storage at temperatures below 12 °C, reducing rind quality and causing major economic loss to the industry,[4-8] which can reach up to 60% of total production in the worst season.[9] These physiological disorders affect the rind and, in general, do not compromise the edible internal portion of the fruit; they do, however, decrease postharvest fruit market value of fresh fruit and lead to increased consumer complaints.[8]

A postharvest physiological rind disorder of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) fruit, commonly referred to as rind breakdown (RBD), is among several commercially important defects affecting the citrus industry.[10-12] The susceptibility of fruit to RBD has been hypothesised to be related to rind quality.[2,10,12] Rind quality of citrus fruit is defined as the sum of many physical and biochemical components including primary and secondary metabolites which influence rind susceptibility to disorders.

The biochemical mechanism of the disorder has been extensively investigated by researchers; however, very limited information has been reported in the literature about the endogenous concentration of plant hormones in relation to their involvement in postharvest rind physiological disorders of citrus fruit.[13,14] As a result, the physiological and hormonal mechanisms involved in susceptibility or tolerance of citrus fruit to rind breakdown disorder is not well understood. However, a recent study by Lafuente *et al.*[15] indicated that rind water status is a key factor affecting the susceptibility of citrus to postharvest rind physiological

disorders at low non-freezing temperatures below 12 °C. Furthermore, it has been hypothesised that canopy position may influence rind water balance, water circulation, biosynthesis of plant growth regulators (PGRs), and growth rate of the fruit,[16] thus affecting water and osmotic potentials in the fruit rind.[2,17]

Preharvest abiotic factors such as fruit position within the canopy may modify the endogenous concentrations of phytohormones such as auxins, cytokinins and abscisic acid (ABA).[18,19] Hence, the relationship between canopy position and evolution of phytohormones during the postharvest life of citrus fruit could be an important step towards understanding fruit susceptibility or tolerance to physiological rind disorders. ABA has been implicated in citrus fruit response to water stress, dehydration and senescence.[20-22] It has been found that changes in the rind water status during postharvest handling of citrus fruit increase the incidence of this physiological disorder.[5,23,24] In 'Pinalate' and 'Navelate' mutants of 'Navel' oranges, Alférez *et al.*[13] reported that ABA levels in the flavedo of a non-chilling sensitive mutant had 6-fold lower ABA levels than tolerant fruit. In the 'Pinalate' mutant, ABA deficiency altered water relations in the flavedo, leading to a decrease in water balance, a reduction in rind firmness and a higher incidence of rind staining.[13] This suggests a possible involvement of ABA, and perhaps its catabolites, in the susceptibility of citrus fruit to rind staining and similar postharvest non-freezing physiological disorders. From these observations, it could be hypothesised that ABA levels in the flavedo may be used as an indicator of fruit susceptibility to postharvest rind physiological disorders.

This study investigated the potential involvement of ABA, its catabolites, and cytokinins in mediating fruit susceptibility of 'Nules Clementine' mandarin to RBD. To do this, the study

evaluated the effects of canopy position and light exposure on the kinetics of endogenous phytohormones during postharvest storage and rind senescence.

MATERIALS AND METHODS

Fruit sampling

This study was conducted during the 2011/12 season using 'Nules Clementine' mandarin (*Citrus reticulata* Blanco.) fruit. A total of 15 uniform trees were marked in a commercial orchard at Citrusdal, Western Cape Province, South Africa (32 ° 25 ' 22 " South, 19 ° 00 ' 53 " East). To evaluate the effect of light exposure on endogenous rind hormones during postharvest storage, ten fruit from outside sun-exposed and ten fruit from inside shaded positions of the tree canopy were selected and tagged in January 2012, after physiological drop and about four months before commercial maturity. Half of the selected fruit (n = 5) from inside and outside position of the canopy were covered with brown opaque paper bags without removing or covering subtending leaves. The study, therefore, consisted of four preharvest treatments, namely, outside canopy, outside bagged, inside canopy and inside bagged.

Postharvest treatments and storage

A total of three hundred fruit (4 treatments x 75 fruits per treatment) were harvested at commercial maturity on 16 May 2012 and received packhouse treatments according to industry practices. Fruit were drenched in a mixture of Thiabendazole (500 mg L^{-1}), Imazalil (500 mg L^{-1}) and 2,4-dichlorophenoxyacetic acid (125 mg L^{-1}) (Farmalinx Pty. Ltd., Bondi Junction, Australia) and coated with Citrashine® wax (Citrashine Pty (Ltd), Decco, Johannesburg, South Africa). Thereafter, fruit were transported in a well-ventilated vehicle to

the Postharvest Laboratory at Stellenbosch University (SU), where they were sorted, separately packed in cartons and air-freighted at ambient temperature the next day to the Plant Science Laboratory at Cranfield University (CU) in the United Kingdom. Fruit arrived at CU within 48 hours and were stored for nine weeks in a cold room at a low non-freezing temperature (8 ± 0.5 °C and 95% relative humidity) as described by Magwaza et al.[2]

Sample preparation

For baseline measurements, a total of 20 fruit (five fruit from each preharvest treatment) were selected upon arrival at CU, weighed, peeled and the rind snap-frozen in liquid nitrogen, and stored at -80 °C. Frozen samples were later freeze-dried in a Labogene ScanVac CoolSafe freeze dryer system (CS55-4, Lynge, Denmark) for 7 days at 0.015 kPa and -55 °C. Lyophilised rind samples were ground using a pestle and mortar into a fine powder. To achieve standard particle size, the ground material was sieved through a 1 mm metal sieve. To increase surface area, large particles remaining on the sieve were further ground until all the material passed through the sieve. Ground samples were returned to storage at -80 °C until extraction and further analysis.

Postharvest fruit quality evaluation

During cold storage, samples (n = 5 per treatment) were taken out of storage every third week. During sampling, all fruit were scored for the presence of rind disorders such as chilling injury and rind breakdown. Different rind disorders were scored on a subjective scale from 0 = no defects to 3 = severe defects. Rind breakdown was then expressed as rind

breakdown disorder index as previously described[25, 26] and calculated according to Eq. 1 for chilling injury and peel pitting.

$$\text{Disorder index} = \frac{\Sigma(\text{disorder } (1-3) \text{ x no. of fruit in each class})}{\text{total no. of fruit}} \quad (1)$$

Samples taken from week 0, 3, 6 and 9 were prepared and analysed for concentration of cytokinins, ABA and catabolites (n = 5 per treatment).

Determination of plant hormones

Chemicals and reagents

Deuterated labelled and unlabelled standards, including [$^2H_4$]-ABA (–)-5,8',8',8'-d4-ABA; [$^2H_3$]-phaseic acid (–)-7',7',7'-d_3-phaseic acid (PA); [$^2H_3$]-dihydrophaseic acid (–)-7',7',7'-d_3-dihydrophaseic acid (DPA); [$^2H_5$]-ABA glucose ester (+)-4,5,8',8',8'-d_5-ABA-GE; [$^2H_4$]-7'-hydroxy-ABA (±)-5,8',8',8'-d_4-7'-hydroxy-ABA; (2)-PA; (2)-DPA; (1)-ABA-GE; (6)-7'-hydroxy-ABA, and [$^2H_6$]-ABA (±)-3',5',5',7',7',7'-d6-ABA were obtained from the National Research Council of Canada-Plant Biotechnology Institute (NRCC-PBI); [$^2H_3$]-dihydrozeatin d_3-DHZ and zeatin (±Z) were purchased from OlchemIm Ltd (Olomouc, Czech Republic); (±)-ABA was purchased from Sigma-Aldrich (UK).

Formic acid and methanol HPLC grade solvents used for sample extraction were purchased from Fisher Scientific (Leic, UK). Formic acid, methanol and acetonitrile used for the preparation of mobile phase LC-MS grade were purchased from Fisher Scientific. HPLC grade water used throughout the study was prepared using a Direct-Q3 ultrapure water system (Milipore Inc., Milford, MA, USA). Ammonium hydroxide (28%-30%) was

purchased from Sigma-Aldrich (Dorset, UK). Sep-Pak® vac tC18 SPE cartridges packed with 500 mg of C18 stationary phase bonded on silica gel matrix, and Oasis® MCX cartridges containing 150 mg of sulfonic acid sorbent were purchased from Waters Corporation (Milford, MA, USA).

Extraction and quantification of plant hormones

Plant growth regulators (abscisic acid [ABA], 7'hydroxy-abscisic acid [7-OH-ABA], ABA glucose ester [ABA-GE], dihydrophaseic acid [DPA] and *t*-zeatin [*t*-Z]) were extracted from freeze-dried rind material according to Ordaz-Ortiz *et al*.[27] with some modifications. Briefly, lyophilised and ground rind powder samples (50 ± 0.5 mg) were added into 5 mL of extraction solution comprised of methanol/water/formic acid mixture (75:20:5, v/v/v). After which, 75 mL of 400 µg L^{-1} solution of an internal standard mixture (deuterated labeled compounds d_4-ABA, d_4-7-OH-ABA, d_5-ABA-GE, d_3-DPA and d_3-DHZ was added to the extraction mixture at the beginning of the extraction procedure. The mixture was vortexed for 20 s, and left to cold-extract for 12 h at -20 °C under protection from light and continuous agitation.

After mixing thoroughly, the extracts were centrifuged at 1936 *g* and -4 °C for 15 min . The supernatant was removed and a second extraction step was performed using 2 mL of extraction solvent for 30 min at -20 °C. Following re-extraction, the extracts were reunified into one fraction, the hormones containing the total extract volume (7 mL) were firstly purified using dispersive solid phase extraction (sSPE) (QuEChERS Dispersive SPE Kit for highly pigmented fruits and vegetables, Agilent) previously conditioned with 5 mL of

methanol, equilibrated with 5 mL of 1 M formic acid solution and filtered with 0.20 μm nylon filters.

The eluate was then purged with gaseous nitrogen at room temperature to evaporate the organic phase and then freeze-dried overnight (Scanvac, Lynge, Denmark) in the dark at -95 °C. The remaining residue was reconstituted with 1 mL of 1 M formic acid. The formic acid solution containing all analytes was then loaded into an Oasis cartridge previously conditioned with 5 mL of methanol and equilibrated with 5 mL of 1 M formic acid solution. The formic acid solution was discarded and then the cartridge was eluted with 2 mL of methanol and 2 mL of 0.35 M NH_4OH with 60% (v/v) methanol, the solution passed through containing the plant hormones was collected and freeze-dried overnight (in the dark at -95 °C).

The final dried material was reconstituted with 400 μL of 0.1% formic acid in ultrapure water. To quantify the five plant hormones, five microliters (μL) of the reconstituted sample was injected into an Agilent 6540 Ultra High Definition Accurate Mass Q-TOF LC-MS System using a Dual ESI Agilent Jet Stream source in a negative and positive mode according to Ordaz-Ortiz et al.[27] A ZORBAX Eclipse Plus C18 column, 2.1 x 50 mm diameter and 1.8 μm particle size, (Agilent, CA, USA, part no. 959757-902) coupled with a 2.1 x 5 mm diameter and 1.8 μm ZORBAX Eclipse Plus C18 guard column (Agilent, CA, USA, part no. 821725-901) was used to separate the individual phytohormones. Quantification endogenous phytohormones were carried out using a nine-point calibration curve (5, 10, 25, 50, 75, 100, 150, 300, 900 μg L^{-1} - each calibration solution level containing 75 μg L^{-1} of each internal standard). The concentrations of hormones were presented on dry mass basis.

Statistical analysis

The data collected were subjected to the analysis of variance (ANOVA) using a statistical software (GenStat 17.1, VSN International, Hemel Hempstead, United Kingdom). Fischer's least significant differences were calculated and used to separate means at 5% level of significance. In an attempt to gain a better insight of the interaction between measured PGRs and RBD, the dataset of fruit from different canopy positions was also subjected to chemometric data analysis. This multivariate data analysis was based on the principal component analysis (PCA) and executed using The Unscrambler chemometric software (The Unscrambler® X v10.5, CAMO SOFTWARE AS, Oslo Science Park, Norway).

RESULTS AND DISCUSSION

Incidence of rind breakdown disorder

The occurrence of rind breakdown was significantly affected by the position within the tree canopy and bagging treatment (Table 1). Bagged fruit from the inside position of the tree canopy had the highest score of RBD (0.88) after 9 weeks of storage under non-freezing conditions, this being three-fold higher than the other canopy position. Although overall, the incidence of RBD was low for all four treatments under study; the symptoms of the disorders were first observed on fruit harvested from inside canopy position after 6 weeks of storage and this trend increased with time. The highest increase of RBD occurred from week 6 to week 9, an indication that RBD is a progressive postharvest physiological disorder that increases with storage time but its incidence and severity is highly dependent on preharvest

canopy position and bagging treatments. This observation is similar to results previously reported by Cronje *et al*.[10] and Magwaza *et al*.[2,28] The results further support the hypothesis that microclimate within the tree canopy affects rind quality during fruit development by modifying the accumulation of photosynthates and nutrients. In bagged fruit harvested from the inside canopy position, this has been reported to contribute to the reduced rind osmotic potential, decreased rind condition, which in turn influences fruit response to postharvest stresses associated with senescence and susceptibility to RBD.[28]

Hormone level in mandarin rind

ABA concentration ranged from 250.5 to 680.5 μg kg^{-1} (Fig. 1), which is similar to those previously reported on citrus by Mahouachi *et al*.[29] and Romero *et al*.[30] Results of the analysis of variance showed significant interaction between preharvest canopy position and storage time for 7-OH-ABA (Fig. 2), ABA-GE (Fig. 3) and DPA (Fig. 4), as well as *t*-zeatin (Fig. 5). Despite the observed interaction, it is worth noting that both fruit position within the canopy and postharvest storage time had significant effects on rind concentration of ABA (Fig. 1), its catabolites (Figs. 2, 3 and 4) and *t*-zeatin (Fig. 5).

Upon arrival at the laboratory, the rind ABA concentrations of bagged fruit, both inside and outside the canopy were significantly higher (*ca*. 593.0 – 680.5 μg kg^{-1}) compared to those from the inside canopy position (462.8 μg kg^{-1}). Although the ABA concentration of inside fruit suddenly increased from the third week, reaching a maximum concentration of 560.9 μg kg^{-1} at 6 weeks of storage, it generally reduced 1.6-folds with storage time. ABA plays an important role as an endogenous messenger in biotic and abiotic stress responses.[31] The positive relationship between ABA and dehydration is well known.[21,22]

Studies by Lafuente and Sala[23] and Lafuente et al.[15] have shown that the water status of citrus peel plays a critical role in the development of postharvest rind disorders at low non-freezing temperatures. This was further supported by results reported by Magwaza *et al*.[2], where bagged fruit inside the canopy had higher moisture loss than outside fruit. Similar to the results obtained in the current study, the previous study by Magwaza *et al*.[28] reported that bagged fruit inside the canopy fruit with higher weight loss were more susceptible to RBD (Table 1). The data herein showed that the concentration of ABA at the time of harvest cannot be directly linked with fruit susceptibility to RBD. Other compounds which may protect fruit against physiological disorders such as antioxidants,[32, 33] and ABA catabolites may also play a role. This is in agreement with the results reported by Kawada[34] who showed that grapefruits were resistant to chilling injury even when the ABA concentration was low at the time of harvest.

Although attempts have been made to improve scientific understanding on the effects of ABA on rind physiological disorders development,[13,30,31] the effects of its metabolites has not received attention. In both inside and inside bagged fruit, the concentration of 7-hydroxy abscisic acid (7-OH-ABA), a metabolic product of ABA oxidation,[35] significantly increased almost 2-folds in week 6, although it significantly dropped for inside fruit at week 9 (Fig. 2). Similar to ABA concentration, the increase of 7-OH-ABA was more prominent in fruit samples from the inside canopy position of the tree. The increase in ABA and 7-OH-ABA of inside fruit observed at week 6 coincided with the first symptoms of RBD on the same fruit. After week 6, a 2-fold decline in ABA and 7-OH-ABA was observed in fruit harvested from the inside position of the canopy. This decrease in both ABA and its oxidized metabolite, 7-OH-ABA, observed in week 9 coincided with a 3-fold increase in the incidence of RBD

observed in the same fruit from the inside position of the canopy. In bagged fruit from the inside canopy position, a significant increase in 7-OH-ABA levels observed in week 6 and further maintenance of this level preceded and coincided with the development of the disorder. This may suggest that an increase in the oxidized form of ABA, 7-OH-ABA, during week 6 observed in the fruit harvested from inside position was in response to the development of the disorder.

ABA-GE is another product of the ABA catabolism, which is readily reversible and known to be a storage and transport form of ABA.[36,37] Overall, the ABA-GE concentration doubled with storage time (Fig. 3). However, unlike ABA and 7-OH-ABA, the increase in ABA-GE was continuous throughout the storage period. The significantly lower ABA-GE concentration in both inside and outside bagged fruit throughout storage, compared to unbagged fruit, showed that blocking sunlight exposure reduced its concentration. Before storage, inside fruit with higher RBD had lower ABA-GE concentration compared to outside fruit. Abrupt variations in relative humidity at colour break have been cited as being responsible for RBD development in citrus fruits.[9] Studies by Zeevaart[38] showed that ABA-GE concentration increased during water stress in *Xanthium strumarium* plants. In the present study, ABA-GE increased over time as fruit continued losing moisture and developed RBD. Previous studies by Magwaza *et al*.[2,28] and Olarewaju *et al*.[33] on 'Nules Clementine' mandarin consistently showed that the rind of fruit borne on the outside position of the canopy had significantly higher concentrations of non-structural carbohydrates (sucrose, glucose and fructose) than the fruit from the inside canopy position.[2,28,33] The higher concentration of ABA-GE and 7-OH-ABA observed in fruit from the inside canopy position in the current study is hypothesised to result from lower solute osmotic potential due to the higher osmoregulatory action of sucrose.[2] This suggests a possible link between fruit

position, rind sugar concentration, ABA metabolites and ultimately the development of the disorder.

Abscisic acid can also be catabolized to phaseic acid, which can be further reduced to dihydrophaseic acid (DPA).[35,39] In this study, DPA concentration was significantly influenced by storage time. For all the fruit samples, DPA concentration increased with storage time and flattened after 6 weeks of storage (Fig. 4). Throughout storage, fruit samples from the inside bagged canopy position had higher DPA compared to other samples. Studies by Lin *et al*.[40] demonstrated that exogenous DPA application led to strong hydroxyl radical scavenging activity and increased membrane integrity by inhibiting lipid peroxidation in litchi pericarp. However, in this study, high RBD index coincided with high DPA concentration. Although the biological activity of DPA has been indicated to be low or non-existence,[41] this study suggests that DPA could be playing an important correlative role in RBD development.

Although there is little information available about the effect of light on carotenoids and ABA biosynthesis in citrus fruit,[24,42] the presence of light (both intensity and quality), is one of the most crucial environmental factors influencing carotenoid accumulation in plant tissues.[43] In citrus species, such as sweet oranges and mandarins, light exposure during fruit ripening generally enhances carotenoid accumulation and external fruit colour.[10] In 'Satsuma' and 'Nules Clementine' mandarins, researchers have shown that bagging fruit with paper bags reduced carotenoid formation in the rind resulting in pale yellow fruit.[2,28,44] Inside fruit remained less yellow compared to the orange colour of outside fruit (data not shown), which was similar to results observed by Cronje *et al*.[10] Mature citrus fruit such as oranges accumulate β-carotene, which is responsible for their typical orange-yellowish colour, while

β-cryptoxanthin is also present in mandarin fruit, contributing to the intense orange coloration displayed by some varieties.[3]

Considering that DPA is a catabolic product of ABA, a downstream metabolite of the carotenoid pathway, it is possible that higher concentration of DPA in bagged fruit harvested from the inside canopy position could explain the lower concentration of carotenoids (the precursor of ABA synthesis) in shaded mandarins, previously reported by Cronje *et al*.[10,45] and Magwaza *et al*.[2,28,46] It is, therefore, feasible to think that higher DPA observed in the current study could be a stress-induced synthesis of ABA, which was later catabolized to a biologically inactive downstream product, DPA. The stress-induced response mechanism is also supported by higher postharvest RBD observed on fruit from the inside canopy position (Table 1). It is, therefore, feasible to hypothesise that oxidized catabolites of ABA, namely ABA-GE and 7-OH-ABA may reduce rind breakdown disorder of 'Nules Clementine' mandarins.

Before storage, a clear difference was observed in the concentration of ABA-GE (a storable and transportable form of ABA) between bagged and non-bagged treatments (Fig. 3). Concurrently, inside-bagged and outside bagged treatments showed significant differences in RBD (Table 1). Therefore, it seems that differences in ABA-GE during storage under non-freezing conditions do not affect susceptibility to RBD. However, those fruit with significant lower concentration of ABA-GE at harvest resulted in the highest susceptibility to RBD at the end of the storage.

The rind concentration of *t*-zeatin remained the same from week 0 to week 6 but suddenly decreased from 6 to 9 weeks of storage (Fig. 5). After 6 weeks of storage, except for outside

canopy, all fruit samples had a *t*-zeatin concentration less than 200 µg kg^{-1}, whilst outside canopy fruit had more than 450 µg kg^{-1}. Remarkably, the significantly higher *t*-zeatin concentration corresponded with zero RBD index in outside canopy position at week 6 and the lowest RBD susceptibility at week 9. Although no previous studies have investigated the effect of *t*-zeatin concentration on RBD of citrus fruit, it could be argued that high *t*-zeatin increases membrane integrity and, therefore, reduces RBD incidence.[19,47]

In order to gain better insight on how the measured PGRs correlated with each other and with RBD incidence, the dataset of fruit from different canopy positions was subjected to PCA. The scores (Fig. 6a) and loadings (Fig. 6b) plots showed that the first two principal components contributed 75% to the observed mapping, with first principal component (PC1) and second principal component (PC2) contributing 50% and 25% of the observed patterns, respectively. PCA showed that the concentration of DPA and 7-OH-ABA were positively correlated with RBD, confirming its significant involvement to the development of the disorder. In accordance with observations in Table 1, where inside and inside bagged fruit were more susceptible to RBD, results in the PCA plots (Fig. 6) also showed that fruit from the inside canopy position were located in the same quadrant as RBD, ABA, 7-OH-ABA and DPA, demonstrating a positive correlation and confirming a significant contribution of these factors to the development of the disorder. These results obtained herein however, contradict those reported on 'Marsh' grapefruit where seasonal levels of ABA in the flavedo were positively correlated with resistance to chilling injury.[34] On the other hand, Lafuente *et al*.[48] and Gosalbes *et al*.[49] demonstrated that in 'Fortune' mandarins, treatments that increased ABA levels favoured the development of chilling injury. This may be an indication that the physiological mechanism for rind physiological disorders is cultivar dependent.

CONCLUSION

This study investigated the effects of fruit canopy position and light exposure to elucidate the potential involvement of ABA and its oxidised catabolites, and the cytokinin *t*-zeatin in mediating the susceptibility of 'Nules Clementine' mandarin to RBD. The study demonstrated that bagging fruit during growth decreased the concentration of ABA-GE, the ABA storable and transportable form. Inside and shaded fruit had the highest concentration of ABA catabolites, namely 7-OH-ABA and DPA, and were more susceptible to RBD, supporting a positively regulated biosynthesis of ABA catabolites. These findings demonstrated that the concentration of DPA could potentially be used as a secondary biochemical marker for fruit susceptibility of RBD. The reduction in the concentration of *t*-zeatin after week 6 coincided with the higher levels of ABA-catabolites and rind breakdown disorder demonstrating a possible antagonistic action of these two groups of hormones.

ACKNOWLEDGEMENTS

This work was supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation. Lembe Magwaza's study visit to Cranfield University was partly funded by the Commonwealth Scholarship Commission of the United Kingdom.

REFERENCES

1 Khalid S, Malik AU, Saleem BA, Khan AS, Khalid MS and Amin M, Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of 'Kinnow'

mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). *Scientia Hortic* **135**: 137–144 (2012).

2 Magwaza LS, Opara UL, Cronje PJR, Landahl S and Terry LA, Canopy position effect on rind biochemical profile of 'Nules Clementine' mandarin fruit during postharvest storage. *Postharvest Biol Technol* **86**: 300–308 (2003).

3 Rodrigo MJ, Alquézar B, Alós E, Lado J and Zacarías L, Biochemical bases and molecular regulation of pigmentation in the peel of Citrus fruit. *Scientia Hortic* **163**: 42–62 (2013)

4 Arpaia M and Kader A, Mandarin/Tangerine: Recommendations for Maintaining Postharvest Quality. Postharvest Technology: Research and Information Center. UC-Davis, California (2002).

5 Alférez F, Agustí M and Zacarìas L, Postharvest rind staining in 'Navel' oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. *Postharvest Biol Technol* **28**: 143–152 (2003).

6 Porat R, Weiss B, Cohen L, Daus A and Aharoni N, Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. *Postharvest Biol Technol* **33**: 35–43 (2004).

7 Cajuste JF and Lafuente TM, Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. *Postharvest Biol Technol* **45**: 193–203 (2007).

8 Alquezar B, Mesejo C, Alferéz A, Agustí M and Zacarìas L, Morphological and ultrastructural changes in peel of 'Navelate' oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. *Postharvest Biol Technol* **56**: 163–170 (2010).

9 Agustí M, Almela V, Juan M, Alferéz F, Tadeo FR and Zacarìas L, Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. *Ann Bot* **88**: 415–422 (2001).

10 Cronje PJR, Barry GH and Huysamer M, Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. *Postharvest Biol Technol* **60**: 192–201 (2011).

11 Cronje PJR, Barry GH and Huysamer M, Fruit position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg, and Ca in the flavedo. *Scientia Hortic* **130**: 829–837 (2011).

12 Magwaza LS, Opara UL, Cronje PJR, Terry LA, Landahl S and Nicolaï BM, Non-chilling Physiological Rind Disorders in Citrus Fruit. *Hort Revs* **41**, 131–176 (2013).

13 Alférez F, Sala JM, Sánchez-Ballesta MT, Mulas M, Lafuente MT and Zacarias L, A comparative study of the postharvest performance of an ABA-deficient mutant of oranges: I. Physiological and quality aspects. *Postharvest Biol Technol* **37**: 222–231. (2005).

14 Sevillano L, Sanchez-Ballesta T, Romojaro F and Flores FB, Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *J Sci Food Agric* **89**: 555–573 (2009).

15 Lafuente MT, Establés-Ortíz B and González-Candelas L, Insights into the molecular events that regulate heat-induced chilling tolerance in citrus fruits. *Frontiers Plant Sci* **8**: 1138. DOI: 10.3389/fpls.2017.01113 (2017).

16 Alferez F, Alquezar B, Burns JK and Zacarias L, Variation in water, osmotic and turgor potential in peel of 'Marsh' grapefruit during development of postharvest peel pitting. *Postharvest Biol Technol* **56**: 44–49 (2010).

17 Ben-Yehoshua SJ, Peretz R, Moran B, Lavie JJ and Kim JJ, Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti' oranges (*Citrus sinensis* Osbeck). Postharvest Biol Technol **22**: 19–27 (2001).

18 Khan MIR, Asgher M and Khan NA, Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). *Plant Physiol Biochem* **80**: 67–74 (2014).

19 Egamberdieva D, Wirth SJ, Alqarawi AA, Abd-Allah EF and Hashem A, Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Front Microbiol* **8**: 2104. DOI:10.3389/fmicb.2017.02104 (2017).

20 El-Otmani M, Lovatt CJ, Coggins Jr CW and Agustí M, Plant growth regulators in citriculture: factors regulating endogenous levels in citrus tissues. *Crit Rev Plant Sci* **14**: 367–412 (1995).

21 Bartels D and Sunkar R, Drought and salt tolerance in plants. *Crit Rev Plant Sci* **24**: 23-58 (2005).

22 Shinozaki K and Yamaguchi-Shinozaki K, Gene networks involved in drought stress response and tolerance. *J Exp Bot* **58**: 221–227 (2007).

23 Lafuente MT and Sala JM, Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rind staning of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biol Technol* **25**: 49–57 (2002).

24 Lado J, Cronje P, Alquézar B, Page A, Manzi M, Gómez-Cadenas Stead AD, Zacarías L and Rodrigo MJ, Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. *Physiol Plantarum* **154**: 469–484 (2015).

25 Alférez F and Burns JK, Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. *Postharvest Biol Technol* **32**: 79–87 (2004).

26 Magwaza LS, Landahl S, Cronje PJR, Nieuwoudt HH, Mouazen AM, Nicolaï BM, Terry LA and Opara UL, The use of Vis/NIRS and chemometric analysis to predict fruit defects and postharvest behaviour of 'Nules Clementine' mandarin fruit. Food Chem **163**: 267–274 (2014)

27 Ordaz-Ortiz JJ, Foukaraki S and Terry LA, Assessing temporal flux of plant hormones in stored processing potatoes using high definition accurate mass spectrometry. *Hort Res* **2**: 1–9, 15002 (2015).

28 Magwaza LS, Opara UL, Cronje PJR, Landahl S, Nieuwoudt HH, Mouazen AM, Nicolaï BM and Terry LA, Assessment of rind quality of 'Nules Clementine' mandarin fruit during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. *Scientia Hortic* **165**: 410–420 (2014).

29 Mahouachi J, Gómez-Cadenas A, Primo-Millo E and Talon M, Antagonistic changes between abscisic acid and gibberellins in citrus fruits subjected to a series of different water conditions. *J Plant Growth Regul* **24**: 179–187 (2005).

30 Romero P, Rodrigo MJ, Alférez F, Ballester AR, González-Candelas L, Zacarías L, et al., Unravelling molecular responses to moderate water stress in sweet orange (*Citrus sinensis* L. Osbeck) by using a fruit-specific ABA-deficient mutant. *J Exp Bot* **63**: 2753–767 (2012).

31 Gascón FR, ABA-deficiency and molecular mechanisms involved in the dehydration response and ripening of citrus fruit. Unpublished doctoral dissertation. Universidad Politécnica De Valencia.Departamento de Biotecnología. (Available online on: riunet.upv.es/bitstream/handle/10251/18105/tesisUPV3996.pdf (2012).

32 Chen J, Mao L, Mi H, Lu W, Ying T and Luo Z, Involvement of abscisic acid in postharvest water-deficit stress associated with the accumulation of anthocyanins in strawberry fruit. *Postharvest Biol Technol* **111**: 99–105 (2016).

33 Olarewaju OO, Magwaza LS, Fawole OA, Tesfay SZ and Opara UL, Role of canopy positions on rind biochemical concentrations and radical-scavenging activities in relation to rind breakdown of 'Nules Clementine' mandarins stored at non-chilling temperature. *Scientia Hortic* **226**: 231–240 (2017).

34 Kawada K, Wheaton TA, Purvis AC and Grierson W, Levels of growth regulators and reducing sugars of 'Marsh' grapefruit peel as related to seasonal resistance to chilling injury. *HortScience* **14**: 446 (1979).

35 Cutler AJ and Krochko JE, Formation and breakdown of ABA. *Trends Plant Sci Rev* **4**: 472–478 (1999).

36 Jiang F and Hartung W, Long-distance signalling of abscisic acid (ABA): the factors regulating the intensity of the ABA signal. *J Exp Bot* **59**: 37–43 (2008).

37 Okamoto M, Kushiro T, Jikumaru Y, Abrams SR, Kamiya Y, Seki M and Nambara E, ABA 9′-hydroxylation is catalyzed by CYP707A in Arabidopsis. *Phytochem* **72**: 717–722 (2011).

38 Zeevaart JA, Metabolism of abscisic acid and its regulation in *Xanthium* leaves during and after water stress. *Plant Physiol* **71**: 477–481 (1983).

39 Kepka M, Benson CL, Gonugunta VK, Nelson KM, Christmann A, Grill E and Abrams SR, Action of natural abscisic acid precursors and catabolites on abscisic acid receptor complexes. *Plant Physiol* **157**: 2108–2119 (2011).

40 Lin S, He J, Jiang Y, Wu F, Wang H, Wu D, Sun J, Zhang D, Qu H and Yang B, Production of nigragillin and dihydrophaseic acid by biotransformation of litchi

pericarp with *Aspergillus awamori* and their antioxidant activities. *J Functional Foods* **7**: 278–286 (2014).

41 Llanes A, Devinar G and Luna V, Role of abscisic acid in legumes under abiotic stress. Legumes under Environmental Stress: Yield, Improvement and Adaptations. John Wiley & Sons , pp. 145–160 (2015).

42 Poiroux-Gonord F, Fanciullino AL, Poggi I and Urban L, Carbohydrate control over carotenoid build-up is conditional on fruit ontogeny in clementine fruits. *Physiol Plantarum* **147**: 417–431 (2013).

43 Pizarro L and Stange C, Light-dependent regulation of carotenoid biosynthesis in plants. *Cienc Invest Agrar* **36**: 143–162 (2009).

44 Hiratsuka S, Yokoyama Y, Nishimura H, Miyazaki T, Nada K, Fruit photosynthesis and phosphoenolpyruvate carboxylase activity as affected by lightproof fruit bagging in Satsuma mandarin. *J Am Soc Hort Sci* **137**: 215–220 (2012).

45 Cronje PJR, Barry GH and Huysamer M, Canopy position affects pigment expression and accumulation of flavedo carbohydrates of 'Nules Clementine' mandarin fruit, thereby affecting rind condition. *J Am Soc Hort Sci* **138**: 217–244 (2013).

46 Magwaza LS, Opara UL, Terry LA, Landahl S, Cronje PJR, Nieuwoudt H, Mouazen AM, Saeys W and Nicolai BM, Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. *Postharvest Biol Technol* **74**: 1–10 (2012).

47 Schmülling T, New insights into the functions of cytokinins in plant development. *J Plant Growth Regul* **21**: 40–49 (2002).

48 Lafuente MT, Martínez-Téllez MA and Zacarías L, Abscisic acid in the response of 'Fortune' mandarins to chilling. Effect of maturity and high-temperature conditioning. *J Sci Food Agric* **73**: 494–502 (1997).

49 Gosalbes MJ, Zacarías L and Lafuente MT, Characterization of the expression of an oxygenase involved in chilling-induced damage in citrus fruit. *Postharvest Biol Technol* **33**: 219–228 (2004).

Figures

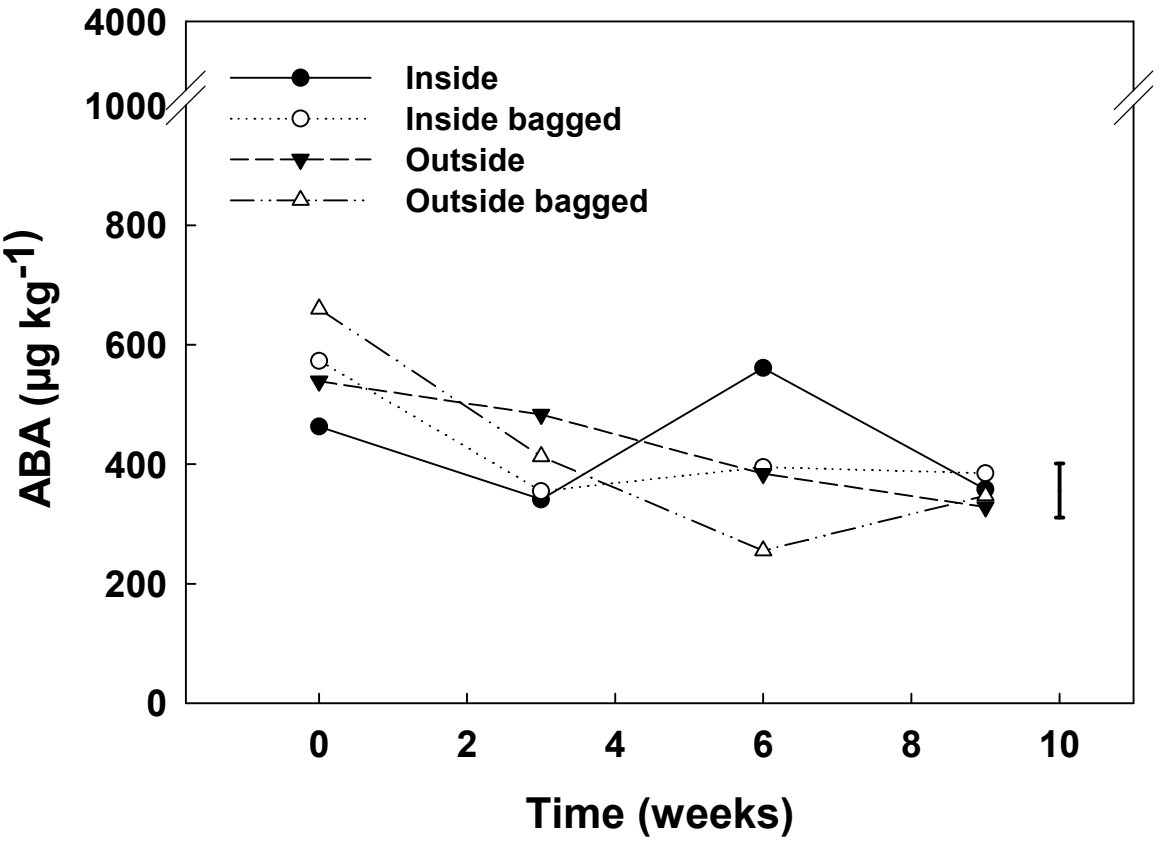

Figure 1. Concentration of abscisic acid (ABA) in the rinds of 'Nules Clementine' mandarins harvested from different canopy positions (inside and outside) and subjected to different light levels (bagged *vs*. non-bagged), and stored at 8 °C for 9 weeks after arrival in UK from South Africa. The concentration was presented on dry weight basis. A least significant difference (LSD) bar, p-value = 0.05, for the interaction storage time x treatment is shown.

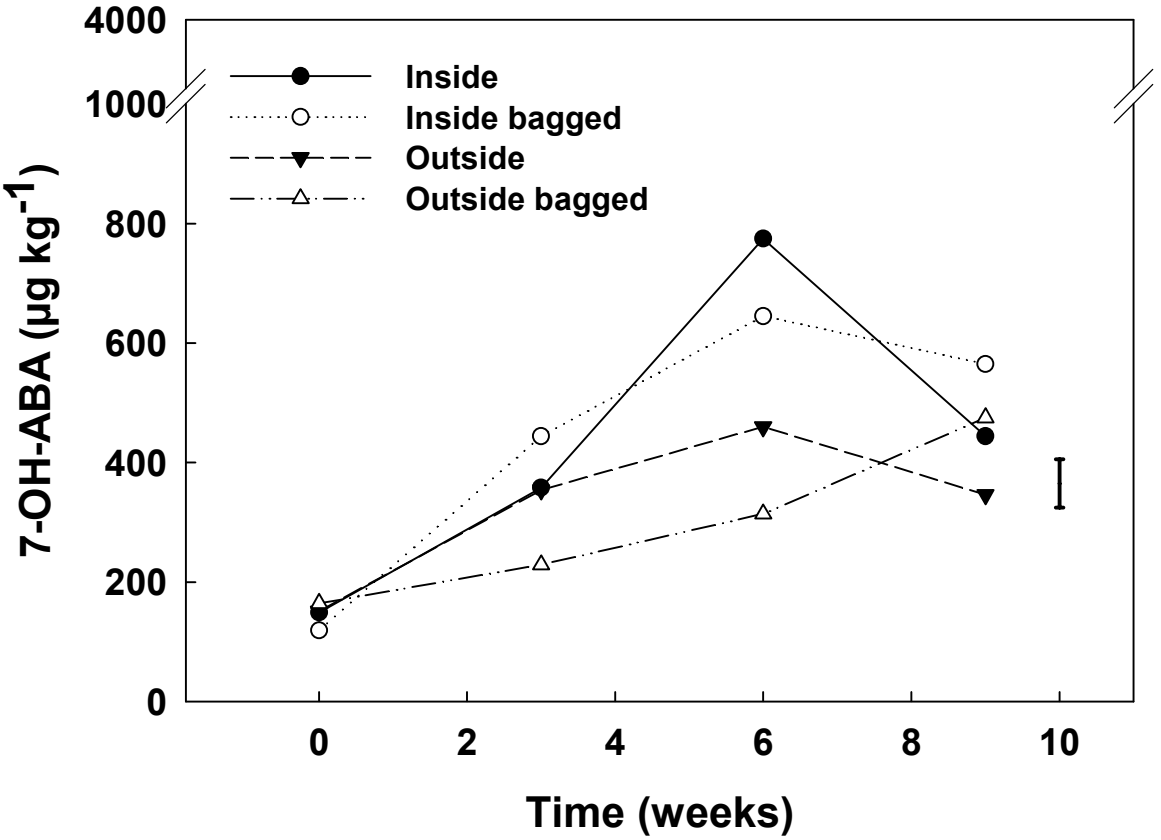

Figure 2. Concentration of 7-hydroxy-abscisic acid (7-OH-ABA) in the rinds of 'Nules Clementine' mandarins harvested from different canopy positions positions (inside and outside) and subjected to different light levels (bagged *vs*. non-bagged), and stored at 8 °C for 9 weeks after arrival in UK from South Africa. The concentration was presented on dry weight basis. A least significant difference (LSD) bar, p-value = 0.05, for the interaction storage time x treatment is shown.

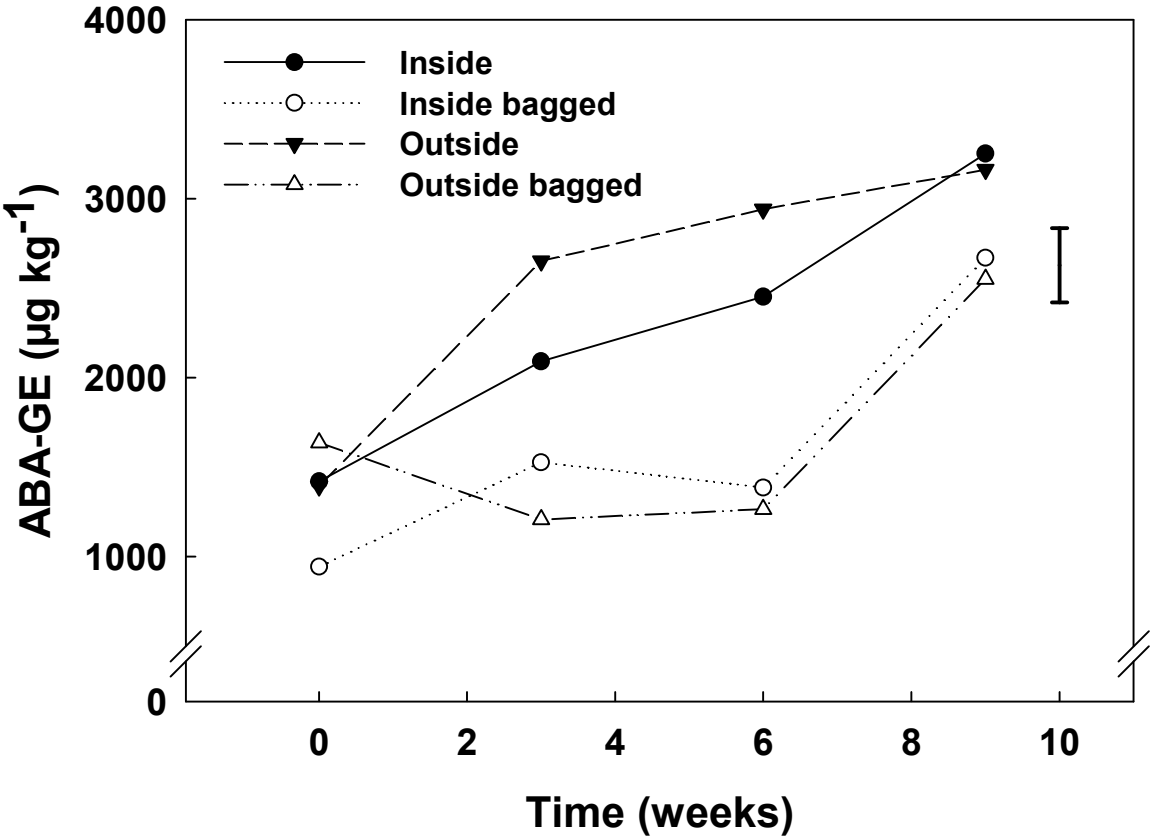

Figure 3. Concentration of abscisic acid glucose ester (ABA-GE) in the rinds of 'Nules Clementine' mandarin fruit harvested from different canopy positions positions (inside and outside) and subjected to different light levels (bagged *vs*. non-bagged), and stored at 8 °C for 9 weeks after arrival in UK from South Africa. The concentration was presented on dry weight basis. A least significant difference (LSD) bar, p-value = 0.05, for the interaction storage time x treatment is shown.

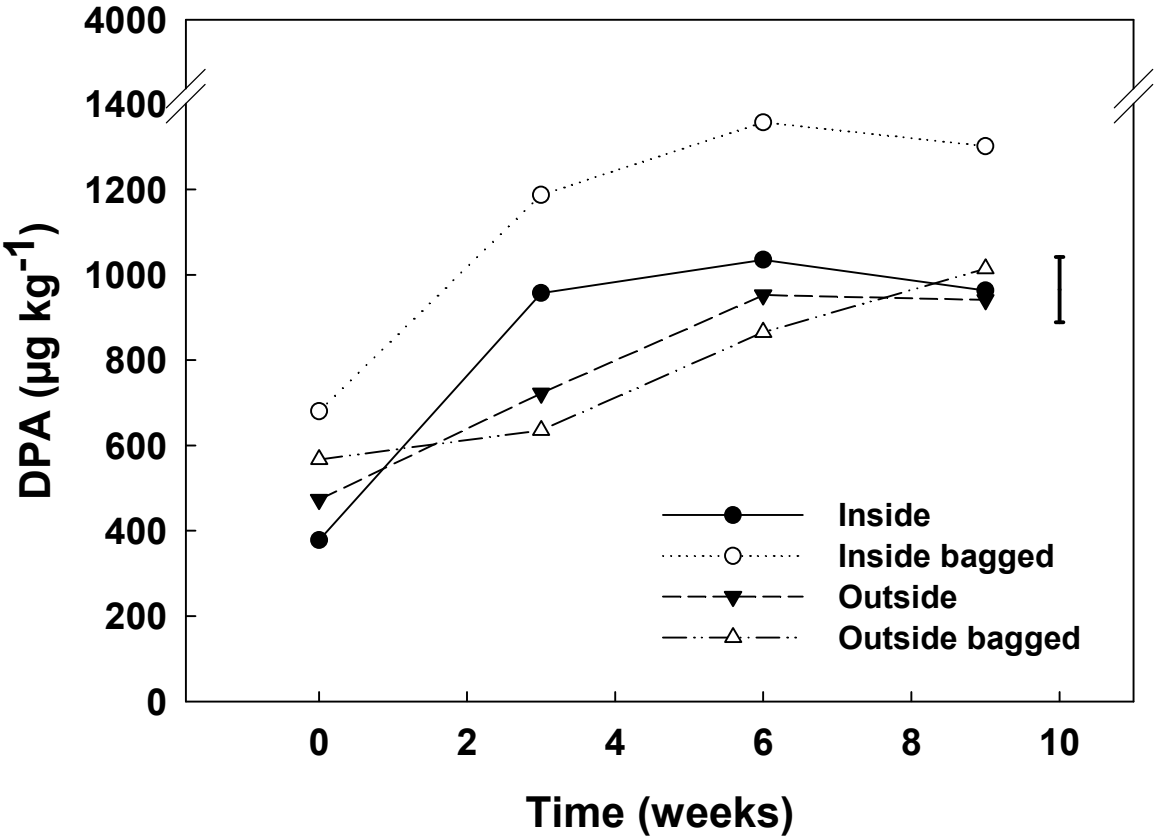

Figure 4. Concentration of dihydrophaseic acid (DPA) concentration in the rind of 'Nules Clementine'mandarin fruit harvested from different canopy positions (inside and outside) and subjected to different light levels (bagged *vs*. non-bagged), and stored at 8 °C for 9 weeks after arrival in UK from South Africa. The concentration was presented on dry weight basis. A least significant difference (LSD) bar, p-value = 0.05, for the interaction storage time x treatment is shown.

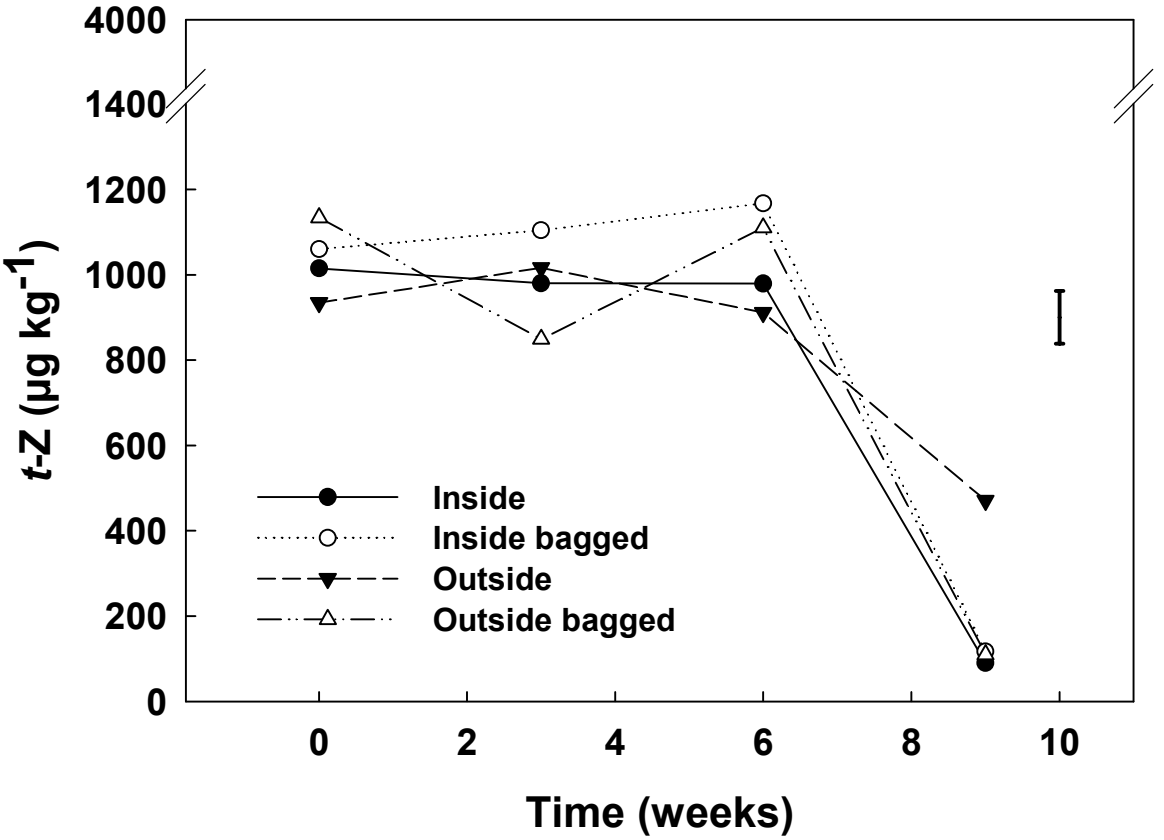

Figure 5. Concentration of *trans*-zeatin (*t*-Z) in the rinds of'Nules Clementine' mandarin fruit harvested from different canopy positions (inside and outside) and subjected to different light levels (bagged *vs*. non-bagged), and stored at 8 °C for 9 weeks after arrival in UK from South Africa. The concentration was presented on dry weight basis. A least significant difference (LSD) bar, p-value = 0.05, for the interaction storage time x treatment is shown.

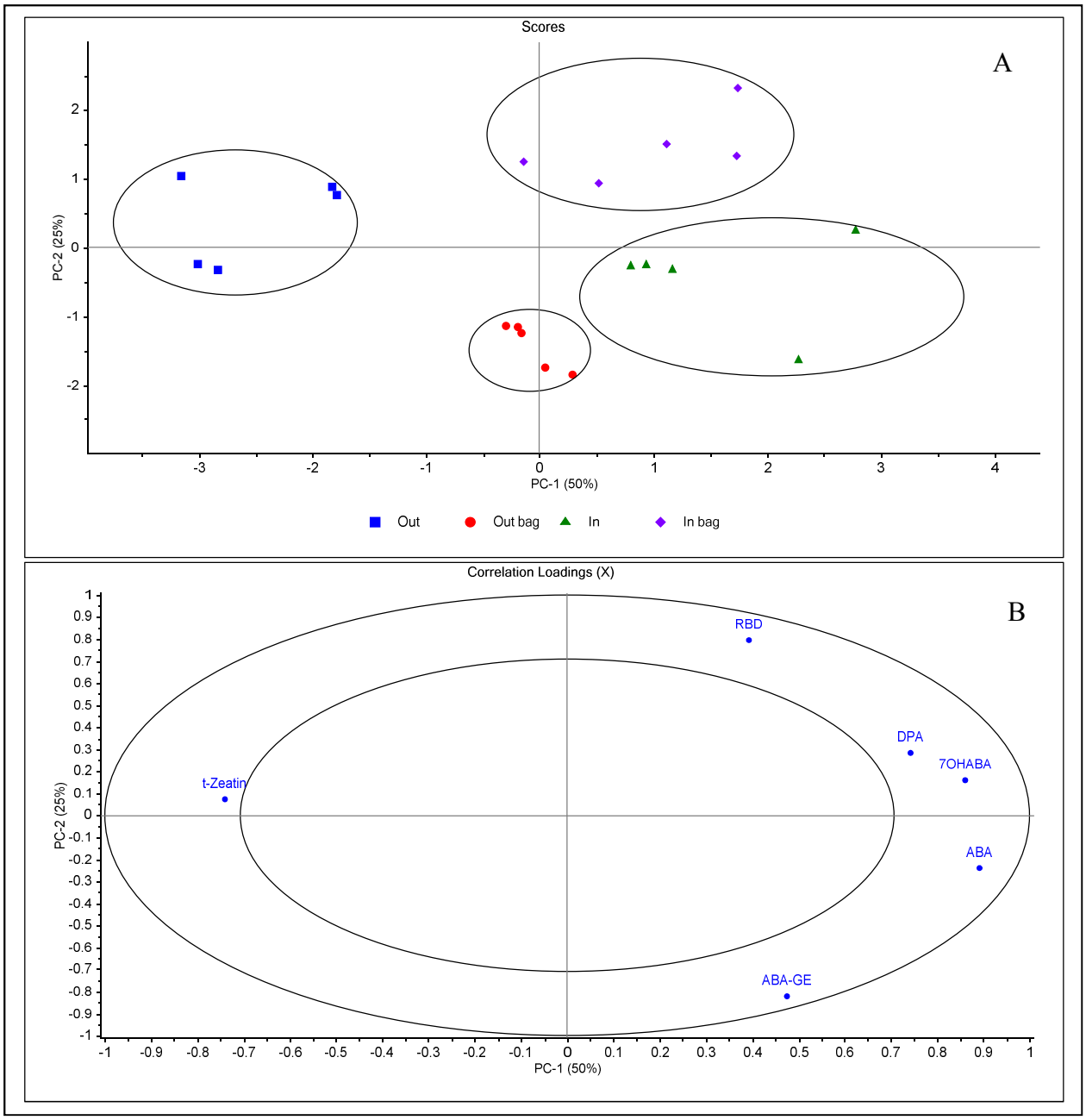

Figure 6. Principal component analysis biplot (scores [A] and loadings plot [B]) showing distribution of fruit from different canopy positions and relationship between measured endogenous hormones and rind breakdown disorder. Out, fruit from outside position; Out bag, bagged fruit from the outside position of the tree canopy; In, fruit from inside position of the canopy; In bag, bagged fruit from the inside position of the tree canopy; RBD, rind breakdown disorder; ABA, abscisic acid; 7-OH-ABA, 7'hydroxy-abscisic acid; ABA-GE, ABA-glucose ester; DPA, dihydrophaseic acid.

Tables

Table 1. Rind breakdown disorder (RBD) index of 'Nules Clementine' mandarin fruit harvested from different canopy positions and stored at 8 °C for 6 and 9 weeks after arrival in UK from South Africa. The RBD index ranges from 0 (no RBD) to 3 (severe RBD).

	Rind breakdown disorder index	
Fruit canopy position	Week 6	Week 9
Outside	0.00	0.12
Outside bagged	0.00	0.32
Inside	0.16	0.50
Inside bagged	0.32	0.88
LSD (0.05)	0.259	0.477
p-value	0.002	0.006

https://dspace.org/

Investigating the involvement of ABA, ABA catabolites and cytokinins in the susceptibility of ‘Nules Clementine’ mandarin to rind breakdown disorder

Magwaza, Lembe Samukelo; Alamar, M. Carmen; Tesfay, Samson Zeray; Mditshwa, Asanda; Opara, Umezuruike Linus ...

2019-02-14
Wiley
https://doi.org/10.1002/jsfa.9644
Magwaza LS, Alamar MC, Tesfay SZ, et al., (2019) Investigating the involvement of ABA, ABA catabolites and cytokinins in the susceptibility of ‘Nules Clementine’ mandarin to rind breakdown disorder. Journal of the Science of Food and Agriculture, Volume 99, Issue 8, June 2019, pp. 4142-4149

Downloaded from a DSpace repository. https://dspace.org/