Appendix 4. PhD thesis by Muhammad Asad Ullah

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AUSTRALIA

Preharvest factors affecting internal disorders in ‘B74' mango

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Abstract

Internal fruit quality is crucial for export markets to maintain consumer satisfaction and repeat purchase. In 2021-22, the Australian mango export accounted for only 7% of the total crop production. For 'B74' (Calypso™) mango, postharvest vapour heat treatment (VHT) remains the preferred fruit fly disinfestation protocol for access to Asian markets.

If fruit are not inherently robust at harvest, then protocol treatments may reduce internal quality. VHT has been associated with expression of physiological disorders in mango, including 'B74' fruit, particularly heat-related injuries like fruit flesh cavities and/or browning. Fruit robustness is determined by production factors including, but not limited to, mineral nutrition, crop load, and seasonal environment variation. Hence, the potential role of preharvest factors on fruit robustness was investigated, with a focus on the putative role of fruit mineral composition, on the susceptibility to internal disorders (IDs) in VHT-treated 'B74' mango. Export quality 'B74' fruit were sourced from three different growing regions, these being in the Northern Territory (NT), Far-North Queensland (FNQ), and South-East Queensland (SEQ). The study captured two serial fruiting seasons. Fruit were assessed for internal defects in relation to mesocarp mineral status.

Flesh Browning (FB) and Flesh Cavity with White Patches (FCWP) were the principal defects. FCWP presented as dry white honeycomb-like cavities in the inner mesocarp near the seed. It was observed only in VHT fruit. FB was characterised as darkening and discolouration of fruit inner flesh near seed. FB presented in VHT and non-VHT fruit as darkening of the fruit mesocarp at eating ripe. Overall, highest incidence of FB was in NT fruit. Highest incidence of FCWP was in fruit from FNQ. These observations were consistent over the two fruiting seasons. FB was not observed in either season for fruit from SEQ. Consistent significant ($p \leq 0.05$) positive correlations between FCWP and fruit flesh (K+Mg)/Ca ratios and negative correlation between FB and fruit flesh boron concentration (mg/kg D.W.) were discerned by linear correlation and principal component (PCA) analyses. However, correlation strengths and significance varied considerably across regions and seasons. Relative inconsistency suggests that deterministic factors additional to mineral composition likely influence 'B74' susceptibility to IDs.

The putative role of preharvest factors was further explored in a multi-year experiment over three serial fruiting seasons in the SEQ region. It primarily sought to explore effects of N- and Ca-based fertilisers on fruit mineral status and postharvest performance towards high fruit quality for export markets. Five treatments in season 1 and a sixth in seasons 2 and 3 were applied in addition to the farm-wide base fertiliser program. Supplementary N and Ca fertiliser treatments generally did not influence nutrient availability as judged by soil and leaf tests. An exception was that a low-N, high-

Ca combination of 50 g N/tree plus 690 g Ca/tree increased exchangeable soil [Ca]. This combination also increased fruit mineral status and reduced FCWP incidence.

Overall, mineral status of the fruit was evidently driven 'more' by crop load and 'less' or 'not' by supplementary N and Ca supply. Crop load (fruit number/tree) showed linear positive correlations ($p \leq 0.001$) with fruit [Ca mg/kg D.W.] ($r = 0.68$), [Mg mg/kg D.W.] ($r = 0.81$) and [B mg/kg D.W.] ($r = 0.50$), and negative correlations with fruit [K mg/kg D.W.] ($r = -0.57$), K/Ca ($r = -0.74$), and K+Mg/Ca ($r = -0.73$).

Crop load effect was explored further at tree level upon categorising trees into four categories; viz., very low (<50 fruit), low (50-100 fruit), medium (100-150 fruit), and high (>150 fruit) per tree. It was assessed for three seasons over which irregular bearing was observed, with low to medium cropping in season 1, high cropping in season 2, and low cropping in season 3. With data pooled across the three seasons, trees with high crop load (>150 fruit) had a significantly ($p \leq 0.05$) lower incidence (9%) of FCWP disorder in association with higher [Ca mg/kg D.W.], [Mg mg/kg D.W.], and [B mg/kg D.W.] and lower K/Ca and K+Mg/Ca ratios.

Serial 'B74' fruit sampling during growth and development over two fruiting seasons suggested that mineral nutrient ratios were stable. That is, they did not change significantly ($p > 0.05$) after stone hardening stage at *ca*. 5-7 weeks before commercial harvest within the overall growth and development period of ~16 weeks from fruit set.

Dye tracing revealed relatively higher xylem functionality at early development stages than at hard green mature. Xylem functionality declined as fruit matured to the degree of complete cut-off from the fruit mesocarp. X-ray fluorescence microscopy (µXRF) revealed higher [Ca] closer to the fruit skin and proximally, adjacent to the pedicel base.

Collectively considering the findings above, decisions to export 'B74' fruit might consider targeting fruit from high crop load trees with inherently low K+Mg/Ca (≤ 20) toward relatively more robust fruit likely to better tolerate stressful phytosanitary treatment in terms of reduced IDs, like FCWP.

Declaration by author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Contributions by others to the thesis

1. Daryl Joyce – Principal Advisor and UQ Professor: Advising on individual experiments and experimental designs, and critical revision of the thesis draft chapters.
2. Priya Joyce – Thesis advisor and Adjunct Associate Professor UQ: Advising on research activities, critically reviewing and professional English editing the draft chapters.
3. Neil White – Thesis advisor and Principal Scientist QDAF: Advising on data analysis and interpretation.
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6. Donald Irving – Adjunct Associate Professor UNE: Critically reviewing and professional English editing the draft chapters.
7. Lachlan Casey – Analytical X-Ray Specialist UQ: Advising on X-ray fluorescence imaging experiments during fruit developmental study.
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9. Darcy Holmes – Logistics and Technical Officer, Perfection Fresh Limited: Contribution to sourcing fruit from export supply chains and use of vapour heat treatment facility for fruit fly disinfestation.

Statement of parts of the thesis submitted to qualify for the award of another degree

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Dedications

I dedicate this humble effort to my parents, siblings, and fiancée - for they are the most precious people in my life.

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Chapter 1 General introduction

1.1 Research background

Mango is a member of family *Anacardiaceae*, genus *Mangifera* (Bally 2006). *Mangifera indica* is the most commercialised mango species in the world. It originated from Southeast Asia (Indo-Malay) (Mukherjee 1997). Over 103 countries produce *Mangifera indica* cultivars in subtropical and tropical regions (Dillon et al. 2013). Fruit of various cultivars range in weight, colour, size, shape, taste, and aroma (Singh et al. 2013b). Mango fruit have many nutrients, including vitamins A and C, folate, B6, iron, and potassium (Mirta & Baldwin 1997). They are also a source of antioxidants and phytochemicals e.g., mangiferin (Lizada 1993; Ribeiro et al. 2007).

Mango fruit have a kidney or oblong oval-shaped seed in their centre (Rajan 2012). Immature fruit typically have green skin. Depending on cultivar, it turns red, yellow, orange or a combination of colours during maturation and ripening (Vithana et al. 2019). The fruit growth and maturation period is dependent on growing region and cultivar, but is typically 12 to 16 weeks from fruit set to fully mature (Singh et al. 2013a; Singh et al. 2013b). Pre-harvest growth and post-harvest quality of mango fruit reflect the combination of genotype (G) (viz., scion, rootstock), environment (E) (e.g., temperature, humidity, vapour pressure deficit, rain), and management (M) practices (e.g., nutrition, irrigation, soil properties, and root and canopy management practices (Léchaudel & Joas 2007)).

Environmental fluctuations impact mango fruit development and phenology, primarily by affecting growth cycles, viz., flowering, fruit set, and maturation (Hofman 1998). A highly influential factor is temperature, which modulates flowering, fruit set, and fruit qualities, including skin colour development (Kulkarni 1992). Prolonged temperatures below 10 °C and above 33 °C can adversely affect pollination and fruit set and also contribute to sunburn (Maria Gloria Lobo 2017). Certain cultivars are relatively tolerant of high temperatures, including ‘Tommy Atkins’, ‘Kensington Pride’, and ‘Irwin’. By contrast, less tolerant cultivars include ‘Keitt’, ‘Edward’, and ‘Sensation’ (Morton 1987). Mango fruit tend to develop brighter attractive skin colour at 12 - 25 °C under cool nights and hot day conditions (Morton 1987).

Rainfall and humidity also impact fruit development cycle (Rajan 2012). Mango fruit can grow in both low and high-rainfall areas. However, they are afflicted more so by diseases, like anthracnose (*Colletotrichum gloeosporioides*) in high-rainfall regions (Lim & Khoo 1985; Ploetz 2003). Monthly distribution and annual amount of rainfall influence fruit yield and quality (Zagade & Chaudhari 2010). Heavy rains up to and during flowering negatively affect pollination and limit fruit set. Low rainfall and humidity at early stages of flowering, fruit set, and harvest are conductive of good fruit yield and quality (Rajan 2012).

Light exposure also influences fruit growth, development, and quality. Light stimulates production of blush pigments in the fruit skin (viz., anthocyanins), which constitute an attractive quality trait (Montanaro et al. 2007; He et al. 2010). Skin colour and blush are attractive to consumers, being conducive to repeat purchases, and attendant profits.

Irrigation in conjunction with environment is important in determining crop quality and yield. Withholding irrigation before flowering can initiate good flowering and fruit set (Spreer et al. 2009). During fruit development, irrigation is important to promote size and quality, and to prevent fruit drop (Hofman 1998). Canopy management deals with maintaining crop load by optimising the leaf-to-fruit ratio (L: F). High L: F ratio is associated with reduced firmness, higher sugar content, and increased size, but can make fruit relatively more susceptible to internal disorders (IDs) (Simmons et al. 1996; Simmons et al. 1998b; Singh et al. 2006b). Irregular, biennial, or alternate bearing is the tendency of mango trees to bear no or very little crop in one year and a heavy crop in the following year (El-Motaium et al. 2019). The condition occurs more when a cultivar is not fit for a region or climate. In turn, biennial bearing affects annual vegetative growth and fruit set (El-Motaium et al. 2019).

These pre-harvest environmental and management factors directly or indirectly affect nutrient availability to and uptake by fruit (Léchaudel & Joas 2007). Combined effects of pre-harvest factors result in the development of intrinsic characteristics at the time of harvest that determine fruit quality. Analysing single-factor effects in relation to yield and quality is insufficient to predict outturn quality for consumers (Rehman et al. 2015).

In Australia, around 68,600 tonnes of mango, worth approximately $217.9 million (AUD), are produced annually (Hort. Innovation Australia 2023). The main cultivars are ‘R2E2’, ‘Honey Gold’, ‘Kensington Pride’, and ‘B74’. Only 7% of total Australian production (4,747 tonnes) was exported in 2022 (Hort. Innovation Australia 2023).

Mango producing regions in Australia span several provinces, including New South Wales, Queensland, Western Australia, and the Northern Territory (Hort. Innovation Australia 2023). Major mango growing regions include Bundaberg, Bowen, and Mareeba in Queensland, Katherine and Darwin in the Northern Territory, and Kununurra in Western Australia (Hort. Innovation Australia 2021) (Figure 1.1). ‘Kensington Pride’ is the predominant mango cultivar, comprising about 43% of total mango production in 2019/2020 (Hort. Innovation Australia 2021). The second cultivar is ‘B74’, marketed under the trade-name ‘Calypso’ (Marques et al. 2016).

‘B74’ originated in Queensland from a cross between the Australian cultivar ‘Kensington Pride’ and the American cultivar ‘Sensation’. It was registered and commercialised in 1999 (Hofman et al.

2010b). In 2019/2020 season, ‘B74’ was 25% of total production, with consistent supply from September to February (Hort. Innovation Australia 2021). At eating ripe, ‘B74’ has bright yellow skin colour with red blush on ‘shoulders’ (Figure 1.2). Other compelling features are its good texture, flavour, small seed, and fibre-free flesh (Hofman et al. 2010b).

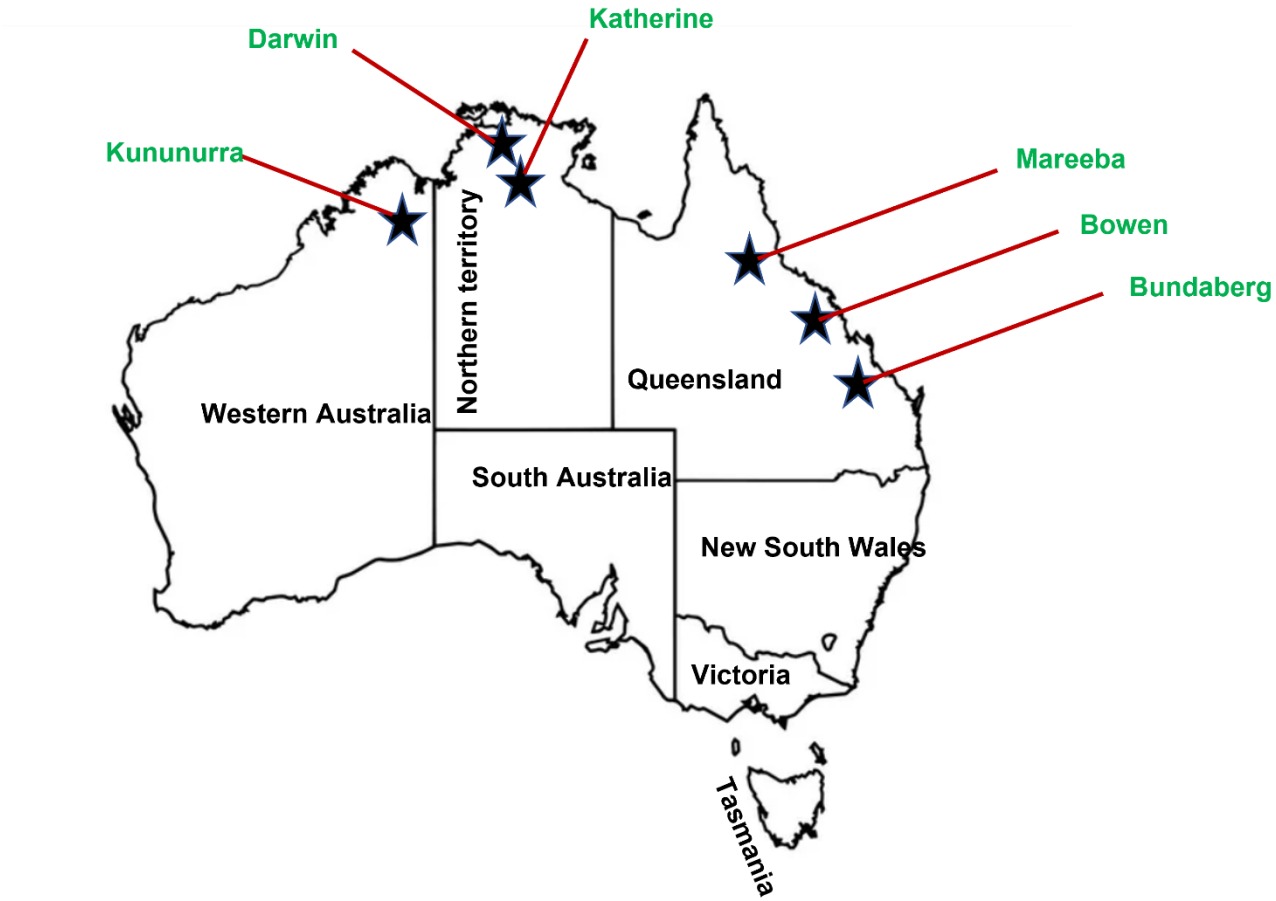


Figure 1.1: Major mango producing regions in Australia.

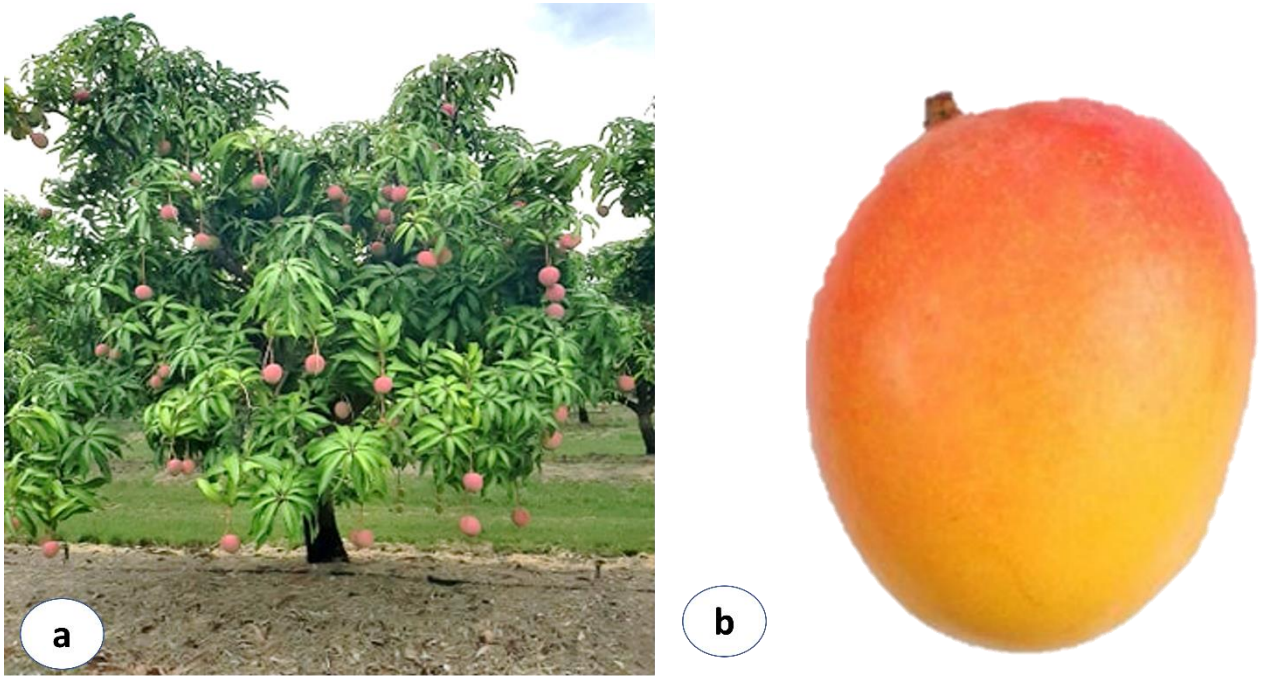


Figure 1.2: a. ‘B74’ mango tree bearing green mature fruit with pink blush over the shoulders, and b. fruit at eating ripe stage.

Mango is a perishable fruit that exhibits relatively short shelf life at room temperature after harvest (Mukherjee 1997). An issue limiting post-harvest life is physiological disorders that develop externally or internally (Sauco 2009). Mango fruit suffer disorders across cultivars and production regions (Wainwright & Burbage 1989). Cultivars vary in susceptibility and symptoms (Cracknell Torres et al. 2004).

Internal disorders (IDs) are primarily the result of failure of normal physicochemical functions pre-harvest and/or post-harvest (Oak et al. 2019). Pre-harvest factors include orchard site characteristics, cultivar, plant nutrient and water status, and environmental fluctuations during fruit development (Ferguson et al. 1999). Post-harvest factors include storage time and temperature, oxygen and carbon dioxide concentrations, and post-harvest treatments, such as surface coatings, hot water treatment, and vapour heat treatment (Wills et al. 1999; Brecht et al. 2020). Unlike the symptoms of pathogenic diseases, causal agents of IDs can be more obscure due to complex interactions of multiple factors (Léchaudel & Joas 2007). Some common IDs of mango include soft nose (Torres & Saúco 2004), spongy tissue (Oak et al. 2019), stem-end cavity (Raymond et al. 1998a), jelly seed (Njuguna et al. 2016), internal browning (Lo'ay 2009), and insidious fruit rot (Tarmizi et al. 1993). It is uncertain whether these are all different disorders or represent different or variously advanced symptoms of some common disorders. In any case, correct identification of a particular disorder is essential in determining the role of key contributing factors involved in triggering and/or inhibiting the disorder

expression.

Mango disorders may present as uneven and/or premature mesocarp ripening (Raymond et al. 1998a). IDs were reported early by Cheema and Dani (1934). A comprehensive work was published on 'Tommy Atkins' by Malo and Campbell (1978). Wainwright and Burbage (1989) described symptomology and changes associated with internal physiological disorders in various other cultivars. Incidence and severity of physiological disorders vary among cultivars and geographical regions (Subraman et al. 1971; Malo & Campbell 1978; Torres & Saúco 2004). For 'B74' mango, the present study is focused on causal factors associated with IDs expressed in export supply chains wherein phytosanitary treatment, like vapour heat treatment (VHT), can be mandatory (Jacobi & Giles 1997; Singh & Saini 2014).

1.2 Research approach

The objective of this study was to better understand the role of environmental and management factors to determine 'B74' mango susceptibility to IDs. Environmental influences cannot easily be controlled, but can typically be monitored with appropriate measures then taken to maintain fruit quality, including robustness, at harvest (Chagne et al. 2014).

Mineral nutrients like calcium (Ca) have roles in maintaining cellular integrity (Shear 1975a; Fallahi et al. 1997; Hocking et al. 2016). In the context of producing fruit that are more robust, better understanding of how complex relationships across the soil-plant-atmosphere-continuum play out towards enhanced fruit quality, including tolerance of supply chain rigours, like VHT.

The objectives of this work on 'B74' mango were:

1. Generate a detailed literature of the physiochemistry of IDs and key contributing factors in maintaining mango fruit quality.
2. Characterise IDs in 'B74' mango fruit in relation to mineral nutrient levels across different regions and growing seasons.
3. Modulate 'B74' mineral nutrient status through pre-harvest fertiliser applications at early fruit development stages to reduce IDs.
4. Characterise fruit quality and mineral nutrient status at tree level towards minimising variation.
5. Determine the optimal fruit sampling time to predict mineral status at-harvest.

1.3 Thesis structure

Chapter 1 offers a general introduction to mango fruit, including status in Australia, and the research

problem focusing on thesis aims and objectives. Chapter 2 offers a literature review on mango physiology, including the biochemistry of internal physiological disorders and other quality attributes. Chapter 3 presents a systematic analysis of the published literature on IDs of mango across the world and factors governing disorder expression and management. Chapter 4 assesses 'B74' mango fruit quality and mineral nutrient variation across regions and growing seasons in association with incidence of IDs. Chapter 5 examines fertilisation, particularly nitrogen (N) and Ca, in relation to fruit quality, including physiological disorders. Chapter 6 explores fruit quality and mineral variation at orchard level and within canopy position of fruit. Chapter 7 considers fruit growth dynamics and Ca uptake capacity of 'B74' mango in relation to fruit development and xylem functionality. Finally, chapter 8 concludes the thesis with general discussion and conclusions with recommendations for future research.

Chapter 2 Literature review: Internal disorders of mango fruit - physiology, biochemistry, and role of mineral nutrients

2.1 Abstract

Mango (*Mangifera indica* L.) is a popular fruit in tropical and subtropical regions with distinctive aroma, flavour, and nutrition properties. Annual global mango production is > 50 million tonnes (FAO, 2019). Major producers of mango are India, Bangladesh, China, Mexico, Pakistan, Indonesia, Brazil, Thailand, and the Philippines. Harvested mango fruit are highly perishable, with short shelf life. Physiological disorders are among the major factors limiting the post-harvest quality and shelf life, including when fruit need phytosanitary treatments, such as hot water treatment, vapour heat treatment, and irradiation. This review focuses on important internal physiological disorders of mango flesh, including physiology and biochemistry. It discusses factors contributing to development and/or exacerbation of the disorders. Improved production practices, including pruning, nutrient application, irrigation, and environmental factors (temperature, humidity, and vapour pressure deficit) monitoring can enhance fruit robustness to help tolerate post-harvest operations. Considerable demand for mangoes on international markets is compromised by internal quality issues and so threatens exports. Optimum quality is crucial to retain existing markets and to gain new consumer markets around the world.

Keywords: *Mangifera indica* L., internal quality, flesh disorders, internal breakdown, nitrogen, calcium, irrigation, maturity

2.2 Introduction

Fruit quality refers to a set of valuable characteristics that augment the worth of fruit. The 'concept' differs from perspectives of producers and consumers. Producers favour enhanced shelf life, yield, disease resistance, and low susceptibility to disorders as key characteristics of fruit quality, while consumers are interested in flavour and visual appeal of fruit (Kiloes et al. 2022). Reduced shelf life, skin damage, lenticel spotting, development of off-flavours, post-harvest diseases (e.g., anthracnose, stem-end rot) (Xu et al. 2017), and flesh breakdown are some major problems that compromise fruit quality (Mishra et al. 2016). They negatively affect fruit texture, visual appeal, marketability, and consumer satisfaction.

In mango, generalised physiological disorders affecting commercially important mango cultivars are blacktip (Zhang et al. 1995), fruit splitting (Lim & Khoo 1985), internal necrosis (Ram 1985), and internal breakdown (Ma et al. 2018). Internal breakdown (or disorders) covers more specific symptoms like soft nose (Torres & Saúco 2004), spongy tissue (Oak et al. 2019), stem-end cavity (Raymond et al. 1998a), jelly seed (Njuguna et al. 2016), and internal browning (Lo'ay 2009). Internal disorders (IDs) often lack any external symptoms which make them difficult to detect (Bally & Owens 2001).

Various factors have been identified as being associated with development of IDs, including management practices, environmental and edaphic factors, fruit characteristics (size, weight, specific gravity), and nutritional imbalance (Krishnamurthy 1980; Raymond et al. 1998b; Njuguna et al. 2016). Nutrient deficiency is considered a key contributing factor to physiological dysfunction at pre- and/or post-harvest (Oak et al. 2019). Measures to identify susceptible fruit are desired towards better understand the causes of physiological disorders and their prevention.

2.3 Common IDs of mango

Major IDs of mango fruit include soft nose, jelly seed, stem-end cavity, flesh cavity, flesh browning, and spongy tissue (Figure 2.2).

- Soft nose was characterized by Young (1960) as flesh softening and yellowing at the fruit apex due to mesocarp breakdown (Figures 2.1 and 2.2a). In soft nose, the 'beak end' of the fruit appears over-ripe, indicating rapid localised ripening (Burdon et al. 1992). Soft nose may be assessed by touching the beak end which would be softer than the rest of the fruit (Mead & Winston 1989). Soft nose incidence is cultivar dependent and is less prevalent in alkaline soils than in acidic sandy soils (De la Cruz Medina & Garcia 2002).
- Stem-end cavity is characterized by cavity formation in the proximal area between the

peduncle and the mesocarp, primarily due to vascular tissue deterioration (Figures 2.1 and 2.2b) (Raymond et al. 1998a). In the early stages of development, tissues at the proximal end of affected fruit turn brown, followed by cavity formation and necrosis (Mead & Winston 1989). In advanced stages, necrosis around the cavity affects the internal mesocarp with symptoms like spongy tissue and jelly seed. Microscopic analysis revealed xylem deterioration, procambium tissue damage, and calcium oxalate crystal accumulation in the cavity (Raymond et al. 1998a).

- Jelly seed occurs as flesh breakdown in the mesocarp around the fruit seed/endocarp (Figure 2.2c). It produces a jelly-like mass around the fruit stone with complete tissue disintegration. In extreme cases, tissue browning and watery translucent appearance arise (Van Lelyveld & Smith 1979). The onset of jelly seed symptoms varies between cultivars. 'Van Dyke' and 'Tommy Atkins' showed jelly seed symptoms after 8 weeks from fruit set while, symptoms in 'Irwin' appeared 12 weeks after fruit set (Raymond et al. 1998a). To avoid jelly seed incidence, harvesting early fruit is recommended (Singh et al. 2011).

- Internal flesh browning is one of the more important physiological disorders of fleshy fruit (Figure 2.2d). It is associated with long-term storage-related physiological changes in fruit (Selvarajah et al. 2001; Pusittigul et al. 2012; Mogollon et al. 2020). It is considered a result of cell wall and membrane degradation with release of enzymes associated with browning, namely polyphenol oxidase and phenylalanine ammonia-lyase, and of phenolic compounds. These enzymes and phenolics are normally localised in different cellular compartments to prevent mixing. Upon cell degradation, phenolic compounds are oxidised into coloured *O*-quinones (Huang et al. 2020). Cell wall weakening is often associated with low Ca concentration in fruit mesocarp (Chitarra et al. 1997). Lo'ay and Ameer (2019) discerned positive effects of ascorbic acid blended Ca nanoparticles towards alleviation of internal flesh browning in low temperature stored 'Hindi Be-Sennara' mango by inhibition of cell wall degrading enzymes.

- Spongy tissue disorder is most common in Indian mango cultivars, particularly 'Alphonso'. Affected tissue displays symptoms similar to soft nose and jelly seed, with spongy mesocarp, off-odour, and pale-yellow or off-white flesh, with or without cavities (Katrodia 1979; Katrodia 1988). Early studies implicated nutritional imbalance and environmental factors in this disorder (Rane et al. 1976; Katrodia 1988), which ultimately results in shift from aerobic to anaerobic respiration. Attendant include an acidic pH shift, cell wall degradation, and free radicals synthesis (Ravindra & Shivashankar 2004).

Similarities among symptoms of jelly seed, spongy tissue, stem-end cavity, and soft nose disorders suggest commonalities at some level(s). Raymond et al. (1998a) concluded that they all commence with cellular disorganisation and cell wall degradation. Nevertheless, the temporospatial distribution of symptoms in different parts of the fruit distinguishes each disorder, hence their specific symptomologies. Progressive expression of visual symptoms makes early detection of these disorders difficult (Oosthuyse 1993).

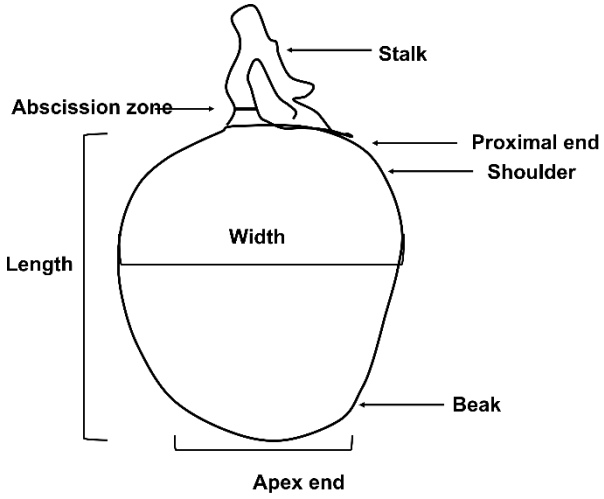


Figure 2.1: Morphological features of the mango (Cultivar 'B74')



Figure 2.2: Internal disorders in ‘B74’ mango: a. Soft nose, b. Stem-end cavity, c. Jelly seed, d. Internal flesh browning (From Hofman et al. (2010a).

2.4 Anatomy and biochemistry of IDs

Macroscopic differences have been observed among different IDs based on spatial distribution of symptoms and their visual appearance. Information in the literature on the anatomy of different IDs in mango is otherwise limited. Generally, however, cell disintegration and cell wall rupture appear to be the first indication of the onset of such disorders. Histological studies showed differences among cultivars affected by different disorders. Torres and Saúco (2004) observed severe xylem deterioration and cell wall disintegration in the internal breakdown-affected mesocarp of highly susceptible ‘Tommy Atkins’ as compared to less susceptible ‘Lippens’, which showed translucent mesocarp with intact cell walls.

Raymond et al. (1998a) characterised early symptoms of stem end cavity in cultivars ‘Tommy Atkins’, ‘Irwin’, and ‘Van Dyke’ as tannin deposition in resin ducts and xylem that leads to the discolouration of flesh around the cavity. Continuous accumulation of tannins eventually causes toxicity and necrosis in advanced stages of stem-end cavity (Raymond et al. 1998a). Ca accumulates in stem-end cavity in the form of oxalate crystals and creates localised deficiency in the cells because it is not available for biochemical functions even though total pulp Ca concentrations may be high

(Raymond et al. 1998a; Hocking et al. 2016). This vascular disruption markedly limits the nutrient supply to inner mesocarp cells and weakens the flesh cellular integrity, proximal to the seed.

Biochemical changes associated with physiological disorders in mango fruit have been studied by Chitarra et al. (1997) and De Oliveira Lima et al. (1999). Mesocarp of spongy tissue-affected 'Alphonso' mango fruit showed increased pectin methylesterase and malic enzyme activities (Murthy 1981) and reduced invertase and amylase activities. A study on 'Dashehari' and 'Langra' mango revealed higher activities of polymethyl esterase and cellulase in jelly seed-affected flesh (Krishna et al. 2020). Enzymes associated with biochemical changes in mango flesh affected by various IDs are presented in Table 2.1.

Previous studies also indicated a role of gas exchange in development of IDs (Lammertyn et al. 2001). In particular, the shift in metabolic processes from aerobic to anaerobic due to the low availability of oxygen inside mango fruit (Mitcham & McDonald 1993). Consequently, cell membrane integrity may decrease to result in internal quality defects, including cavities, flesh gelation, and tissue browning. Over-mature fruit tend to accumulate more anaerobic metabolites, such as acetaldehyde and ethanol, in the mango fruit flesh over time (Jacobi et al. 2001). Accumulation of such metabolites in muskmelon fruit has been associated with reduced sugar content and water-soaked tissue appearance (Nishizawa et al. 1998). A similar response in late-harvested litchi fruit compared to early-harvested under modified atmosphere storage has been reported (Pesis et al. 2002). It was ascribed to fermentation leading to flesh deterioration. Reduced mitochondrial activity due to membrane damage may render cells incapable of producing sufficient energy to sustain normal cell activity (Pesis 2005).

Table 2.1. Enzyme activities in mango flesh afflicted by various IDs.

Cultivar	Disorder	Enzyme	Activity	Reference
'Alphonso'	Spongy tissue	Superoxide dismutase, Catalase, Peroxidase, Polyphenol oxidase	Lower	(Nagamani et al. 2010)
'Tommy Atkins'	Spongy tissue	Amylase	Lower	(Lima et al. 2001)
'Alphonso'	Spongy tissue	α-amylase, Invertase	Lower	(Katrodia 1979)
'Tommy Atkins'	Spongy tissue	Peroxidase, Polyphenol oxidase	Higher	(De Oliveira Lima et al. 1999)
'Chiin Hwang'	Jelly seed, Lumpy tissue, Soft nose,	α-amylase activity	Lower	(Lin et al. 2013)
'Langra'	Jelly seed	α-amylase, Pectin methyl esterase, Cellulase, Polyphenol oxidase	Higher	(Krishna et al. 2020)
'Dashehari'	Jelly seed	α-amylase, Pectin methylesterase, Cellulase, Polyphenol oxidase	Higher	(Krishna et al. 2020)
'Amrapali'	Jelly seed	Pectin methyl esterase, Pectate lyase, Polygalacturonase	Higher	(Shivashankar et al. 2016)

'Alphonso'	Internal breakdown	Pectin methyl esterase, Malic enzyme	Higher	(Murthy 1981)

2.5 Factors affecting fruit quality and robustness

Fruit quality attributes are largely determined and influenced by pre-harvest factors and early fruit development conditions (Rehman et al. 2015). Fruit susceptibility to physiological disorders depends upon cultivar type, untimely environmental fluctuations, poor management practices, and post-harvest handling conditions (Hofman et al. 2012). Table 2.2 lists factors that directly and/or indirectly influence fruit quality and physiology.

Table 2.2. Pre-harvest factors affecting mango fruit quality (adapted from Joyce (2001)).

Genotype	✓ Cultivar (rootstock, scion)
Environment	✓ Soil type (pH, cation exchange capacity, water holding capacity) ✓ Temperature (heat injury, chilling injury) ✓ Moisture (rain, relative humidity, precipitation) ✓ Radiation (day-length, wavelength, intensity)
Management	✓ Canopy management (pruning, thinning, plant growth regulators) ✓ Nutrition (calcium, nitrogen, potassium, boron) ✓ Irrigation (time, amount, frequency) ✓ Harvest (maturity, timing, method)

2.5.1 Role of mineral nutrients in fruit flesh

Imbalance in mineral nutrients such as nitrogen (N), Ca, potassium (K), magnesium (Mg), and boron (B) is a major contributing factor in fruit susceptibility to IDs (Table 2.3). Many studies conclude that low [Ca], high [N], and high N/Ca ratio in fruit flesh predispose fruit to disorders. There are also conflicting reports implicating fruit flesh [K], [Mg], [P], and [B] in disorders (Table 2.3). Irrespective of differences in type of disorders, if fruit are inherently robust in terms of optimal mineral balance, they are likely to tolerate postharvest rigours and susceptibility to IDs.

In view of Table 2.3, the following discussion primarily focuses on the roles of Ca and N in internal quality of mango fruit. It explores challenges associated with their transport, accumulation, and analysis in the soil-plant-fruit pathway. Because of variability across cultivars and regions, there are no specific threshold values for these elements ratios to be used as standards for fruit quality (Tarmizi et al. 1993; Torres & Saúco 2004; Bally 2006).

Table 2.3. Mineral nutrients associated with increased (+) or decreased (-) expression of some major IDs in mango flesh. '+/-' denotes associations with both increased and decreased defect expression.

Defect	N	Ca	K	P	Mg	B	N/Ca	Reference

Spongy tissue	+	-			+		+	(Katrodia 1979; Selvaraj et al. 2000; Cracknell Torres et al. 2004)
Jelly seed	+	-					+	(Cracknell Torres et al. 2004; Lin et al. 2013; Bitange et al. 2020)
Internal flesh breakdown	+	-	+/-	+	+/-	-	+	(Murthy 1981; Hermoso et al. 1997; Torres et al. 2004a; Torres & Saúco 2004; Bally 2006; Ma et al. 2018)
Soft nose	+	-			-		+	(Burdon et al. 1991, 1992; Lin et al. 2013)

Balance among nutrients improves fruit robustness at harvest and subsequent attendant ability to withstand the rigours of post-harvest handling. Innate fruit robustness cannot be improved after harvest. It is, therefore, important to focus on pre-harvest best management strategies. Studies on pre-harvest N and Ca application on internal fruit breakdown are collated in Table 2.4. Application of N and Ca nutrient solutions reduced internal breakdown in 'Tommy Atkins' mango and the incidence was relatively low when both flesh [N] and [Ca] were high versus high [N] and low [Ca] (Torres & Saúco 2004). Silva et al. (2008) applied foliar Ca as $CaCl_2$ during fruit development in 'Tommy Atkins'. Their analysis in peel and flesh tissues at eating ripe stage revealed an inverse correlation between [Ca] and incidence of internal breakdown. Correlations of fruit N and Ca concentrations have been associated with the incidence and severity of IDs. However, most studies do not take important aspects of N and Ca analysis into consideration. These are discussed below.

Table 2.4. Mineral nutrition and internal breakdown in mango cultivars. "+" Ca, N, and Mg in the results column indicate a higher concentration in flesh as a response to their application. "+ or - disorder" indicates an increase or decrease in disorder expression, respectively.

Cultivar	Disorder	Nutrient	Source	Method	Time of application	Results	Reference
'Sensation'	Spongy tissue, Jelly seed	N, Ca	NH_4NO_3, $CaSO_4 \cdot 2H_2O$	Drip irrigation	Continuous throughout growing season	+N = +Disorder +Ca = -Disorder	(Cracknell Torres et al. 2004)
'Van Dyke'	Jelly seed	Ca	$CaCl_2$, $Ca(NO_3)_2$	Foliar	At fruit set	+Ca = -Disorder	(Bitange et al. 2020)
'Tommy Atkins'	Internal flesh breakdown	N, Ca	-	Drip irrigation	Continuous throughout growing season	+N = +Disorder +Ca = -Disorder	(Torres et al. 2004a)
'Sensation'	Internal flesh breakdown, Stem-end cavity	Ca, Mg, K, B	$CaCl_2$, $MgCl_2$, KCl, Solubor® (20% B)	Foliar	Weekly from 3 weeks after fruit set to 3 weeks before harvest	No effect	(Hermoso et al. 1997)
'Sensation'	Internal flesh breakdown, Stem-end	Ca	Ca-EDTA	Stem injection	One month before harvest	+Ca = -Disorder	(Hermoso et al. 1997)

	cavity,						
'Chaunsa'	Soft nose	Ca, Mg	$CaCl_2$, $CaSO_4$, $Ca(OH)_2$, $MgCl_2$	Foliar	2 weeks before harvest	+Ca = - Disorder +Mg = - Disorder	(Amin et al. 2007)
'Keitt'	Watery pulp breakdown	N, Ca	NH_4NO_2, $CaSO_4 \cdot 2.H_2O$	Soil	Pre- and post-flowering	No effect	(Bally 2006)

2.5.1.1 Calcium

Ca is critical in fruit development, cell wall strengthening, and signal transduction pathways (Kamthan et al. 2015). It's deficiency is associated with increased fruit susceptibility to physiological disorders (Wainwright & Burbage 1989; Burdon et al. 1991; Zhao & Wang 2015). These include bitter pit in apple (Kalcsits et al. 2019), blossom end rot in tomato (Ho et al. 1993), and cork spot and end spot in pear and avocado, respectively (Shear 1975a; Monselise & Goren 1987; Ferguson et al. 1994). Ca deficiency in mango fruit can weaken mesocarp cell walls and result in jelly seed (Raymond et al. 1998a) and spongy tissue disorders (Gunjate et al. 1979b). Ca import and accumulation in fruit varies temporally during development (Joyce et al. 2001; Hirschi 2004; Montanaro et al. 2015). Given the importance of Ca in development and signal transduction, its availability, concentration, and transport to specific cells need to be regulated and managed (Dodd et al. 2010; Gilliham et al. 2011). Excess Ca can result in developmental abnormalities, cell wall rigidity, and cellular phytotoxicity (Cybulska et al. 2011; Hocking et al. 2016). Ca deficiency due to low availability and/or limited transport can result in cell membrane breakdown (e.g., leakiness) and disorders (Tonetto de Freitas & Mitcham 2012).

Ca also acts as secondary messenger facilitating abscisic acid, auxin, and gibberellic acid hormone signalling that regulate biochemical, cellular and morphological functions, including fruit set, and cell division expansion up to and including fruit ripening and senescence (Yu et al. 2006; Hocking et al. 2016). Ca nutrition can influence phytohormones that modulate its distribution at tissue level. Auxins are pivotal in developmental pathways, including fruit set, cell division, and expansion processes that involve Ca as a secondary messenger (Banuelos et al. 1987). Auxin export from developing tissues increases Ca allocation and partitioning (Witney et al. 1990; Else et al. 2004; Sorce et al. 2011). Reduced uptake of Ca during fruit development upon exogenous application of auxin transport inhibitor (2,3,5 tri-iodobenzoic acid (TIBA)) was reported for tomato and avocado fruit (Cutting & Bower 1989; Brown & Ho 1993). Light-induced higher hydroxycinnamic acid levels in kiwifruit increased Ca uptake by reducing auxin degradation (Montanaro et al. 2007).

Tonetto de Freitas et al. (2014) reported that exogenous application of abscisic acid enhanced Ca accumulation by increasing xylem sap flow to fruit, resulting in less susceptibility to blossom-end rot

in tomato. Abscisic acid may redirect Ca flow towards fruit by reducing stomatal conductance in leaves. Foliar application of abscisic acid increased Ca partitioning into and lessened bitter pit in apple fruit (Falchi et al. 2017). Transcriptional analysis of response to foliar abscisic acid application revealed down-regulation of MdCAX, MdACA8, and MdCDPK. These three genes are involved in the regulation and partitioning of Ca in apples (Falchi et al. 2017).

2.5.1.1.1 Ca translocation

Ca uptake, translocation, and accumulation within the canopy among leaves and fruit is controlled or regulated by factors depicted in Figure 2.3. Fruit levels depend on the complex interplay of availability in the soil, uptake through roots, including competition with other minerals in root system, mobility in xylem sap, and competition between vegetative (foliage) and reproductive (fruit) sinks (Figure 2.3). Ca in the soil can be tightly bound to negatively charged particles or soluble in the soil solution. Plant roots take up Ca that's present in the soil solution (McLaughlin & Wimmer 1999; Taylor & Locascio 2004). Soil solution Ca is influenced by soil type and can change with soil acidity and fertiliser application. Root uptake is modulated by root growth rate and it's transport into xylem vessels via symplastic and apoplastic pathways and mass flow of water, triggered by fruit growth and transpiration (White 2001; Karley & White 2009). Ca availability in interaction with other cations (e.g., K, Mg) affects uptake rate and movement along the soil-root-plant continuum (Taylor & Locascio 2004).

Ca loaded into xylem vessels is transported towards the shoot by the mass flow of water due to more negative water potential in the canopy as generated by growth and transpiration (Hirons & Thomas 2018). Ca partitioning within the canopy and between leaves and fruit is modulated by xylem cation exchange capacity, xylem sap Ca content, leaf and fruit growth rates and transpiration into the surrounding air (Hocking et al. 2016). Leaves are relatively strong sinks that tend to accumulate more Ca due, by virtue of high surface area to volume ratio and high stomatal frequency, to higher transpiration rates than fruit (Rogiers et al. 2000). Fruit generally exhibit relatively high transpiration during early development stages and then the rate diminishes as they approach harvest maturity (Joyce et al. 2001; Montanaro et al. 2015). As fruit reach maturity, water uptake decreases also due to the loss of xylem functionality, stomatal conversion into lenticels, and development of waxy cuticles on fruit surface (Bally 1999; De Freitas et al. 2011; Nordey et al. 2015).

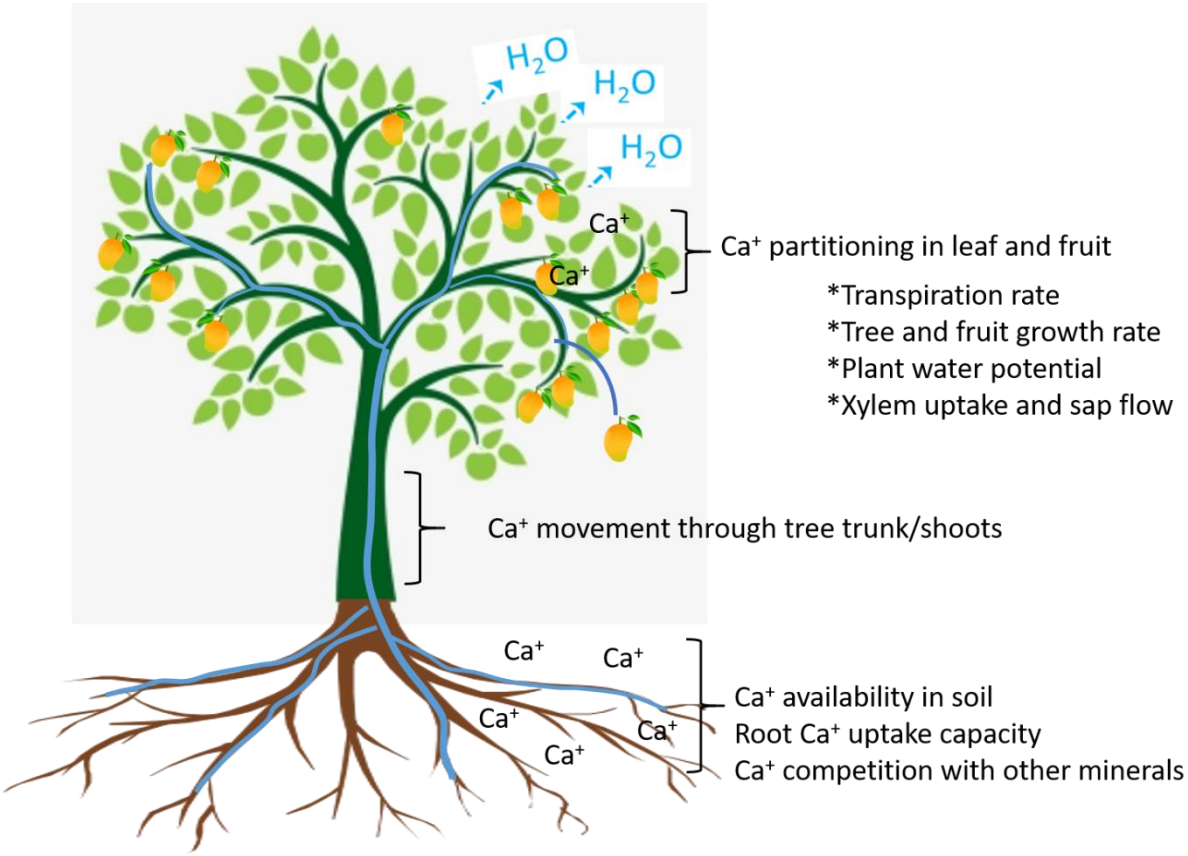


Figure 2.3: Processes of and influences on Ca uptake, translocation, and partitioning in fruit trees.

2.5.1.1.2 Ca monitoring in mango fruit

2.5.1.1.2.1 Sampling

To determine fruit mineral status, it is advised to collect fruit from the middle part of the tree to secure a representative sample and avoid higher and lower parts of the canopy (Ferguson & Triggs 1990; Saure 2005; Kalcsits et al. 2019). In general, fruit from middle-inside of canopy are more susceptible to disorder and disease incidence and has lower [Ca] levels (Woolf et al. 1999; Willingham et al. 2004; Kimeu et al. 2010; Kalcsits et al. 2019). Disease incidence could be linked to pathogen load in the orchard (Everett et al. 2003) and orchard conditions (humidity, rain, tree vigour) (Everett et al. 2007). In vigorous canopies, fruit tend to develop more disease from canopy middle due to low air, light, and spray penetration and higher moisture and humidity which provide ideal conditions for pathogens to thrive. Hence, sourcing fruit from canopy middle is ideal to get an estimate of overall orchard health and fruit quality. Ferguson and Triggs (1990) suggested analysing fewer fruit from many trees than many fruit from fewer trees towards minimising fruit-to-fruit variability. The sampling strategy is an important factor to consider in minimising the variability and representative estimation of nutrient levels e.g., variation of Ca levels in individual apple fruit from one tree may be two-to-three fold (Perring & Jackson 1975).

Fruit mesocarp analysis has typically been an indicator of fruit Ca status. However, differences within whole fruit are evident in localised Ca levels. For example, temperature differences across fruit may exceed *ca*. 10 °C between sun and non-sun exposed sides of avocado fruit (Woolf et al. 1999). Accordingly, sun-exposed tissue may experience different physiology (e.g., transpiration rate) compared to other parts of the fruit. As Ca is xylem mobile, it's partitioning within the fruit depends, in part at least, on transpiration rate. Temperature differences of ~5 °C between sun-exposed and unexposed sides in mango fruit were reported by Katrodia and Sheth (1988) that may differentially influence localised Ca partitioning.

A common means of sampling fruit flesh for mineral analysis is to use fruit corer to take samples from skin to seed at the equatorial region (Bally 2006). However, mineral analyses on individual parts of mango flesh have shown that skin trends to accumulate more [Ca] than inner flesh. Joyce et al. (2001) found 523 mg/kg DW Ca in skin versus 50 mg/kg DW Ca in inner mesocarp of 'Kensington Pride' mango. Burdon et al. (1991) analysed [Ca] in mango 'Kent' and 'Beverly' and found that outer mesocarp had higher Ca content (9.52 and 7.21 mg/100g FW, respectively) compared to inner mesocarp (5.00 and 4.51 mg/g FW, respectively). Similarly, longitudinal spatial variation in Ca accumulation is seen from proximal to distal ends of fruit. The proximal end of 'Kensington Pride' mango showed higher Ca (94 mg/kg DW) levels compared to the distal end (71 mg/kg DW) (Joyce et al. 2001). Burdon et al. (1991) also reported such variation for [Ca] from stem-end to apex in 'Sensation' fruit. In 'Alphonso', Gunjate et al. (1979a) reported variation in Ca from 122 to 110 mg/100g DW between stem-end and apex, respectively. Localised Ca deficiency in the inner mesocarp and the distal part of the fruit may cause internal breakdown and soft nose, respectively (Burdon et al. 1991, 1992; Whangchai et al. 2001). Overall, a general trend is observed in spatial variation of Ca accumulation of skin > outer mesocarp ≥ middle mesocarp > inner mesocarp (Table 2.5) (Shorter & Joyce 1998; Joyce et al. 2001). This variation could be due to physiological difference in cell type, size, and Ca movement from the inner mesocarp to the outer mesocarp through vascular bundles, partially regulated by transpiration mass flow.

It is difficult to compare Ca concentrations among different reports due to inadequacies or discrepancies in the information provided. For example, region(s) of the fruit sampled and unit of measurement (converting mg/g FW to mg/g DW is problematic without moisture percentage of sample). It is incumbent to specifically and consistently state flesh mineral sampling position. It is also important in the context of predicting post-harvest quality and/or expression of physiological disorders.

Table 2.5. Ca concentrations reported in different parts of various mango cultivars.

Cultivars	Skin	Outer flesh	Middle flesh	Inner flesh	Proximal flesh	Distal flesh	Reference
'Beverly'	-	7.21 mg/100g FW	-	4.51 mg/100g FW	-	-	(Burdon et al. 1991)
'Dashehari'	4570 mg/kg dw	-	-	460 mg/kg dw	-	-	(Singh et al. 1993)
'Glen'	1900 mg/kg dw	-	-	700 mg/kg dw	-	-	(Singh 2005)
'Haden'	2300 mg/kg dw	-	-	700 mg/kg dw	-	-	(Singh 2005)
'Irwin'	3600 mg/kg dw	-	-	600 mg/kg dw	-	-	(Singh 2005)
'Keitt'	3900 mg/kg dw	-	-	1000 mg/kg dw	-	-	(Bally 2006)
'Kent'	-	9.52 mg/100g FW	-	5.00 mg/100g FW	-	-	(Burdon et al. 1991)
'Kent'	3600 mg/kg dw	-	-	900 mg/kg dw	-	-	(Singh 2005)
'Kensington Pride'	523 mg/kg dw	123 mg/kg dw	55 mg/kg dw	50 mg/kg dw	94 mg/kg dw	71 mg/kg dw	(Joyce et al. 2001)
'Kensington Pride'	1670 mg/kg dw	-	-	580 mg/kg dw	-	-	(Hofman et al. 1999)
'Kensington Pride'	1740 mg/kg dw	560 mg/kg dw	400 mg/kg dw	410 mg/kg dw	-	-	(Shorter & Joyce 1998)
'Kensington Pride'	2400 mg/kg dw	-	-	600 mg/kg dw	-	-	(Singh 2005)

2.5.1.1.2.2 Ca fractions in fruit

Ca in fruit tissues can be sub-divided into various fractions based on solubility, availability, and biochemical or physiological activity. Exchangeable Ca (adsorbed on proteins and pectin) and soluble Ca (associated mainly with nitrates, chlorides, and organic acid) are generally considered active forms as compared to tightly bound forms, like Ca oxalate, carbonate, and phosphates (Franceschi & Horner 1980; Franceschi & Nakata 2005; Hocking et al. 2016). Ca deposited in the form of oxalate crystals in parenchyma of developing fruit showed low or no solubility and mobility (Hocking et al. 2016). The presence of insoluble Ca may create localised deficiencies of physiologically active Ca, which can lead to physiological disorders (Raymond et al. 1998a).

Pavicic et al. (2004) determined that bitter pit development in apples was associated with low soluble Ca concentrations in affected fruit. Studies on cracking resistant and susceptible cultivars of litchi determined higher level of cell wall Ca present in the pericarp of resistant cultivars (Huang et al. 1999; Yuan et al. 2002). However, the presence of Ca-rich bodies in cracking susceptible litchi cultivars, as detected by X-ray microanalysis, suggested that Ca deficiency might be due to the presence of the insoluble Ca form rather than available Ca (Huang et al. 2001). Accordingly, it can

be important to state the Ca form or pool being analysed in reporting associations with physiological disorders.

2.5.1.2 Nitrogen

2.5.1.2.1 Sources and translocation

N is an essential component of amino acids, proteins, enzymes, and chlorophyll. It plays a key role in cell division during early growth of young tissues, including leaves, buds, and flowers, particularly at the onset of flowering and fruit set (Tagliavini et al. 1997; Menino et al. 2007; Neto et al. 2008; Stellacci et al. 2013; Zekri & Obreza 2013). N is highly mobile and mostly present as proteins in cells (Zekri & Obreza 2013). Fruit trees acquire N for vegetative growth and reproduction by external uptake through the roots and internal N cycling. In trees experiencing N deficiency, proteins in older cells undergo proteolysis and resultant amino acids move to newer cells (Mengel et al. 2001). Proteolysis results in a decline in leaf chlorophyll content and attendant chloroplast collapse. Hence, N deficiency is evident as yellowing appears first in older leaves.

Roots uptake N in both nitrate (NO_3^-) and ammonium (NH_4^+) forms. However, NO_3^- is their preferred source in well-aerated soils (Tromp & Ovaa 1979). Both forms are usually derived from applied mineral fertilisers or native N mineralization. After absorption, N is translocated to different plant organs as required. Sometimes trees do not respond to N fertilisers due to adequate built-up reserves in perennial organs from previous years (Millard 1993; Tagliavini et al. 1997; Menino et al. 2007; Stellacci et al. 2013). Documentation of seasonal changes in xylem sap amino acid composition in apple, grape, and cherry allows distinguishment between N remobilisation and recently absorbed N (Marschner 2012; Whiteside et al. 2012). N^{15} labelled fertilisers have been used to determine the fate of recently applied and stored N in fruit trees. N reserves from previous year are generally used to support tree N demand and vegetative growth in the following year. Remobilisation depends on the amount of N stored, tree size, and age. In apples and other deciduous trees, there typically is intense N withdrawal from ‘autumn’ leaves for translocation to perennial root, trunk, and stem storage organs (Tagliavini et al. 1997; Grassi et al. 2003; Stellacci et al. 2013). According to Niederholzer et al. (2001), about 50% of the N is withdrawn from the leaves of peach trees before their abscission. This N is stored in tree trunk or roots. Upon either remobilisation from storage or root uptake, N is preferentially allocated to newly developing vegetative and reproductive organs (Roccuzzo et al. 2017).

2.5.1.2.2 Effect of N on fruit quality

An increase in N supply promotes tree vigour and, if managed carefully, can enhance yield (Neilsen et al. 1999; Fallahi et al. 2001; Neto et al. 2008). Limited availability slows tree growth and adversely

affects crop load (Zekri & Obreza 2013). On the other hand, excess N supply can negatively affect fruit quality post-harvest (Musacchi & Serra 2018). The optimum N levels mediate fruit skin colour, size, yield, and flavour (Hofman et al. 2012). It is vital to apply appropriate N form to avoid potential antagonistic effects on root mediate uptake of other important plant mineral e.g., Bonomelli et al. (2021) reported that ammonium excess application significantly inhibited and nitrate excess stimulated Ca uptake in tomato seedlings.

Fruit N concentration is generally high during early development stages and decreases thereafter. Fruit response to N application is influenced by timing, method, rate, source, tree phenological stage, and edaphic and climate conditions (Bally 2009). Increased tree vigour in response to high N supply typically leads to higher leaf-to-fruit ratio and fruit size, and lower fruit Ca concentrations (Crisostol et al. 1997; Simmons et al. 1998b). Positive correlations between fruit size and occurrence of IDs have been reported for mango (Young et al. 1962; Torres et al. 2004b) and other fruit, such as avocado (Marques et al. 2003; Willingham et al. 2006).

Young (1960) reported that increased N fertilisation was associated with increased soft nose incidence in 'Kent' mango. Inconsistencies, in later study, were ascribed to unaccounted seasonal effects (Young et al. 1962). Murthy (1981) also questioned the pivotal involvement of Ca and/or N in ID development with findings on 'Alphonso' mango suggesting that high [P] and low [K] were responsible for pulp tissue breakdown. However, Tarmizi et al. (1993) discerned higher N/Ca in 'Haramanis' mango with greater incidence of insidious fruit rot. In mango fruit ontology studies from fruit set to ripening, Raymond et al. (1998b) found no difference in mineral composition between healthy and disordered fruit. Nevertheless, Torres et al. (2004a) determined a positive correlation between internal breakdown and N concentration in the 'Tommy Atkins' fruit mesocarp and an inverse relationship with fruit Ca concentrations. Similarly, in comparing flesh mineral concentrations for healthy and disordered 'Keitt' mango, Ma et al. (2018) found higher N and lower Ca concentrations in the disordered fruit.

How high N concentration contributes towards IDs in mango fruit remains uncertain. It is possible that high vegetative growth in response to excess N redirects Ca flow to leaves, rendering developing fruit vulnerable to Ca deficit, worsened upon the co-incident increase in fruit size leading to Ca dilution in the flesh (Cull 1991; Willingham et al. 2006). It follows that rapidly growing fruit with limited Ca supply are more prone to Ca-mediated physiological disorders during post-harvest handling and storage (Ferguson 1984; Monselise & Goren 1987; Ferguson et al. 1994; Simmons et al. 1998b; Simmons et al. 2015).

Such findings suggest that optimising N is as important as is Ca supply to achieve robust high-quality

long shelf life fruit (Torres et al. 2004a). Improved understanding around the complex interplay of orchard management practices and pre-harvest mineral analyses regarding post-harvest fruit quality, storage, and shelf life potential is enabling industry to make more informed decisions.

In an experiment to determine the long-term effects of N application on apple fruit quality, Fallahi et al. (2001) determined that optimum leaf N content should be in the range of 2.05 - 2.30% for acceptable quality in 'Fuji' apples. Mango orchards in Spain with leaf N of 15 - 18 g/kg DW and Ca of 17 - 20 g/kg DW were considered prone to fruit internal flesh breakdown and deterioration (Torres et al. 2004b).

Mineral analysis of leaves has been extensively used in the industry to predict outturn fruit quality (Casero et al. 2004; Wang et al. 2015). However, studies question reliability due to poor predictive potential and suggest that fruit mineral analysis is the more reliable diagnostic tool for quality prediction (Fallahi et al. 1988; Marcelle 1989; Fallahi & Simons 1996; Hamilton et al. 2017). If fruit can be characterised by industry as likely to be high or low in certain quality attributes, then it will certainly help to make more informed decisions and can enhance profitability (Fallahi et al. 1997). In this context, appropriate timing of leaf and possibly inflorescence and/or immature fruit sampling is an important consideration (Campisi-Pinto et al. 2017).

2.5.2 Irrigation and fruit quality

Depending on amounts and timing during fruit development, rainfall and irrigation play a prominent role in fruit growth and sizing (Spreer et al. 2009; Schulze et al. 2013). Water deficit stress from flowering up to halfway through the mango rapid cell division period markedly affected growth rate and final fruit size (Simmons et al. 1995). Due to lower cell number, a reduction of 34% in fruit size was observed by Simmons et al. (1998a) for water-stressed 'Kensington Pride' mango trees as compared to non-stressed fruit. Thus, limited water supply may lead to lower Ca concentrations in developing fruit and can adversely affect post-harvest quality attributes. Additionally, water deficit conditions are likely to increase competition between highly transpiring leaves and young developing fruit for xylem sap uptake, which may ultimately contribute to decrease in Ca import into the fruit rendering it susceptible to postharvest disease and disorders.

On the other hand, an oversupply of water close to fruit maturity (viz., cell expansion phase) leads to increase fruit size and yield and also makes them more susceptible to physiological disorders (Spreer et al. 2009; Santos & Martinez 2013). This late-phase fruit expansion leads to Ca dilution which negatively affects post-harvest fruit quality (Simmons et al. 1998b; Shivashankara & Mathai 1999).

Studies on pears, apples, and mangoes reported mixed effects on post-harvest fruit quality of deficit irrigation during critical growth phases (Mitchell et al. 1989; Caspari et al. 1994; Ebel et al. 1995;

Dos Santos et al. 2015). Nonetheless, managing fruit size through best management practice irrigation could help manage fruit Ca and overall quality.

2.5.3 Temperature

Temperature modulates fruit metabolism, growth, maturation, and ripening (Paull & Chen 2000; Adams et al. 2001; Fonseca et al. 2002). Both pre-harvest and post-harvest storage temperature contributes to final fruit quality to consumers, including susceptibility to physiological disorders (Siddiqui 2015). Pre-harvest temperatures over the fruit development cannot be controlled but can be monitored such that measures can be taken to maintain fruit robustness and quality (Chagne et al. 2014). Orchard temperatures predominantly affect fruit physiology by influencing water loss through transpiration in association with prevailing vapour pressure deficits (Leonardi et al. 2000; Montanaro et al. 2012). Transpiration-mediated water loss influences fruit growth rate, diurnal shrinkage, and final quality at harvest (Génard & Bruchou 1992; Guichard et al. 2005), including in terms of fruit size, taste, and aroma (Génard & Bruchou 1992; Hewett 2006).

The internal temperature of growing fruit reflects energy exchange associated with fruit metabolic activity, energy conductance, and evapotranspiration. Collectively, these define the heat budget of a fruit (Cellier et al. 1993). Tertiary factors affecting a fruit's heat budget include environmental factors of temperature, humidity, air speed, and solar radiation and physical properties of fruit volume, skin colour and reflectance, pulp density, and skin permeability across serial developmental stages (Saudreau et al. 2007).

Optimum post-harvest handling, treatment and storage temperatures and durations are important at each step from farm to consumer in order to maintain quality. Exposure to elevated temperatures, especially for relatively prolonged durations, can hasten ripening, inactivate enzymes, inhibit protein synthesis, and alter membrane integrity. In afflicted fruit, reactive oxygen species buildup increases followed by fruit quality deterioration, including flesh discolouration (Durner 2013), internal breakdown, and cavity formation (Ferguson et al. 1999; Bautista & Esguerra 2007). Moreover, tropical fruit, including mangoes, are sensitive to low-temperature storage and can develop chilling injuries. Maintaining balance in storage time and temperature for specific commodities helps preserve quality and longevity of fresh produce (Wills & Golding 2016b). Recent investigations on mango fruit postharvest storage indicated low temperature storage (< 10 °C) for extended periods of time can promote likelihood of chilling injury and internal browning (de Freitas & Pareek 2019).

2.5.4 Harvest maturity

Optimum fruit maturity at harvest is essential to post-harvest quality, shelf life, and consumer

preferences (Wills & Golding 2016a). Fruit maturity is classified as physiological maturity and commercial maturity. A fruit that has attained maximum growth and development stage, is physiologically mature. Commercial maturity is the stage of fruit development when consumers' preferred characteristics have been attained in a commodity (Bautista 1990). 'Desert' mango varieties must be harvested green mature to ripen fully. 'Salad' cultivars are harvested and consumed 'green' for culinary purposes. Physiologically mature mango fruit are harvested before the onset of their ethylene and/or respiratory climacteric rises (Hofman & Whiley 2010). Early harvest physiologically immature desert fruit develop poor flavour and are more susceptible to mechanical damage and physiological disorders from post-harvest operations, like vapour heat treatment, hot water treatment, and irradiation (Jacobi et al. 2001). On the other hand, fruit harvested over-mature can exhibit off-odours, poor texture, and short shelf life. The severity of IDs, such as jelly seed and watery pulp breakdown in mango (Bally 2006) and flesh browning in avocado (Cutting & Bower 1987), is characteristically high in over-mature fruit.

2.6 Control measures and recommendations to minimise IDs

Mineral nutrient levels change throughout fruit development stages. Towards optimum levels, Young and Miner (1961) recommend maintaining <1.2% leaf N and >2.5% leaf Ca concentrations prior to flowering with a view to minimising susceptibility to disorders. Hofman and Whiley (2010) developed a best management practice guide for 'B74'mango. They recommended maintaining N at 1.0 - 1.5% and Ca at 2.0 - 3.5% in young mature leaves at the bud swelling stage for optimum quality fruit. It is considered important to maintain a balance in N and Ca levels to achieve optimum fruit quality. Antagonistic effects of N fertilisation on Ca uptake and accumulation in fruit have been researched in various fruit crops (Shear 1975b; Lewis et al. 1977; Cracknell Torres et al. 2004). Depending on pH, lime or gypsum are recommended to increase Ca levels in low and high pH soils in order to improve cation exchange percentage in the soil (Silva et al. 2005).

Subraman et al. (1971) found IDs in mango fruit to be more prevalent in coastal areas in India compared to inland orchards, possibly due to prevailing high humidities. Gunjate et al. (1982) suggested that prolonged sun exposure following harvest could alter physiology in favour of expression of disorders. Katrodia and Rane (1988) presented a heat convection theory that fruit from lower branches were more likely to develop physiological disorders due to heat convection from soil to surrounding air near the lower branches of the canopy which results in high-temperature conditions. In this regard, orchard management practices such as mulching can lower soil temperatures and reduce the incidence of IDs (Katrodia & Sheth 1988; Lad et al. 1992).

In complex interplay, a range of other factors can influence fruit quality. For example, fruit weight

and density are potential contributing factors (Figure 2.4). ‘Alphonso’ mango fruit harvested at 1.0 – 1.2 relative density were less likely to manifest IDs than fruit at >1.2 relative density (Krishnamurthy 1980). Harvesting at the green mature stage is recommended to reduce IDs, but it may result in poorer eating quality in some cultivars. Late-harvested fruit are more susceptible to internal flesh browning and so immediate shift to cooling chambers after harvest is recommended to preserve quality and shelf life (Baloch & Bibi 2012). Noticeable differences have been observed in susceptibility and tolerance to IDs in different cultivars of mango (Cracknell Torres et al. 2004; Srivastav et al. 2015). Selection of compatible rootstock and scions could be another way to reduce disorders as certain rootstocks can also improve nutrient uptake and accumulation in fruit (Iyer & Subramanyam 1992; Iyer & Degani 1997).

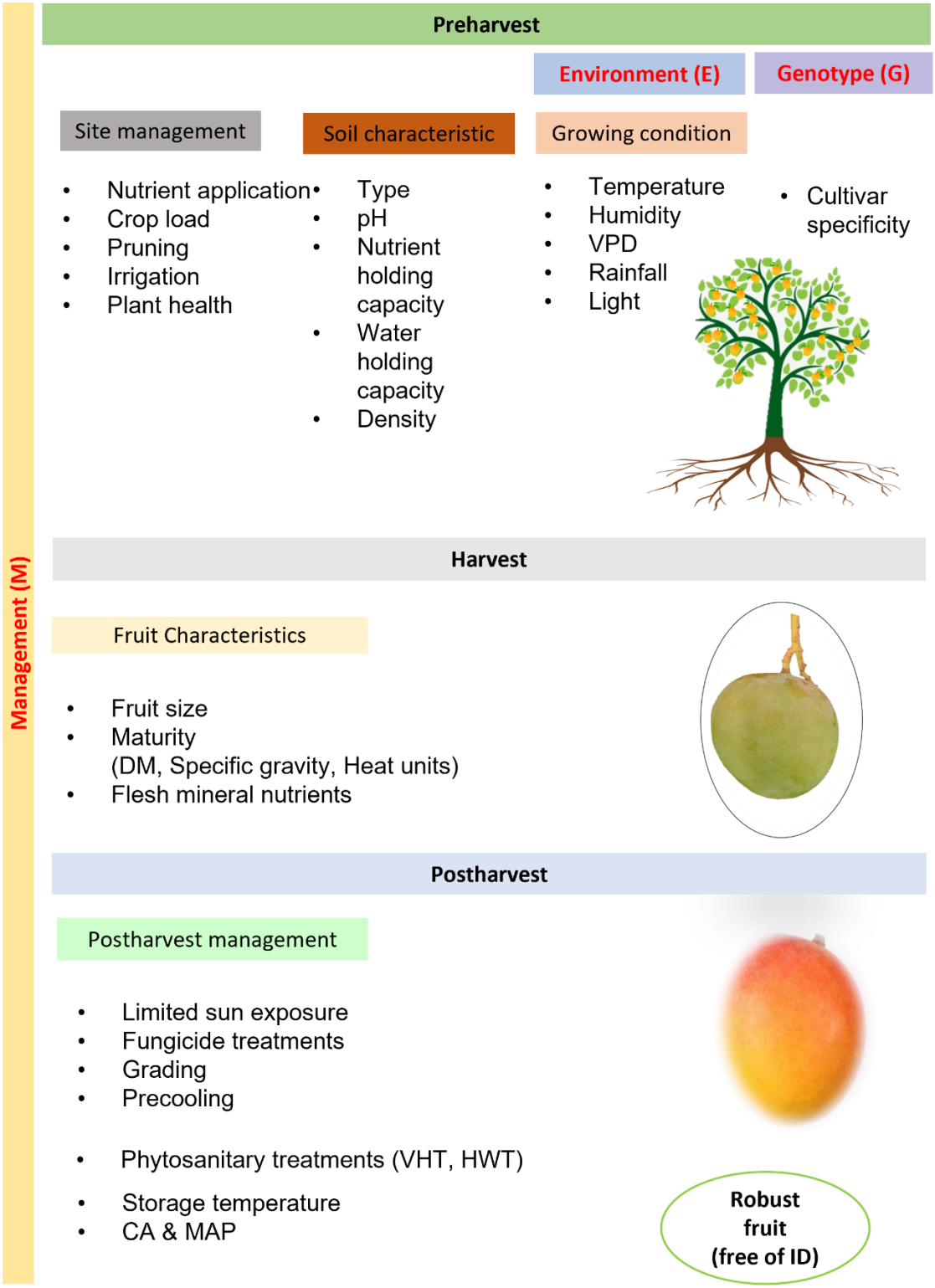


Figure 2.4: Major considerations at pre-harvest, harvest, and post-harvest stages across the 'genotype x environment x management' spectrum to produce robust mango fruit.

2.7 Conclusion

Fruit robustness at harvest and post-harvest quality and shelf life are predominantly linked to pre-harvest nutrient status. Nutrient imbalance like high fruit [N] and low [Ca] can negatively affect fruit

quality and the incidence of post-harvest disorders. Increasing Ca concentrations in fruit flesh is a challenge as applying more and more Ca to the soil does not guarantee its accumulation in fruit. Controlling tree vigour by pruning, managing crop load by thinning, carefully timing irrigation, and small frequent nutrient applications can potentially improve Ca levels in fruit, especially during the early developmental stages when the Ca accumulation rate is high. While N manipulation is achievable due to its mobility, application strategies need to limit fruit N without negatively affecting fruit development and yield. Careful monitoring of soil, leaf, and fruit nutrient status is recommended and desirable. When sampling and analysing fruit and leaf samples, adoption of recommended strategies to minimise variation and avoid ambiguity in results is advised. Other factors including temperature, humidity, light radiation, and vapour pressure deficit likely differentially influence fruit development and physiology. Considering complex interplays of variables, consistent production of robust quality mango fruit requires a holistic approach. Mango fruit quality is an output of a dynamic system that integrates influences of growing environment, genotype, and associated management practices at pre-harvest, harvest, and post-harvest stages.

Chapter 3 Factors contributing to internal disorders of mango (*Mangifera indica*) fruit – A systematic literature review

3.1 Abstract

The expression of internal disorders is a complex phenomenon due to the interaction of genotype, environment, and management practices at the pre-harvest, harvest, and post-harvest stages. This study aims to provide a comprehensive overview of factors associated with, and have been investigated in relation to, the susceptibility and expression of internal disorders (IDs) in mango. Six hundred and fifty-seven studies were sourced from three databases. Of these, 72 were relevant after screening based on exclusion-inclusion criteria. An additional nine relevant studies were identified through manual searches and included in the final data synthesis. From the 81 studies, the prevalent disorders were spongy tissue and jelly seed, followed by flesh browning and soft nose. 'Alphonso' expressed only spongy tissue disorder. By contrast, 'Tommy Atkins' simultaneously expressed multiple disorders, viz., soft nose, jelly seed, and stem-end cavity. Mineral composition of flesh and exposure to heat post-harvest (viz., sunlight, phytosanitary heat treatment, and elevated storage temperature) were prominent in association with IDs expression. Relatively high [N], N/Ca, Mg/Ca, and K/Ca ratios and relatively low [Ca] and [B] were related to most disorders. With [K] and [Mg], studies inconsistently suggested positive and inverse relationship with a particular disorder. While individual studies suggest casual relationships, there was a relative dearth of pre-harvest work on soil characteristics, growing conditions (e.g., temperature, rain, vapour pressure deficit), crop physiology (e.g., age, biennial bearing) and management practices (e.g., fertilisation, irrigation) in the literature. To address this, future studies might seek to gather and report comprehensive data on edaphic and environmental characteristics and post-harvest conditions. Such more comprehensive data sets relating to fruit quality would contribute towards better understanding of implications for mango fruit quality, including IDs.

Keywords: Flesh browning, mineral nutrition, physiological disorders, post-harvest quality,

3.2 Introduction

Mango (*Mangifera indica L*., family *Anacardiaceae*) is an economically important fruit crop grown in tropical and subtropical regions of Asia, Australia, Africa, and Latin America (Litz 2009; Kiloes et al. 2022). Mango is known for its sweet taste, distinct aroma, and high carotenoid content (Sivakumar et al. 2011; Sivankalyani et al. 2016).

World mango production is increasing, for example, from 25 million tonnes in 2000 to over 57 million in 2021 (FAOSTAT 2022). Furthermore, the volume of mango exports from 2000 to 2021 increased from 0.62 to 2.3 million tonnes, with an average annual increase of 26% (FAOSTAT 2022). This rise in global demand for mangoes presents challenges associated with fruit quality. For example, the internal quality of the fruit towards the end of the supply chain, particularly for export markets. Fruit internal quality is often compromised by internal physiological disorders, which frequently arise during pre-harvest fruit development and post-harvest storage (Raymond et al. 1998a; Raymond et al. 1998b; Mogollón et al. 2020). Such disorders lessen the internal quality of the fruit, potentially rendering it unsuitable for human consumption, and posing a problem for the mango industry (Ma et al. 2022).

Mangoes in most of the world's major production areas are susceptible to various physiological disorders. They include spongy tissue, jelly seed, soft nose, and insidious fruit rot, to name a few (Raymond et al. 1998a; Andrade et al. 2022; Ma et al. 2022). Internal disorders (IDs) are not visible to the naked eye, making them nearly impossible to detect at early stages. Although IDs have been extensively studied in the literature, underlying mechanisms and causes are dynamic. A range of factors across pre-harvest, harvest, and post-harvest stages have been associated with expression of IDs (Léchaudel & Joas 2007; Sivakumar et al. 2011).

This systematic literature review was conducted towards a comparative comprehensive understanding of individual disorders affecting mango fruit quality and potential contributing factors. It aimed to inform and facilitate future management of these disorders. It also summarises varying terminologies used to describe different or similar disorders based on symptoms or pictorial references with the intent to establish a ‘common language’ for academic and industry sectors of the mango community. The strict inclusion criteria used in this review ensured that the article selection fits the study's objective and minimised literature biasness.

3.3 Methodology

Methodology utilised in this study followed the standard guidelines of systematic review outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines

(PRISMA) (Nightingale 2009). The PRISMA workflow effectively shows information flow through different phases. A general workflow of PRISMA involves the following steps: (i) Scoping: formulating the study objectives and expected outcomes, (ii) Planning: developing a search strategy using specific terminologies and identifying suitable databases for the study, (iii) Identification: identifying and examining resulting papers, (iv) Screening and Eligibility: removing duplications, irrelevant titles, or abstracts and further elimination based on exclusion/inclusion criteria, (v) Data Extraction: extracting relevant data from selected papers in a structured format according to the study objectives, and (vi) Interpretation: providing a descriptive summary of the findings, interpretations, and limitations.

Three databases were selected for the initial search: ScienceDirect, Scopus, and Web of Science. The search was conducted in November 2022. Search terms used for each database and the results are shown in Table 3.1. The search identified 657 potential papers for initial screening. The results were saved in RIS format and imported into the Covidence platform, an online screening and data extraction tool used for systematic review management (https://www.covidence.org/). Of the full total, 123 papers were removed due to duplication across databases. Further 416 papers were excluded in the initial screening because the study title and/or abstract was irrelevant to this study's objectives. During the second phase of screening, which involved full-text screening, 46 studies were excluded as not meeting the inclusion criteria (Table 3.2). Additional searches were performed manually to identify and include papers that might have been missed from previous searches. Nine papers relevant to the study objectives were identified through manual search and subsequently included in the final set of papers for data extraction. The final result was 81 papers (Figure 3.1) that included scientific articles, short communications, and conference papers, if sufficient information was available to meet requirements, in an inclusion timeline of 1971 - 2023.

For data extraction, each paper was read thoroughly and included in a structured extraction sheet coded as Title, authors, study year, publication year, country of study, cultivar, publication type, study type, post-harvest treatments (if applicable), study length, fertiliser application (if applicable), type of disorder studied, and reason of defect (if applicable).

In the final step, the data was interpreted in a structured report consisting of a descriptive summary of the findings, interpretation of findings, discussion, and limitations. Disorders were classified through written or pictorial references of symptoms along with factors responsible for their expression in mango fruit. Descriptive figures were created by Microsoft Excel (365). 'Sankey' diagrams depicting association of IDs with cultivars and mineral concentrations were produced in R (4.0.2) using 'd3Network' package.

Thereafter, a causal loop diagram (CLD) was developed based on secondary data extracted from selected literature to discern the nature of disorder expression in terms of multifactorial associations. CLD involves the extensive mapping of the feedback loops within a defined system (Haraldsson 2004). IDs in mango were considered an output of a complex system and direct and indirect effects of various factors positively or negatively influencing the expression of IDs were mapped using Stella® Architect systems modelling software considering its user-friendly interface and recognised iconography (Walters et al. 2016). Cause-and-effect relationships were represented between variables using arrows within the CLD. A positive sign (+) at arrowhead indicated a proportional relationship between cause and effect, and a negative sign (-) indicated an inverse relationship.

Table 3.1. Database searched using specific terminologies.

Database	Search terms	Results
ScienceDirect	("mango") and ("Internal disorders" OR "Internal breakdown" OR "physiological disorders" OR "internal browning" OR "jelly seed" OR "stem end cavity" OR "spongy tissue" OR "soft nose")	393
Scopus	(TITLE-ABS-KEY ("mango" OR "Mangifera indica") AND TITLE-ABS-KEY ("Internal disorders" OR "Internal breakdown" OR "flesh breakdown" OR "physiological disorders" OR "flesh browning" OR "internal browning" OR "jelly seed" OR "stem end cavity" OR "flesh cavity" OR "spongy tissue" OR "soft nose" OR "internal darkening"))	133
Web of Science	(TITLE-ABS-KEY ("mango" OR "Mangifera indica") AND TITLE-ABS-KEY ("Internal disorders" OR "Internal breakdown" OR "flesh breakdown" OR "physiological disorders" OR "flesh browning" OR "internal browning" OR "jelly seed" OR "stem end cavity" OR "flesh cavity" OR "spongy tissue" OR "soft nose" OR "internal darkening"))	131

Table 3.2. The exclusion and inclusion criteria for systematic literature review.

Criteria	Inclusion	Exclusion
Document type	Journal article Conference papers Short communications	Reviews Book chapters Thesis
Language	English	Non-English
Study objective	Discussing any aspect of internal disorders of mango	Not discussing internal disorders of mango
Full text	Available through web or library resources	Not available

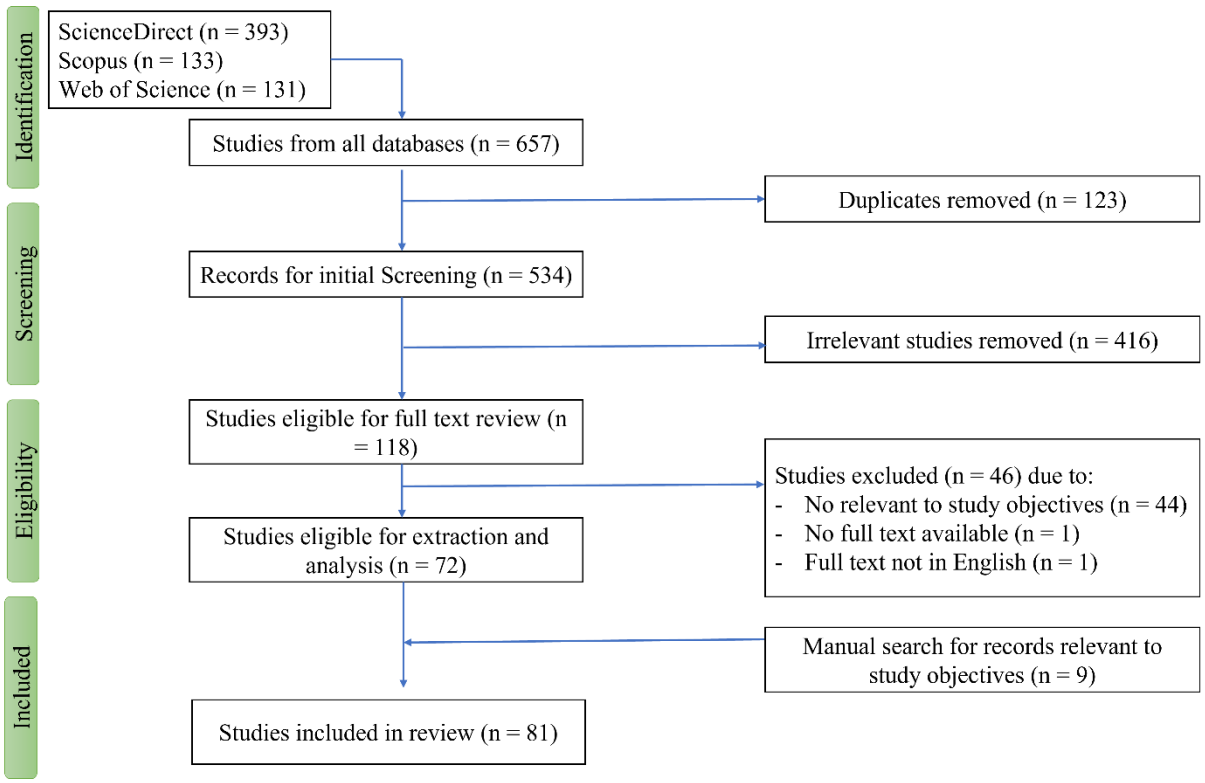


Figure 3.1: Step-by-step literature selection process based on PRISMA flow diagram (Nightingale 2009).

3.4 Descriptive analysis

In general, three types of publications were included for data extraction and analysis: scientific papers, short communications, and conference papers. Figure 3.2a breakdowns number of publications over the past 5 decades, discussing one or more aspects of IDs. An increase in published studies was observed after 2000, which may have been associated with the global increase in mango production and export (FAO 2022; FAOSTAT 2022). World mango export volume increased from 0.62 to 1.65 million tonnes over the 2000–2013 period (Evans et al. 2017). Such increase in export demand drives increase in quality control management as 'long' export supply chains often result in reduced shelf life, quality deterioration, and physiological disorders (Le et al. 2022). The highest number of publications (n = 40) discussing IDs of mango was conducted in India, followed by Brazil (n = 13), Spain (n = 6), China (n = 3), Philippines (n = 3), and USA (n = 3) (Figure 3.2b). When studies were categorised based on cultivar, 'Alphonso' was the most studied one for expression of IDs (n = 29), followed by 'Tommy Atkins' (n = 12), 'Keitt' (n = 10), and 'Dashehari' (n = 7) (Figure 3.3). Some studies discussed more than one cultivar for the development of a particular disorder, e.g., Cracknell Torres et al. (2004) studied incidence of internal flesh breakdown in 28 different mango cultivars and Srivastav et al. (2015) evaluated incidence of jelly seed disorder in 20 mango genotypes. Eleven publications highlighted incidence of IDs in 'not-so-popular cultivars' as represented by "other

cultivars" in Figure 3.3.

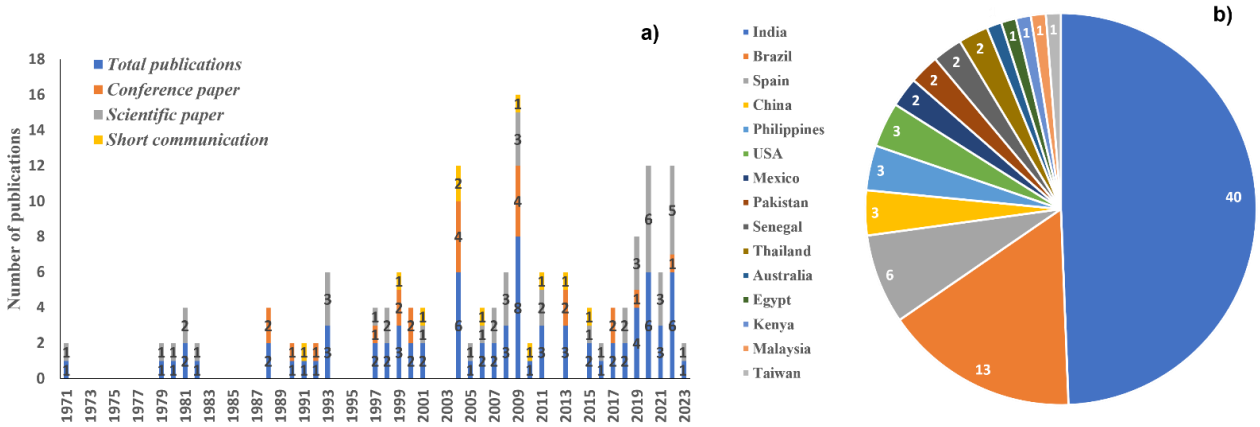


Figure 3.2: a. Studies published over five decades discussing the IDs of mango and b. number of studies categorised based on country of study around the world.

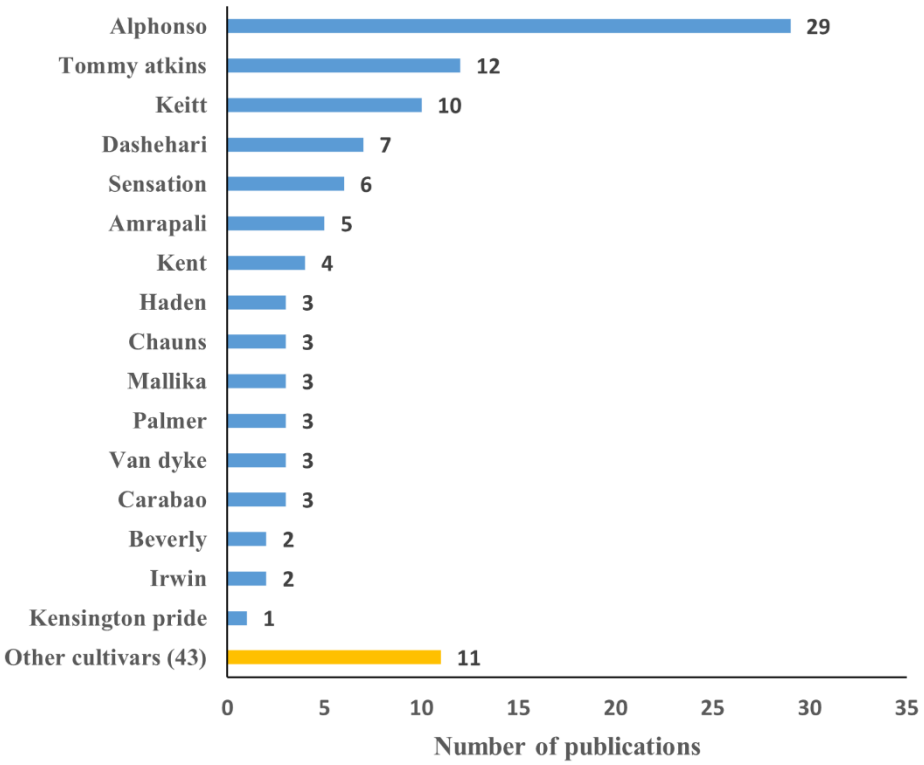


Figure 3.3: Number of studies discussing the IDs in major mango cultivars over five decades. Some studies discussed more than one cultivar for single or multiple disorders within a single publication.

3.5 Internal disorders: Symptoms and classification

IDs in mango are also called flesh breakdown or internal physiological disorders. They are characterised by any disintegration/abnormality in fruit mesocarp that is non-pathogenic in nature (Wainwright & Burbage 1989). Literature on mango IDs uses a range of specific or general

terminologies to describe one or more types of defects. Table 3.3 presents a range of general and specific terminologies used in the literature and their manifestation as a disorder.

Specific terminologies are based on nature/appearance and/or location of the disorder within the mango fruit flesh. Soft nose is over softening and yellowing of fruit flesh at the distal end of the fruit (Figure 3.4b) (Burdon et al. 1991; Raymond et al. 1998a). Spongy tissue is brown/yellowish/white corky spots and sponginess in the flesh with or without cavities or air pockets and off-odour (Figure 3.4c) (Gunjate et al. 1979b). Stem-end cavity is characterised by the presence of a cavity or air pocket in the fruit's proximal region in the mesocarp adjacent to the peduncle (Figure 3.4d) (Raymond et al. 1998a). Jelly seed is associated with watery and jelly-like appearance of the mesocarp near and around fruit seed (Figure 3.4e) (Andrade et al. 2022). Finally, flesh browning is characterised by brown/blackish discolouration of the fruit mesocarp around the seed (Figure 3.4f).

However, the literature is rich with general terminologies, such that when symptoms are compared as to their written or pictorial reference, flesh browning is apparently also described as black flesh (Mogollón et al. 2020; Andrade et al. 2022), internal browning (Gabriëls et al. 2020), internal darkening (Acosta et al. 2000), internal necrosis (Saran & Kumar 2011), and insidious fruit rot (Tarmizi et al. 1993). Another general terminology is internal breakdown/defects that collectively describe more than one type of disorder in individual fruit e.g., Raymond et al. (1998b) referred soft nose, stem-end cavity, and jelly seed as generic term 'internal breakdown' in 'Tommy Atkins' mango.

Such discrepancies could create confusion when seeking an understanding of the causal factors of IDs and their effective management. Soft nose was mostly attributed to mineral nutrient imbalance in the fruit flesh (Burdon et al. 1991, 1992), stem-end cavity was associated with fruit weight at harvest (Hermoso et al. 1997; Torres et al. 2004b; Torres et al. 2009), and flesh browning a response to post-harvest storage temperature and duration (Acosta et al. 2000; Pina et al. 2000) and to post-harvest heat treatment at eating ripe stage (Jabbar et al. 2011) (Figure 3.6). Moreover, some cultivars are more susceptible to a specific disorder expression than are others. For instance, 'Alphonso' and 'Dashehari' are highly susceptible to spongy tissue and jelly seed respectively, and 'Sensation' is prone to stem-end cavity and spongy tissue disorders (Figure 3.6). 'Beverly', 'Kent', and 'Chauns' are susceptible to soft nose. 'Keitt' and 'Tommy Atkins' express almost all disorders, viz., soft nose, jelly seed, spongy tissue, flesh browning, and stem-end cavity (Figure 3.6).

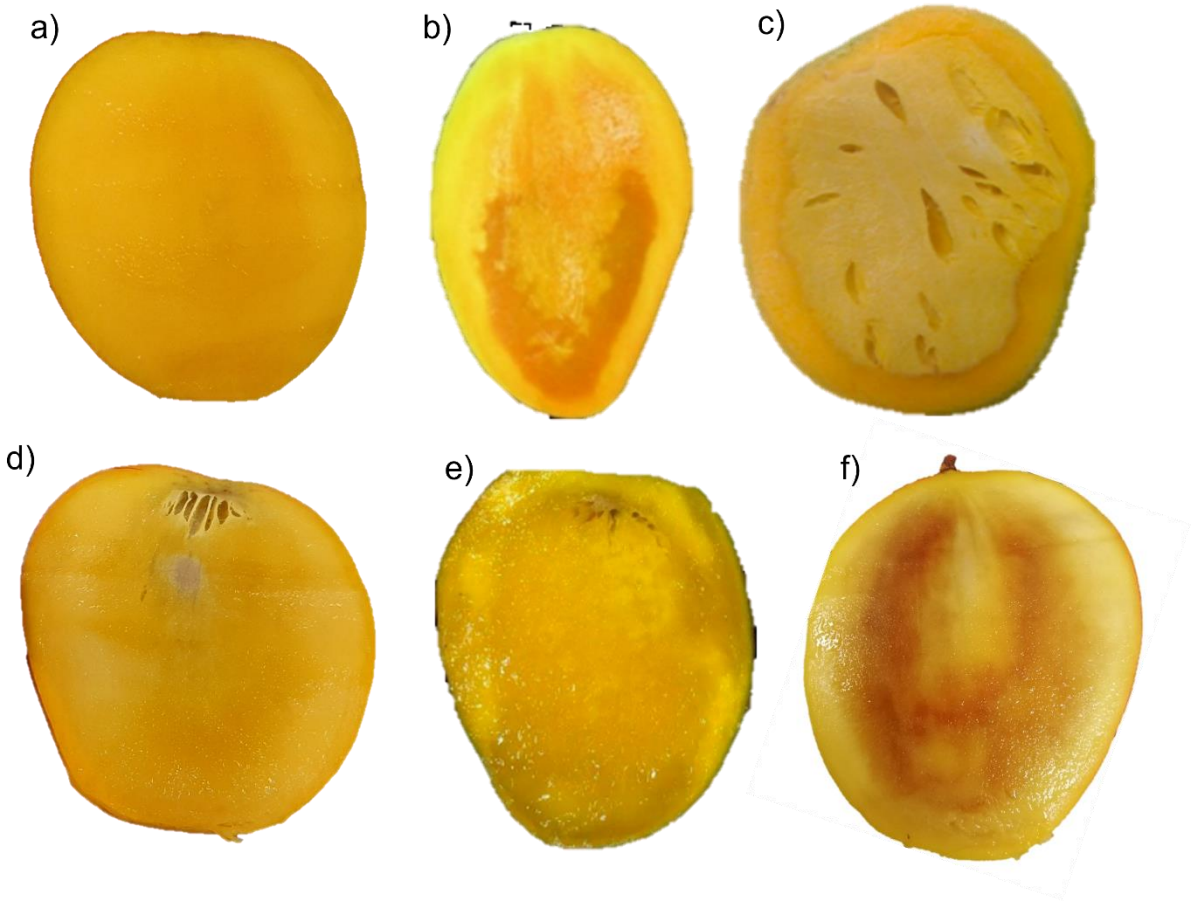


Figure 3.4: Internal disorders of mango. a. No disorder (control), b. Soft nose disorder (Lin et al. 2013), c. Spongy tissue disorder (Oak et al. 2019), d. Stem-end cavity disorder (Asad et al. unpublished), e. Jelly seed disorder (Andrade et al. 2022), and f. Flesh browning disorder (Asad et al. *unpublished*).

Table 3.3. General and specific terminologies and symptoms as mentioned in the literature to describe different IDs of mango.

General terminologies	Specific terminology	Symptoms	Similarity	References
----	Spongy tissue	Brown/yellowish/white corky patches with or without air pockets/cavities and sponginess in flesh	----	(Gunjate et al. 1979b; Krishnamurthy 1980; Gunjate et al. 1982; Katrodia & Rane 1988; Katrodia & Sheth 1988; Thomas et al. 1993; De Oliveira Lima et al. 1999; Shivashankara & Mathai 1999; Lima et al. 2001; Ravindra & Shivashankar 2004; Torres et al. 2004b; Ravindra & Shivashankar 2006; Shinde et al. 2006; Singh et al. 2006a; Vasanthaiah et al. 2006; Shivashankar et al. 2007; Janave & Sharma 2008; Vasanthaiah et al. 2008; Burondkar et al. 2009; Janave 2009; Raja 2009; Shinde et al. 2009; Nagamani et al. 2010; Ravindra et al. 2010; Mehta et al. 2013; Shivashankar et al. 2015; Oak et al. 2019; Shivashankar & Sumathi 2019; Andrade et al. 2022; Oak et al. 2022; Ma et al. 2023)
----	Jelly seed	Deeper yellow-orange colour/ watery and jelly appearance of the mesocarp tissue	----	(Raymond et al. 1998a; Whangchai et al. 2001; Torres et al. 2004b; Torres et al. 2009; Lin et al. 2013; Srivastav et al. 2015; Shivashankar et al. 2016; Krishna et al. 2019; Seshadri et al. 2019; Bitange et al. 2020; Krishna et al. 2020; Mogollón et al. 2020; Andrade et al. 2022)
----	Soft nose	Over softening/breakdown of flesh near distal end	----	(Burdon et al. 1991, 1992; Raymond et al. 1998a; Amin et al. 2007; Jabbar et al. 2011; Lin et al. 2013)
----	Stem-end cavity	Cavity and discolouration at stem-end in the flesh	----	(Hermoso et al. 1997; Raymond et al. 1998a; Torres et al. 2004b; Torres et al. 2009)
Internal breakdown/ flesh breakdown/ Internal necrosis	----	Pale yellow/white flesh with air pockets/cavities	Spongy tissue	(Subraman et al. 1971; Esguerra et al. 1990; Hermoso et al. 1997; Hofman et al. 1997b; Kitma & Esguerra 2009; Lizada & Rumbaoa 2017; Ma et al. 2018; Bizzani et al. 2020; Ma et al. 2022)
		Brown-black flesh and flesh discolouration	Flesh browning	(Saran & Kumar 2011; Bizzani et al. 2020; Ma et al. 2022; Seehanam et al. 2022)
		Tissue collapse and watery areas	Jelly seed	(Oldoni et al. 2022)
Internal browning/ darkening/ discolouration/ black flesh	----	Browning in the mesocarp of fruit	Flesh browning	(Tarmizi et al. 1993; Acosta et al. 2000; Pina et al. 2000; Jabbar et al. 2011; Gabriels et al. 2019; Lo'ay & Ameer 2019; Gabriëls et al. 2020; Mogollón et al. 2020; Andrade et al. 2022)

3.6 Major contributing factors in the expression of internal disorders

Contributing factors associated with the expression of IDs in mango fruit in selected literature are shown in Figure 3.5. Mineral nutrient imbalance in terms of access and/or deficiency was the dominant factor in disorder expression (n = 22). Other factors were post-harvest temperature management, including the storage temperature (n = 5), post-harvest quarantine heat treatment (n = 4), and sun exposure after harvest in the field (n = 3). Some studies mention multiple reasons for the expression of IDs in a single publication. For example, Esguerra et al. (1990) asserted fruit maturity and post-harvest vapour heat treatment as being collectively responsible for spongy tissue in 'Carabao' mango. Similarly, Raja (2009) considered pre-harvest high temperatures and humidity and mineral nutrient imbalance in the leaves as potential contributing factors to spongy tissue disorder in 'Alphonso' mango. A number of studies in the selected literature (n = 31) had focused mainly on molecular and biochemical aspects and/or non-destructive detection of IDs (Thomas et al. 1993; Raymond et al. 1998a; Oak et al. 2019; Mogollón et al. 2020; Oak et al. 2022). These did not identify nor discuss a factor, *per se*. Spongy tissue was associated directly and/or indirectly with all of the factors discussed in the literature (Figure 3.6). However, jelly seed was only linked to mineral nutrient imbalance in the fruit flesh, fruit weight at harvest, and premature seed germination (Torres et al. 2004b; Torres et al. 2009; Singh & Swati 2011; Shivashankar et al. 2016; Andrade et al. 2022) (Figure 3.6). The role of each contributing factor is discussed in detail in the following sections.

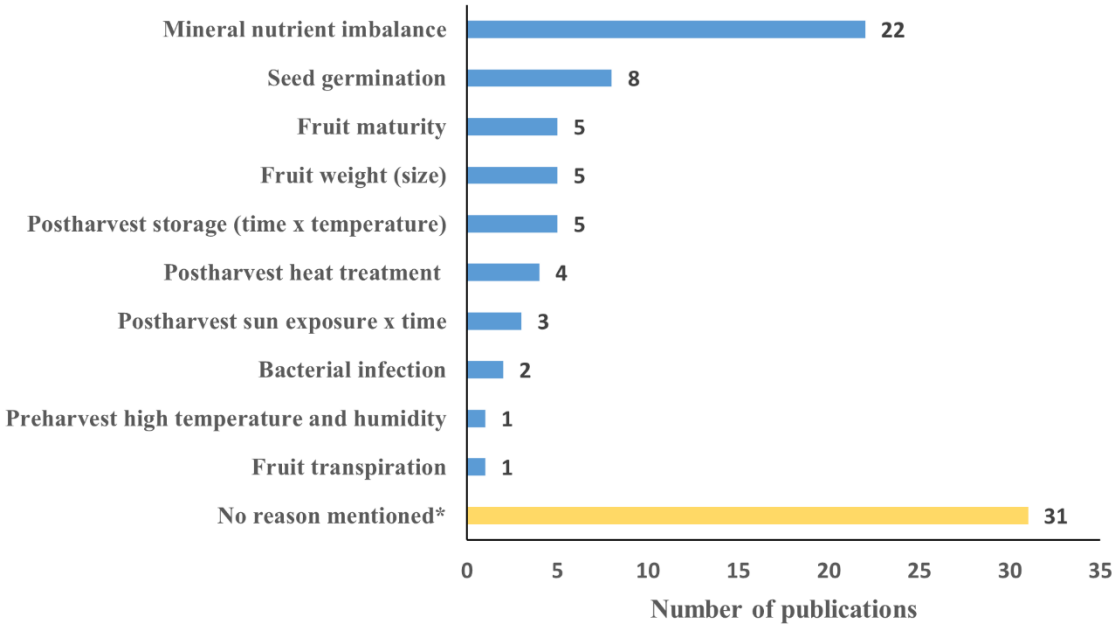


Figure 3.5: Breakdown of factors discussed in selected literature for expression of mango IDs. '*' denotes studies discussing molecular, biochemical aspects, and non-destructive detection of IDs.

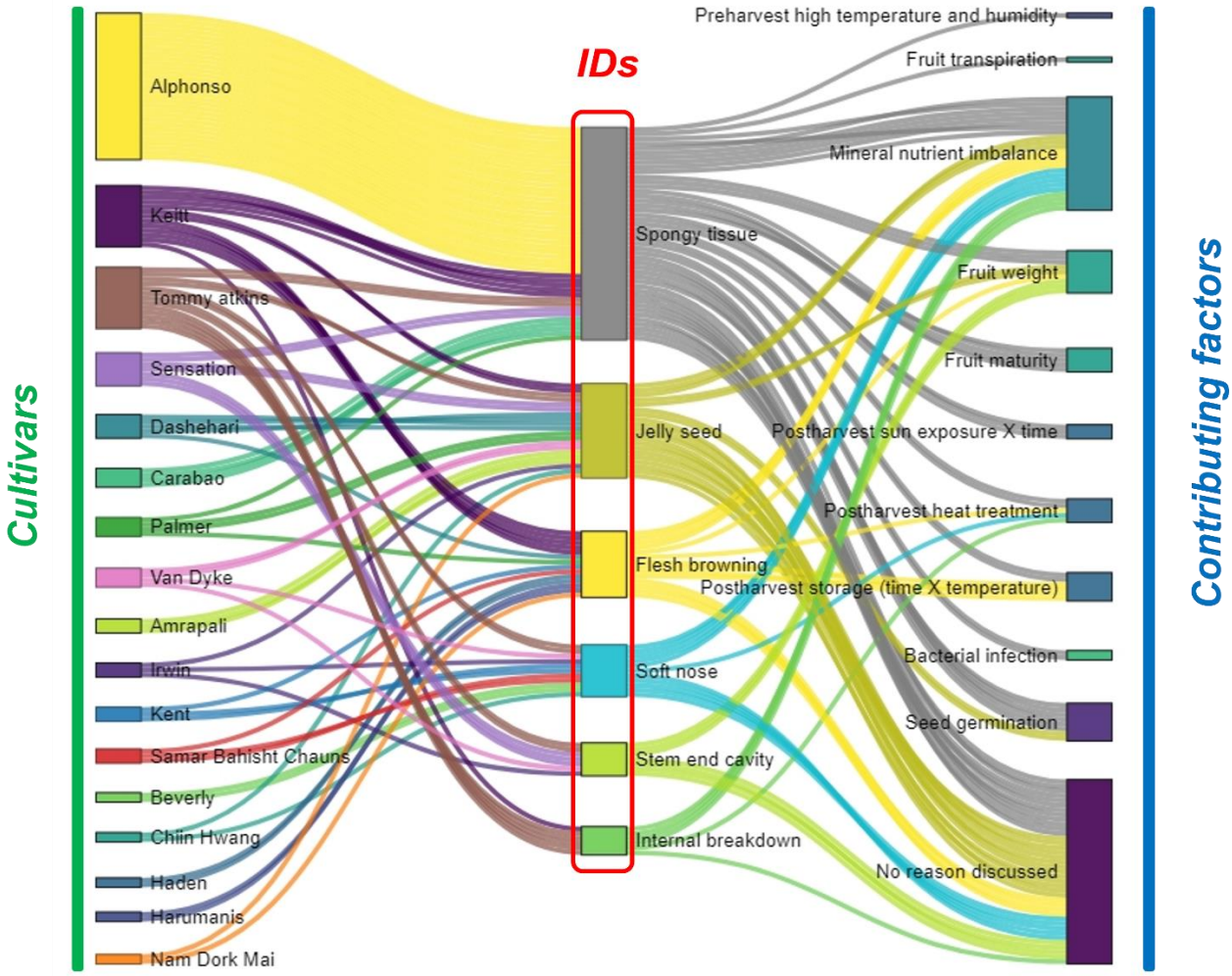


Figure 3.6: Classification and association of cultivar vs. IDs and respective contributing factors in the selected literature. Herein, the term 'internal breakdown' refers to the presence of multiple disorders all at once in single fruit, i.e., soft nose, jelly seed, stem-end cavity, and/or flesh browning.

3.6.1 Mineral nutrients

One of the most important and well-discussed contributing factors in literature to mango fruit ID expression is mineral nutrient imbalance, either deficiency or access (n = 22) (Figures 3.5 and 3.6). Mangoes require a range of nutrients to grow and develop properly (Bally 2009). Obtaining an optimum balance of mineral nutrients is likewise important to define fruit quality, in the context of varying genetic (G), management (M), and environmental (E) influences at pre-harvest fruit development and post-harvest handling stages (Léchaudel & Joas 2007; Bally 2009).

Ca deficiency is the mineral most studied and associated with expression of IDs in mango. Ca deficiency has been linked to spongy tissue in 'Alphonso' (Subraman et al. 1971; Gunjate et al. 1979b; Raja 2009) and 'Keitt' (Ma et al. 2018; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023), flesh browning (Tarmizi et al. 1993; Andrade et al. 2022), soft nose (Burdon et al. 1991, 1992), and jelly seed (Singh & Swati 2011; Bitange et al. 2020; Andrade et al. 2022) (Figure 3.7, Appendix A1). Ca

provides membrane stability and cell wall strength through cross-linkages of pectin and carboxylic groups (Hocking et al. 2016). Therefore, Ca deficiency weakens the cell wall and renders fruit susceptible to breakdown during post-harvest rigours and stresses (Tonetto de Freitas & Mitcham 2012). Ca concentrations in fruit flesh depends on fruit growth rate and transpiration (Hocking et al. 2016). Fruit transpires at relatively high rates per unit fresh mass at early development stages, particularly during rapid cell division. They thus accumulate much of their Ca during early growth phase (Joyce et al. 2001; Montanaro et al. 2010). As fruit develop and mature, they enter the predominantly cell expansion stage and Ca intake decreases drastically (Joyce et al. 2001; Montanaro et al. 2010). This decline in Ca at later stages is attributed to reduction in xylem sap uptake due to the loss of xylem functionality and hydrostatic gradient between fruit and pedicel, generated by fruit growth and transpiration (Nordey et al. 2015). Transpiration is restricted at later stages as functional stomata turns into lenticels and with the formation of thick cuticle wax on mango fruit surface (Bally 1999). Therefore, adequate availability of Ca during critical early growth stages may make a major difference in overall fruit Ca concentrations and post-harvest quality.

Boron (B) deficiency was associated with browning and cracking-like symptoms in mango fruit flesh (Saran & Kumar 2011), along with spongy-like cavity formation in 'Keitt' (Ma et al. 2018). B is important for fruit set by regulating pollen tube growth, pollen germination, and flower development (Bally 2009). Saran and Kumar (2011) reported significantly ($p \leq 0.05$) lower levels of B in fruit flesh expressing flesh browning symptoms in 'Dashehari' mango fruit. Foliar application of B as disodium octaborate tetrahydrate was effective in improving B levels, yield, and reducing disorder.

Relatively high concentrations of N and phosphorus (P) were associated with mango fruit expressing a number of IDs (Subraman et al. 1971; Burdon et al. 1991; Torres et al. 2004a; Andrade et al. 2022; Ma et al. 2023). N tends to stimulate vegetative growth and increase fruit set, retention, and weight (Nguyen et al. 2004; Bally 2009; Ibell et al. 2017). Untimely application of N may stimulate new growth flush during early fruit developmental stages, consequently redirecting Ca influx from fruit towards young leaves with high transpiration rates. P is involved in root tip elongation, cell division and growth and is an essential component of sugar phosphates that regulate respiratory and other cellular energy metabolism mechanisms (Bally 2009).

The influences of mineral nutrition can be modulated by G x E x M. 'Keitt' mango from Brazil had Ca concentrations of 1000 mg/kg D.W. in healthy and ~200 mg/kg D.W. in spongy tissue-affected fruit (Andrade et al. 2022). Ma et al. (2023) reported Ca concentrations of ~175 mg/kg D.W. in healthy and ~125 mg/kg D.W. in spongy tissue affected 'Keitt' fruit grown in China (values were estimated from graphs). There were conflicting reports of magnesium (Mg) and potassium (K) in terms of their associated with spongy tissue disorders (Figure 3.7, Appendix A1). For example,

Andrade et al. (2022) reported [Mg] deficiency and Ma et al. (2023) reported [Mg] excess in spongy tissue-affected 'Keitt' mango from Brazil and China, respectively. Reasons for such discrepancies in the literature are elusive and make it impractical to name optimum nutrient levels for robust fruit. However, mineral nutrient ratios, like N/Ca, K/Ca, and Mg/Ca, may be more reliable indicators of ID susceptibility in mango. There has been a consistent trend of higher ratios of these minerals in fruit affected with IDs compared to healthy fruit (Tarmizi et al. 1993; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023). Nevertheless, and as for individual element concentrations, specific ratios are elusive. Hence, predicting fruit quality based on mineral nutrient concentrations and ratios may need to be optimised for G x E x M.

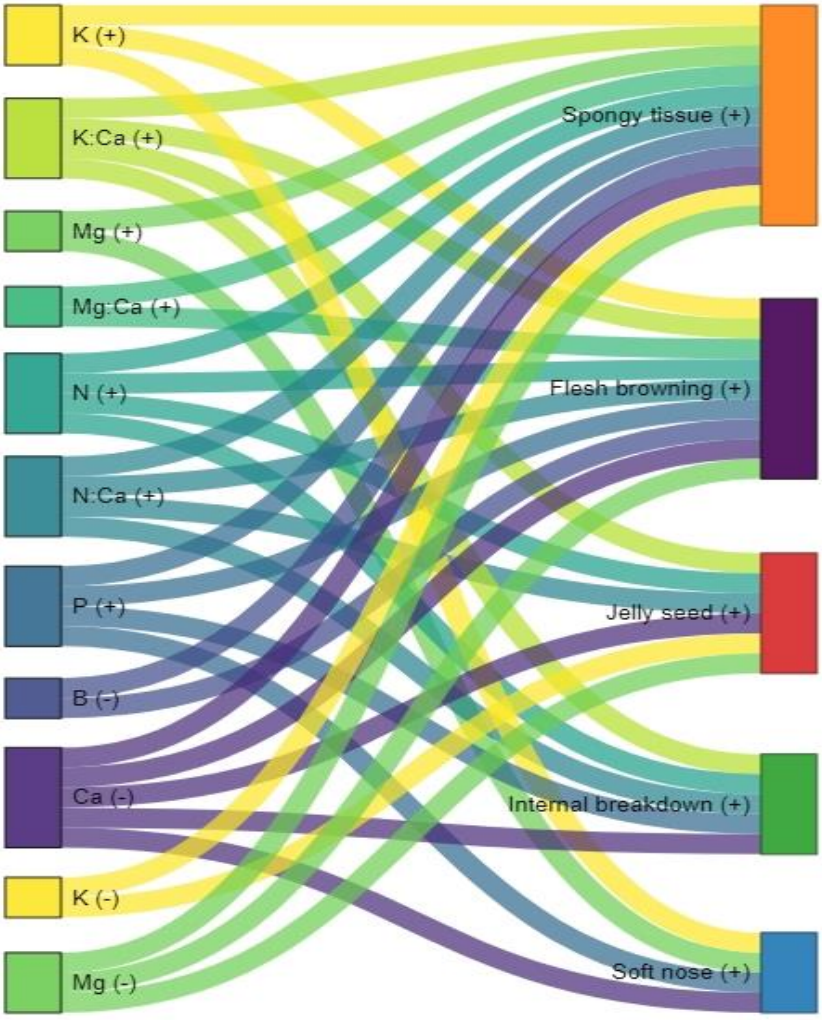


Figure 3.7: Association of the individual mineral element in mango flesh and their ratios with the expression of internal disorders. '+' sign in disorder column indicates an increase in disorder with an increase (+) and decrease (-) in mineral element concentrations in mango flesh. Here, the internal breakdown term refers to the expression of the combination of soft nose, jelly seed and stem-end cavity disorder in single fruit at once. For detailed information on individual studies, see Appendix A1.

3.6.2 Temperature exposure

Temperature regulates critical development processes, including growth, maturation, and ripening, and mediates post-harvest product quality and shelf life (Paull & Chen 2000; Fonseca et al. 2002). Raja (2009) reported a marked increase in spongy tissue in 'Alphonso' mango in humid climate (humidity: 60-70%, rainfall: 2500 mm/year) with higher growing temperatures compared to a semi-arid climate (humidity: 25-30%, rainfall: 650 mm/year). Pre-harvest temperatures modulate developmental aspects of mango fruit physiology through transpiration rate and water loss that determines nutrient movement, diurnal shrinkage, growth rate, and ultimately fruit quality and susceptibility to IDs (Shivashankara & Mathai 1999; Montanaro et al. 2006; Singh et al. 2006b). Influences of temperature on the development of important quality traits, such as fruit size, taste, and aroma are well documented (Génard & Bruchou 1992; Hewett 2006).

Post-harvest temperature management includes limited exposure to field temperatures and sunlight at harvest, post-harvest phytosanitary heat treatments as a control measure for fruit fly infestation for export, and low-temperature storage in transit (Gunjate et al. 1982; Esguerra et al. 1990; Acosta et al. 2000) (Figure 3.6). Mango fruit exposed to heat after harvest can alter its physiology such as hindering normal physiological processes and ripening (Gunjate et al. 1982; Katrodia & Sheth 1988). Studies in the 1980s highlighted the critical roles that sunlight and field temperatures play in the development of IDs in mango. Gunjate et al. (1982) exposed harvested 'Alphonso' mango to a direct sunlight-induced internal fruit temperature range of 37.5 – 49 °C for 5 – 240 min and observed a linear increase of the incidence of spongy tissue disorder from 10% to 100% in control (no exposure) to 240 min sunlight exposed fruit. Similarly, the sun-exposed side with internal temperature of 43.8 °C of an 'Alphonso' mango manifested 100% spongy tissue disorder incidence as compared to 0% for the unexposed side (39.2 °C) (Katrodia & Sheth 1988). Such studies paved the way for early management guidelines to limit direct sunlight exposure and to precool at the earliest convenience after harvest.

Post-harvest phytosanitary heat treatments, like vapour heat treatment (VHT) and hot water treatment (HWT), are important for mango exports to specific protocol countries as a quarantine treatment for fruit fly and other pests, like mango seed weevil. In post-harvest heat treatments, the fruit mesocarp temperature is raised to 47 °C and maintained for 15 min by hot moist air (VHT) or hot water dips (HWT) (Jacobi et al. 2001). Temperature range and duration can vary depending upon a cultivar's susceptibility to heat injury. Both treatments kill fruit fly eggs and larvae, but can induce disorders depending upon the cultivar and inherent fruit robustness (Esguerra et al. 1990; Kitma & Esguerra 2009). Jabbar et al. (2011) reported internal discolouration (flesh browning) and soft nose disorder in HW-treated 'Chauns' mango. Esguerra et al. (1990) sorted fruit based on maturity using 1% salt solution and observed white starchy lesions, termed spongy tissue, in immature (floaters) 'Carabao'

mango fruit subjected to VHT. Kitma and Esguerra (2009) reported a similar disorder in 'Carabao' mango upon HWT treatment. However, conditioning the fruit using hot air prior to HWT significantly ($p \leq 0.05$) reduced the incidence of white starchy lesions or spongy tissue disorder. Since phytosanitary treatments are essential for mango export to protocol countries, appropriate measures (e.g., resilient maturity stage, conditioning) to ensure that fruit remain free of IDs that otherwise would be revealed at the consumer's end of the supply chain.

Post-harvest storage and transport, including time and temperature, is also pivotal in maintaining fruit quality throughout the supply chain, especially for longer, in terms of time and/or distance, export supply chains. Prolonged periods of storage at low temperatures and/or in modified atmosphere packaging can lead to internal hypoxia (oxygen starvation) precipitating mesocarp degradation, internal breakdown, and browning (Pina et al. 2000; Lizada & Rumbaoa 2017). Acosta et al. (2000) reported that the incidence of internal darkening (flesh browning) increased from 6 to 78% and 56 to 94% with an increase in storage duration from 22 to 28 days at 13 °C and 18 °C, respectively, in 'Haden' mango. Fruit stored at 8 °C didn't show any such symptoms until day-26 but did suffer low temperature-induced chilling injury after 24 days storage.

Incidence of IDs can be minimised by limiting sun exposure, choosing appropriately mature fruit for post-harvest heat treatment, and maintaining a balance in storage time and temperature with respect to cultivar so as to preserve the quality and longevity of mango fruit (Wills & Golding 2016b).

3.6.3 Fruit characteristics (size, maturity)

Fruit characteristics such as maturity and weight at harvest are essential to meet consumers' preferences, and can markedly alter post-harvest fruit physiology, quality and shelf life (Wills & Golding 2016a).

Mango fruit are usually harvested at the hard green mature stage before the onset of the climacteric respiration rise (Hofman & Whiley 2010). Immature and overmature fruit tend to develop poor texture, flavour, and aroma. While early harvest immature fruit exhibit longer shelf life, they tend to express disorders upon post-harvest heat treatments (Esguerra et al. 1990; Jacobi et al. 2001). On the other hand, overmature fruit have shorter shelf life but are also more susceptible to express disorders (Bally 2006). Subraman et al. (1971) and Burondkar et al. (2009) reported a higher incidence of spongy tissue in middle-to-late harvested 'Alphonso' mango compared to early harvest. The 'Alphonso' mango were sorted into three maturity classes based on specific gravity, viz., < 1.00, 1.00 – 1.02, and > 1.02 (Krishnamurthy 1980, 1982). The percent incidence of spongy tissue was 4% in <1.00 specific gravity fruit compared to 33% and 63% in 1.00-1.02 and >1.02 respectively, indicating higher susceptibility in more mature fruit.

Fruit weight is another particularly important characteristic of mango fruit. Along with external appearance (e.g., colour, blemish), weight is most often used as an index for fruit grading category for target domestic and/or export markets. The relationship between mango fruit size and susceptibility to IDs depends on the cultivar and type of disorder. For example, Subraman et al. (1971) reported that an increase in fruit weight from > 200 to > 300 g significantly ($p = 0.1$) increased the spongy tissue disorder in 'Alphonso' mango from 18.2% to 44.5%, respectively. A similar trend was noted for 'Harumanis' mango for insidious fruit rot (flesh browning) disorder, with fruit having < 350 g weight had 7.4% and 451 – 500 g fruit had 35.1% incidence of disorder (Tarmizi et al. 1993). Also, jelly seed disorder was noted to be positively correlated with fruit size in N-fertilised experimental trees, but not in unfertilised control trees (Torres et al. 2004b; Torres et al. 2009).

Contradictory reports were noted in 'Sensation' mango between stem-end cavity disorder and fruit weight (Hermoso et al. 1997; Torres et al. 2004b; Torres et al. 2009). Hermoso et al. (1997) found negative correlation and Torres et al. (2009) reported positive correlation between fruit weight with stem-end cavity disorder in 'Sensation' mango. However, in later studies (Torres et al. 2004b; Torres et al. 2009), 'Sensation' trees were heavily N fertilised with biennial/alternate crop bearing pattern for the entire duration of experiment which can most likely influence fruit characteristics and incidence of IDs respectively. Thus, it is very difficult to separate whether the incidence of stem-end cavity disorder was entirely due to fruit weight or if crop load and/or N fertilisers also contributed to fruit's susceptibility during development and maturation.

3.6.4 Premature seed germination

Premature seed germination causing IDs was first explored in the Indian cultivars 'Alphonso' and 'Amrapali' for spongy tissue and jelly seed disorders, respectively (Ravindra & Shivashankar 2004; Shivashankar et al. 2016).

Comparative physiological, biochemical, and mineral analysis indicated that seeds of spongy tissue-affected 'Alphonso' mango fruit had higher respiration rates, higher amylase, and lipase activities, moisture, and greater Ca concentrations compared to seeds in healthy fruit (Ravindra & Shivashankar 2004). The results implied that withdrawal and remobilization of minerals and water from fruit mesocarp into the seed caused spongy tissue symptoms (Ravindra & Shivashankar 2004, 2006; Ravindra et al. 2010). The early onset signs of spongy tissue were linked to a burst of ethylene production and an increase in cytokinin and gibberellins in seed that signalled the onset of germination (Shivashankar et al. 2015; Shivashankar & Sumathi 2019).

Pre-harvest application of paclobutrazol markedly reduced the incidence of spongy tissue and jelly seed in 'Alphonso' and 'Amrapali' mangoes, respectively (Shivashankar et al. 2007; Ravindra et al.

2010; Shivashankar et al. 2016). This was interpreted to be by downregulating seed metabolism and amylase activity. In contrast, gibberellic acid aggravated the disorders upon upregulating general metabolic and amylase activity (Shivashankar et al. 2007; Ravindra et al. 2010; Shivashankar et al. 2016). Seshadri et al. (2019) recommended a pre-harvest spray of a salt formulation that primarily consisted of NaCl, $CaCl_2$, KCl, and low concentrations of H_3BO_3, $CuSO_4$, $ZnSO_4$, $FeSO_4$, $MnSO_4$, and EDTA as an effective preventive measure for jelly seed in 'Amrapali'. This formulation delayed seed germination by eliciting abscisic acid and germination-inhibiting phenolics in the seed with reduced jelly seed disorder (Seshadri et al. 2019).

3.6.5 Bacterial infection

Two studies in the selected literature noted the presence of gram-positive *Staphylococcus xylosus* bacteria as a potential causative factor for spongy tissue disorder in 'Alphonso' mango (Figure 3.5) (Janave & Sharma 2008; Janave 2009). Previous work by Subraman et al. (1971) on spongy tissue-affected 'Alphonso' had not given any clear indication of the presence of a pathogen and its role as the primary causative agent of internal disorder. They discussed isolation of *Bacillus sp.* from disordered tissue but did not establish pathogenicity. Hence, further investigations are required to corroborate this prospect.

3.7 Interacting factors influencing expression of internal disorders

From above, it is evident that expression of IDs is intricate due to multiple positive and negative predisposing interacting factors across pre-harvest, harvest, and post-harvest stages (Figure 3.8). The majority of aforementioned studies in the selected literature highlight one to a few causal factors.

Accordingly, characterising the complex interplay of individual factor effects on IDs of mango is challenging, including because of interactions across the dynamic G x E x M continuum.

A first step in managing fruit susceptibility to IDs is perhaps to recognise that the interactive relationships may be overlooked during reductive scientific practice. A second may be recognising pre-harvest fruit growth and development and postharvest ripening and senescence as a dynamic continuum.

The majority of relevant literature identified in the course of this systematic literature review focused on fruit minerals concentrations and their association with IDs and typically recommended fertiliser applications to improve fruit nutrient levels (Krishnamurthy 1982; Tarmizi et al. 1993; Torres et al. 2004a; Shinde et al. 2006; Shinde et al. 2009; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023). However, efficacy needs to account for factors that mediate nutrient uptake from the growth substrate and transport through the tree and into the fruit (Gilliham et al. 2011; Tonetto de Freitas & Mitcham

2012; Hocking et al. 2016). These deterministic variables include soil properties, like pH, moisture, and nutrient holding capacity (Jackson & Meetei 2018; Marschner & Rengel 2023), root properties, like morphology, distribution, and health (Mengel 1995), tree properties, like canopy volume, fruit set, and crop load (Bally 2006; Zabedah et al. 2008; Robinson & Lopez 2010; Serra et al. 2016; Isarangkoo et al. 2019), and environmental conditions, like temperature, VPD and rainfall (Gunjate et al. 1982; Ho & Adams 1994; Montanaro et al. 2006). Moreover, all such factors change constantly with time across any one crop cycle, affecting the flowering, fruit set and development cycle and fruit characteristics at harvest, like size, maturity, and robustness (Léchaudel & Joas 2007).

Considering this complexity, a causal loop diagram is proposed to organise and help understand the interplay of factors affecting fruit qualities at harvest and attendant postharvest susceptibility to disorders (Figure 3.8). The prototype association of deterministic variables is presented with arrowheads having positive and negative signs indicating direct and inverse relationships, respectively. Pre-harvest factors suggest monitoring temperature, rainfall and VPD during fruit set and development stages, also taking appropriate management decisions (e.g., fertiliser application, irrigation scheduling, canopy pruning) into account. For example, to maintain optimum soil moisture levels for nutrient availability in the soil and root health for uptake via feeder roots. In this regard, an ideal rootstock-scion combination can improve nutrient uptake and distribution in the tree (Iyer & Subramanyam 1992; Iyer & Degani 1997). Dynamic interactions of environmental conditions and management practices needs to be understood and ideally monitored and managed on a seasonal basis to ensure consistency of fruit quality.

Likewise, fruit physicochemical attributes such as weight and maturity, are important factors determining a fruit's susceptibility to IDs (Krishnamurthy 1980; Hermoso et al. 1997). However, the timeline is dynamic across pre-harvest through harvest into postharvest, with pressures associated with fruit treatment, handling, and distribution, like for domestic versus overseas markets. For instance, fruit harvested at higher maturity tend to have greater incidence of spongy tissue (Krishnamurthy 1980) or, where post-harvest VHT treatment is involved, the less mature fruit may be more likely to develop spongy tissue disorder (Esguerra et al. 1990) (Figure 3.8). Towards understanding how fruit are likely to behave, it is instructive to consider what stresses it may endure in the supply chain, including phytosanitary treatments (VHT, HWT, irradiation) and low-temperature storage conditions and durations, including for relatively long export supply chains. Conceptual comprehension of entire farm orchard to retail store systems, as facilitated by CLDs should inform the production of relatively more robust fruit through enlightened management of factors otherwise likely to increase the incidence of IDs.

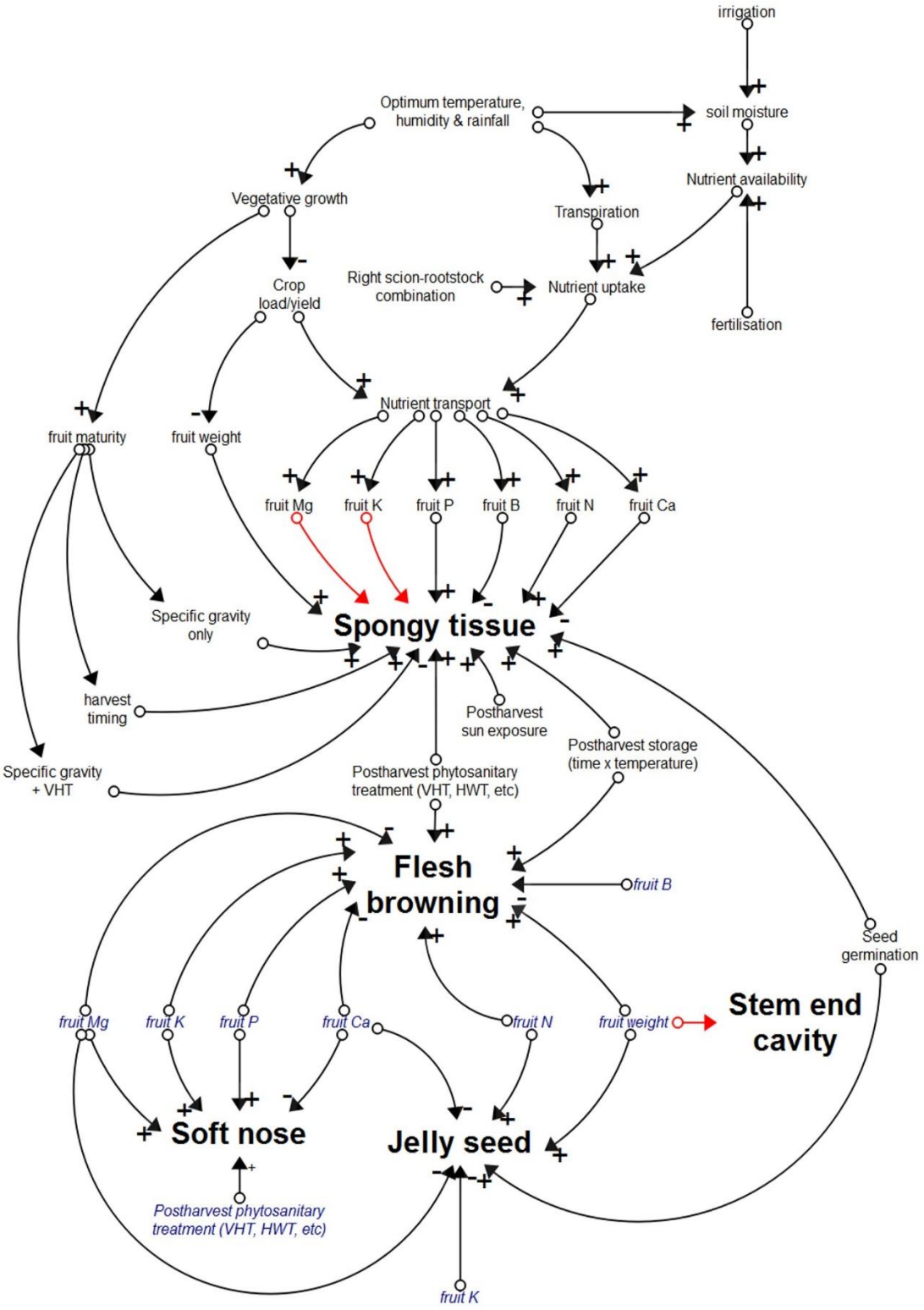


Figure 3.8: A notional high-level causal loop diagram (CLD) encompassing pre-harvest, harvest, and post-harvest factors associated with G x E x M practices in the context of predisposition to expression of various internal disorders in mango fruit flesh. '+' and '-'on arrowheads indicate positive or negative effects, respectively, on subsequent factors in loops. '±' effects were indicated with the red coloured arrows. Blue italicised variables are 'shadow variables' of the original variables presented as such to avoid overlapping arrows.

3.8 Conclusion

The current study systematically reviews progression of research on underlying causes of internal physiological fruit disorders in mango cultivars. From systematic literature review, mineral nutrition imbalance in fruit flesh was the single most studied factor, along with fruit size and maturity characteristics at harvest, and post-harvest management practices that include storage conditions and phytosanitary treatments. The analysis suggests that these variables directly and/or indirectly profoundly influence mango fruit quality in terms of susceptibility to internal disorders. It was discerned, however, 'the literature' is substantially lacking in important background information on soil characteristics (e.g., texture, pH, nutrient holding capacity), growing conditions (temperature, humidity, and rainfall), and other orchard characteristics that include fertilisation and irrigation management practices, biennial cropping effect, and insight into how these interacting factors mediate fruit quality and disorder susceptibility. This review provides a benchmark against which future research might adopt holistic approaches to contiguously ascertain predisposition of mango fruit to internal disorders across the pre-harvest, harvest, and post-harvest continuum.

Chapter 4 Variation in mineral nutrient composition across seasons and regions with regards to internal disorders expression in vapour heat-treated 'B74' mango fruit

4.1 Abstract

Supply of premium quality fruit to export markets is important for consumer satisfaction and repeat purchase. For ‘B74’ mango fruit export, vapour heat treatment (VHT) is protocol for fruit fly control for market access. However, VHT has been associated with internal physiological disorders in ‘B74’ fruit. Susceptibility of this cultivar to internal disorders (IDs) was investigated in association with VHT and flesh mineral composition. Fruit were sourced from three varying climatic regions (Northern Territory (NT), Far-north Queensland (FNQ), and South-east Queensland (SEQ)) over two successive seasons. They were assessed for IDs associations with mesocarp minerals. Flesh browning (FB) and flesh cavity with white patches (FCWP) were the main defects observed. Over two seasons, highest FB incidence was observed in NT fruit (20%) and highest FCWP in FNQ fruit (65%). FB was not seen in fruit from SEQ in either season. The flesh [Ca] and [Mg] were highest in NT fruit. [N], [B], N/Ca, K/Ca, and (K+Mg)/Ca were higher ($p \leq 0.05$) in fruit from SEQ. Overall, significant ($p \leq 0.05$) positive correlations between FCWP with N/Ca, K/Ca, and (K+Mg)/Ca ratios and negative correlation between FB with [B] was discerned by linear correlation and principal component analysis (PCA). However, correlation strength and significance level varied across regions and seasons. This inconsistency suggests other deterministic factors also influence VHT-induced IDs in ‘B74’ mango.

Keywords: Export, flesh nutrients, postharvest quality, physiological defects, VHT.

4.2 Introduction

Mangoes, a climacteric perishable fruit, are susceptible to external and internal disorders that include lenticel spots, under-skin browning, soft nose, jelly seed, flesh cavity, and internal browning (Wainwright & Burbage 1989; Kulkarni 1992; San et al. 2014; Mishra et al. 2016; San et al. 2019). Such physiological defects affect fruit texture, visual appeal, marketability, and consumer satisfaction (Oldoni et al. 2022). Susceptibility of mangoes to physiological disorders depends upon a variety of factors that include cultivar, seasonal environment changes, mineral nutrition, and post-harvest practices (Schaffer 1994a; Hofman et al. 2012). Defects are problematic for the mango industry, including occurring in many cultivars (Raymond et al. 1998b; Krishna et al. 2020; Mogollón et al. 2020).

Effects of pre- and/or post-harvest factors have been investigated to determine cause and effect in respect to fruit's predisposition to IDs (Subraman et al. 1971; Rane et al. 1976; Katrodia 1979; Gunjate et al. 1982; Singh et al. 2006b; Léchaudel & Joas 2007). Previous research has linked mineral nutrient deficit with development of IDs, e.g., Ca and B (Singh et al. 2013a; Ma et al. 2018; Bitange et al. 2020).

Ca plays a critical role in fruit development, cell wall strengthening, and signal transduction pathways (Kamthan et al. 2015). Ca deficiency during early fruit development leads to physiological disorders such as bitter pit in apple (Kalcsits et al. 2019), blossom end rot in tomato (Ho et al. 1993), and internal breakdown in avocado (Hofman et al. 2002). Upon weakening mesocarp cell walls, Ca deficiency in mango fruit is associated with jelly seed (Raymond et al. 1998b), stem-end cavity (Raymond et al. 1998a), and spongy tissue (Ma et al. 2023).

While individual mineral nutrients, like Ca, evidently have a role in fruit development and resilience, nutrient balance (e.g., N/Ca, K/Ca) is a potentially useful index for robustness and quality (Torres & Saúco 2004; Bally 2006; Ma et al. 2018).

Robustness at harvest denotes the innate ability to withstand harvest and post-harvest stresses. For export, fruit for protocol markets undergo phytosanitary treatments. Particularly for long export supply chains, disinfestation treatment may increase fruit susceptibility to physiological disorders that dimmish the quality to consumers (Jacobi & Giles 1997).

For 'B74' mango export to certain Asian markets, fruit must undergo vapour heat treatment (VHT) for fruit fly infestation (Jacobi & Giles 1997; Singh & Saini 2014). Thereby, fruit core temperature is raised to and maintained at 47 °C for 15 min using forced moisture-saturated air, before being cooled down to storage temperature (e.g., 18 °C) (Jacobi et al. 2001). If fruit are not sufficiently robust at harvest, then VHT may adversely affect fruit physiochemistry and elicit IDs (Mitcham &

McDonald 1993).

While demand for mangoes in international markets is strong, compromised internal quality poses a threat. The present study investigates the prevalence of IDs in export quality ‘B74’ fruit and the putative role of fruit minerals in susceptibility to VHT-induced IDs for ‘B74’ mangoes from several production regions and two successive growing seasons.

4.3 Materials and Methods

4.3.1 Description of experimental sites

‘B74’ mango fruit were sourced from three distinct growing regions (Northern Territory (NT), Far-North Queensland (FNQ), and Southeast Queensland (SEQ)) over two fruiting seasons (2020 and 2021) (Figure 4.1). The annual crop production cycle in the NT typically starts with flowering in late winter/early spring, and harvest starts in late September/October, depending upon temperature fluctuations (AMIA 2022b). The annual crop production cycles for FNQ and SEQ start from July to late-August, with harvests beginning in late November and late January/early February, respectively (AMIA 2022a). NT fruit were sourced mainly from farms near Katherine (14.479^{o}S, 132.266^{o}E) and Mataranka (14.925^{o}S, 133.066^{o}E), FNQ fruit from near Dimbulah (17.158^{o}S, 145.099^{o}E), and SEQ fruit from near Childers area (25.136^{o}S, 152.372^{o}E). The orchards were 10-16 years old trees on ‘Kensington Pride’ rootstock. They were maintained under standard commercial practice (Appendix A2Appendix A6). Average monthly maximum and minimum temperatures and rainfall data were obtained from The Australian Bureau of Meteorology website for the closest weather stations; viz., Katherine (14.52^{o}S, 132.38^{o}E), Dimbulah (17.34^{o}S, 144.93^{o}E), and Childers (25.26^{o}S, 152.28^{o}E). Soil mineral analysis data were collected from each production region from the growers. Soil samples were taken by growers as part of routine nutrient management programs and were analysed by commercial soil analysis laboratories.



Figure 4.1: Map of Australia showing regions (approximate) and location of the VHT facility where 'B74' fruit were harvested and treated, respectively.

4.3.2 Fruit sampling

Fruit were commercially harvested, de-sapped, and treated with fungicide as per standard commercial practice. They were then transported to the VHT facility at Rocklea, Brisbane after harvest and within 5-6 days of harvest from NT (~3400 km) and 3-4 days from FNQ (~1700 km). Transport was in refrigerated trucks at avg. 16 °C. Fruit from SEQ were transported to the VHT facility within 1-2 days (~350 km) of harvest in an airconditioned vehicle (Figure 4.1). Although SEQ fruit are not generally exported due to inconsistent quality across growing seasons (S. Grabbe, *pers. comm.*, 2020), they were included towards assessing if mineral nutrient imbalance is a contributing factor to inconsistent postharvest quality. Detailed information on blocks and fruit samples from regions is presented in Table 4.1. Fruit were sampled multiple times throughout each season from multiple supply chains at the VHT facility. At each sampling event, they were sorted into six commercial packing trays and each containing 15 individual fruits. Each tray was one replicate. Three trays per sampling interval were subjected to VHT, whereby the core temperature was raised to 47 °C and maintained for 15 min. Relative humidity was > 90%. The whole process takes around 4-6 h. Afterwards, fruit were immediately placed at 18 °C room temperature for 6-8 h and transferred to the post-harvest laboratory at The University of Queensland, Gatton campus, about 100 km away. The remaining three trays were non-VHT and kept at room temperature (20 ± 2 °C) in the VHT facility. Thereat, post-harvest assessments and ripening were conducted at 20 °C without ethylene treatment.

Table 4.1. Number of fruit assessed for IDs from each region in two seasons.

Region	Blocks	Total fruit assessed	Number of individually assessed fruit	
			Season 2020	Season 2021

			+VHT	-VHT	+VHT	-VHT
NT	3	1602	246	242	576	538
FNQ	2	1652	216	150	648	638
SEQ	1	990	225	225	270	270

4.3.3 Fruit assessments

The fruit ripening stage was determined subjectively using a hand firmness rating system after Hofman et al. (2010a): viz., 0 = Hard (no 'give' in the fruit), 1 = Rubbery (slight 'give' in the fruit), 2 = Sprung (flesh deforms by 2 - 3 mm with extreme thumb pressure), 3 = Firm soft (whole fruit deforms with moderate hand pressure), and 4 = Soft (whole fruit deforms with slight hand pressure). Incidence and severity of IDs were assessed by visual inspection at ripe (firmness stage 4).

Longitudinal cuts were made through the fruit flesh immediately adjacent to the seed. Incidence % was (number of fruit with a disorder / total number of fruit) × 100. Severity for individual fruit was rated 0 - 3: where, 0 = Healthy (no sign of disorder); 1= Slight (<25% area of mesocarp affected); 2 = Moderate (25-50% area of mesocarp affected); and 3 = Severe (> 50% area of mesocarp affected).

4.3.4 Fruit mineral analysis

For mineral analysis, mesocarp sections (~2 × 2 × 2.6 cm) from the widest part of the fruit cheek were obtained for five fruit per tray after VHT and duly combined as one composite sample per tray. Additionally, mesocarp sections from individual healthy or disorder fruit were separated, dried at 65 °C in a fan-forced oven for 3 days, and ground to fine powder using a laboratory scale mini ball mill (Retsch Mixer Mill MM 400). Total N was measured using LECO C/NS928 carbon/nitrogen combustion analyser at combustion temperature 1250 °C (Flanagan et al. 2022). Other minerals, including Ca, Mg, K, P, and B, were measured using inductively coupled plasma-optical emission spectrometry (ICPOES: Thermo iCAP PRO XP) (Altundag & Tuzen 2011). Tissue samples were digested in an open vessel with a 5:1 mixture of nitric and perchloric acid heated to 200 °C. The forward power for the ICPOES plasma was 1200 W. Samples were aspirated into the spectrometer using a concentric nebuliser with a Tracey spray chamber. The instrument was operated in radial mode for macronutrients and axial mode for micronutrients. All results were expressed as mg/kg on dry weight basis.

4.3.5 Statistical analysis

Incidence and severity data on IDs and mesocarp mineral concentrations were analysed by two-way analysis of variance (ANOVA). Since multiple supply chains originated from a single block and blocks were adjacent to each other and managed under similar management practices, blocks were aggregated, and data was analysed using region and season as factors with uneven replicates. Fruiting

season was factor A and region factor B, and means were compared at a 5% confidence level by Tukey's HSD test (Ferreira et al. 2014). Data normality distribution was determined by the Shapiro-Wilk normality test (Razali & Wah 2011). Pearson's correlation coefficients (r) were determined to relate mineral nutrients to IDs (Schober et al. 2018). Multivariate analysis was performed using principal component analysis (PCA) (Biermann et al. 2022). All statistical analyses were performed in R (4.0.2) and graphs were prepared using R-packages, viz., ggplot2 (Wickham 2011) and gplots (Warnes et al. 2009).

4.4 Results

4.4.1 Weather and soil conditions

Weather and soil data were collected from each production region over two consecutive seasons. NT experienced higher temperatures than the two other regions in both years, with average maximum temperatures reaching 40 °C around harvest in summer (Figure 4.2). Site-specific differences in soil properties were evident between regional farm sites. The soil at the farm in the NT was mildly alkaline (pH 8.8) with high cation exchange capacity (CEC), Ca (meq/100g), and Mg (meq/100g) compared to the SEQ farm site (Table 4.2). The cationic balance by base saturation of CEC for Ca % was lower at the SEQ site. On the other hand, K % was high at the SEQ and FNQ sites compared to the NT farm site (Table 4.2). Na % was much higher in SEQ than NT soil.

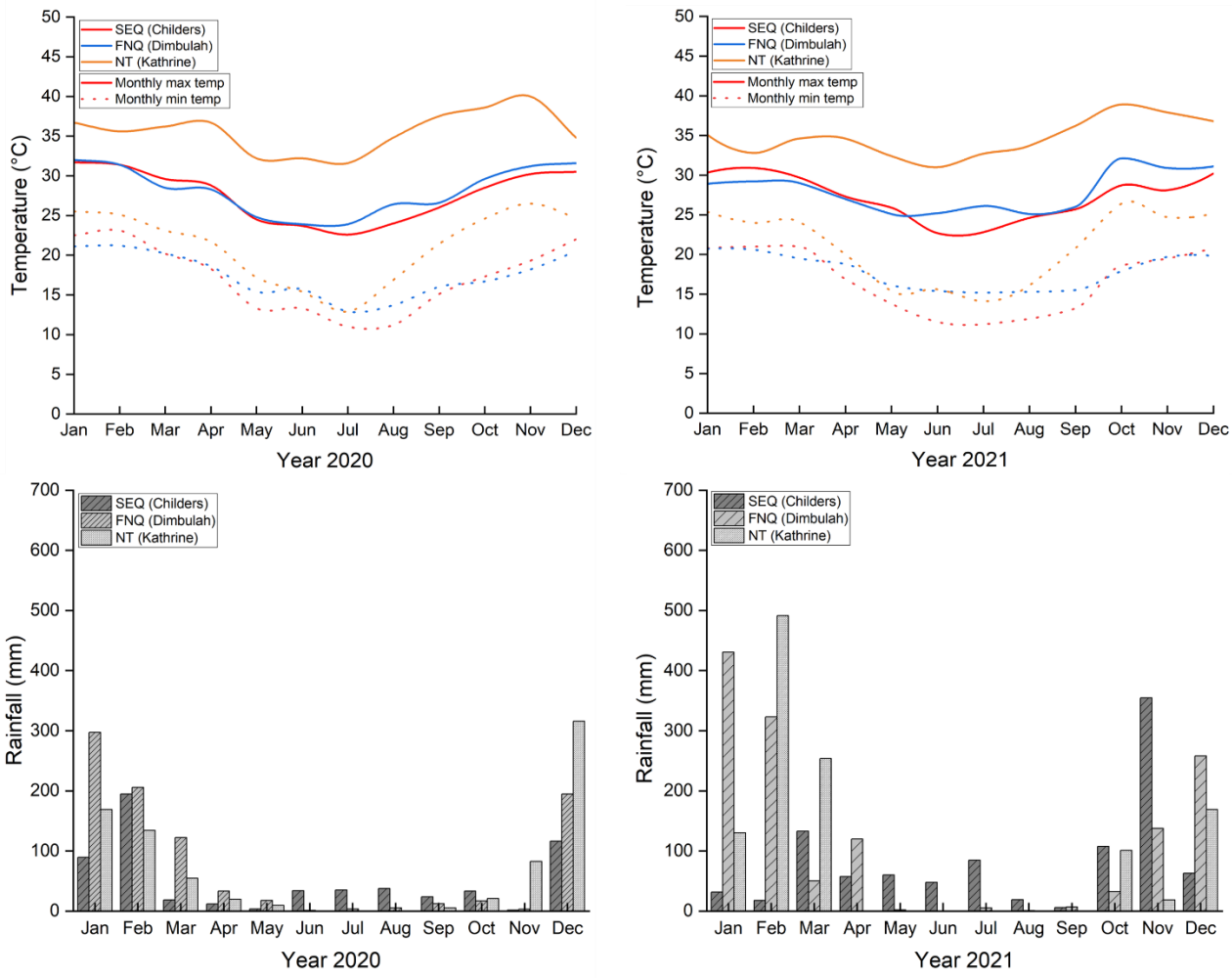


Figure 4.2: Meteorological data from the three sites from which fruit were sourced for quality assessments and mesocarp mineral analyses in two consecutive seasons. The top two figures show maximum and minimum (solid and dotted lines, respectively) average monthly temperatures for 2020 and 2021. The bottom two figures show the monthly average rainfall over two seasons for each of the selected sites. The fruit production cycles from flowering to harvest were April to September/October for NT, June/July to November/December for FNQ and August/September to January/February for SEQ, respectively.

Table 4.2. Soil characteristics and mineral concentrations at 30 cm depth taken at the end of Jan 2021 and 2022 mango seasons for the three sampling regions, i.e., NT, FNQ, and SEQ. "-" denotes data not available.

Parameters	Unit	NT		FNQ		SEQ	
		2020	2021	2020	2021	2020	2021
pH (H_2O)	-	8.77	8.85	-	6.07	7.50	7.23
CEC	meq/100g	9.87	7.72	-	3.40	3.97	3.21
Ca	meq/100g	7.27	5.45	-	2.56	2.62	2.29
Mg	meq/100g	2.30	2.16	-	-	0.94	0.61
K	meq/100g	0.20	0.08	-	0.18	0.21	0.19
Na	meq/100g	0.10	0.01	-	-	0.16	0.07

Ca base saturation	% of CEC	73.87	70.64	-	75.60	66.01	69.94
Mg base saturation	% of CEC	24.67	28.24	-	-	23.88	19.58
K base saturation	% of CEC	2.00	1.10	-	5.24	5.41	6.11
Na base saturation	% of CEC	0.87	0.00	-	-	4.16	2.42
Ammonium - N	mg/kg	1.04	-	-	-	1	3.56
Nitrate - N	mg/kg	2.73	-	-	-	<1	< 1
B	mg/kg	0.30	0.40	-	0.27	0.48	0.58
P	mg/kg	54.00	57.59	-	-	43.67	53.83
S	mg/kg	5.67	5.53	-	-	9.67	8.41

4.4.2 Occurrence and severity of internal disorders

Two distinct IDs were observed in export quality 'B74' mango, they being flesh cavity with white patches (FCWP) and flesh browning (FB) (Figure 4.3). FCWP was characterised by dry-white honeycomb-like cavities in the inner mesocarp near the seed (Figure 4.3b). FCWP was distinguished from spongy tissue disorder as there was no off-odour (Oak et al. 2019). It was only present in fruit subject to VHT.

FB was characterised by darkening and discolouration in the fruit mesocarp at eating ripe (Figure 4.3c). It presented in both VHT and non-VHT fruit. Figure 4.4 shows the regional distribution of % incidence and the severity of both FCWP and FB disorders in VHT-treated fruit over two seasons.

Incidence of both disorders varied significantly ($p \leq 0.05$) for fruit sourced from the different production regions and seasons. The mean values of FCWP incidence ranged from 4.8% to 69.5% (Table 4.3). Overall, FCWP incidence was significantly ($p \leq 0.001$) lower in 2021 (29.2%) compared to 2020 (52.4%). NT fruit consistently showed a significantly ($p \leq 0.001$) lower incidence of FCWP (22.4%) compared to SEQ (31.5%) and FNQ (64.9%) at the ripe stage when two years of data were pooled (Table 4.3). There was no significant ($p > 0.05$) difference in FCWP incidence in fruit from FNQ in 2020 (50.2%) and 2021 (69.5%) and fruit from SEQ in 2020 (63.6%). However, in 2021, SEQ fruit had a significantly ($p \leq 0.001$) lower incidence of FCWP disorder (4.8%).

The incidence of FB was significantly ($p \leq 0.001$) higher in NT fruit (20.0%), followed by FNQ (8.2%) (Table 4.3). No incidence of FB was noted in SEQ fruit in any season. The severity of FCWP and FB across all regions and seasons followed a similar trend to the incidence of these disorders. Overall, NT fruit had a significantly ($p \leq 0.001$) higher incidence of FB, and FNQ fruit showed the highest FCWP and moderate FB incidence and severity in both seasons.

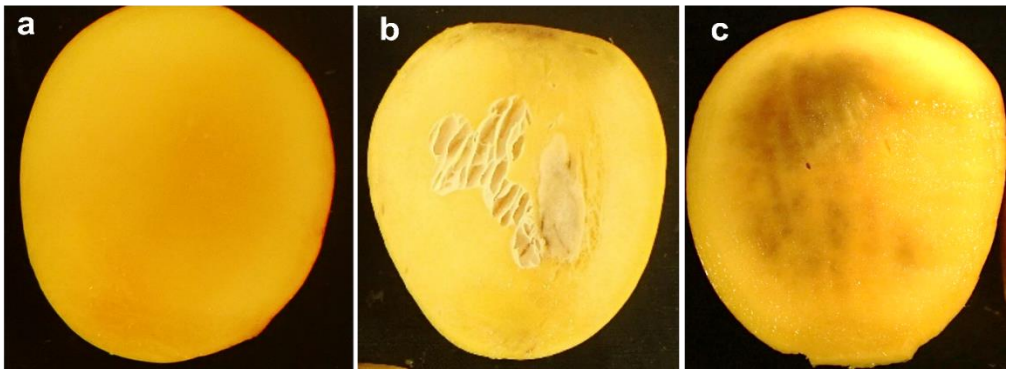


Figure 4.3: Photographs showing a cross-section of ripe 'B74' mango fruit exhibiting IDs. a. Healthy (no disorder), b. Flesh cavity with white patches (FCWP), and c. Flesh browning (FB).

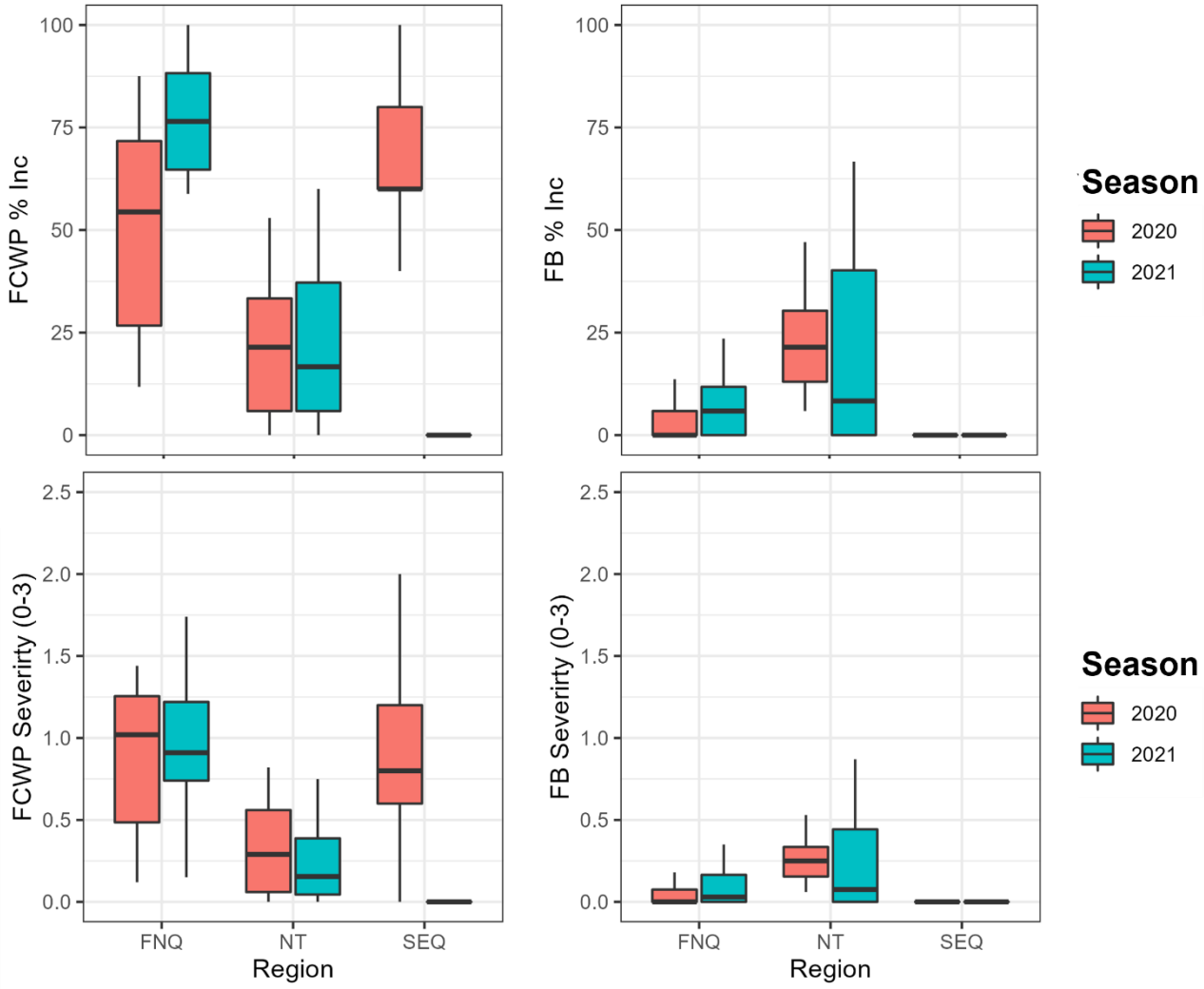


Figure 4.4: Box and Whiskers plots of percent incidence (% Inc) and severity (0-3) of FCWP and FB disorders in 'B74' mango from different production regions over the two seasons. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

Table 4.3. Statistical analyses of occurrence and severity of IDs of 'B74' mango from different

production regions. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test. FCWP: flesh cavity with white patches, FB: flesh browning.

	Internal disorders at eating ripe stage			
Season (S)	**FCWP % Incidence**	**FCWP Severity**	**FB % Incidence**	**FB Severity**
2020	52.41 [a]	0.79 [a]	5.35	0.06
2021	29.19 [b]	0.37 [b]	8.71	0.10
Significance	*<0.001*	*<0.001*	*0.06*	*0.73*
Region (R)				
NT	22.44 [c]	0.27 [c]	20.01 [a]	0.24 [a]
FNQ	64.98 [a]	0.99 [a]	8.23 [b]	0.08 [b]
SEQ	31.52 [b]	0.44 [b]	0.00 [c]	0.00 [c]
Significance	*<0.001*	*<0.001*	*<0.001*	*<0.001*
S × R				
2020 × NT	20.72 [bc]	0.34 [b]	23.17 [a]	0.26 [a]
2020 × FNQ	50.21 [a]	0.87 [a]	3.13 [bc]	0.05 [bc]
2020 × SEQ	63.56 [a]	0.91 [a]	0.00 [c]	0.00 [c]
2021 × NT	23.07 [b]	0.25 [b]	18.88 [a]	0.23 [a]
2021 × FNQ	69.53 [a]	0.96 [a]	9.80 [b]	0.10 [b]
2021 × SEQ	4.81 [c]	0.04 [b]	0.00 [c]	0.00 [c]
Significance	*<0.001*	*<0.001*	*0.01*	*0.01*

4.4.3 Mesocarp mineral nutrient concentrations of 'B74' mango

Mesocarp mineral analyses also showed considerable variation in mineral concentrations of 'B74' mangoes across regions and seasons (Figure 4.5). Fruit from SEQ had significantly ($p \leq 0.01$) higher [B], N/Ca, K/Ca, and (K+Mg)/Ca over the two fruiting seasons than did those from the NT and FNQ (Table 4.4). However, Ca and Mg concentrations were significantly ($p \leq 0.001$) higher in NT fruit compared to those in FNQ and SEQ fruit. There was no significant ($p > 0.05$) difference in flesh K concentrations across the three regions and the two seasons (Table 4.4). Overall, seasonal influences on mineral concentrations across all regions were significant ($p \leq 0.01$), except for Ca. For region × season interaction, [Ca] was significantly ($p \leq 0.001$) higher in both NT and FNQ fruit in 2020 compared to 2021. However, there was no significant interaction ($p > 0.05$) in Ca concentrations of SEQ fruit in both years. A similar trend of no significant interaction ($p > 0.05$) was observed in Mg concentrations for NT and FNQ, but not for SEQ fruit (Table 4.4, Figure 4.5). On the other hand, [B] was consistently and significantly ($p \leq 0.001$) higher in SEQ for both seasons and for FNQ fruit in 2021. Overall, NT fruit had significantly ($p \leq 0.001$) lower concentrations of B, compared to FNQ and SEQ fruit (Figure 4.5). K/Ca and (K+Mg)/Ca were significantly ($p \leq 0.001$) higher in SEQ fruit in 2020, and N/Ca was highest in 2021.

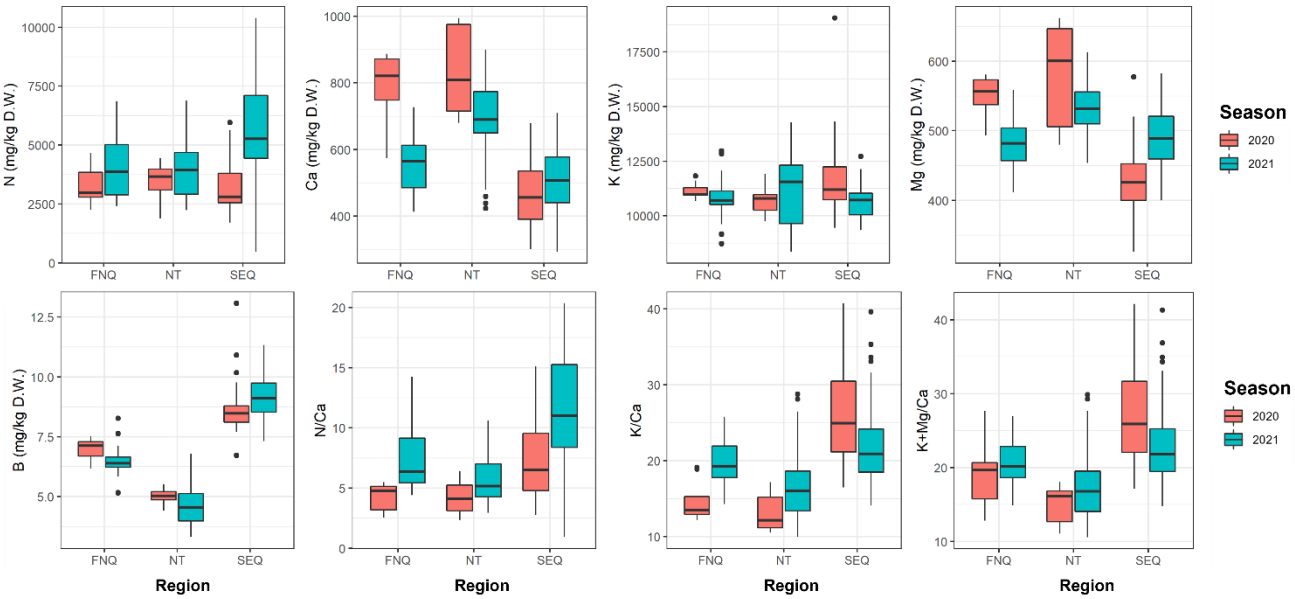


Figure 4.5: Mineral nutrient concentrations in mesocarp of 'B74' mango sourced from different production regions over the two fruiting seasons. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

Table 4.4. The differences in mineral nutrient concentrations across regions and seasons of 'B74' mango flesh. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Mesocarp mineral concentrations at harvest (mg/kg D.W.)								
Season (S)	**N**	**Ca**	**K**	**Mg**	**B**	**N/Ca**	**K/Ca**	**(K+Mg)/Ca**
2020	3287 ^{b}	593.9	11350 ^{a}	480.5 ^{b}	7.62 ^{a}	6.26 ^{b}	21.4 ^{a}	23.06 ^{a}
2021	4627 ^{a}	579.7	10852 ^{b}	502.2 ^{a}	6.96 ^{b}	8.46 ^{a}	19.69 ^{b}	20.59 ^{b}
Significance	*<0.001*	*0.34*	*0.004*	*0.002*	*<0.001*	*<0.001*	*0.01*	*<0.001*
Region (R)								
NT	3771 ^{b}	734.8 ^{a}	11022	544.0 ^{a}	4.73 ^{c}	5.38 ^{b}	15.65 ^{c}	16.69 ^{c}
FNQ	3935 ^{ab}	607.9 ^{b}	10921	498.8 ^{b}	6.54 ^{b}	6.80 ^{b}	18.63 ^{b}	20.28 ^{b}
SEQ	4502 ^{a}	486.2 ^{c}	11080	463.3 ^{c}	8.94 ^{a}	9.48 ^{a}	23.81 ^{a}	24.79 ^{a}
Significance	*0.01*	*<0.001*	*0.967*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
S × R								
2020 × NT	3410 ^{b}	835.9 ^{a}	10711 ^{ab}	578.7 ^{a}	5.02 ^{d}	4.20 ^{c}	13.12 ^{c}	14.88 ^{c}
2020 × FNQ	3228 ^{b}	779.5 ^{ab}	11131 ^{ab}	548.6 ^{ab}	6.97 ^{c}	4.24 ^{c}	14.66 ^{c}	18.81 ^{bc}
2020 × SEQ	3261 ^{b}	463.7 ^{d}	11621 ^{a}	429.6 ^{d}	8.66 ^{b}	7.48 ^{b}	25.96 ^{a}	26.92 ^{a}
2021 × NT	3900 ^{b}	698.7 ^{b}	11133 ^{ab}	534.1 ^{b}	4.63 ^{d}	5.81 ^{bc}	16.55 ^{c}	17.34 ^{c}
2021 × FNQ	4153 ^{b}	555.1 ^{c}	10857 ^{b}	483.5 ^{c}	6.40 ^{c}	7.59 ^{b}	19.86 ^{b}	20.74 ^{b}
2021 × SEQ	5535 ^{a}	504.9 ^{cd}	10630 ^{b}	490.9 ^{c}	9.17 ^{a}	11.16 ^{a}	22.01 ^{b}	23.02 ^{b}
Significance	*0.004*	*<0.001*	*0.003*	*<0.001*	*<0.001*	*0.01*	*<0.001*	*<0.001*

4.4.4 Correlation of mineral nutrients and IDs in 'B74' mango

Associations between individual mineral nutrient concentrations and IDs expression were inconsistent, with inconsistent correlations across regions and fruiting seasons (Table 4.5). On the other hand, their ratios as N/Ca, K/Ca, and (K+Mg)/Ca were relatively consistently correlated with

IDs (Table 4.5).

FCWP incidence showed significant positive correlations with N concentrations in fruit from the NT ($r = 0.59$, $p < 0.05$), FNQ ($r = 0.67$, $p < 0.05$), and SEQ ($r = 0.42$, $p < 0.01$) in 2020. In contrast, such correlations were not evident in the 2021 fruiting season (Table 4.5). Individually [K] consistently showed a significant ($p \leq 0.05$) positive association with FCWP incidence in NT and SEQ fruit over two fruiting seasons but not with FNQ fruit. [Ca] did not significantly ($p > 0.05$) correlate with FCWP incidence over the two fruiting seasons except for fruit from NT ($r = -0.33$, $p < 0.05$) and FNQ ($r = -0.55$, $p < 0.05$) in 2021 and 2020, respectively (Table 4.5). [Mg] was not correlated with FCWP in FNQ and SEQ fruit in any season. Conversely for NT fruit, there was a strong positive correlation ($r = 0.87$, $p < 0.001$) in 2020 season and negative correlation ($r = -0.34$, $p < 0.05$) in 2021. On the other hand, [B] only and negatively correlated with FCWP ($r = -0.59$, $p < 0.05$) in 2020 season for NT fruit. N/Ca, K/Ca, and (K+Mg)/Ca showed significant ($p \leq 0.05$) positive correlations with FCWP incidence in either first and/or second season across all regions. The scatter plots clearly indicated the positive trend in FCWP incidence with N/Ca, K/Ca, and (K+Mg)/Ca ratios in NT and FNQ fruit across both fruiting seasons (Figure 4.6). However, for SEQ fruit, the FCWP incidence increased with an increase in flesh K/Ca and (K+Mg)/Ca ratio but not with N/Ca (Figure 4.6). Strong correlations were observed for N/Ca in FNQ-2020 ($r = 0.88$, $p < 0.001$), for (K+Mg)/Ca in NT ($r = 0.72$, $p < 0.01$), and FNQ ($r = 0.83$, $p < 0.001$) in 2020 season. SEQ showed moderate correlation with mineral nutrient ratios in 2020 and no significant ($p > 0.05$) correlation with any of the mineral nutrient ratio in 2021 (Table 4.5).

Incidence of FB disorder was positively correlated with [N] in only FNQ-2020 fruit ($r = 0.63$, $p < 0.05$), with [K] in NT-2021 ($r = 0.68$, $p < 0.001$) and FNQ in both seasons (2020: $r = 0.46$, $p < 0.05$, 2021: $r = 0.62$, $p < 0.001$). Like FCWP, a negative correlation of [B] with incidence of FB was evident in FNQ-2021 fruit only (Table 4.5). Mineral nutrient ratios were not as significantly ($p > 0.05$) involved in FB incidence as in FCWP disorder. However, in 2021, NT fruit showed significant positive correlation between FB incidence and N/Ca ($r = 0.37$, $p < 0.05$), K/Ca ($r = 0.52$, $p < 0.001$), and ($r = 0.51$, $p < 0.001$). On the other hand, fruit from SEQ had no FB over the two fruiting seasons.

The distribution of FCWP and FB across three regions over the two seasons in relation to mineral nutrients was further illustrated by PCA. PCA is used to dimmish biasness in multivariate dataset and to form an objective evaluation by compressing data variability into a few key principal components (Biermann et al. 2022). The pooled data were plotted and clustered based on production regions for the first two principal components explaining 63.8.% of the total variation for FCWP disorder and 64.4% of the variation for FB disorder (Figures 4.8 and 4.9). The vectors indicating the % incidence of FCWP were located on the same axis as mineral nutrient ratios, consistent with the strong positive

correlation and higher contribution to this disorder. The data grouping indicated higher susceptibility of SEQ fruit towards FCWP due to potential higher concentrations of K/Ca and (K+Mg)/Ca (Figure 4.8). NT fruit appeared to be more susceptible to FB disorder and data distribution from FNQ fruit showed equal susceptibility towards both disorders (Figures 4.8 and 4.9). Furthermore, vectors pointing in opposite directions for [Ca] and [Mg] vs FCWP, and [B] vs FB indicated a negative correlation of [Ca] and [Mg] and [B] with each disorder, respectively (Figures 4.8 and 4.9). It was evident from the scatter plot of [B] against FB incidence from all three regions over two seasons (Figure 4.7).

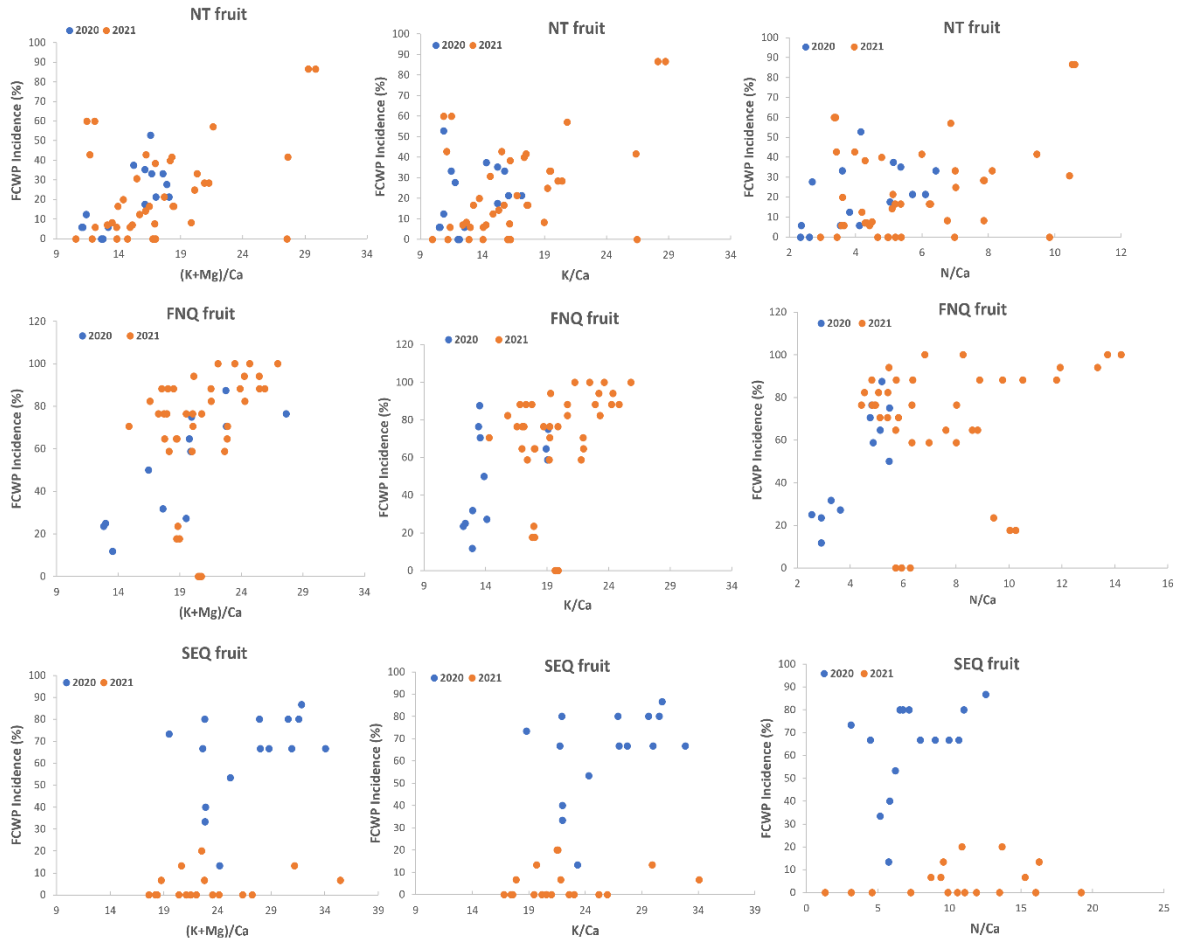


Figure 4.6: Scatter plots of incidence of FCWP disorder versus mineral nutrient ratios in 'B74' mango flesh across three different production regions over two seasons. Each data point represents the % incidence of 15 sample fruit.

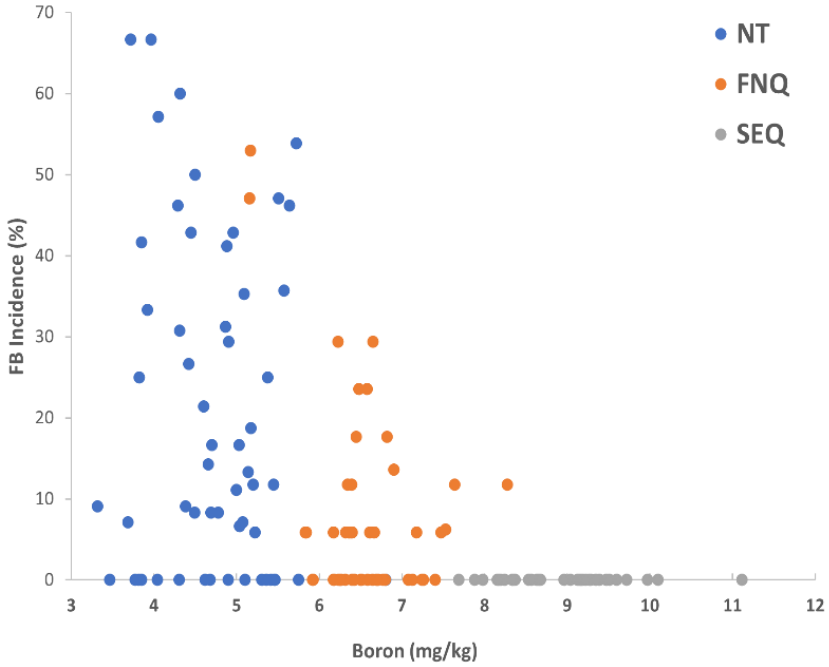


Figure 4.7: Scatter plot of FB disorder versus B concentration in 'B74' mango flesh across three different production regions over two seasons. Each data point represents an average of 15 sample fruit.

Table 4.5. Correlation coefficients (r) between 'B74' mango flesh mineral concentrations at harvest and incidence of IDs at ripe stage for each region and season. '* , **, ***' denotes the level of significance of correlation at $p \leq 0.05$, 0.01 and 0.001, respectively (Tukey's HSD test).

Nutrients	FCWP % Incidence						FB % Incidence					
	NT		FNQ		SEQ		NT		FNQ		SEQ	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
N	0.59*	ns	0.67*	ns	0.42**	ns	ns	ns	0.63*	ns	-	-
Ca	ns	-0.33*	-0.55*	ns	ns	ns	ns	ns	ns	ns	-	-
K	0.52*	0.31*	ns	ns	0.32*	0.30*	ns	0.68***	0.46*	0.62***	-	-
Mg	0.87***	-0.34*	ns	ns	ns	ns	ns	ns	ns	ns	-	-
B	-0.59*	ns	ns	ns	ns	ns	ns	ns	ns	-0.44**	-	-
N/Ca	ns	0.37*	0.88***	ns	0.46**	ns	ns	0.37*	ns	ns	-	-
K/Ca	ns	0.48**	0.46*	0.32*	0.44**	ns	ns	0.52***	ns	ns	-	-
(K+Mg)/Ca	0.72**	0.47**	0.83***	0.32*	0.44**	ns	ns	0.51***	ns	ns	-	-

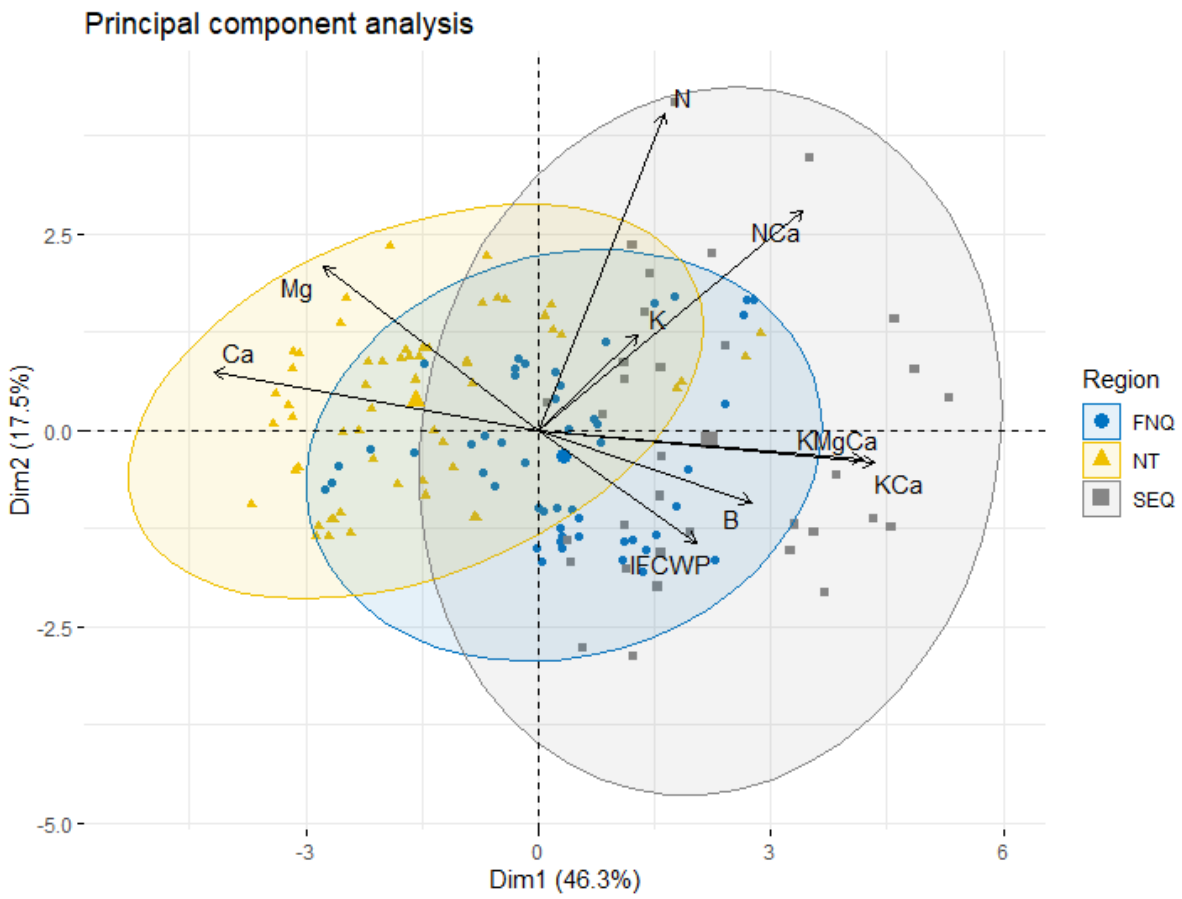


Figure 4.8: Principal component analysis (PCA) fruit nutrient concentrations and FCWP in 'B74' mango. Variance of component 1 (Dim1) = 46.3% and that of component 2 (Dim2) = 17.5%. Here, IFCWP = % incidence of FCWP, NCa = nitrogen to calcium ratio, KCa = potassium to calcium ratio, and KMgCa = potassium + magnesium to calcium ratio.

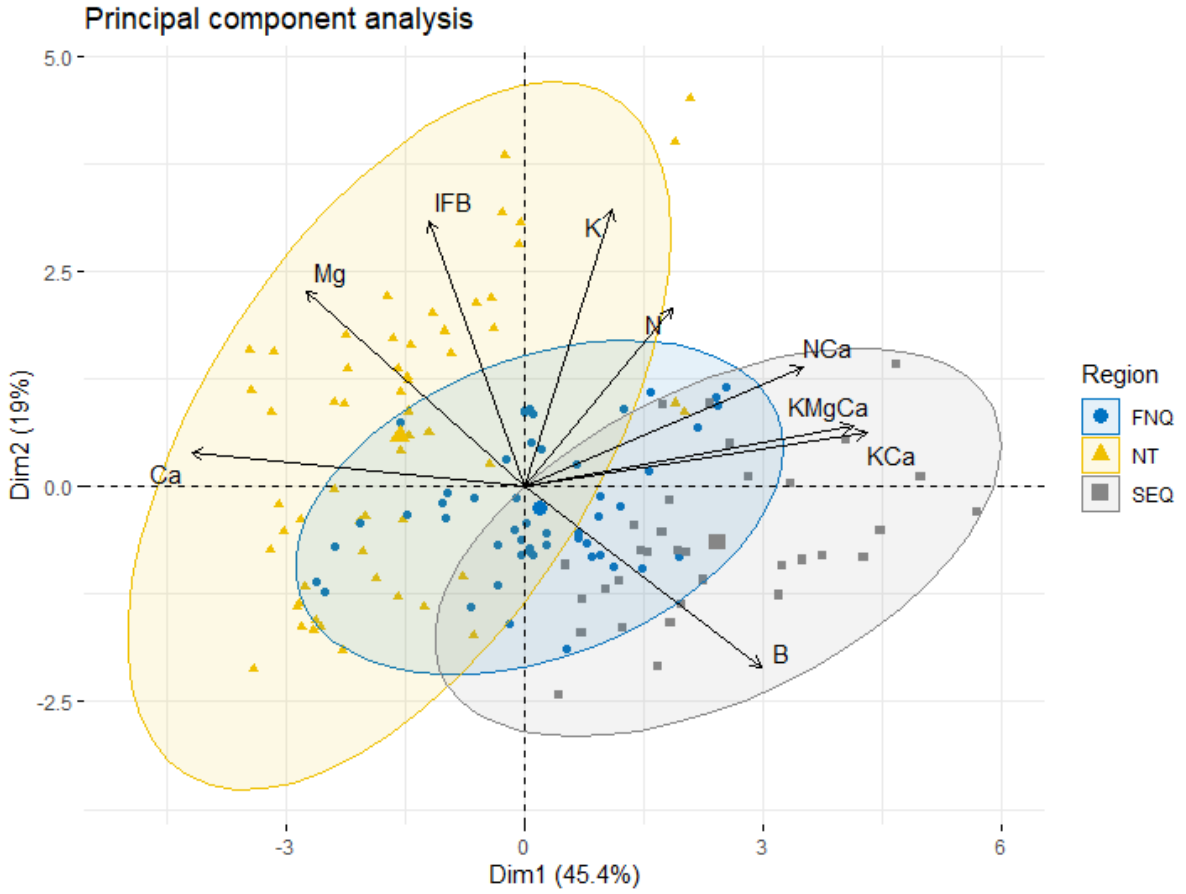


Figure 4.9: Principal component analysis (PCA) fruit nutrient concentrations and FB in 'B74' mango. Variance of component 1 (Dim1) = 45.4% and that of component 2 (Dim2) = 19%. Here, IFB = % incidence of FB, NCa = nitrogen to calcium ratio, KCa = potassium to calcium ratio, and KMgCa = potassium + magnesium to calcium ratio.

4.4.5 Mineral concentrations in healthy versus FCWP fruit

The flesh of individual healthy and FCWP disorder fruit with different severity levels was assessed for mineral analysis (Table 4.6). N concentrations were significantly ($p \leq 0.05$) higher in disordered fruit from FNQ compared to healthy fruit. No significant ($p > 0.05$) differences in [N] were observed between healthy and FCWP fruit from SEQ and NT regions. [Ca] was significantly ($p \leq 0.01$) higher in healthy fruit across all regions except FNQ. A trend like [Ca] was observed in Mg concentrations except for NT where healthy fruit had lower [Mg] compared to FCWP disordered fruit (Table 4.6). [P], [Mn], [Cu], and [B] were not significantly ($p > 0.05$) different between healthy and FCWP fruit from any production region. Mineral nutrient ratios viz., N/Ca, K/Ca, and (K+Mg)/Ca were significantly ($p \leq 0.05$) different between healthy and FCWP fruit across all regions except for K/Ca in FNQ, and the highest values were noted in severely FCWP-affected, and the lowest in healthy fruit (Table 4.6).

Table 4.6. Mineral concentrations in the mesocarp of healthy and FCWP disordered 'B74' mango

fruit from different production regions (n = 3). Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to the Tukey's HSD test.

Region	FCWP Severity	Mesocarp mineral concentrations (mg/kg D.W.)						
		N	Ca	K	Mg	N/Ca	K/Ca	(K+Mg)/Ca
SEQ	Healthy	3690.9	527.52 [a]	11691.9 [b]	460.54 [a]	7.04 [c]	22.29 [c]	23.17 [c]
	Slight	4084.3	487.77 [a]	12891.8 [a]	440.74 [b]	8.38 [b]	26.44 [b]	27.34 [b]
	Moderate	3983.7	485.82 [a]	12490.8 [a]	445.18 [b]	8.20 [b]	25.71 [b]	26.63 [b]
	Severe	3695.5	389.51 [b]	11440.6 [b]	402.65 [c]	9.49 [a]	29.37 [a]	30.41 [a]
	Significance	*ns*	**	***	***	**	***	***
FNQ	Healthy	3812.6 [b]	630.21	11230.9 [b]	503.55 [ab]	6.06 [b]	17.88	18.68 [b]
	Slight	4004.8 [ab]	568.37	12308.3 [a]	534.96 [a]	7.17 [ab]	21.94	22.89 [a]
	Moderate	4278.6 [ab]	565.07	11776.7 [ab]	488.08 [ab]	7.58 [a]	20.83	21.70 [ab]
	Severe	4627.6 [a]	580.45	12037.7 [ab]	480.66 [b]	7.98 [a]	20.75	21.58 [ab]
	Significance	*	*ns*	*	*	*	*ns*	*
NT	Healthy	3460.7	791.97 [a]	9167.3	535.44	4.38 [b]	11.72 [b]	12.41 [b]
	Slight	3953.2	528.97 [b]	9145.6	544.55	7.53 [a]	17.34 [a]	18.37 [a]
	Moderate	4331.9	575.59 [b]	9945.1	532.73	7.56 [a]	17.30 [a]	18.23 [a]
	Severe	3480.8	522.81 [b]	9331.3	561.23	6.69 [ab]	17.95 [a]	19.04 [a]
	Significance	*ns*	**	*ns*	*ns*	*	**	**

Here, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: not significant.

4.5 Discussion

The relative deficiency or excess of one or more mineral nutrients can cause developmental abnormalities and metabolic disorders in fruit during post-harvest storage and ripening (Baugher et al. 2017; Donahue et al. 2021; Andrade et al. 2022). For instance, low [Ca] and high K/Ca concentrations in apple peel were associated with bitter pit disorder (Kalcsits et al. 2019; Donahue et al. 2021). Also, deficiency of [K], [B], and [Zn] in citrus peel can result in fruit cracking (Juan & Jiezhong 2017).

In the present study concerning phytosanitary (VHT) treatment, incidence and severity of IDs in 'B74' mango were assessed for fruit from three distinct regions of Australia over two consecutive growing seasons and with regard to flesh mineral concentrations. FCWP and FB were the most prominent defects observed and their prevalence varied significantly ($p \leq 0.05$) across both regions and seasons (Table 4.3).

Overall, fruit from FNQ and SEQ were more susceptible to FCWP compared to NT fruit. The disorders were evidently associated with lower Ca, Mg, and higher N concentrations in the mesocarp over the two seasons, except for SEQ in the 2021 season (Tables 4.3 and 4.4). Involvement of Ca and Mg deficiencies in fruit predisposition to range of IDs (i.e., soft nose, spongy tissue, and browning) has been reported for other mango cultivars (Burdon et al. 1991; Assis et al. 2004; Sharma & Singh 2009; Ma et al. 2018). These associations were supported by mineral analysis of individual healthy and defective fruit, where healthy fruit had relatively higher Ca and Mg, while FCWP fruit had

relatively higher N and K concentrations (Table 4.6). High Mg and low K concentrations have previously been related to less internal defects and diseases in other fruit crops, namely avocados (Witney et al. 1990).

Lower concentrations of Ca and Mg in FCWP fruit may relate to competition for uptake with K by the roots level, as well as relatively low mobility of these nutrients in the plant during the active fruit growth period (Marschner 1995; Tonetto de Freitas & Mitcham 2012; Hocking et al. 2016). Edaphic and environmental factors, including temperature and humidity during active fruit growth and development, are also likely modulators of Ca uptake and movement within the plant (Hewett 2006; Léchaudel & Joas 2007). NT soils had more available [Ca] with higher CEC and % base saturation in both seasons compared to FNQ and SEQ soils (Table 4.2). The NT region also experienced higher average temperatures throughout the year compared to FNQ and SEQ (Figure 4.2). As Ca predominantly moves through xylem under transpiration pull, relatively higher temperatures may have driven VPD, which facilitated Ca uptake and distribution to NT fruit via high transpiration rates (Shivashankara & Mathai 1999).

The mineral ratios N/Ca, K/Ca, and (K+Mg)/Ca were consistently significantly ($p \leq 0.05$) positively correlation with FCWP disorder across all seasons and regions (Table 4.5). Imbalance of [Ca], [N], [K], and [Mg] reportedly plays a vital role in fruit susceptibility to post-harvest disorders and defects (Assis et al. 2004; Torres & Saúco 2004). High N/Ca and K/Ca ratios are related to IDs of mango (Assis et al. 2004; Andrade et al. 2022; Ma et al. 2022). Assis et al. (2004) reported significantly ($p \leq 0.05$) higher N/Ca at 31:1 in 'Tommy Atkins' fruit flesh affected with a disorder compared to non-defected fruit (17.1:1). Similarly, mineral nutrient ratios were significantly ($p \leq 0.05$) lower in healthy 'Keitt' mango flesh (N/Ca: ~7.5:1, K/Ca: 25:1) compared to fruit with spongy tissue (N/Ca: 30:1, K/Ca: ~95:1), jelly seed (N/Ca: 15:1, K/Ca: ~65:1), and flesh darkening (N/Ca: ~25:1, K/Ca: ~70:1) disorder (Andrade et al. 2022).

Principal component analysis supported positive correlation between mineral nutrient ratios and incidence of FCWP when all data across two seasons and three regions were pooled (Figure 4.8). However, the range in ratios between healthy and disordered fruit differed across regions. For instance, the mean (K+Mg)/Ca ratio in fruit with severe disorders from NT was 19:1, which was higher than that for healthy fruit from SEQ (23.2:1) (Table 4.6). These differences in ratio values between healthy and disordered fruit highlights production region as a contributor to variability in numerical values of ratios alone for use as a predictive index. Another possible reason for NT fruit to have lower incidence of FCWP could be long transit times between harvest and VHT (5-7 days) compared to FNQ (3-4 days) and SEQ (1-2 days). Time before VHT may 'condition fruit' by, for example, starch hydrolysis into sugars and even activation of heat shock proteins that may further

assist with tolerating subsequent heat treatment (Paull & Chen 1990; Joyce & Shorter 1994; Brecht et al. 2020)

Data separation by PCA for region suggested that NT fruit were more susceptible to FB than FNQ and SEQ fruit (Figure 4.9, Table 4.3). PCA also indicated negative correlation between incidence and severity of FB with B concentration (Figure 4.9). SEQ fruit had significantly ($p \leq 0.001$) higher [B] in both seasons, followed by FNQ and NT (Table 4.4). A role of [B] in maintaining internal fruit quality and/or susceptibility to internal breakdown in mango has been documented (Sharma & Singh 2009; Saran & Kumar 2011; Ma et al. 2018; Andrade et al. 2022). [B] in plant cells contributes to the network of pectic polysaccharides in the primary cell wall through borate-diester bonds (Matoh 1997; Bariya et al. 2014), which is distinct from hemicellulose and cellulose components of the cell wall (Matoh 1997). Formation of the pectin network provides cell wall stability, including in growth of reproductive cells, flower initiation, fruit retention, and in maintaining fruit internal quality (Lovatt 1990; Whiley et al. 1996; Smith et al. 1997b; Sharma & Singh 2009; Bariya et al. 2014).

However, in addition to mineral nutrients, other pre- and postharvest factors, such as crop load, maturity, and time and temperature in the supply chain are also potential modulators of fruit predisposition to physiological disorders (Jacobi et al. 2001; East et al. 2009; Sivakumar et al. 2011; Rehman et al. 2015). In general, fruit robustness at harvest should mitigate adverse effects of post-harvest rigours on fruit quality and thereby diminish development of internal defects (Joyce 2021; Joyce et al. 2023; Kiloes et al. 2023). Seasonal variation needs to be characterised, including with regard to soil minerals availability, crop load, and environmental dynamics (Katrodia & Rane 1981, 1988; Hofman 1995; Bally 2006; Payne et al. 2014; Anderson et al. 2017; Wang et al. 2018; Wang et al. 2019b).

4.6 Conclusion

Incidence of IDs in ‘B74’ mango varied appreciably across production regions and fruiting seasons. Fruit from FNQ and SEQ were relatively more susceptible to express FCWP post VHT. This appeared to be due to lower [Ca] and [Mg] and higher [N], K/Ca, and (K+Mg)/Ca ratios as compared to NT fruit. The flesh mineral involvement with FCWP was further affirmed by assessing individual mineral concentrations of healthy and FCWP-affected fruit from all regions. Notably, the (K+Mg)/Ca ratio could be used as a predictive index for the likelihood of FCWP developing in the fruit after VHT due to its consistent correlation across regions and seasons. In contrast, NT fruit were more prone to develop FB disorder, evidently in association with lower fruit flesh [B]. This observation suggests that improving fruit [B] status could improve resistance to FB. Nonetheless, as correlations of mineral concentrations with the prevalence of IDs were inconsistent with seasonal variations, disorder

expression is most likely a multi-variable issue. Hence, there is imperative for both more comprehensive as well as in-depth studies on a broad range of pre-harvest factors in addition to nutrition with a view to develop reliable predictive model for consistent supply of robust quality fruit to export markets, including in terms of tolerance to phytosanitary treatments like VHT.

Chapter 5 Improving ‘B74’ mango fruit quality for export through pre-harvest mineral nutrition

5.1 Abstract

A field experiment over three consecutive cropping seasons investigated effects of N- and Ca-based fertiliser applications on ‘B74’ mango fruit mineral status and post-harvest quality for export. Fertiliser treatments in season 1 were: 1. control (no additional N or Ca); 2. low-N, nil-Ca (N: 50 g/tree); 3. high-N, nil-Ca (N: 100 g/tree); 4. low-N, high-Ca (N: 50 g/tree + Ca: 690 g/tree); and 5. high-N, high-Ca (N: 100 g/tree + Ca: 690 g/tree). N was applied as urea and Ca as microfine gypsum. A sixth treatment in the second and third season was: 6. very high-N, high-Ca (N: 429 g/tree + Ca: 526 g/tree) as calcium nitrate. In general, no significant ($p > 0.05$) effects of fertiliser applications over and above standard commercial practice were observed on soil and leaf mineral concentrations. The sole exception was for the low-N and high-Ca combination treatment, which increased exchangeable soil [Ca]. This treatment also improved fruit mineral status and reduced incidence of flesh cavity with white patches (FCWP) disorder. Irregular bearing for the trial site was recorded in low to medium crop in season 1, high crop in season 2, and, expectedly, low crop in season 3. This pattern was irrespective of fertiliser treatments. Overall, fruit mineral status was determined by crop load and neither by fertiliser application nor nutrient availability in the soil. Crop load was significantly ($p < 0.001$) positively correlated with fruit [Ca] ($r = 0.68$), [Mg] ($r = 0.81$), and [B] ($r = 0.50$), and was negatively correlated with [K] ($r = -0.57$), K/Ca ($r = -0.74$), and (K+Mg)/Ca ($r = -0.73$). Considered collectively, findings suggest that management decisions around fruit quality export would best be made based on orchard and tree conditions, namely from high crop load trees, and at-harvest fruit mineral status collectively.

Keywords: Disorders, fertiliser, VHT, quality.

5.2 Introduction

Post-harvest fruit quality, including expression of internal disorders (IDs), is affected by various factors. These including growing temperatures and VPD, edaphic characteristics, canopy management practices, and mineral nutrition (Hofman 1998). Ca is commonly implicated in physiological and pathological diseases of fruit crops (Shear 1975a, 1975b; Ferguson et al. 1994; Zocchi & Mignani 1995; Zhang et al. 1999; White & Broadley 2003; Taylor & Locascio 2004; Tonetto de Freitas & Mitcham 2012). In avocados, strong inverse correlations have been found between high fruit [Ca] and reduced rots (Hofman et al. 2002) and IDs, including flesh discolouration, vascular browning, and pulp spots (Eaks 1985; Thorp et al. 1997; Penter & Stassen 2000). For example, Ca is associated with improving papaya fruit firmness (Qiu et al. 1995) and shelf life in avocados (Witney et al. 1990). Plant cells, including those in fruit mesocarp tissues, are bonded by the middle lamella predominantly composed of Ca-bound pectin (Hocking et al. 2016). It usually breaks down during fruit ripening and softening and the process is typically faster under Ca deficit, which can lead to cellular breakdown and expression of disorders (Hocking et al. 2016).

Conversely, high N concentrations have been associated with more rot and ID in avocado and mango fruit (Arpaia et al. 1996; Penter & Stassen 2000; Marques et al. 2003; Kruger et al. 2004; Torres et al. 2004a; Torres et al. 2004b). In this context, it is considered important to maintain [N] and [Ca] balance to achieve optimum fruit quality.

Effects of N and Ca fertilisers on fruit quality have been extensively studied for fruit crops (Shear 1975b; Lewis et al. 1977; Cracknell Torres et al. 2004). For example, Schaffer (1994b) observed reduced internal flesh breakdown in ‘Keitt’ mango upon soil application of calcium carbonate plus foliar spray of calcium nitrate. Nevertheless, repeated foliar applications of $CaCl_2$, KCl, and $MgCl_2$ from fruit set to harvest failed to either improve fruit [Ca] or reduce internal breakdown incidence in ‘Sensation’ mango (Hermoso et al. 1997). ‘Tommy Atkins’ showed higher incidence of internal breakdown in mango fruit mesocarp having higher N and lower Ca concentrations in response to fertiliser applications (Torres et al. 2004a). However, in a multi-year field trial with ‘Sensation’ mango, increased internal fruit breakdown along with increased fruit weight were attributed to supplementary ammonium nitrate fertilisation (Torres et al. 2004b). Also, it is important to avoid any antagonistic effects of applied N form on root mediated Ca uptake e.g., Bonomelli et al. (2021) reported that ammonium excess application significantly inhibited and nitrate excess stimulated Ca uptake in tomato seedlings.

Nevertheless, strategically timed N applications can increase fruit number and yield, ultimately improving fruit quality overall (Lahav & Whiley 2002). For Ca and depending on soil characteristics,

lime (for acid soils) or gypsum (for neutral or alkaline soils) are recommended to increase Ca availability and to improve Ca cation exchange percentage in the soil (Silva et al. 2005).

Optimum balance of mineral nutrients is critical in maintaining fruit robustness, especially when targeting export markets where fruit needs to withstand postharvest rigours (Corcoran et al. 2000; Bally 2006; Sivakumar et al. 2011). Certain protocol markets (e.g., China, Japan, New Zealand) require specific phytosanitary treatments, including vapour heat treatment, hot water treatment, and irradiation (Jacobi et al. 2001; Brecht et al. 2020). If fruit lack inherit robustness at harvest, phytosanitary treatments are likely to adversely affect internal and/or external fruit quality (Jacobi & Giles 1997; San et al. 2014; Singh & Saini 2014; Marques et al. 2016)

The present study explores 'B74' mango fruit quality in response to varying rates of N and Ca fertilisers and phytosanitary vapour heat treatment. The specific objectives were to understand: 1. whether soil application of N and Ca at early fruit development stages alone and/or in combination influence fruit mineral composition and quality, and 2. the potential interplay of N- and Ca- fertiliser application and post-harvest phytosanitary (VHT) treatment on fruit quality, including expression of IDs.

5.3 Materials and Methods

5.3.1 Experimental site

The field study was conducted on a commercial 'B74' on 'Kensington Pride' rootstock mango orchard at Childers, Queensland (25.136ºS, 152.372ºE) over three consecutive cropping seasons (2020 to 2022, inclusive). The trees were ~16 years old in 2020 at a planting density of 8 m interrow x 4 m in row. The soil texture was loam/sandy loam comprised of 82.5% sand (coarse sand: 39.2%, fine sand: 43.3%), 7.8% silt, and 9.7% clay. The soil electrical conductivity was < 0.15 dS/m and the cation exchange capacity (CEC) was low at 3.3 meq/100g, suggesting limited low capacity to hold nutrients. pH of the top 0 - 30 cm soil profile was neutral (7.5), and the 30 - 60 cm layer was mildly acidic (5.9).

5.3.2 N and Ca treatments

Five treatments (see below) were applied in season 2020 to plots of five trees. A guard tree was between each plot in the randomized complete block design experiment with three replicates and a guard row between each. A sixth treatment was imposed in seasons 2021 and 2022. All standard macro- and micro-nutrients were applied as per commercial management practices in addition to N and Ca treatments (Appendix A6). N, alone and in combination with Ca, was applied as urea (N –

46%, Green Urea, Incitec Pivot, Ltd). Ca was applied as microfine gypsum (Ca – 23%, S – 18%, $CaSO_4.2H_2O$, GreenCal Gypsum (Powder) Swancorp Pty Ltd.) and as calcium nitrate (Ca – 19%, N – 15.5%, B – 0.2% Swancorp Pty Ltd). Low and high N rates (urea; Table 5.1) were split into three applications (40%, 40%, and 20%) in all three seasons from flower bud swelling to mid-fruit development to maintain a relatively continuous supply of N during growth and development and with a view to reduce the risk of leaching (Table 5.2). N was thoroughly mixed in water and distributed evenly under the canopy area from trunk to drip line. For the three consecutive seasons, a high dose of gypsum-Ca was applied by hand at flower bud swell stage in combination with the first half split application of N. A high dose of calcium nitrate was applied in 2021 and 2022 seasons and split into three equal applications 1/3rd at 2 - 3 weeks intervals over early fruit development stages (Tables 5.1 and 5.2). The sprinkler irrigation system was operated on each occasion for 15 mins to help incorporate N and Ca fertilisers into the soil. Trees without any additional N or Ca were treated as controls.

Table 5.1. Elemental N and Ca concentrations applied to 'B74' mango trees in a three-year trial.

Season 2020		Treatments	Seasons 2021 and 2022		Fertilisers
N (g tree^{-1})	Ca (g tree^{-1})		N (g tree^{-1})	Ca (g tree^{-1})	
0	0	Control	0	0	●
50	0	low-N, nil-Ca	50	0	●
100	0	high-N, nil-Ca	100	0	●
50	690	low-N, high-Ca	50	690	● ●
100	690	high-N, high-Ca	100	690	● ●
-	-	high-N, high-Ca	429.4	526	◉

Here, ●: control, ●: urea, ●: micro-fine gypsum, ◉: calcium nitrate

Table 5.2. Timeline of fertiliser application across the three seasons.

Season	Fertilisers	Month of application				
		August	September	October	November	December
2020	Urea	40%	-	40%	20%	-
	Microfine-gypsum	100%	-	-	-	-
	Ca-nitrate	-	-	-	-	-
2021	Urea	40%	40%	20%	-	-
	Microfine-gypsum	100%	-	-	-	-
	Ca-nitrate	-	33.5%	33.5%	33.5%	-
2022	Urea	40%	-	40%	-	20%
	Microfine-gypsum	100%	-	-	-	-
	Ca-nitrate	-	-	33.5%	33.5%	33.5%

5.3.3 Meteorological data, soil, and leaf sampling

The trial site climate was monitored for temperature and humidity data in the field from full bloom to commercial harvest using Tinytag™ data loggers (Tinytag Plus 2, TGP-4500) within Stevenson screens. Rainfall data were collected from the closest weather station in Childers South (lat. 25.26°S,

long. 152.28°E, elevation: 95 m) approximately 20 km from experiment site. There was little difference in maximum and minimum temperatures and in humidity throughout fruit growth cycles across the seasons (Figure 5.1). However, 2021 was a wetter year, with multiple rain events during fruit maturation up to commercial harvest.

Before treatment application in 2020, six soil samples from start and end of each replicate row were collected at 0 - 30 cm and 30 - 60 cm depth, where most feeder roots were present, using a 110 cm hand-held corer tube (Bally 2006). Soil samples were bulked and analysed for mineral concentrations. Subsequently, soil samples were taken from each treatment plot in each replicate at bud swelling in seasons 2021 and 2022 and at fruit harvest time in seasons 2020, 2021, and 2022. Soil samples were oven-dried at 40 °C for 3 - 4 days and sieved to < 2 mm. Soil nitrate-nitrogen and ammonium-nitrogen were measured by extracting samples using 2 M potassium chloride solution. Ammonium-nitrogen was measured colourimetrically after dilution, and nitrate-nitrogen was reduced to nitrite through copperised cadmium column and measured colourimetrically (Rayment & Lyons 2011).

For exchangeable cation concentrations for Ca, Mg, Na, K, and Al, soil samples were extracted using mixture of 0.1 M ammonium chloride and barium chloride at 1:10 ratio and measured using inductively coupled plasma spectroscopy (Rayment & Lyons 2011). Cation results were expressed as meq/100g.

Boron (B) was measured by extracting sample using 0.01 M calcium chloride at 1:4 ratio. The mixture was heated to 90 °C and analysed by inductively coupled plasma spectroscopy (Rayment & Lyons 2011). Plant-available sulphur (S) was determined by extracting soil using 0.25 M potassium chloride solution and measured by inductively coupled plasma spectroscopy (Rayment & Lyons 2011). Plant-available phosphorus (P) was measured by extracting samples with 0.5 M sodium bicarbonate solution (adjusted to pH 8.5) for 16 hours at a 1:100 ratio and measured colourimetrically (Rayment & Lyons 2011).

Leaf mineral analysis was done at pre-flowering (bud swelling stage) and at fruit harvest times in each season. In 2020, 30 young fully expanded mature leaves were collected randomly from each replicate at bud swelling stage and 15 young fully expanded mature leaves from each treatment plot in each replicate were collected at harvest. In 2021 and 2022, 15 young fully expanded mature leaves from each treatment plot in each replicate were collected at pre-flowering (viz., bud swelling stage) and harvest. Leaf samples were immediately placed in polyethylene bag and transferred to laboratory within 3 - 4 hours. Leaf samples were dried in fan-forced oven at 60 °C for 2 days, ground to a fine powder, and analysed for mineral concentrations. Briefly, dried leaf samples were digested with hydrogen peroxide and nitric acid. The acid-digested leaf samples were measured by inductively coupled plasma spectroscopy (McQuaker et al. 1979). Total N was measured using LECO

combustion analyser with combustion temperature of 950 °C (Flanagan et al. 2022). Results were expressed as mg/kg dry weight for B, Mn, Zn, Fe, and Cu, and as % dry weight for N, Ca, K, S, P, and Na. All of the mineral analyses were analysed by commercial 'Soil and plant analysis laboratory' (https://www.csbplab.com.au).

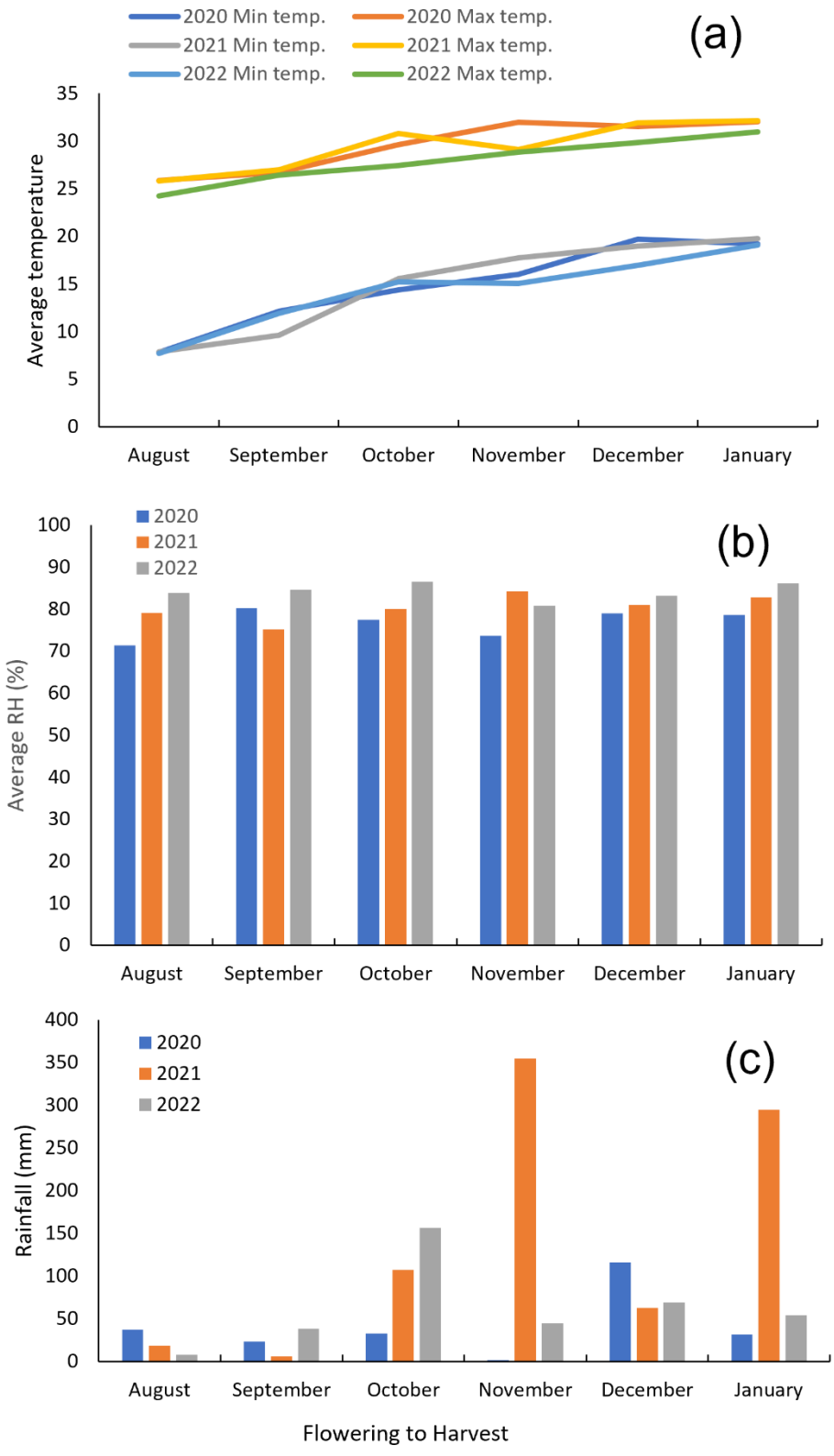


Figure 5.1: Fertiliser trial site weather conditions over three growing seasons from bud swelling to

commercial harvest: flowering and fruit set (August-September), fruit growth and maturation (October-December), and harvest (January). a. maximum and minimum temperatures, b. Average daily relative humidity (RH%), and c. cumulated rainfall (mm).

5.3.4 Crop load, weight, and yield

Fruit were manually counted from each treatment tree/plot/rep 3 days before commercial harvest and expressed as crop load per treatment. Average fruit weight was calculated by weighing 10 fruit randomly from each tree at commercial harvest and subsequently used to estimate tree yield. Accumulated heat sum (degree days = cumulative daily average temperature in °C – 10 °C) from full bloom to harvest were calculated as a guide to fruit maturity and harvest (Hofman & Whiley 2010).

5.3.5 Fruit quality assessments and mineral analyses

Thirty mature green fruit per treatment plot were randomly collected across all four tree quadrants and from outside of the canopy at commercial harvests on 20/01/2021, 17/01/2022, and 24/01/2023 for subsequent quality assessments. They were harvested carefully with long stems in the morning between 8:00 - 10:00 am, packed into foam trays, and transported in an air-conditioned vehicle (ca. 22 °C) to post-harvest research laboratory at the Maroochy Research Facility near Nambour, Queensland. Upon arrival, fruit were labelled, de-stemmed, and de-sapped in mango wash solution (Mango-plus: 1 kg/400L H_2O) for 90 sec, followed by fungicide treatment (Sportak®: 55 ml/100L H_2O) for 30 sec. They were air-dried for 2 hours and stored at 16 °C overnight. The next day, fruit were transported to Brisbane, Queensland in an air-conditioned vehicle for vapour heat treatment (VHT) for Queensland fruit fly control. Of the 30 fruit/treatment/rep, 15 were subject to VHT at 47 °C core temperature and > 90% relative humidity for 15 min. Another fifteen fruit were maintained as non-VHT controls. They were stored at 18 °C for the duration of VHT, while the treated fruit were cooled down to 18 °C post VHT. All fruit were transferred to the post-harvest laboratory at The University of Queensland, Gatton campus, about 100 km away, for post-harvest assessment and ripening at 20 °C.

Fruit weight was measured after VHT. Fruit ripe stage was determined by hand firmness rating and subjective assessment of fruit colour (Hofman et al. (2010a) (section 4.3.3). Incidence and severity of IDs were assessed at eating ripe upon making a longitudinal cut across the fruit flesh near the seed (section 4.3.3). Shelf life was from the day of VHT until eating ripe stage. Fruit flesh was assessed individually for total soluble solids (TSS) using a handheld digital refractometer.

Fruit was analysed for mineral concentrations at mature green stage of commercial maturity. An additional 15 hard green mature fruit from each treatment plot were collected, carefully packed, and transported directly to the post-harvest laboratory at The University of Queensland, Gatton campus, for analysis. Fruit mesocarp sections from widest part of the cheek were taken (~2 x 2.6 cm, L x D) using fruit corer. For mineral analysis, they were dried at 65 °C in fan-forced oven for 3 days and then ground to fine powder using a mini laboratory scale ball mill (Retsch Mixer Mill MM 400). The

dried ground flesh samples were analysed for mineral concentrations (section 4.3.4).

5.3.6 Statistical analysis

For potential differences in soil, leaf, and fruit mineral concentrations, crop load, and yield between treatments, data were analysed by ANOVA for randomised complete block design (Ferreira et al. 2014). Means were separated by the Tukey's HSD test at a 5% level of significance. Fruit quality parameters were analysed by two-factor ANOVA using randomised complete block model with fertiliser application and VHT as the factors and means separated by the Tukey's HSD test at a 5% level of significance (Ferreira et al. 2014). Level of significance are indicated as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$, with ns for: not significant. Mesocarp mineral concentrations were correlated against incidence and severity of IDs using Pearson's correlation coefficient and principal component analysis (Schober et al. 2018; Biermann et al. 2022). All statistical analyses were performed in R (4.0.2).

5.4 Results

5.4.1 Soil and leaf mineral analysis

Soil mineral analysis: Mineral analysis of the soil before implementing the field trial indicated low exchangeable [Ca] at 0 - 30 and 30 - 60 cm depths (Table 5.3). NH_4^+ and NO_3^- concentrations were slightly higher before nutrient application compared to the end of season 2020 (Tables 5.3 and 5.4). However, no significant ($p > 0.05$) increase in [NH_4^+] and [NO_3^-] was noted in response to fertiliser treatments at pre-flowering or post-harvest in any season (Table 5.4). NH_4^+ and NO_3^- ions may have leached below 60 cm due to multiple rain events leading up to commercial maturity (Figure 5.1).

Exchangeable [Ca] levels at harvest in 2020 were not significantly different ($p > 0.05$) among treatments at either sample depths. However, it was slightly higher in gypsum treatments (Table 5.4). There was a trend for [Ca] to decrease with soil analysis depth. In season 2021, [Ca] at 0 - 30 cm depth were significantly higher ($p \leq 0.05$) for the microfine gypsum treatments of low-N, high-Ca and high-N, high-Ca at pre-flowering compared the other treatments. At the end of crop cycle in 2021, most applied Ca was depleted from the low-N, high-Ca treatment (Table 5.4). However, in season 2022, overall [Ca] were higher at pre-flowering than at post-harvest for both depths, and there was no significant ($p > 0.05$) difference at 30 cm (Table 5.4). Other nutrients, including [S], [P], [B], exchangeable [K], and [Mg] were not significantly ($p > 0.05$) affected by any treatment over the three seasons. The % base saturations of exchangeable Ca, Mg, and K were calculated from total soil CEC (Appendix A3). At 0 - 30 cm, %Ca-base saturation was significantly ($p \leq 0.05$) higher for microfine-gypsum treatments of low-N, high-Ca and high-N, high-Ca in seasons 2020 and 2021. In contrast,

%Mg- and %K-base saturations were unaffected by any treatment over the three fruit seasons (Appendix A3).

Leaf mineral analysis: The recommended levels of leaf micro- and macro-nutrients at bud swell stage for good quality 'B74' mango are presented in Appendix A4 (Hofman & Whiley 2010). Leaf [N], [Mg], [K], [Na], [Cu], [Fe], and [Zn] were within desired ranges before commencement of fertiliser applications. However, [Ca] and [S] were low and [B] and [P] levels were high. Overall, trees were healthy and in good condition. Leaf mineral analysis at pre-flowering and harvest in all seasons showed no significant ($p > 0.05$) difference in any mineral concentrations among treatments (Appendix A5).

Table 5.3. Soil nutrient status at the pre-flowering stage in season 2020 before treatment application (n=3).

		Soil mineral status							
	Depth (cm)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	Ex. Ca (meq/100g)	Ex. K (meq/100g)	Ex. Mg (meq/100g)	P (mg/kg)	B (mg/kg)	S (mg/kg)
At pre-flowering	0-30	2.33 ± 0.58	1.33 ± 0.58	2.18 ± 0.14	0.20 ± 0.02	0.71 ± 0.03	33.33 ± 4.04	0.50 ± 0.06	9.93 ± 1.21
	30-60	2.00 ± 0.00	<1 ± 0.00	1.62 ± 0.07	0.14 ± 0.01	0.40 ± 0.06	22.67 ± 4.73	0.43 ± 0.07	30.80 ± 11.72

Table 5.4. Soil NH4+, NO3- and exchangeable Ca concentrations at 0 - 30 and 30 - 60 cm depths over three seasons (n=3). Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Depth (cm)	Treatment	NH_4^+ (mg/kg)					NO_3^- (mg/kg)					Ex. Ca (meq/100g)				
		Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3
0-30	Control	1	2.0	3.3	1.5	<1	<1	<1	<1	<1	2.0	2.46	2.7 ^{b}	3.0 ^{a}	4.8	2.9
	low-N, nil-Ca	1	1.7	3.7	1.3	<1	<1	<1	1	<1	2.0	2.80	2.5 ^{b}	1.7 ^{bc}	3.2	2.3
	high-N, nil-Ca	1	2.0	3.3	1.3	<1	<1	<1	<1	<1	3.5	2.14	2.6 ^{b}	2.9 ^{a}	3.5	3.8
	low-N, high-Ca	<1	1.7	4.3	2.0	<1	<1	<1	<1	<1	2.5	2.92	3.1 ^{a}	1.5 ^{c}	4.8	2.7
	high-N, high-Ca	1	2.0	3.3	1.3	<1	<1	<1	<1	<1	3.5	2.79	3.1 ^{a}	2.9 ^{a}	3.2	3.8
	v. high-N, high-Ca	-	2.0	3.3	1.0	<1	-	<1	<1	<1	1.67	-	2.7 ^{b}	1.8 ^{bc}	4.2	2.6
	Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*	**	*ns*	*ns*
30-60	Control	<1	1.5	4.0	<1	2	<1	<1	<1	<1	<1	1.57	1.9	2.6 ^{a}	1.3	1.8 ^{b}
	low-N, nil-Ca	<1	1.7	4.0	<1	1	<1	<1	<1	<1	<1	1.56	1.7	1.2 ^{c}	1.6	1.2 ^{b}
	high-N, nil-Ca	<1	1.3	3.3	<1	2	<1	<1	<1	<1	<1	1.41	1.9	2.5 ^{ab}	1.7	1.8 ^{b}
	low-N, high-Ca	1	1.0	4.0	<1	2	<1	<1	<1	<1	<1	1.66	2.2	1.3 ^{c}	2.2	1.7 ^{b}
	high-N, high-Ca	<1	1.5	3.7	<1	1.5	<1	<1	<1	<1	<1	1.51	1.8	2.8 ^{a}	1.4	2.4 ^{a}
	v. high-N, high-Ca	-	1.5	3.3	<1	3	-	<1	<1	<1	<1	-	1.9	1.4 ^{bc}	2.2	1.5 ^{b}
	Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	***	*ns*	*

Here, Pre: pre-flowering, Post: Post-harvest, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

5.4.2 Fruit mineral concentrations

Fruit mesocarp mineral analysis was performed at harvest to determine the effects of fertiliser application on mineral nutrient concentrations. Flesh [N], N/Ca, K/Ca, and (K+Mg)/Ca were all significantly ($p \leq 0.05$) lower in response to the low-N, high-Ca combination treatment in season 2020 (Tables 5.5 and 5.6). There was no effect of fertiliser application on K concentrations in 2020 and 2021 seasons, nor on [Ca] and [Mg] in 2020 season. However, all Ca combination treatments (i.e., low-N, high-N, and v. high-N) significantly ($p \leq 0.05$) improved [Ca] and [Mg] in fruit flesh in season 2021 (Table 5.5). No significant ($p > 0.05$) difference in the mineral nutrient ratios K/Ca and (K+Mg)/Ca were discerned for any treatment in 2021 and 2022, except for low-N, nil-Ca in 2021 (Table 5.6).

Table 5.5. Individual mineral nutrient concentrations in fruit mesocarp at harvest. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Treatments	N (% dw)			K (% dw)			Ca (mg/kg dw)			Mg (mg/kg dw)		
	S-1	S-2	S-3	S-1	S-2	S-3	S-1	S-2	S-3	S-1	S-2	S-3
Control	0.34^{a}	0.67^{a}	0.41^{c}	1.13	1.05	1.21^{bc}	463.5	489.2^{ab}	497.2^{a}	438.1	497.2^{ab}	381.2
low-N, nil-Ca	0.37^{a}	0.62^{a}	0.45^{bc}	1.14	1.04	1.14^{c}	445.8	428.2^{b}	378.7^{b}	419.9	452.7^{c}	374.6
high-N, nil-Ca	0.31^{ab}	0.59^{ab}	0.43^{bc}	1.18	1.03	1.18^{c}	432.9	489.4^{ab}	409.6^{b}	442.8	469.5^{bc}	367.3
low-N, high-Ca	0.23^{b}	0.42^{c}	0.47^{b}	1.08	1.07	1.28^{ab}	518.9	565.7^{a}	420.0^{b}	412.2	496.4^{ab}	370.7
high-N, high-Ca	0.37^{a}	0.45^{bc}	0.46^{b}	1.26	1.05	1.30^{ab}	457.4	519.8^{a}	426.8^{b}	435.2	497.3^{ab}	394.1
v. high-N, high-Ca	-	0.55^{abc}	0.53^{a}	-	1.17	1.19^{bc}	-	537.3^{a}	371.0^{b}	-	531.8^{a}	406.1
Significance	*	**	***	*ns*	*ns*	**	*ns*	*	**	*ns*	**	*ns*

Here, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

Table 5.6. Mineral nutrient ratios in fruit mesocarp at harvest. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Treatments	N/Ca			K/Ca			(K+Mg)/Ca		
	S-1	S-2	S-3	S-1	S-2	S-3	S-1	S-2	S-3
Control	7.82^{a}	13.61^{a}	8.57^{c}	25.31^{ab}	21.89^{b}	25.08	26.18^{ab}	22.92^{b}	25.87
low-N, nil-Ca	8.73^{a}	15.01^{a}	12.57^{ab}	26.54^{a}	26.93^{a}	31.29	27.52^{a}	28.07^{a}	32.33
high-N, nil-Ca	7.66^{a}	12.49^{ab}	11.05^{bc}	28.14^{a}	21.35^{b}	29.79	29.19^{a}	22.32^{b}	30.71
low-N, high-Ca	4.67^{b}	7.65^{c}	11.57^{b}	21.36^{b}	19.38^{b}	31.99	22.17^{b}	20.27^{b}	32.91
high-N, high-Ca	8.50^{a}	8.74^{c}	10.93^{bc}	28.55^{a}	20.82^{b}	30.70	29.54^{a}	21.81^{b}	31.63
v. high-N, high-Ca	-	9.41^{bc}	14.92^{a}	-	21.68^{b}	33.42	-	22.72^{b}	34.55
Significance	*	***	***	*	*	*ns*	*	*	*ns*

Here, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

5.4.3 Yield and post-harvest quality

Recommended heat units of ≥1578 in the Childers region were used as a guide for fruit harvest maturity towards acceptable flavour of ripened 'B74' mango (Hofman et al. 2010b). Green mature fruit were harvested at 1606 heat units in 2020, 1775 in 2021, and 1571 in 2022. Their average DM

range as measured in-field by handheld NIR was 14-16% (F750 produce quality meter (Felix instruments, Camas, WA, USA)).

Crop load differed significantly ($p \leq 0.05$) among fertiliser treatments in seasons 2020 and 2022, but not 2021 (Table 5.7). Yield was only significantly ($p \leq 0.05$) different in the 2020 season. Fruit weight was unaffected by fertiliser treatment applications in any season. Biennial bearing was observed, with seasons 2020 and 2022 being off-years with medium to low crop load and yield (Table 5.8). Season 2021 was an on-year with significantly ($p \leq 0.001$) higher crop load and yield (Table 5.8).

Fertiliser treatments did not significantly ($p > 0.05$) affect post-harvest quality parameters, such as shelf life and TSS. Fruit subjected to phytosanitary VHT had significantly ($p \leq 0.001$) lower shelf life in seasons 2020 and 2021 compared to non-treated controls. However, there was no such effect of VHT in 2022 (Table 5.9). TSS was not affected by VHT treatment compared to the control across all seasons.

Table 5.7. Effect of fertiliser application on fruit number per tree, average fruit weight, and tree yield across three seasons. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Treatments	Crop load (fruit number/tree)			Fruit weight (g)			Estimated yield (kg/tree)		
	S-1	S-2	S-3	S-1	S-2	S-3	S-1	S-2	S-3
Control	82.6^{ab}	203.2	36.2^{ab}	441.1	526.1	459.4	36.3^{ab}	106.6	17.2
low-N, nil-Ca	50.0^{b}	134.2	26.8^{abc}	447.6	545.5	472.2	22.5^{b}	72.3	12.9
high-N, nil-Ca	76.2^{ab}	173.3	27.0^{abc}	458.0	518.4	492.6	34.1^{ab}	88.6	13.6
low-N, high-Ca	105.3^{a}	210.0	44.4^{a}	456.3	529.2	468.0	46.7^{a}	109.5	21.7
high-N, high-Ca	96.6^{a}	233.2	16.3^{bc}	438.4	524.6	504.2	41.4^{a}	122.5	8.2
v. high-N, high-Ca	-	223.8	13.8^{c}	-	529.2	503.5	-	118.1	7.0
Significance	*	*ns*	*	*ns*	*ns*	ns	*	*ns*	*ns*

Here, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

Table 5.8. Seasonal differences in fruit number, average fruit weight, and tree yield. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Seasons	Avg. fruit weight (g)	Avg. crop load (fruit/tree)	Avg. yield (kg/tree)
2020	448.3^{c}	82.1^{b}	36.2^{b}
2021	528.9^{a}	196.3^{a}	102.9^{a}
2022	483.3^{b}	27.4^{c}	13.4^{c}
Significance	***	***	***

Table 5.9. Effect of post-harvest VHT treatment on shelf life and TSS across three consecutive seasons. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$

by Tukey's HSD test.

Seasons	Treatment	Avg. shelf life	Avg. TSS
2020	VHT	15.32 [b]	13.32
	Non VHT	19.78 [a]	13.25
	Significance	***	*ns*
2021	VHT	17.35 [b]	13.47
	Non VHT	20.96 [a]	13.44
	Significance	***	*ns*
2022	VHT	17.61	13.94
	Non VHT	17.12	13.46
	Significance	*ns*	*ns*

5.4.4 Incidence and severity of IDs

Three prominent IDs were observed in 'B74' fruit flesh (Figure 5.2), namely flesh cavity (FC), flesh cavity with white patches (FCWP), and riceyness (R) in season 2020, and FC and FCWP but not R in seasons 2021 and 2022. FCWP was characterised by dry white honeycomb-like cavities in the mesocarp around the seed (Figure 5.2c). FC was characterised as cracking in the flesh and R as white starchy spots in the fruit flesh. Incidence of FC was equally distributed irrespective of fertiliser and VHT treatments. However, FCWP and R were present only in VHT fruit. FC and R severity were very low, such that fruit were considered marketable. On the other hand, FCWP was severe, with potential to cause losses in commercial markets.

In season 2020, incidence and severity of FCWP in VHT-treated fruit were significantly ($p \leq 0.05$) influenced by fertiliser application. The lowest incidence (40%) and severity (0.57) were observed in the low-N, high-Ca combination fertiliser treatment (Table 5.10). However, fruit quality was better in 2021, with very low expression of FCWP disorder and no significant ($p > 0.05$) effect of fertiliser treatments on FCWP incidence and severity (Table 5.10). In season 2022, the internal quality was poorer than in 2021, but better than 2020. Lowest incidence of FCWP disorder (0%) was observed in the low-N, high-Ca combination treatment (Table 5.10).

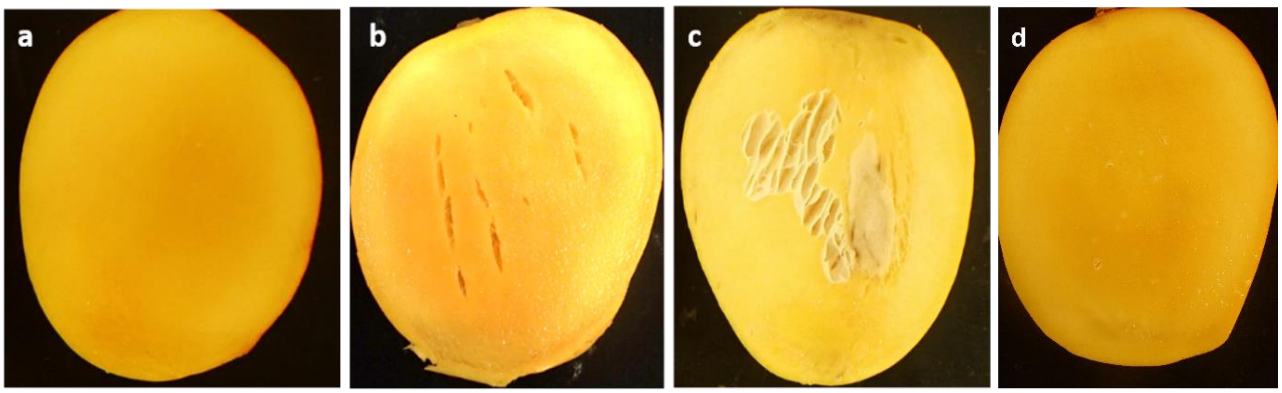


Figure 5.2: Internal disorders of 'B74' mango. a. Healthy, b. flesh cavities (FC), c. Flesh cavity with

patches (FCWP), and d. Ricyness (R).

Table 5.10. Incidence (%) and severity (0 = healthy, 3 = >50 affected flesh) of FCWP disorder in VHT-treated 'B74' mango fruit at eating ripe stage in response to fertiliser applications. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

Treatments	% Incidence of FCWP			Severity of FCWP (0-3)		
	S-1	S-2	S-3	S-1	S-2	S-3
Control	71.1[a]	0.0	8.9[bc]	1.04[a]	0.0	0.1[bc]
low-N, nil-Ca	71.1[a]	6.7	13.3[ab]	1.00[a]	0.05	0.1[ab]
high-N, nil-Ca	62.2[a]	6.7	20.0[a]	0.66[b]	0.04	0.2[a]
low-N, high-Ca	40.0[b]	0.0	0.0[c]	0.57[b]	0.00	0.0[c]
high-N, high-Ca	73.3[a]	8.9	15.6[ab]	1.26[a]	0.06	0.2[ab]
v. high-N, high-Ca	-	6.7	20.0[a]	-	0.07	0.2[a]
Significance	*	*ns*	**	**	*ns*	**

Here, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

5.4.5 Relationship between crop load, fruit quality and mineral nutrients

The relationship between fruit mineral concentrations and crop load at harvest with FCWP incidence was assessed by Pearson's correlation coefficient. Strength and significance levels of correlation coefficients varied across seasons, perhaps due to alternate crop bearing. In season 2020, [N], [K], N/Ca, K/Ca, and (K+Mg)/Ca were significantly ($p \leq 0.05$) and positively correlated with FCWP incidence (Table 5.11). Correlation coefficients improved when data were pooled across seasons 2020 and 2021, with significant positive relationships between incidence of FCWP and [K] ($r = 0.69$, $p < 0.001$), K/Ca ($r = 0.50$, $p < 0.01$), and (K+Mg)/Ca ($r = 0.49$, $p < 0.01$). Negative correlations were discerned with [Mg] ($r = -0.58$, $p < 0.001$), crop load ($r = -0.68$, $p < 0.001$), and yield ($r = -0.72$, $p < 0.001$) (Table 5.11). Seasonal variability was evident when pooled data across the three seasons were correlated with incidence of FCWP in that most significant correlations observed in individual seasons disappeared except for N/Ca ($r = 0.30$, $p < 0.05$), crop load ($r = -0.28$, $p < 0.05$), and yield ($r = -0.33$, $p < 0.05$) (Table 5.11).

On the other hand, crop load showed strong positive correlations with fruit [Ca] ($r = 0.68$, $p < 0.001$), [Mg] ($r = 0.81$, $p < 0.001$), and [B] ($r = 0.50$, $p < 0.001$) and negative correlations with [K] ($r = -0.57$, $p < 0.001$), K/Ca ($r = -0.74$, $p < 0.001$), and (K+Mg)/Ca ($r = -0.73$, $p < 0.001$) (Table 5.12). These correlations highlighted crop load as a major driver for fruit flesh mineral nutrient concentrations. Principal component analysis was conducted on pooled data from all three seasons irrespective of fertiliser treatments (Figure 5.3). Data plotted against the first two principal components explained 68.7% of total variation for FCWP disorder from data grouped based on the season. The data

distribution in PCA plot indicated clear differences in fruit quality and mineral nutrient concentrations across seasons (Figure 5.3).

Fruit from the 2021 season were superior overall in quality, with higher yield, crop load, fruit weight, shelf life, flesh [Mg], [N], [Ca], and N/Ca and lower % incidence of FCWP (Figure 5.3). In contrast, fruit in 2020 and 2022 seasons had higher incidence and severity of FCWP, with higher fruit flesh [K], K/Ca, and (K+Mg)/Ca.

Table 5.11. Pearson correlation coefficients between mesocarp mineral concentrations, crop load, yield at harvest and incidence and severity of FCWP at eating ripe stage. '*' denotes the significance of correlation at $p \leq 0.05$ by Tukey's HSD test.

Variables	% FCWP Incidence		
	Season 2020	**Season 2020 + 21**	**Season 2020 + 21 + 22**
Fruit			
N	0.43*	n.s.	n.s.
Ca	n.s.	n.s.	n.s.
Mg	n.s.	-0.58***	n.s.
K	0.52*	0.69***	n.s.
B	n.s.	n.s.	n.s.
N/Ca	0.42*	n.s.	0.30*
K/Ca	0.43*	0.50**	n.s.
(K+Mg)/Ca	0.42*	0.49**	n.s.
Crop load	n.s.	-0.68***	-0.28*
Yield	n.s.	-0.72***	-0.33*

Table 5.12. Pearson correlation coefficients between mesocarp mineral concentrations and crop load at harvest (all three fruit seasons). '*' denotes the significance of correlation at $p \leq 0.05$ by Tukey's HSD test.

Crop load	**N**	**Ca**	**K**	**Mg**	**B**	**N/Ca**	**K/Ca**	**(K+Mg)/Ca**
	0.17	0.68	-0.57	0.81	0.50	-0.22	-0.74	-0.73
Significance	n.s.	***	***	***	***	n.s.	***	***

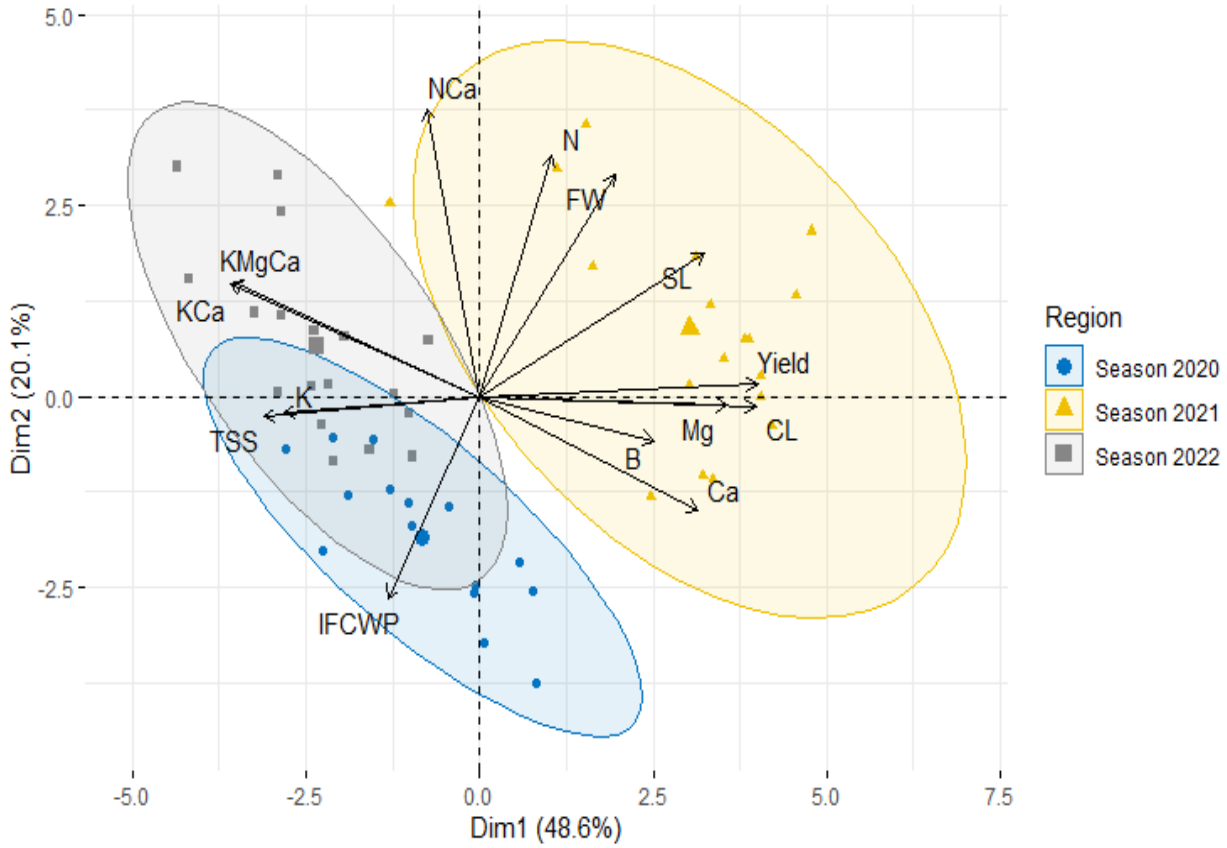


Figure 5.3: Principal component analysis (PCA) of flesh nutrient concentrations, harvest, and post-harvest parameters and FCWP in 'B74' mango. The variance of component 1 (Dim1) = 35.3% and that of component 2 (Dim2) = 21.5%. Here, IFCWP = % incidence of FCWP, NCa = nitrogen to calcium ratio, KCa = potassium to calcium ratio, KMgCa = potassium + magnesium to calcium ratio, CL = crop load (fruit number per tree), SL = shelf life, FW = fruit weight, and TSS = total soluble solids.

5.5 Discussion

Mineral nutrients play a critical role in maintaining fruit robustness at harvest and post-harvest in a range of horticultural crops, including apple, avocado, and pear (Brunetto et al. 2015; Gomez & Kalcsits 2020; Joyce et al. 2021). In the present study, N- and Ca-based fertilisers were applied at early fruit development stages for three consecutive cropping seasons to alter fruit flesh mineral concentrations towards improved overall 'B74' mango fruit quality for export, where phytosanitary treatment is mandatory for some markets.

The effects of fertilisers on soil and leaf mineral concentrations were not significant ($p > 0.05$) in terms of differences between control (no additional application) and fertiliser treatments across the three serial seasons at pre-flowering or post-harvest sampling stages. Nevertheless, soil exchangeable [Ca] was uniquely improved at pre-flowering soil sampling stage for 0 - 30 cm soil depth in season

2021 by low and high-N, high-Ca combination treatments (Table 5.4). Nguyen et al. (2004) similarly reported no significant ($p > 0.05$) effect of N application in the 50 – 200 g/tree range on 'Kensington Pride' mango leaf N concentrations and tree yield. However and undesirably, the fruit were visibly greener at ripe stage with a higher incidence of anthracnose disease (Nguyen et al. 2004; Bally et al. 2006).

Fertiliser application did not improve leaf N concentrations (Appendix A5), with no significant ($p > 0.05$) difference between fertiliser treatments and control. Overall, leaf N concentrations were within optimal recommended range (1.0 – 1.5% dw) across all seasons at both pre-flowering and post-harvest sampling stages across control and fertiliser treatments (Appendix A4Appendix A5). Hence, the N applied may have leached beyond 60 cm in soil profile below feeder roots before trees could uptake and accumulate in in their leaves (Bojappa & Singh 1975; Rao & Mukherjee 1985). Also, fertiliser treatment versus control may have not been evident due to the application of calcium nitrate, potassium nitrate, and gypsum as part of standard commercial practice on the trial site (Appendix A6).

Fruit N concentrations were consistently low in the low-N, high-Ca combination treatment across all seasons, however, in season 2022, very high-N, high-Ca treatments significantly ($p \leq 0.05$) increased N concentrations in fruit flesh compared to control (Table 5.5). There was no correlation of %Ca-base saturation in the soil at pre-flowering stages with fruit Ca concentrations at harvest (Appendix A7), indicating Ca availability in the root alone does not guarantee its uptake and accumulation in fruit. Overall, low-N, high Ca combination treatment was very effective with consistently lower mineral nutrient ratios N/Ca, K/Ca, and (K+Mg)/Ca in fruit flesh across all seasons (Table 5.6).

The FCWP incidence was not significantly ($p > 0.05$) affected by fertiliser application except for the low-N, high-Ca combination treatment (Table 5.10). Lower incidence and severity of FCWP could be associated with higher Ca availability in soil at pre-flowering stage in this treatment and subsequently lower mineral nutrient ratios in fruit at harvest. In general, Ca concentration increases rapidly in mango during early fruit development and gradually decreases during cell expansion and maturation phases (Joyce et al. 2001). This means higher availability of Ca at flowering and early developmental stages could likely improve overall fruit Ca status at harvest and its innate ability to withstand post-harvest rigours, but this is not always the case, as shown in Appendix A7. The variability of mineral nutrient association with FCWP % incidence across seasons was attributed to the orchard conditions during the trial period. A biennial pattern of crop load was noted across the trial site with season 2020 bearing low/medium crop, followed by a heavy cropping season in 2021 and again a low crop load in 2022 (Table 5.8).

Crop load appeared to be the major driver of fruit flesh mineral concentrations, with significant positive ($p < 0.001$) correlations with flesh [Ca], [Mg], and [B] and negative correlations with [K], K/Ca, and (K+Mg)/Ca (Table 5.12). A similar trend was observed in apples where fruit from low crop load trees had lower flesh [Ca], (K+Mg+N)/Ca, and higher [K] (Serra et al. 2016). On the contrary, crop load did not affect mineral concentrations in 'B10' and 'Num Dok Mai' mango fruit on a dry weight basis (Zabedah et al. 2008; Isarangkoo et al. 2019). High tree vigour has considerable negative impact on fruit quality and susceptibility to IDs (Witney et al. 1990). Katrodia and Rane (1981) found 50% spongy tissue disorder in highly vigorous trees compared to 9% in less vigorous trees. The vigorous trees had a higher leaf-to-fruit ratio in the canopy compared to low vigour trees. As crop load increases, the number of leaves supporting nearby developing fruit decreases and reduces the overall leaf-to-fruit ratio. This redirects the xylem flow carrying Ca and other essential mineral elements towards fruit and improves fruit nutrient concentrations which results in improved post-harvest quality (Simmons et al. 1998b).

Data segregation based on seasons using PCA further highlighted the seasonal variability in terms of mineral concentrations, crop load, yield, and susceptibility to IDs (Figure 5.3). A clear on- and off-cropping seasonality was evident where higher crop load and yield (season 2021) was followed by higher flesh [Ca], [B] and [Mg], shelf life and lower incidence of FCWP disorder. Similarly, lower cropping years (season 2020 and 2022) promoted higher flesh [K], K/Ca, and (K+Mg)/Ca with higher incidence of FCWP (Figure 5.3).

These results suggest that fruit mineral concentrations were not directly influenced by the application and availability of fertilisers and were not the sole indicator of fruit susceptibility to IDs. Mineral uptake and spatial distribution in the mango tree is greatly influenced by orchard conditions such as pruning and crop load management (Bally 2006; Zabedah et al. 2008; Robinson & Lopez 2010; Serra et al. 2016; Isarangkoo et al. 2019). Proper crop load management helps maintain cropping consistency across seasons and subsequently drives and improve mineral nutrient concentrations, robustness, and post-harvest quality. Hence, management decisions on fruit consignment for a particular market, e.g., domestic (no VHT required) or export (VHT required), should be derived and informed by monitoring orchard conditions and crop load across growing seasons in conjunction with at-harvest fruit mineral concentrations.

5.6 Conclusion

In the present study, N and Ca fertilisers as urea, calcium nitrate, and microfine gypsum were applied during the early stages of mango fruit development, to determine potential effects on fruit quality for export lines. Application of low-N, high-Ca treatment (N: 50 g/tree + Ca: 690 g/tree) was more

effective in increasing soil and fruit [Ca] compared to control and rest of fertiliser treatments. The low-N, high-Ca fertiliser combination treatment was also the most effective in lowering FCWP incidence. Seasonal variability in crop load at the trial site was the major driver of fruit flesh mineral concentrations and susceptibility to FCWP disorder upon post-harvest phytosanitary treatment (VHT). High crop load improved fruit flesh [Ca], [Mg], and [B] and reduced mineral ratios K/Ca and (K+Mg)/Ca, which ultimately reduced FCWP incidence. Future studies should look closely on crop load effect at individual tree level on fruit mineral concentrations and subsequent fruit quality, including FCWP incidence after VHT. Careful monitoring of crop load such that harvesting and marketing fruit for export on 'on-year' cropping season, in conjunction with mineral nutrient status at harvest is recommended to produce optimum quality 'B74' mango and to inform decisions for export.

Chapter 6 Effect of crop load and fruit position in the canopy on ‘B74’ mango quality and mineral nutrients

6.1 Abstract

Consistent mango fruit quality is pivotal to consumer confidence, repeat purchase, and profitability.

A trial was conducted across three consecutive fruiting seasons on a ‘B74’ mango farm to determine crop load effect on consistency for fruit quality and mineral concentrations. Trees were assigned into four crop load categories based on fruit number per tree: very low (<50 fruit); low (50-100 fruit); medium (100-150 fruit); and, high (>150 fruit). Ten fruit randomly sampled per tree were subject to VHT treatment, ripened, and assessed for post-harvest qualities and mineral nutrient concentrations. In season 1, 91% of trees had very low (22% trees), low (47% trees), and medium (22% trees) crop loads, only 9% had high crop load. In season 2, 70% of trees had high crop load and 11%, 9%, and 9% had medium, low, and very low crop loads, respectively. In the third season, 85% of trees had very low crop load compared with 11%, 4%, and 0% with low, medium, and high crop loads, respectively. In pooled data across the three seasons, FCWP incidence at 9% was lowest in high crop load trees compared with medium at 33%, low at 48%, and very low at 22% crop loads. The mineral nutrient ratio (K+Mg)/Ca was significantly ($p \leq 0.01$) lower in fruit from high (21.8) and medium (23.4) crop loads as compared to low (27.7) and very low (31.6) crop load categories.

A single-season trial was conducted in 2022 to ascertain effects of fruit position in the canopy on fruit quality and mineral nutrient concentrations. Five high crop load (>150 fruit) trees were selected from three blocks. Fruit were sampled from five positions: Outer canopy top, inner canopy bottom (breast height), shaded inner canopy (middle), sun-exposed outer canopy (East), and sun-exposed outer canopy (West). Shaded fruit from bottom and middle canopy positions had higher ($p \leq 0.001$) flesh [N], [Mg], and [K] than sun-exposed fruit. However, mineral ratios were not influenced ($p > 0.05$) by fruit position. No incidence of FCWP disorder in response to VHT was noted in this trial which further highlights fruit robustness from high crop load trees to disorder expression with lower mineral ratios.

Thus, to get better quality ‘B74’ mango fruit, selective harvest from trees with higher crop loads is highly recommended.

Keywords: Export, flesh nutrient concentrations, internal quality, vapour heat treatment, VHT.

6.2 Introduction

Delivering consistent fruit quality is a challenge that any fruit industry faces, including when targeting export markets (Oosthuyse 1993; Sivakumar et al. 2011; Rivera et al. 2017; Escobar et al. 2021). Fruit quality is the result of a variety of pre-harvest factors, including nutrient application, irrigation, and soil and canopy management (Léchaudel & Joas 2007). Such factors play a role in producing robust fruit that cope with post-harvest rigours and maintain quality towards consumer satisfaction. Canopy characteristics such as crop load, vegetative to reproductive ratio, light penetration, sun exposure, and fruit position may affect fruit physiology, mineral concentrations, and quality attributes at and after harvest (Bally 2006; Serra et al. 2016; Isarangkoo et al. 2019; Kalcsits et al. 2019).

Mineral elemental nutrient distribution in trees and concentrations in fruit are dependent on canopy characteristics (Simmons et al. 1998b). For instance, once absorbed by the roots, the fate of Ca is influenced by vegetative to reproductive ratios in the tree canopy. These govern nutrient, water, and photo-assimilate partitioning that impinges on fruit quality (Witney et al. 1990; Hofman 1995; Hofman et al. 1997a).

Ca moves predominantly through the xylem via transpirational pull and mass flow of water (Montanaro et al. 2014). Hence, leaves typically accumulate more than do fruit by virtue of their higher transpiration rates (Shivashankara & Mathai 1999; Singh et al. 2006b). Nevertheless, fruit act as a strong sink during their early development stages and may accumulate most of their Ca then (Joyce et al. 2001; Montanaro et al. 2015). Accordingly, managing partitioning through pruning can potentially direct Ca to fruit and ultimately improve post-harvest quality (Simmons et al. 1998b; Bally 2006).

Ca deficiency in fruit flesh can render them more susceptible to physiological and pathological disorders (Ferguson & Triggs 1990; Hofman et al. 2002; Brunetto et al. 2015; G. Pandey et al. 2017; Escobar et al. 2021; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023). This may be particularly relevant for ‘long’ export supply chains wherein stressful phytosanitary treatments (e.g., VHT) may be requisite (Jacobi & Giles 1997; Escobar et al. 2021).

In this context, the study sought to determine influence of crop load per tree and fruit position in canopy for ‘B74’ mango fruit quality, including susceptibility to VHT-induced disorders (e.g., flesh cavity with white patches).

6.3 Materials and Methods

A commercial ‘B74’ mango orchard at Childers, South-east Queensland (25.136^{o}S, 152.372^{o}E) was used in this study. Trial site characteristics are outlined in section 5.3.1.

6.3.1 Experiment 1: Effect of crop load on fruit quality at individual tree level

Fruit quality and mineral nutrient concentration variations within one orchard block were determined at individual tree level. Forty-five trees were randomly selected and tagged for serial sampling in season 2020, 2021 and 2022. An additional 9 trees were included in the latter two seasons. Fruit numbers on each tree were counted 3 days prior to commercial harvest. The trees were assigned into 4 crop load categories: very-low (<50 fruit/tree), low (50-100 fruit/tree), medium (100-150 fruit/trees), and high (>150 fruit/tree). Tree trunk cross sectional area (TCSA) was also measured at harvest and fruit density (fruit number/cm^2 TCSA) was calculated in each crop load category. Ten fruit per tree were randomly collected at commercial harvest. Five of these fruits were assessed for post-harvest quality. The remained were aggregated for a single composite sample for flesh mineral analysis.

6.3.2 Experiment 2: Fruit quality and mineral variations based on position in the canopy

Five trees were selected considering uniformity in vigour and crop load (>150 fruit per tree) from three different orchard blocks (B2, B3, and B4) at Childers, South-east Queensland (25.136^{o}S, 152.372^{o}E). The trees were ~18 years old at a planting density of 8 m interrow x 4 m in row. Canopy uniformity was assessed by measuring canopy area. Radius measurements from tree trunk to canopy edge from four sides were taken at harvest and area was calculated using average radius values. There was no significant difference ($p > 0.05$) in tree canopy area across sampling blocks. Each tree was sampled for five canopy positions; viz., top, bottom (breast height), shaded/inside, and sun-exposed/outside East and West (Figure 6.1). At commercial maturity (≥1578 heat units and 14-16% DM, section 5.4.3), a fruit from each position/tree that was free from damage and defects was harvested. Overall sampling was: 1 × 5 positions/tree = 5 fruit; 5 × 5 trees = 25 fruit/block; 25 x 3 blocks = 75 fruit in total. Fruit was individually assessed for post-harvest quality and mineral nutrient concentrations.

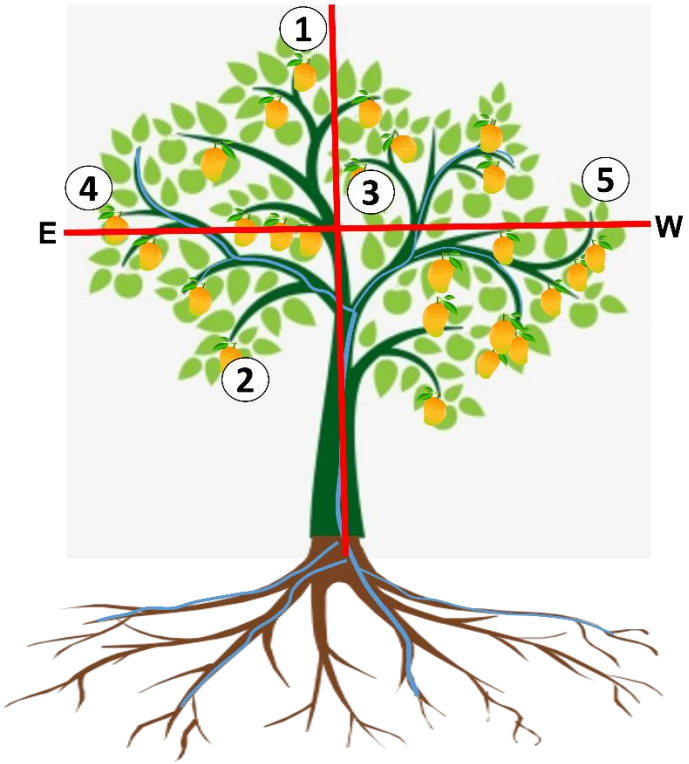


Figure 6.1: 'B74' mango fruit sampling towards 'mapping' mineral concentrations and quality variation within the canopy. Number represents a sampling point: 1. canopy top, 2. canopy bottom (breast height), 3. Shaded and inside the canopy, 4. sun exposed and outside the canopy-East, and 5. sun exposed and outside the canopy-West).

6.3.3 Fruit quality assessments and mineral analysis

Fruit were harvested at commercial maturity in the morning (8:00 - 10:00 am), packed onto foam fruit trays, and transported in an air-conditioned vehicle (ca. 22 °C). Details for VHT treatment and post-harvest assessments are in section 5.3.5. Fruit dry matter was measured using a handheld F-750 produce quality meter. Shelf life was from the day of VHT to eating ripe stage (section 5.3.5). Individual fruit flesh was assessed for total soluble solids (TSS) using a digital pocket refractometer (Atago^{TM} Refractometer PAL-1, Japan). Fruit mesocarp sections were analysed for mineral concentrations as described in section 4.3.4.

6.3.4 Statistical analyses

Data from the crop load experiment were analysed by one-way analysis of variance (ANOVA) and means compared at a 5% confidence level by Tukey's HSD test (Ferreira et al. 2014). Bar graphs (Microsoft Excel 365) were produced to represent frequency (%) of trees in each crop load category for the three seasons.

Data from the canopy position experiment were analysed by two-way analysis of variance (ANOVA) (Ferreira et al. 2014). Canopy position (viz., top, middle, bottom, east and west) was considered a treatment unit with trees as replicates within each orchard block treatment unit. Canopy position and orchard block were analysed as factors and means compared at 5% confidence level by Tukey's HSD test. Also, data were reassigned based on sun exposure; viz., exposed (top + east + west) and shaded (middle + bottom fruit) and such that sun exposure became a treatment unit with trees as replicates within each block. Sun exposure and orchard block were analysed as factors. Means were compared at 5% confidence level by Tukey's HSD test. Level of significance are indicated as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s.: not significant. All statistical analyses were performed in R (4.0.2).

Fruit mineral analysis data from the canopy position experiment were used for sample size calculation for N/Ca, and (K+Mg)/Ca ratios. The whole data set from this experiment was used regardless of canopy position or orchard block as there were no significant ($p > 0.05$) differences by ANOVA (Table 6.4). Cochran's formula was used to estimate minimum sample size based on standard deviation of the population; viz., $n = (z/d)^{\wedge 2} \times SD^{\wedge 2}$ (Cochran 1977), where n is the minimum sample size required, z is the critical value for desired level of confidence (e.g., 1.96 for 95% confidence), d is the desired level of precision (i.e., the maximum allowable difference between the sample mean and the true population mean), and SD is the standard deviation of the population.

6.4 Results

6.4.1 Effect of crop load on fruit quality at the individual tree level

Season 2020 was an 'off-year' or low cropping season, with 47% of sampled trees in low crop load, 22% in both very-low plus medium crop load categories, and only 9% in high crop load. The 2021 season was an 'on-year' high cropping season where 70% of trees had high crop load, 11% medium, and 9% low crop load (Figure 6.2). The 'off-year' pattern was repeated in season 2022, with 85% trees in very-low, 11% in low, and 4% in medium crop load categories. No trees produced >150 fruit in season 2022 (Figure 6.2).

There was no significant ($p > 0.05$) effect of high and very-low crop load on 'B74' mango fruit shelf life. However, TSS and fruit weight were significantly ($p \leq 0.05$) higher in very-low and high crop load categories, respectively (Table 6.1). Incidence of FCWP disorder was significantly ($p \leq 0.05$) higher at 48% and significantly ($p \leq 0.05$) lower at 9% in low and high crop load category fruit, respectively. No significant ($p > 0.05$) effect of crop load was noted on % incidence of FCWP in very low and medium crop load categories (Table 6.1).

Flesh mineral analysis revealed that [Ca] and [Mg] increased significantly ($p \leq 0.01$) with the increase

in crop load. [Ca] rose from 406 mg/kg to 523 mg/kg and Mg from 388 mg/kg to 494 mg/kg between very low and high crop load fruit, respectively (Table 6.2). Crop load did not have any significant ($p > 0.05$) effect on fruit flesh [B], except in high crop load fruit. An inverse trend in fruit flesh [K] was observed, with higher concentrations in very-low crop load fruit. N concentrations were not statistically different ($p > 0.05$) between very low and high crop load fruit, however, there was a trend of increase in [N] with the increase in crop load (Table 6.2). Mineral nutrient ratios of K/Ca and (K+Mg)/Ca were significantly ($p \leq 0.01$) higher in very-low crop load fruit. They tended to decrease as crop load increased (Table 6.2).

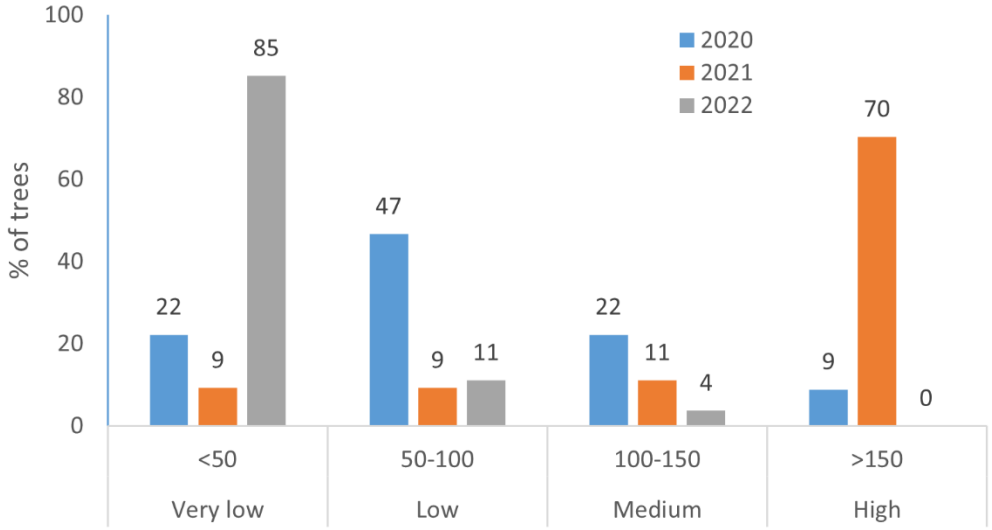


Figure 6.2: The proportion (%) of 'B74' mango trees (n = 153) with very low, low, medium, and high crop load across the 2020, 2021 and 2022 cropping seasons.

Table 6.1. Statistical analyses of 'B74' mango fruit quality attributes from trees of four different crop load categories. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to the Tukey's HSD test.

Crop load	Fruit density (number/cm^2 TCSA)	Fruit weight (g)	Shelf life (d)	TSS (%)	Incidence of FCWP (%)
Very-low	0.045 ^{d}	490.9 ^{b}	17.2 ^{a}	14.1 ^{a}	22 ^{b}
Low	0.164 ^{c}	477.6 ^{b}	16.4 ^{ab}	13.3 ^{b}	48 ^{a}
Medium	0.288 ^{b}	482.1 ^{b}	15.8 ^{b}	13.3 ^{b}	33 ^{b}
High	0.508 ^{a}	522.9 ^{a}	17.1 ^{a}	13.2 ^{b}	9 ^{c}
Significance	**	*	*	**	*

Table 6.2. Statistical analyses of 'B74' mango fruit flesh mineral analysis from trees of four different crop load categories. Means followed by the same letter in each column are not significantly different

at $p \leq 0.05$ according to the Tukey's HSD test.

Crop load	N (% dw)	K (% dw)	Ca (mg/kg dw)	Mg (mg/kg dw)	B (mg/kg dw)	N/Ca	K/Ca	(K+Mg)/ Ca
Very-low	0.45 [ab]	1.20 [a]	406.49 [c]	388.97 [c]	8.03 [b]	11.64 [a]	30.57 [a]	31.56 [a]
Low	0.40 [b]	1.18 [ab]	460.69 [bc]	435.46 [b]	8.80 [b]	9.22 [ab]	26.69 [b]	27.67 [b]
Medium	0.42 [ab]	1.11 [bc]	508.94 [ab]	448.19 [b]	8.93 [b]	8.45 [b]	22.46 [c]	23.36 [c]
High	0.51 [a]	1.06 [c]	523.03 [a]	494.20 [a]	9.08 [a]	9.71 [ab]	20.82 [c]	21.79 [c]
Significance	*	*	**	*	*	*	**	**

6.4.2 Fruit-fruit variability based on position in the canopy

6.4.2.1 Fruit quality variations

'B74' mango fruit weight and DM at harvest and post-harvest shelf life, TSS, and weight loss were assessed across three different orchard blocks in relation to fruit position within the canopy. IDs, such as FCWP, was not observed in any sampled fruit from any block. Quality parameters for canopy position (top, middle, bottom, east, and west position) and sun exposure (exposed vs. shaded) across all sampled blocks are presented in Figures 6.3 and 6.4. For canopy position, fruit weight, shelf life, and TSS did not differ significantly ($p > 0.05$). DM at harvest and % weight loss at eating ripe were significantly ($p \leq 0.01$) higher in top and east-sided fruit compared to middle and bottom-positioned fruit (Table 6.3). However, when data was arranged for exposure, sun-exposed fruit had significantly ($p \leq 0.05$) higher weight, DM, and TSS compared to shaded fruit from middle and bottom canopy (Table 6.3). Irrespective of canopy position, orchard block effect was significant ($p \leq 0.05$) for all the quality parameters, except fruit weight at harvest. B3 block showed higher DM, weight loss, and TSS values, and lower shelf life than other two blocks (Table 6.3). Interactions of canopy position vs. orchard block and of sun exposure vs. orchard block were not significant ($p > 0.05$) for any quality parameter.

Table 6.3. Statistical analyses of 'B74' mango fruit quality attributes for the position in the canopy and orchard blocks. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to the Tukey's HSD test.

	Fruit weight (g)	DM (%)	Fruit weight loss (%)	Shelf life (d)	TSS (%)
Canopy position					
Top	478.31	15.72 [a]	10.60 [b]	19.47	14.57
Middle	463.59	14.71 [bc]	11.48 [ab]	18.93	13.93
Bottom	434.89	14.61 [c]	12.30 [a]	19.93	13.35
East	488.43	15.53 [a]	10.24 [b]	19.27	14.10
West	468.33	15.30 [ab]	10.28 [b]	19.20	14.49
Sun exposure					
Exposed	478.35 [a]	15.52 [a]	10.38 [b]	19.31	14.39 [a]
Shaded	449.24 [b]	14.66 [b]	11.89 [a]	19.43	13.64 [b]
Orchard block					
B2	457.94	14.89 [b]	11.12 [b]	21.04 [a]	13.50 [b]

B3	458.82	15.56 [a]	11.65 [a]	17.80 [b]	14.62 [a]
B4	483.36	15.07 [b]	10.17 [c]	19.24 [ab]	14.23 [ab]
Significance					
Canopy position	n.s.	***	**	n.s.	n.s.
Sun exposure	*	***	***	n.s.	*
Orchard block	n.s.	**	**	**	*
Canopy position × Orchard block	n.s.	n.s.	n.s.	n.s.	n.s.
Sun exposure × Orchard block	n.s.	n.s.	n.s.	n.s.	n.s.

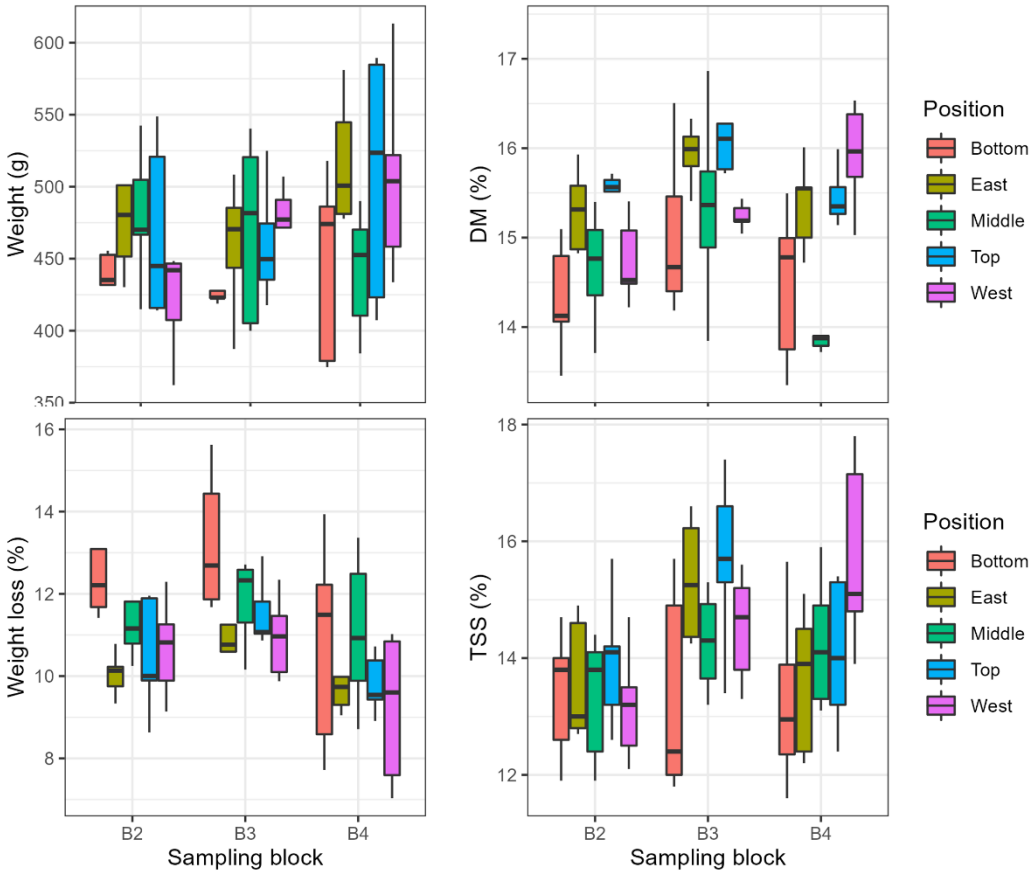


Figure 6.3: Box and whiskers plots of 'B74' mango fruit quality parameters at harvest (fruit weight and DM) and at eating ripe stage (fruit weight loss and total soluble solids (TSS)) of 'B74' mango fruit at different positions within the canopy across different orchard blocks. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

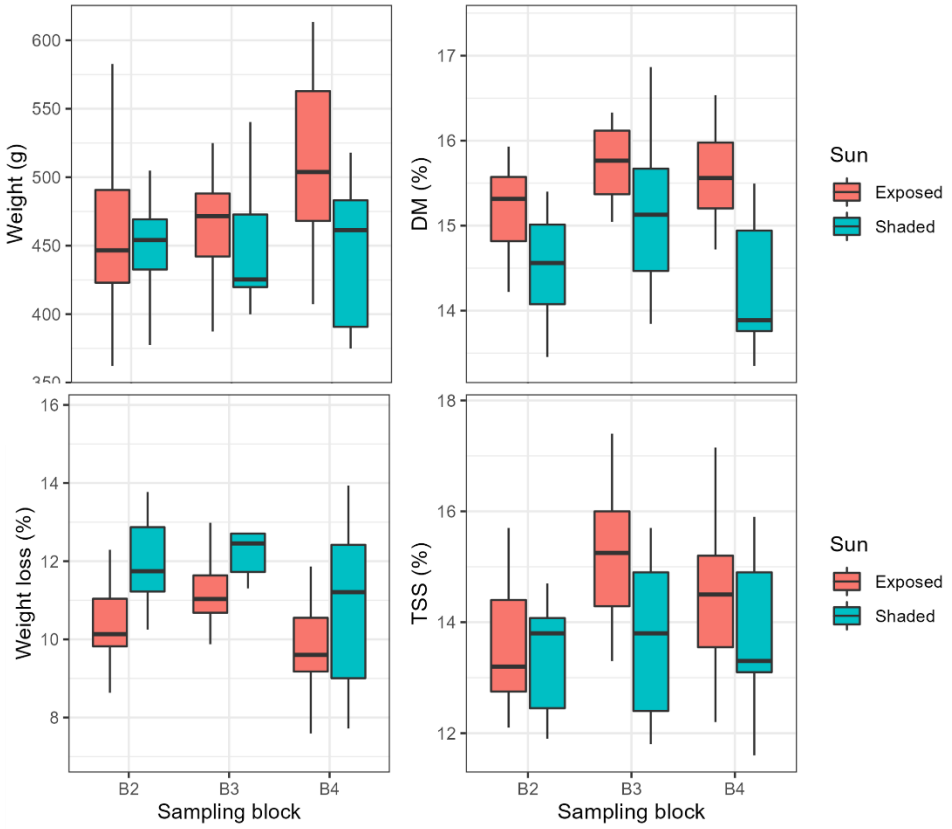


Figure 6.4: Box and whiskers plots of fruit quality parameters at harvest (fruit weight and DM) and at eating ripe stage (fruit weight loss and total soluble solids (TSS)) 'B74' mango fruit for sun exposure (exposed or shaded) across different orchard blocks. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

6.4.2.2 Variability in mineral nutrients within the canopy

Mineral nutrient variability in 'B74' mango flesh was evident for fruit from different parts of the canopy and across orchard blocks (Figures 6.5 and 6.6). Fruit from middle and bottom canopy had significantly ($p \leq 0.05$) higher fruit flesh [N] and [K] compared to the top of the canopy (Table 6.4). Only fruit from the canopy bottom had significantly ($p \leq 0.05$) higher N, Ca, K, and Mg and lower B concentrations compared to those at the canopy top. Similarly, shaded fruit showed a similar trend, with significantly ($p \leq 0.001$) higher [N], [K], and [Mg] (except [Ca]), and lower [B] than sun-exposed fruit (Table 6.4). The orchard block factor was significant ($p \leq 0.05$) for mineral concentrations, with B2 having lower N and higher Ca and K concentrations. B4 had higher N and B concentrations (Table 6.4). Mg concentrations were not significantly ($p > 0.05$) different in any block (Table 6.4). Mineral nutrient ratios (N/Ca, K/Ca, and ((K+Mg))/Ca) were not influenced by fruit position in the canopy nor sun exposure in any of the three orchard blocks. However, there was a suggestion of lower mineral

ratios in sun-exposed fruit (Table 6.4).

Table 6.4. Statistical analyses of 'B74' mango fruit flesh mineral concentrations from different positions in the canopy and orchard blocks. Means followed by the same letter in each column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test. Here, * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s.: not significant.

	Flesh mineral analysis (D.W.)							
	N (%)	**K (%)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**B (mg/kg)**	**N/Ca**	**K/Ca**	**K+Mg/Ca**
Canopy position								
Top	0.47 ^{b}	1.09 ^{b}	496.07 ^{b}	403.73 ^{c}	10.27 ^{a}	10.21	23.75	24.63
Middle	0.56 ^{a}	1.31 ^{a}	533.64 ^{ab}	490.57 ^{ab}	8.86 ^{b}	10.95	25.60	26.56
Bottom	0.55 ^{a}	1.30 ^{a}	649.64 ^{a}	527.50 ^{a}	8.88 ^{b}	9.19	21.29	22.15
East	0.50 ^{ab}	1.15 ^{b}	613.20 ^{ab}	444.87 ^{bc}	10.20 ^{a}	8.57	19.60	20.35
West	0.50 ^{ab}	1.17 ^{ab}	581.27 ^{ab}	432.60 ^{c}	10.10 ^{a}	9.24	21.28	22.07
Sun exposure								
Exposed	0.49 ^{b}	1.14 ^{b}	563.51	427.07 ^{b}	10.19 ^{a}	9.34	21.54	22.35
Shaded	0.55 ^{a}	1.30 ^{a}	591.64	509.04 ^{a}	8.87 ^{b}	10.07	23.45	24.36
Orchard block								
B2	0.49 ^{b}	1.26 ^{a}	627.08 ^{a}	471.32	10.01 ^{a}	8.46	21.42	22.22
B3	0.52 ^{ab}	1.15 ^{b}	521.92 ^{b}	437.67	8.85 ^{b}	10.55	23.64	24.53
B4	0.54 ^{a}	1.20 ^{ab}	571.71 ^{ab}	466.00	10.18 ^{a}	9.90	21.80	22.65
Significance								
Canopy position	**	***	*	***	**	n.s.	n.s.	n.s.
Sun exposure	***	***	n.s.	***	***	n.s.	n.s.	n.s.
Orchard block	*	*	*	n.s.	**	n.s.	n.s.	n.s.
Canopy position × Orchard block	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sun exposure × Orchard block	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

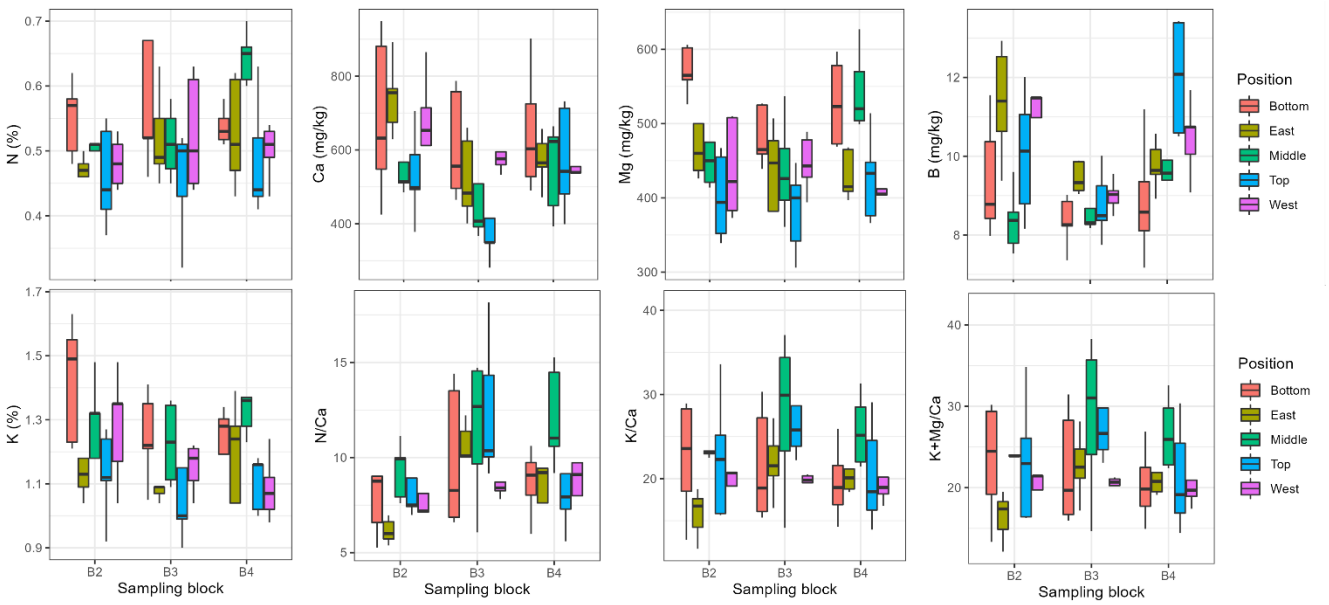


Figure 6.5: Box and whiskers plots of 'B74' mango fruit flesh mineral concentrations at different positions within the canopy across different orchard blocks. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

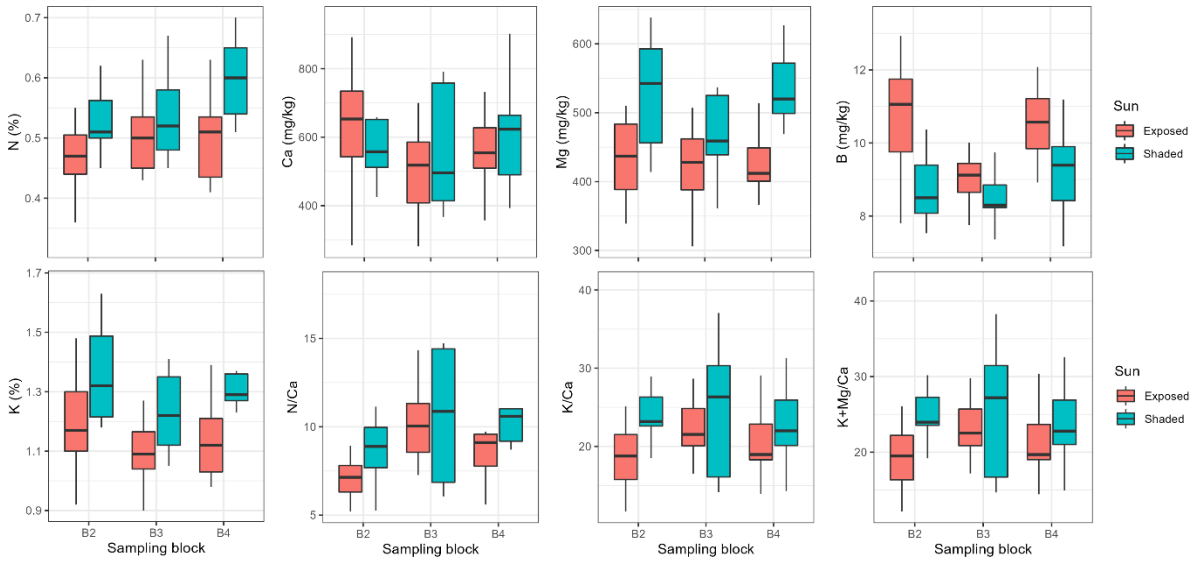


Figure 6.6: Box and whiskers plots of 'B74' mango fruit flesh mineral concentrations for sun exposure (exposed or shaded) across different orchard blocks. The upper and lower whiskers represent 25% and 25% data. The coloured boxes represent the intermediate 50% data and the transvers lines within the boxes is the median separating the value in two equal parts.

6.4.2.3 Sample size estimation for mineral nutrient concentrations

Mineral nutrients are critical in determining fruit robustness at harvest (Joyce 2021). Nevertheless, it is not clear how many fruits should be sampled for representative values of the measures or ratios. Sample size calculation for 'B74' flesh mineral nutrient ratios is presented herein. A like for sample size estimation approach can be used for other quality parameters. By example, there was no significant ($p > 0.05$) effect of fruit position in the canopy and interaction of orchard block vs. canopy position on mineral nutrient ratios (Table 6.4). Hence, standard deviations of N/Ca and (K+Mg)/Ca across the whole data set, irrespective of block or fruit position, were calculated and used for sample size calculation (Table 6.5). Representations of sample size influences for N/Ca and (K+Mg)/Ca are presented in Figure 6.7. The x-axis represents the difference in the mean that can be detected for a given standard deviation and y-axis represents number of samples required to detect mean difference at 3 significance levels, like $p = 0.01$, $p = 0.05$, and $p = 0.1$. For N/Ca, to detect a difference of two between two means, seven samples are required at $p = 0.1$, 10 samples at $p = 0.05$, and 16 samples at $p = 0.01$ (Figure 6.7). Associated with higher standard deviation in (K+Mg)/Ca, sample size requirements are 27 fruit at $p = 0.1$, 41 at $p = 0.05$, and 67 at $p = 0.01$ in order to detect the difference of two between the two means of (K+Mg)/Ca. The sample number increases with an increase in the significance level for the detection of a specific mean difference from a population.

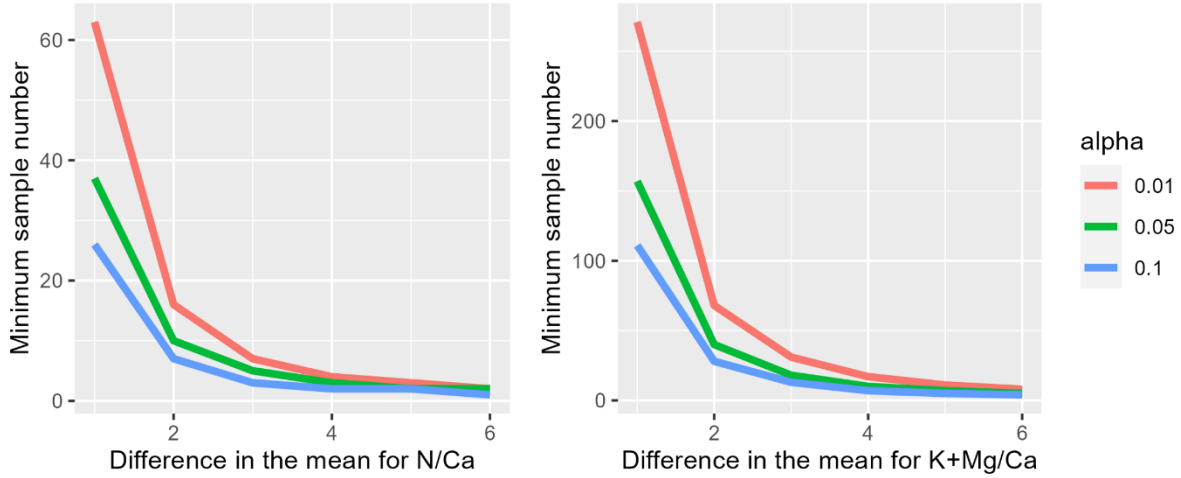


Figure 6.7: Example sample size estimation by Cochran's method using standard deviations from the collected dataset for N/Ca and (K+Mg)/Ca values at three significance levels; $p = 0.01$, $p = 0.05$ and $p = 0.1$.

Table 6.5. Fruit mineral sample parameters for 'B74' mango sourced from 3 different blocks at different positions in the canopy. There was no significant interaction of fruit position x sampling

blocks (Table 6.4).

	Data pooled by blocks and fruit position in the canopy							
Parameters	**B (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**K (%)**	**N (%)**	**N/Ca**	**K/Ca**	**(K+Mg)/Ca**
Mean	9.68	574.30	458.51	1.20	0.51	9.62	22.27	23.12
Standard deviation	1.49	149.92	71.76	0.16	0.08	3.08	6.17	6.39
Variance	2.22	22475.77	5149.81	0.03	0.01	9.50	38.11	40.87
Maximum	13.43	949.00	638.00	1.67	0.70	18.15	40.93	42.41
Minimum	7.17	281.00	306.00	0.90	0.32	5.20	11.66	12.14

6.5 Discussion

For any fruit industry, producing consistent quality is paramount to maintain consumer satisfaction, repeat purchases and potentially expand into new markets. Considering orchard management practices and environmental conditions, individual trees seldom produce fruit with consistent quality characteristics (Ferguson & Triggs 1990). In the present study, tree-to-tree variability within a single 'B74' mango orchard block was examined over three consecutive seasons to ascertain how quality characteristics and fruit mineral associates with crop canopy load. The influence on variability of fruit position within the canopy in terms of fruit quality and mineral accumulation was also assessed. Additionally, implication of sampling position in the canopy were explored.

The 'B74' trees were assigned into four crop load categories for three cropping seasons. An off-on-off pattern of biennial bearing crop load was observed (Figure 6.2). Biennial cropping in 'B74' mango trees is not evident during early cropping years. Whiley et al. (2006) determined consistency in tree yield and fruit quality for up to 6 years old 'B74' trees. Biennial bearing may become more evident with orchard age. Tree-to-tree crop load variability was observed in 12-year (Anderson et al. 2019) and 17-year-old 'B74' trees (Figure 6.2).

Biennial bearing can be put down to management (pruning) or environmental (temperature) influences on photo-assimilate production and storage to support the fruit crop in the following year (Monselise & Goren 1987). Early understanding of orchard variability can be gleaned through early flowering assessment (flowering patterns and % flowering panicle on the tree) (Wang et al. 2017) and/or fruit counting (after stone hardening stage past final fruit drop) (Payne et al. 2014). This can be through manual counting, which is labour-intensive, or by using machine learning, through image acquisition (Qureshi et al. 2017; Wang et al. 2018; Wang et al. 2019b; Koirala et al. 2020). Such techniques have been used in orchard management toward optimising yield, maturity, and harvest timing. They can also help maintain fruit harvest and post-harvest quality consistency and robustness, including in respect to managing the incidence of disorders.

The present results indicated higher FCWP incidence in fruit from low crop load trees (~50%)

compared to fruit from high crop load trees (9%) (Table 6.1). Similarly, fruit from high crop load trees had higher [Ca], [Mg], and [B] and lower [K], K/Ca, and (K+Mg)/Ca in fruit flesh (Table 6.2). A crop load management trial on 'Keitt' mango revealed significant ($p \leq 0.05$) increase in the incidence of watery pulp disorder when 70% of fruiting terminals were removed during early development stages to maintain a low crop load treatment (30%) (Bally 2006). The crop load effect for 70 – 100 fruit per tree on nutrient accumulation was not significant ($p > 0.05$) for 'Num Dok Mai Sithong' mango (Isarangkoo et al. 2019). However, higher crop load improved Ca and Mg, and decreased K and N concentrations in 'Honeycrisp' apple (Serra et al. 2016). Crop load effect in terms of leaf to fruit ratio at branch level indicated similar pattern, with higher L:F ratio (low crop load) significantly reduced [Ca] and increased [K] in 'Kensington pride' mango fruit flesh (Simmons et al. 1996, 1998a; Simmons et al. 1998b). Canopy management deals with maintaining crop load by optimising the leaf-to-fruit ratio (L: F). High L: F ratio is associated with reduced firmness, higher DM, sugar content, and increased size, but can make fruit relatively more susceptible to IDs (Simmons et al. 1996; Simmons et al. 1998b; Singh et al. 2006b). Decisions around consigning fruit for export and/or domestic markets can be better informed by monitoring and managing orchard variability across growing seasons. For 'B74' mango export, sourcing fruit from high crop load trees (>150 fruit per tree) can potentially reduce disorder incidence through lower mineral ratios (Tables 6.1 and 6.2).

Although, 'B74' fruit quality characteristics (viz., weight, shelf life, and TSS) were not significantly ($p > 0.05$) influenced by fruit position within the canopy, sun-exposed fruit had significantly ($p \leq 0.05$) higher weight, DM, and TSS compared to shaded fruit (Table 6.3). Whiley et al. (2006) also reported higher DM in sun-exposed fruit, but there was no significant ($p > 0.05$) effect on fruit weight at harvest for 'B74' mango. The upper canopy of 'B74' mango fruit showed significantly ($p \leq 0.05$) higher DM compared to the inner and lower canopy fruit (Table 6.3). This is consistent with Ketsa et al. (1991) and Kawphaitoon et al. (2014) finding for 'Nam Dok Mai' mango fruit. Similarly, higher DM and sugar contents were observed in sun-exposed fruit compared to shaded within canopy 'Cogshall' mango fruit (Joas et al. 2010).

There was no incidence of FCWP in fruit from any canopy position after VHT, possibly because these fruit were sourced from high crop load (>150) trees and had lower (K+Mg)/Ca mineral nutrient ratios that may otherwise have improved fruit robustness (Table 6.4). Fruit flesh [N], [K], and [Mg] were significantly ($p \leq 0.05$) higher in shaded middle and bottom fruit compared to fruit from the top of the canopy (Table 6.4). Effects of canopy position have been reported for other fruit crops, like 'Honeycrisp' apple (Kalcsits et al. 2019). Bitter pit incidence and flesh Ca concentrations were significantly ($p \leq 0.05$) influenced by fruit position in the canopy, with upper canopy fruit

accumulating higher [Ca] and having lower incidence of bitter pit compared to lower canopy fruit (Kalcsits et al. 2019). 'B74' mineral nutrient ratios were not significantly ($p > 0.05$) influenced in fruit from any canopy position. However, and on average, sun-exposed fruit had lower concentrations than shaded fruit (Table 6.4). This further highlighted the stability of mineral ratios and their potential use as indicators for fruit robustness instead of using individual mineral concentrations. Overall, there can be considerable differences in fruit physiology due to position in the canopy (Witney et al. 1990; Ketsa et al. 1991; Joas et al. 2010; Kawphaitoon et al. 2014; Coyago-Cruz et al. 2017). Such differences are likely to influence post-harvest quality and susceptibility to IDs, including when growing fruit for protocol (e.g., VHT) markets.

6.6 Conclusion

The effects of crop load at a tree level on 'B74' fruit quality attributes and mineral concentrations were determined for 153 trees over three cropping seasons. Trees with high crop load (>150 fruit) had significantly ($p \leq 0.05$) lower incidence of FCWP disorder (9%) in association with higher [Ca], [Mg], and [B] and lower mineral ratios (K/Ca and (K+Mg)/Ca). These ratios evidently constitute useful indices of fruit robustness. Decisions around whether or not to export 'B74' fruit might consider targeting fruit from high crop load trees (>150) within the orchard with low flesh mineral nutrient ratios (e.g., (K+Mg)/Ca: ≤ 20) at harvest with a view to enhance the likelihood of tolerance to phytosanitary treatment and lessen the risk of FCWP disorder. The data suggest that fruit can be sampled for minerals from any position in the canopy of high crop load trees. Nevertheless, more extensive studies are needed to better characterise varying crop load effects on fruit mineral concentrations and susceptibility to IDs with regard to fruit position in the canopy and quality parameters.

Chapter 7 Growth dynamics and spatial mineral distribution in developing ‘B74’ mango fruit

7.1 Abstract

Mineral nutrients, especially Calcium (Ca) and its balance with other cations such as potassium and magnesium, play a critical role in cell wall strength, fruit robustness, and post-harvest quality. An early indication of nutrient deficiency ahead of harvest can be useful in making informed marketing decisions i.e., for domestic or international markets. In the present study, a serial sampling strategy was implemented from flowering to harvest to analyse mineral nutrient concentrations in ‘B74’ mango fruit. The pathway of xylem inflow carrying mineral nutrients and its functionality was determined over fruit development period using safranin dye tracing method. The spatial distribution of Ca in fruit flesh was mapped through micro-X-ray fluorescence imaging (µXRF). The results indicated that daily fruit growth rate was highest during early development stages as determined by higher daily diameter changes and decreased gradually as fruit matured. Fruit underwent more rapid growth in length compared to width and thickness, with maximum length attained 5-6 weeks before commercial maturity. Fruit flesh mineral analysis indicated a gradual decrease in most mineral nutrients as fruit reached maturity, except for K/Ca and (K+Mg)/Ca ratios. Mineral nutrient ratios were stable and did not change significantly ($p > 0.05$) after stone hardening stage. Dye trace analysis showed higher xylem functionality at early stages of development than at mature-green stage. As fruit matured, xylem supply was completely cut-off from the middle and basal sections of fruit. This loss of functionality impacts nutrient distribution in fruit flesh, and it was evident through µXRF mapping of Ca. The Ca deposition was higher closer to fruit skin and proximal end near the pedicel base, following xylem functionality pathway. In conclusion, the present study provides a detailed understanding of ‘B74’ growth patterns and mineral distribution in fruit flesh during development and highlights the importance of understanding the physiology of fruit development for efficient cultivation practices (e.g., fertiliser application, irrigation) and ultimately to improve fruit robustness.

Keywords: Fruit development, mineral accumulation, X-ray fluorescence imaging, µXRF

7.2 Introduction

Calcium (Ca) plays an irreplaceable function as a structural constituent of the cell wall and in signal transduction in response to internal and/or external stimuli (Hocking et al. 2016). Localised Ca deficiency during fruit development period can cause physiological disorders that eventually lead to quality and yield loss in fresh produce (Huang et al. 2005; Saure 2005; Tonetto de Freitas & Mitcham 2012).

The application of Ca-based fertilisers in the soil does not guarantee its uptake in the fruit, due to several internal and/or external factors involved in its transport, allocation to fruit, and further distribution at subcellular level (Bally 2006; Tonetto de Freitas et al. 2014; Hocking et al. 2016). Fruit [Ca] collectively depend on its availability in soil during particular stages of fruit growth, uptake through roots, competition with other cations in root system, mobility via xylem sap, and competition among leaf and fruit (Tonetto de Freitas & Mitcham 2012).

Fruit, as reproductive organs, are more vulnerable to Ca deficiency than vegetative organs (White & Broadley 2003). Being architecturally isolated organs, fruit are attached to the tree through the pedicel and essential nutrients are transported to developing fruit through the phloem and xylem network in the pedicel. Ca transport to fruit almost exclusively happens through apoplast/xylem pathway (Zocchi & Mignani 1995; Saure 2005; Hocking et al. 2016). The rate of xylem inflow is a key determinant of Ca delivery to fruit and is primarily regulated by fruit growth and transpiration rates (Montanaro et al. 2014; Tonetto de Freitas et al. 2014). In Kiwi fruit, Montanaro et al. (2015) reported higher import of transpiration-coupled Ca in fruit at high vapour pressure deficit (VPD), whereas, at low VPD, fruit growth was a dominant factor in Ca import.

Xylem inflow is determined by xylem functionality and hydraulic conductivity in pedicel and in developing fruit. Studies on kiwi, grape, and apple reported loss of xylem functionality and limited Ca import at later stages of fruit development (Dichio et al. 2003; Choat et al. 2009; Miqueloto et al. 2014b). Miqueloto et al. (2014b) related loss of xylem functionality with Ca deficiency and susceptibility to bitter pit disorder in apples. Song et al. (2018) studied the anatomical features of the xylem in pedicel in relation to Ca uptake rate in apple, litchi, grape, citrus, and pear and concluded that xylem functionality markedly decreased at maturation phases.

There is a lack of information on the growth dynamics of ‘B74’ mango to identify critical stages of active mineral accumulation and the role of dynamic xylem functionality in developing ‘B74’ mango fruit concerning Ca import and allocation. This information would be valuable in identifying mango fruit developmental phases with higher xylem functionality which eventually translates into a higher Ca import rate in fruit. In current study, fruit growth dynamics in relation to mineral nutrient uptake

will be explored to determine and identify critical growth stages in terms of xylem functionality and Ca distribution in developing fruit through dye tracing and X-ray fluorescence imaging, respectively.

7.3 Material and methods

7.3.1 Experimental site

Trial was conducted on a commercial 'B74' mango orchard in seasons 2021 and 2022 at Childers, Queensland (25.136ºS, 152.372ºE). Trial site details are presented in section 5.3.1.

7.3.2 Fruit sampling for growth and nutrient concentrations

Fruit growth was determined by continuous diameter measurements at 5 min intervals using dendrometers (FI-M, Implexx Sense, Melbourne, Australia). Two fruit dendrometers were used once the fruit diameter (thickness) was ~25 mm, large enough for dendrometer clipper to hold onto the fruit. The dendrometers consisted of a linear variable displacement transducer (LVDT) mounted in a special clip and a DC-powered signal conditioner connected to CR-300 data logger (Campbell Scientific, Inc., Logan, UT, USA) (Figure 7.1). Fruit dendrometer and datalogger were powered by 20W in-field installed solar panels. The changes in diameter were monitored throughout fruit growth and development up to harvest. The daily fruit growth was calculated by subtracting the maximum daily diameter from the maximum diameter from the previous day.

To determine the mineral accumulation pattern, a serial harvesting strategy was implemented, starting from inflorescence emergence to green-mature fruit. Nine trees were selected from the orchard block and branches were tagged for uniformity and serial harvesting. Inflorescence samples were collected at 3 stages; 1. Bud burst (BB) – first floral primordial just visible and panicle development begins (BBCH phenological stage: 513 after Rajan et al. (2011)), 2. Inflorescence emergence (IE) – when flowers were visibly separated and secondary axes began to elongate (BBCH phenological stage: 515 after Rajan et al. (2011)), and 3. at full flowering (FF) – when more than 50% of panicle flowers were open (BBCH phenological stage: 615 according to Rajan et al. (2011)) (Figure 7.2). Following inflorescence sampling, nine fruit at different stages of development from fruit set to commercial harvest were collected every 2-3 weeks, carefully packed, and transported to the post-harvest laboratory at the University of Queensland, Gatton campus for fruit length, width and thickness, weight (g), and mineral nutrient analysis. Fruit length, width, and thickness was measured manually using ditial vernier caliper. Fruit flesh samples from the widest part of the cheek on both sides were separated using a fruit corer (Bally 2006). Flesh samples were dried in a fan-forced oven at 65 °C to constant weight and ground to fine powder using a laboratory scale mini ball mill (Retsch Mixer Mill

MM 400). Dried ground flesh samples were analysed for mineral concentrations as outlined in section 4.3.4.

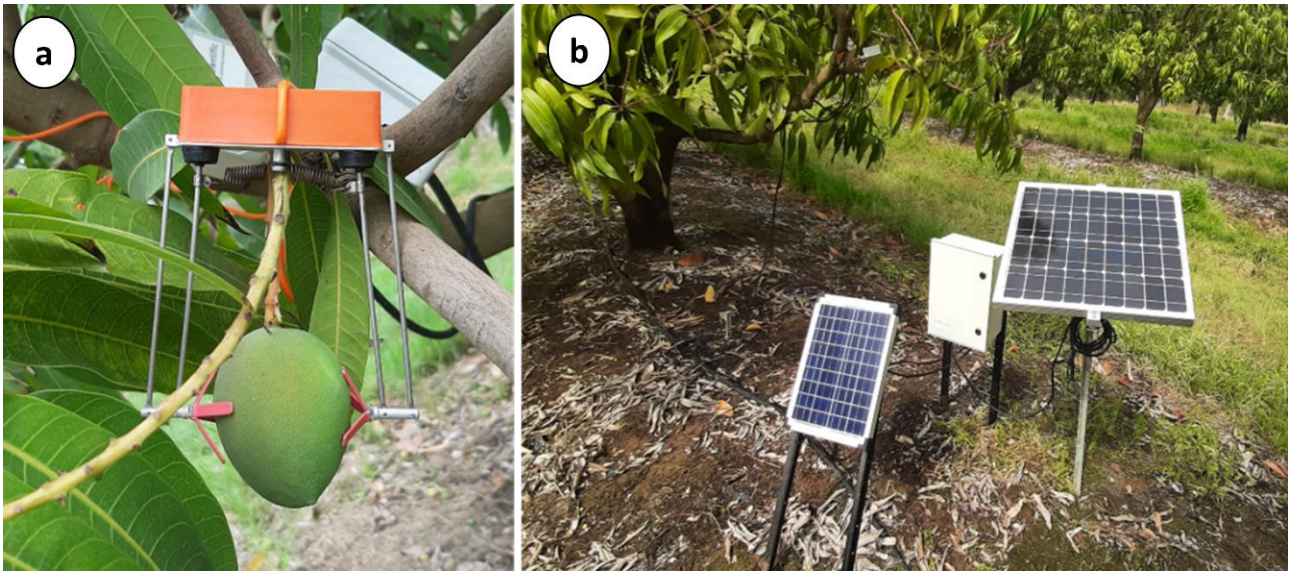


Figure 7.1: a. Fruit dendrometer, b. datalogger and in-field solar panel to monitor continuous mango fruit growth.

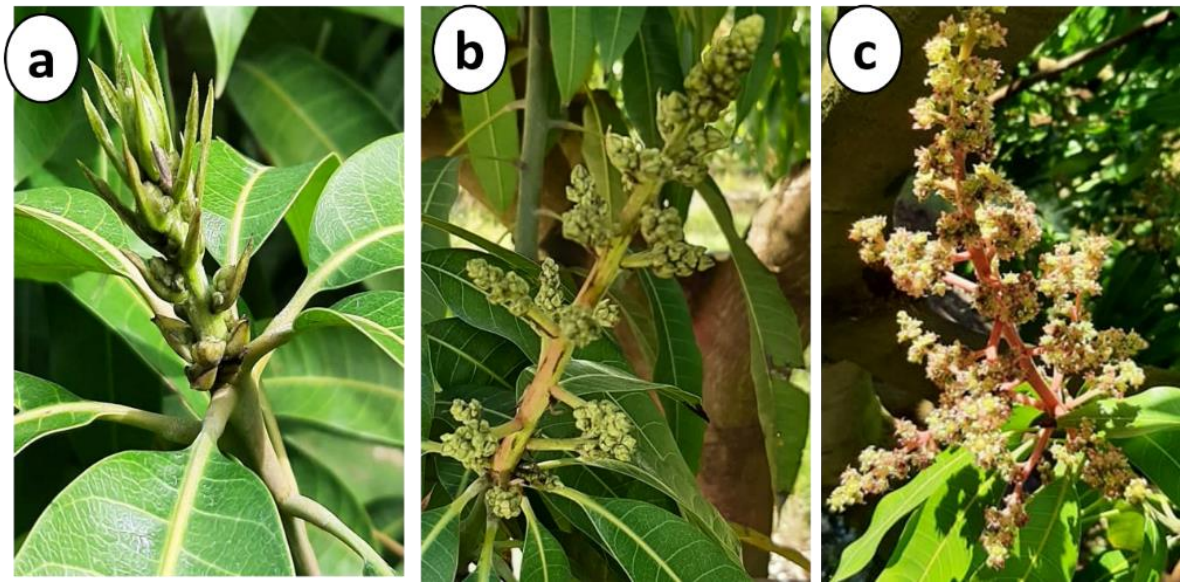


Figure 7.2: Flowering sampling stages from, a. Bud burst, b. Inflorescence emergence, and c. Full flowering.

7.3.3 Xylem functionality using dye tracing

The xylem functionality pattern was evaluated in developing mango fruit using the dye feeding technique, as Ca is translocated via the xylem stream with water inflow. Briefly, five fruit with long pedicels were collected at different growth stages until maturity (Figure 7.3). The details of the

sampling stages and the fruit physical characteristics are outlined in Table 7.1. At each sampling time, fruit were collected early morning to keep the xylem water potential close to zero. The pedicel cut end was wrapped with parafilm and fruit were immediately sealed in polyethene bags. Fruit were placed in an ice cooler to minimise transpiration and transported to laboratory in an air-conditioned vehicle. Upon arrival, pedicel was recut underwater to 40 mm length from the proximal end with a sharp blade to expose fresh surface and placed in a vial containing 1% w/v safranin dye solution (Song et al. 2018). The long stem helped avoid potential blockage of xylem vessels from sap release that typically occurs when stem is cut too close to the pedicel abscission zone (Gosbee et al. 1998). The whole setup was placed under constant air flow (0.5 m/s) at 20.5 °C and ~57% relative humidity to increase dye uptake by stimulating transpiration mass flow. After 16 hours of dye feeding, fruit were cut longitudinally (along the pedicel axis) and transversely (at the proximal, middle, and distal end), and dye distribution reflecting xylem functionality was assessed and photographed using a digital camera (Figure 7.4).

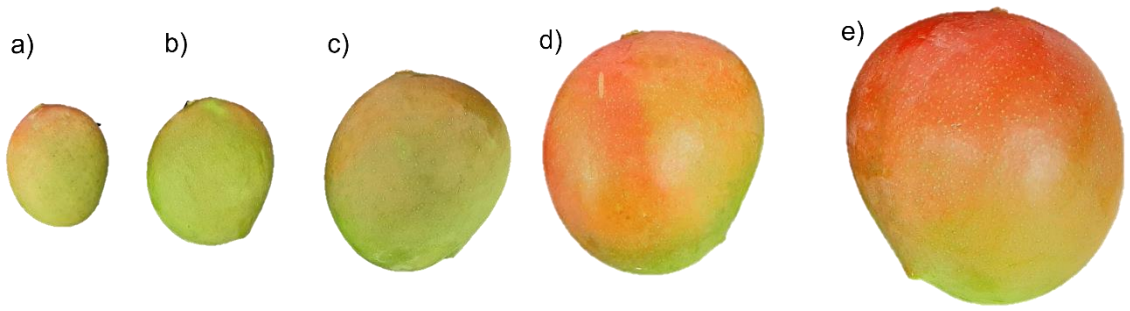


Figure 7.3: The serial sampling stages for dye infiltration. a. 82 DBH, b. 76 DBH, c. 62 DBH, d. 53 DBH, e. 26 DBH. Here, DBH = Days before commercial harvest.

Table 7.1. Fruit physical characteristics before dye infiltration. Each value represents the average of five fruit. DBH: days before commercial harvest.

Sampling (DBH)	Avg. fruit weight (g)	Avg. fruit length (mm)	Avg. fruit width (mm)	Avg. fruit thickness (mm)	Avg. pedicel diameter (mm)
88	74.1	60.8	52.6	45.7	4.1
76	126.2	73.4	62.5	54.8	4.6
62	272.4	94.4	80.6	71.5	5.0
53	338.5	97.9	89.2	77.3	5.3
26	441.5	107.7	93.9	87.1	5.6

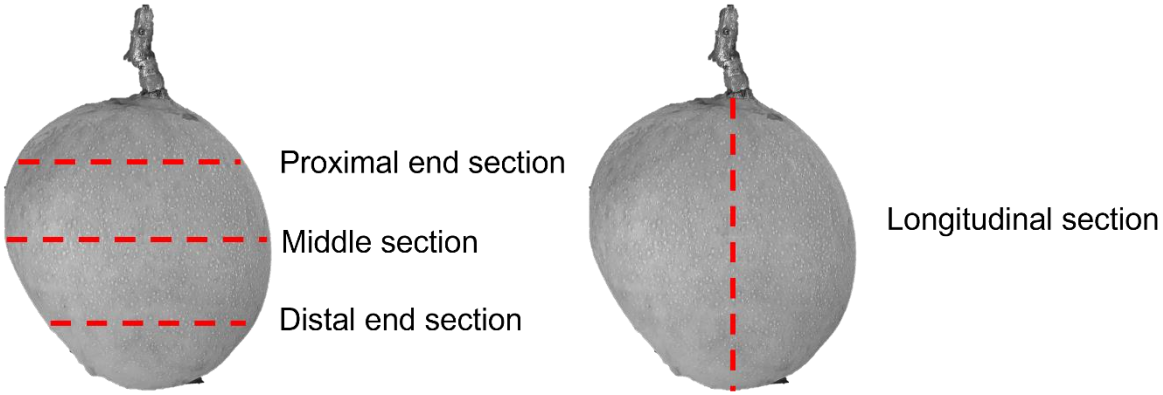


Figure 7.4: Schematics of mango fruit sectioning at the end of the dye feeding period (16 h).

7.3.4 Elemental mapping in developing fruit by micro-X-ray fluorescent (µXRF) imaging

The spatial nutrient distribution of Ca and K in developing mango fruit was further imaged by µXRF imaging. The UQ µXRF facility is a custom-built system manufactured by IXRF (Austin, TX 78748, United States) that consists of two 50 kV–1000 mA sources fitted with polycarpellary focusing optics: XOS microfocus Mo-target tube producing 17.4 keV X-rays (flux of 2.2 × 108 photons s^{-1}) focusing to 25 mm and a Rh-target tube producing 20.2 keV (flux of 1.0 × 107 photons s^{-1}) focusing to 5 mm. The system is fitted with two KETEK silicon drift detectors (SDD) of 170 mm^2 collimated to 150 mm^2 (with 25 mm Be-window) with a CUBE preamplifier coupled to an XIA Mercury X4 signal processing unit. It has an energy resolution of <133 eV with an input count rate of up to 2 M counts per second. The fast motion stage can address areas up to 300 × 300 mm (Harvey et al. 2020).

In current study, the 25 µm Mo-anode with a per-pixel dwell time of 100 ms was used. For mineral distribution in developing mango fruit, three fruit of representative size were collected at different growth stages until maturity (Table 7.1). Fruit were cut at longitudinal and middle sections (Figure 7.4), mounted on a scaffold of double-sided tape for limited movement and covered with 6µm ultralene thin film in a tight sandwich to limit evaporation, and analysed at ~20 °C. The beam spot was 200 µm for analysis. The coloured maps of µXRF scans were generated based on intensities of minerals using Fiji software (ImageJ 2.9.0).

7.3.5 Statistical analysis

Data on fruit physical characteristics, viz., weight, length, width, and thickness were presented as mean ± standard deviation and plotted in Origin Pro-2023. Fruit flesh mineral concentrations were analysed by one-way analysis of variance (ANOVA), and means were compared at 5% confidence

level by Tukey's HSD test. All statistical analyses were performed in R (4.0.2). The µXRF spectra were acquired from the UQ micro-XRF facility in mapping mode using the instrument control package, Iridium (IXRF systems), from the sum of counts at the position of the principal peak for each element. The spectra were further exported as TIFF 8-bit greyscale images in Fiji software (ImageJ 2.9.0) and displayed as Fiji's 'Fire' lookup tables.

7.4 Results

7.4.1 Fruit growth and development

'B74' mango showed a typical sigmoidal growth pattern. Fruit growth in length was rapid compared to that in width and thickness and attained maximum length 5 - 6 weeks before commercial maturity (Figure 7.5a). The width and thickness kept increasing up to harvest indicating a gradual deposit of carbohydrates as fruit matured. Fruit weight followed the same pattern as of fruit width and thickness (Figure 7.5b).

The continuous measurement of fruit thickness using an LVDT dendrometer further complemented the manual assessments of fruit physical characteristics, with a steady increase from early growth stages until fruit maturity (Figure 7.5c). The daily growth rate was higher during early development stages with higher daily diameter changes and decreased gradually as fruit reached maturity (Figure 7.5d). The daily increase in fruit diameter over a 24 h period was assessed on selected days throughout fruit developmental stages. At 90 days before harvest (DBH), the daily increase in diameter in 24 h was ~0.6 mm, with exponential increase till 75 DBH (~1.15 mm) and then slowly returned to ~0.6 mm at 60 DBH (Figure 7.6). The 90 to 60 DBH period was characterised to be the highest growth period, followed by a gradual decline (Figures 7.5d and 7.6). As fruit matured, the trend of increase in diameter over 24 h period decreased until there were no changes noted ~3 weeks before maturity (Figures 7.5c and 7.6).

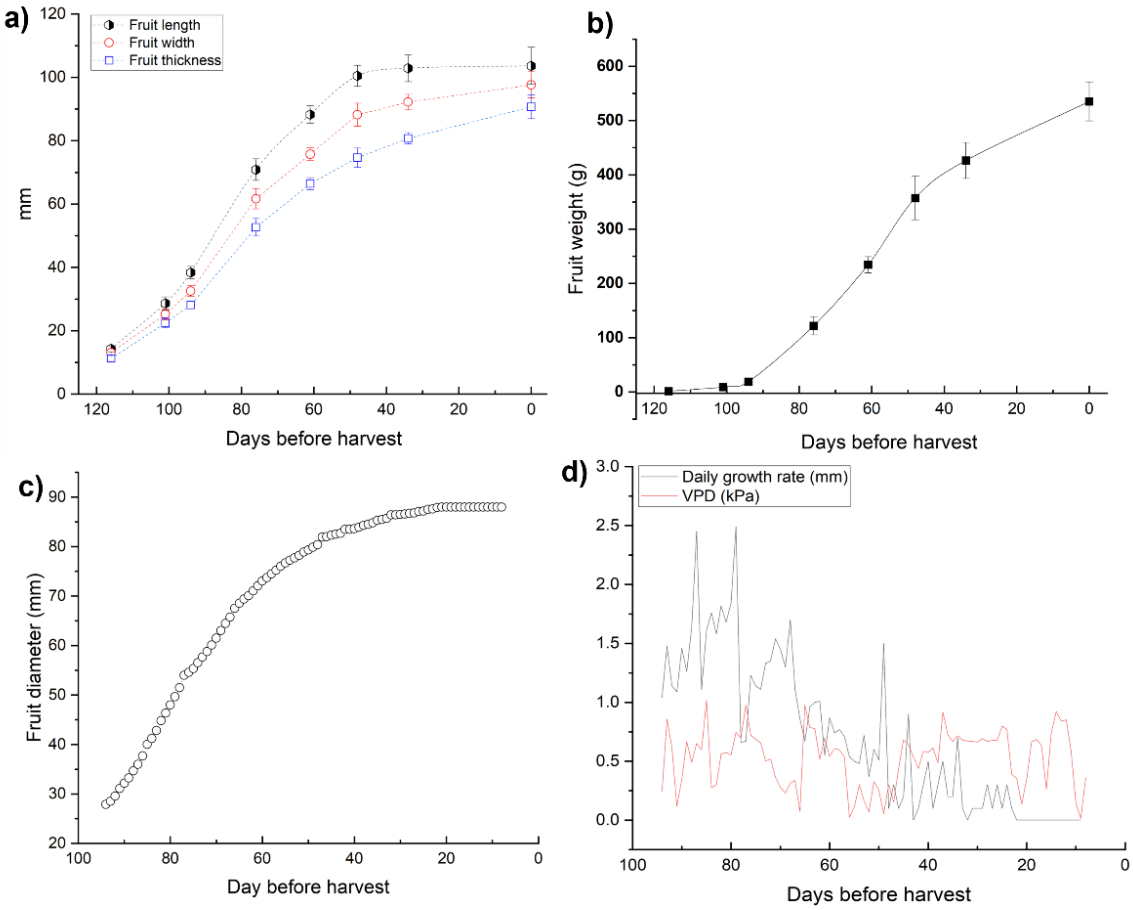


Figure 7.5: The fruit physical properties. a. fruit length, width, and thickness at different sampling intervals (n=9), b. fruit weight (n=9), c. fruit diameter (thickness) measurements using dendrometers, and d. Daily growth rate and vapour pressure deficit (VPD) throughout fruit growth and development.

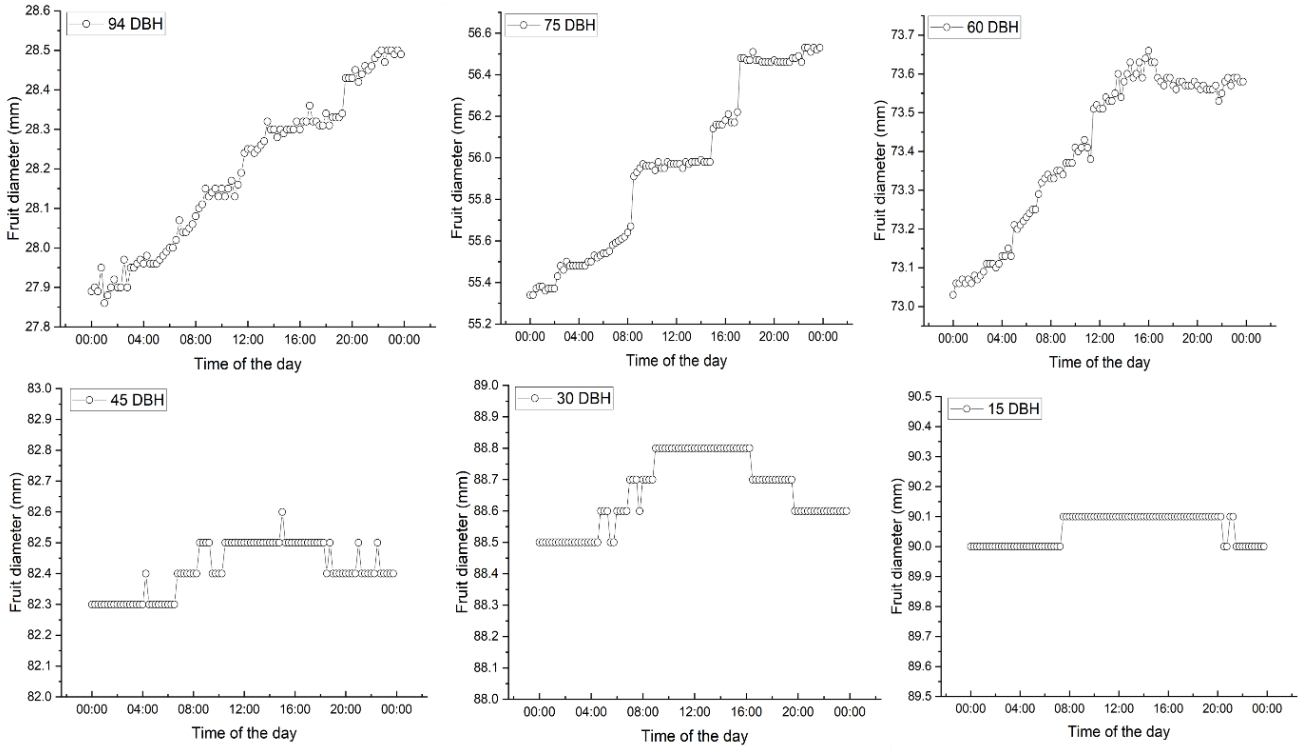


Figure 7.6: Continuous diameter measurements using fruit dendrometers at 15 min intervals on selected days during mango fruit growth and development. DBH: Days before harvest.

7.4.2 Fruit mineral nutrients

The dynamics of mineral nutrient accumulation during mango flowering and in mesocarp of developing fruit were determined through serial harvesting, starting from BB until harvest maturity. There were significant ($p \leq 0.05$) differences in the 3 flowering stages, with higher [N], N/Ca, K/Ca, and (K+Mg)/Ca ratios at BB stage, and higher [Ca], [K], [B], and [Mg] at FF stage (Table 7.2). The K/Ca and (K+Mg)/Ca ratios were not significantly ($p > 0.05$) different between IE and FF stages.

Fruit flesh mineral analysis showed a gradual decrease in most mineral nutrients as fruit reached maturity except for K/Ca and (K+Mg)/Ca ratios (Table 7.2). The concentrations of N, Ca, B, and Mg were highest during early fruit set stage (116 DBH) and decreased significantly ($p \leq 0.05$) thereafter until 48 DBH (~7 weeks before harvest) and remained unchanged until fruit maturity. In comparison, K concentration peaked at 94 DBH (~13th week before harvest) and then decreased significantly ($p \leq 0.05$) until harvest maturity (Table 7.2). N/Ca ratio, however, showed no significant ($p > 0.05$) difference between IE and at harvest.

Since mineral nutrient ratios (N/Ca, K/Ca, and (K+Mg)/Ca) are potentially a reliable indicator of fruit robustness (Chapters 4 and 5), sampling fruit at stone hardening stage (5-7 weeks before commercial maturity) can provide a stable estimate of at-harvest fruit mineral nutrient concentrations.

Table 7.2. The mineral concentrations on a dry weight basis from inflorescence development until fruit maturity. Each data point represents the mean value of triplicate analyses. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to the Tukey's HSD.

	N (% dw)	K (% dw)	Ca (mg/kg dw)	Mg (mg/kg dw)	B (mg/kg dw)	N/Ca	K/Ca	(K+Mg)/Ca
Flowering stage								
BB	1.73^{a}	1.17^{de}	1550.01^{c}	1050.15^{d}	13.46^{de}	11.13^{a}	7.51^{e}	8.19^{e}
IE	1.67^{a}	1.22^{d}	2320.09^{b}	1633.33^{b}	38.92^{b}	7.30^{bc}	5.31^{f}	6.02^{f}
FF	1.50^{b}	1.39^{c}	3531.70^{a}	1900.00^{a}	68.62^{a}	4.30^{d}	3.99^{f}	4.53^{f}
Fruit sampling								
116 DBH	1.74^{a}	1.53^{b}	1705.79^{c}	1675.32^{b}	25.80^{c}	10.19^{a}	9.01^{d}	9.99^{e}
101 DBH	1.37^{c}	1.40^{bc}	1398.35^{cd}	1398.02^{c}	15.31^{d}	10.11^{ab}	10.33^{cd}	11.35^{de}
94 DBH	1.19^{d}	1.68^{a}	1195.13^{d}	1444.32^{c}	13.03^{de}	10.08^{ab}	14.21^{b}	15.43^{bc}
76 DBH	0.73^{e}	1.36^{c}	1054.45^{de}	867.48^{e}	11.27^{def}	6.94^{cd}	12.97^{bc}	13.80^{cd}
61 DBH	0.53^{f}	1.12^{e}	731.10^{ef}	616.42^{ef}	9.26^{ef}	7.27^{bc}	15.33^{ab}	16.18^{bc}
48 DBH	0.48^{f}	1.11^{e}	625.88^{ef}	556.84^{f}	8.42^{f}	7.83^{bc}	17.98^{a}	18.87^{a}
34 DBH	0.48^{f}	1.10^{e}	620.40^{ef}	516.68^{f}	9.51^{ef}	7.73^{bc}	17.85^{a}	18.69^{ab}
0 DBH	0.49^{f}	1.01^{e}	556.15^{f}	480.24^{f}	9.08^{ef}	8.79^{bc}	18.38^{a}	19.26^{a}

Here, BB: Bud burst, IE: Inflorescence emergence, FF: Full flowering, DBH: Days before harvest.

7.4.3 Xylem functionality and Ca mapping in developing fruit

Xylem continuity in mango fruit was analysed using the safranin dye tracing method at 1% w/v under controlled laboratory conditions. The results showed greater dye penetration in mango fruit at early stages of development than at mature-green stage (Figure 7.7-left). Dye penetration was restricted to pedicel end of the fruit at later stages. No penetration in the middle section was observed after stone hardening stage (5-7 weeks before harvest). The longitudinal sections showed a continuous supply of water to tissues around the seed even after stone hardening and maturation. However, xylem supply to the adjacent middle flesh was undetectable, indicating a complete cut-off from the middle and basal sections of the fruit (Figure 7.7-left). The dye uptake did not cease altogether, as proximal end at pedicel base showed considerable dye presence, however, it was limited to the seed coat and skin tissues. This corresponds to a decrease in hydraulic conductance as the fruit goes through the expansion and maturation phases.

The transport of essential mineral nutrients and their accumulation is associated with active xylem functionality throughout development stages. The distribution of Ca and K was mapped in young and mature green fruit in conjunction with dye penetration and concentration gradients were generated using false colours based on their intensities (Figure 7.7-middle and right).

Enriched Ca deposition across whole fruit at early development stage (88 DBH) was evident in longitudinal and middle cross sections. As fruit developed, the longitudinal Ca deposition gradient diminished and became restricted to the proximal end near pedicel base and fruit skin near the

‘shoulder’ sun-exposed area. A similar trend was observed in the transverse middle section of the fruit, with prominent Ca presence in fruit skin but less in flesh. This trend was consistent between the dye pattern and destructive mineral analysis of fruit flesh (Figure 7.7, Table 7.2). Limited water flow to the middle and distal end of fruit and limited Ca deposition indicated limited uptake and dilution of existing Ca during rapid fruit expansion stages leading up to harvest (Figure 7.7).

The K deposition intensity in fruit flesh increased during development and it peaked at 62 DBH during the cell expansion stage (Figure 7.7, Table 7.2), followed by a decline in the later stage, as observed in fruit’s middle section. However, K mainly deposited and accumulated in the inner flesh during later stages of fruit development as opposed to Ca which was mainly in the outer flesh right next to fruit skin (Figure 7.7). The wrinkle impressions in fruit flesh at 26 DBH were due to dehydration during analysis.

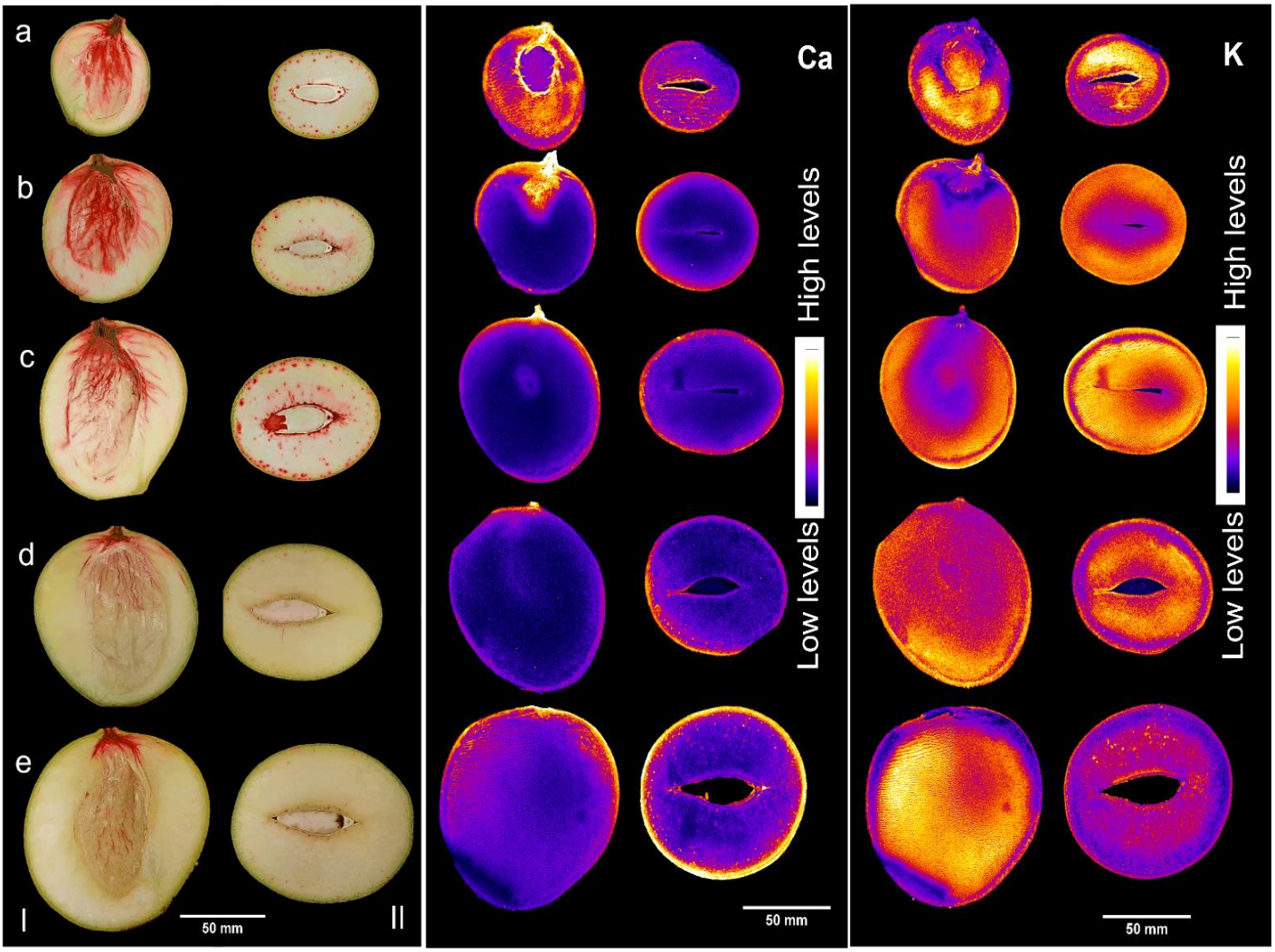


Figure 7.7: The changes in xylem functionality in developing 'B74' mango fruit fed with 0.1 % w/v safranin dye solution for 16 h under simulated transpiration conditions (left), and laboratory µXRF maps of Ca (middle) and K (right) in hydrated mango fruit sections at different development stages. a. 88 DBH, b. 76 DBH, c. 62 DBH, d. 53 DBH, e. 26 DBH. Here, DBH = Days before harvest, I = Longitudinal section, II = Middle section.

7.5 Discussion

Mineral nutrient accumulation is dynamic in developing fruit and is attributed to the particular stage of growth and development (Joyce et al. 2001; Sinha et al. 2017). It is vital, from production point of view, to characterise active growth phases of mineral uptake and accumulation in developing fruit. It allows for early estimation of fruit mineral concentrations, which can potentially inform nutrient management strategies to optimise fruit growth, quality, and robustness.

The present study investigated growth patterns of 'B74' mango fruit in relation to mineral nutrient accumulation throughout development stages from flowering to harvest. Fruit length dimension

showed a marked increase compared to width and thickness as previously characterised in 'Tommy Atkins', 'Keitt', and 'Sensation' mango, showing a typical sigmoidal curve (Joyce et al. 2001; Carella et al. 2021). Daily increase in diameter over a 24 h period provided insight into fruit growth rate during different development stages. The daily growth rate was higher during early development, with higher daily diameter changes and decreased gradually as fruit reached maturity (Figure 7.6). Highest fruit growth rate was observed between 90 and 60 days before harvest, followed by a gradual decline (Figures 7.5 and 7.6) (Carella et al. 2021). The LVDT-based fruit dendrometers are very efficient in measuring on-tree fruit growth non-destructively and can be used to correlate with the effects of dynamic micro-climate variations in and around the canopy on water inflow and growth patterns (Carella et al. 2021; Atay et al. 2023).

Mineral concentrations varied throughout flowering and fruit development. At flowering stages, K, Ca, Mg, and B concentrations increased significantly ($p \leq 0.05$) from BB to FF stage which may be attributed to higher transpiration rates by flowering panicles (Table 7.2) (Whiley et al. 1988; Higuchi & Sakuratani 2005). Previously, about 13% of the total transpiration water loss from tree canopies was attributed to the flowering event in 'Fuerte' avocado and during flowering, the canopy surface area for transpiration-mediated water loss increased to 90% (Whiley et al. 1988). Higuchi and Sakuratani (2005) measured sap flow of mango peduncle at shoot level and found that 22% of the total shoot water consumption was linked to inflorescence. The cation mobility is predominantly linked with xylem water flow, hence, accumulated higher concentrations of Ca and Mg at flowering stages compared to later fruit development stages.

Mineral ratios (N/Ca, K/Ca, and (K+Mg)/Ca) were observed to be stable at stone hardening stage (5-7 weeks before harvest) (Payne et al. 2014) and no significant ($p > 0.05$) difference was noted from stone hardening to commercial maturity (Table 7.2). During fruit growth and development, mineral accumulation was higher at early stages of fruit development and declined with fruit maturity. This is linked to the shift from cell division to cell elongation that can cause nutritional dilution, especially for Ca in mango fruit (Joyce et al. 2001). The anatomical study on 'Kensington Pride' and 'Sensation' reported a marked increase in cell length and breadth in the middle and inner mesocarp of the fruit during fruit development up to maturity (Joyce et al. 2001). Ca is involved in cell wall formation during active cell division, for membrane stability, and strength during cell expansion stages (Hocking et al. 2016).

The pathway of Ca import and accumulation in developing fruit was further mapped through safranin dye infiltration to analyse xylem continuity and water flow. The results indicated a complete cut-off of xylem inflow from middle and basal section of fruit at later stages, suggesting that water and

essential mineral nutrients may not be evenly distributed across fruit (Figure 7.7).

The µXRF analysis further confirmed this suggestion showing higher deposition of Ca close to the fruit skin compared to the inner and distal mesocarp sections (Figure 7.7). Ca mineral analysis of various parts of mature 'Kensington Pride' mango has previously shown ~10-fold higher Ca concentrations in skin (0.53% D.W.) and ~7-fold increase in outer mesocarp (0.12% D.W.) compared to inner flesh (0.05% D.W.) (Joyce et al. 2001). In 'B74', Ca was almost evenly distributed at early fruit development (88 DBH) but during cell elongation and expansion, it was diluted in the mesocarp due to loss of xylem functionality (Figure 7.7). The loss of xylem functionality is linked to decrease in hydraulic conductivity of xylem vessels. Nordey et al. (2015) reported a similar trend of higher xylem flow in mango at early stages but a decline during late developmental stages due to xylem embolism in fruit pedicel which resulted in decrease in hydraulic conductivity of xylem vessels. Similar observations were made in grapes by Knipfer et al. (2015) indicating decline in xylem uptake and hydraulic conductivity post-veraison was due to xylem blockage.

Another possible explanation for the loss of xylem functionality could be the physical rupture of xylem vessels in fruit flesh caused by growth stress and strain during exponential mesocarp cell expansion at later stages (Grimm et al. 2017), but further studies are required to test and validate this hypothesis in mango fruit. The fruit growth stress associated with xylem rupturing during later stages of development has previously been noted in sweet cherry fruit through acid fuchsin dye tracing and gadoteric acid-mediated magnetic resonance imaging (Grimm et al. 2017). Water inflow via xylem is dependent on intact xylem vessels and hydrostatic gradient between peduncle and fruit (generated by fruit growth and transpirational pull). In grapes, Bondada et al. (2005) reported intact functional xylem vessels, however, the loss of driving force (hydrostatic gradient) post-veraison reduced xylem inflow into the grape berry. Similarly, a surface morphological study on 'Kensington Pride' mango indicated a gradual increase in thick epicuticular wax development and rupture of stomata into lenticels (after >38 g fruit weight) as fruit matured (Bally 1999). This could limit the transpiration-mediated water pull into the fruit along with xylem deterioration and can collectively contribute to loss of xylem functionality in developing mango fruit. The pattern of xylem and phloem functionality during fruit development is very dynamic among fruit types (Hou et al. 2021). In any case, the loss of xylem functionality (either due to loss of driving force, ruptured xylem vessel, or embolism) leads to lower mineral transport and accumulation in the developing fruit and makes it more susceptible to post-harvest disorders, as noted by blossom end rot disorder in tomato (Riboldi et al. 2022). The xylem functionality studies, and mineral mapping gradients provide useful insights in understanding nutrient requirements of mango fruit and optimising cultivation practices.

7.6 Conclusion

In present study, fruit growth and mineral nutrient accumulation pattern of ‘B74’ mango was investigated throughout flowering and fruit developmental stages. The fruit growth rate was higher at early developmental stages with a higher daily increase in diameter over a 24 h period. Mineral nutrient ratios were stable at the stone hardening stage (5-7 weeks before harvest) and could be used as a reliable indicator to estimate the at-harvest fruit mineral status. Furthermore, this study provides insights into role of xylem functionality and continuity in nutrient transport and spatial distribution within fruit flesh. These findings highlight the importance of understanding physiology of fruit development for efficient cultivation practices (i.e., fertiliser application, irrigation) and improving fruit quality. Optimal cultivation practices can help meet changing nutrient requirements of mango fruit during development, leading to improved fruit quality and productivity.

Chapter 8 General discussion, conclusion, and future research directions

8.1 Introduction

Australian mango exports currently stand at ~7% of total production (Hort. Innovation Australia 2023). There is a huge potential to drive export to overseas markets. However, 'long' supply chains and requisite post-harvest disinfestation protocols negatively influence quality, shelf life, and marketability (Jacobi & Giles 1997; Jacobi et al. 2001; Brecht et al. 2020; Le et al. 2022).

'B74' mango has considerable demand from Asian markets. For example, from China, for which VHT is a mandatory phytosanitary treatment for fruit fly infestation (Hort. Innovation Australia 2023). VHT has been associated with compromised 'B74' internal quality in export consignments that result in monetary loss to the industry (D. Holmes, *pers. comm*., 2020, Technical and logistics officer, Perfection Fresh Limited.). Therefore, the present project aimed to investigate the potential causes of IDs in 'B74' mango fruit and potential factors affecting fruit robustness against stressful post-harvest VHT in export supply chains. Growing robust fruit may lessen the incidence of IDs in 'B74' mango.

A systematic literature review highlighted a potential role for mineral nutrient concentrations in mango fruit flesh at harvest in mediating susceptibility to IDs. In particular, low [Ca], high [N], and high N/Ca ratios regarding spongy tissue, soft nose, and jelly seed disorders (Chapter 3). Mineral nutrient associations with IDs have been studied for various mango cultivars (Gunjate et al. 1979b; Katrodia 1979; Raymond et al. 1998b; Bally 2006; Ma et al. 2018; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023). Disorder occurrence and intensity vary between cultivars and seasons. Seasonal environmental effectors likely fall under growing conditions and pre-harvest and post-harvest practices (Hewett 2006; Léchaudel & Joas 2007). Consequently, a single simple solution to manage disorder problem is improbable. Rather, cultivar (genetic) and region (environmental) specific identification and understanding of influences pre-harvest and post-harvest is likely to offer the best recipe for management of IDs in 'B74' mango fruit.

The most prevalent IDs in VHT export quality 'B74' mango were flesh cavity with white patches (FCWP) and flesh browning (FB) (Figure 4.3, Chapter 4). FCWP featured cavities with dense white boundaries in the flesh near the seed. This symptom is similar to spongy tissue disorder, a widely recognised ripening disorder in Indian cultivars (Katrodia 1988; Oak et al. 2022). However, FCWP was distinguished from spongy tissue in that it lacked off-odour at eating ripe and was present only

in VHT fruit (Chapters 2 and 4). On the other hand, FB presented as browning or darkening of flesh near the seed and was evident in both VHT and non-VHT fruit.

8.2 Link between IDs and mineral nutrients in 'B74' mango

The link between mineral concentrations and the incidence of IDs in VHT-treated 'B74' mango was explored with fruit sourced from commercial supply chains over two consecutive seasons from three major 'B74' production regions of Australia, i.e., NT, FNQ, and SEQ (Chapter 4).

NT fruit proved relatively more prone to develop FB disorder, possibly due to lower B concentrations in fruit flesh. SEQ fruit had higher flesh [B] and exhibited no FB (Figure 4.7, Tables 4.3 and 4.4). Improving B status in fruit may improve tolerance to FB. For 'Dashehari' mango, Saran and Kumar (2011) reported reduced incidence of internal necrosis (browning) coincident with a significant ($p \leq 0.05$) increase in fruit [B] as a response to foliar application of 0.10% disodium octaborate tetrahydrate. Previously, foliar application of B has been reported to improve quality, yield, and shelf life for 'Dashehari' (Arvind et al. 2012) and 'Palmer' mango fruit (Oldoni et al. 2018). Combined foliar application of B and humic acid on 'Zebda' mango markedly reduced alternate bearing in concert with improved fruit size and total soluble solids (El-Hoseiny et al. 2020). B play a critical role in cell wall stability, including regarding growth of reproductive cells, flower initiation, fruit retention, and internal quality (Lovatt 1990; Whiley et al. 1996; Smith et al. 1997b; Sharma & Singh 2009; Bariya et al. 2014). Soil-applied B fertiliser increased pollen viability and fruit set and size in 'Hass' avocado (Smith et al. 1997a; Smith et al. 1997c).

Fruit from FNQ and SEQ were more susceptible to FCWP after VHT, with higher FCWP incidence associated with lower flesh [Ca] and [Mg] and higher [N], K/Ca, and (K+Mg)/Ca ratios (Tables 4.3 and 4.5) as compared to NT fruit. This was supported by mineral analyses of healthy and FCWP-affected fruit from all regions (Table 4.6). Higher [Ca] in NT fruit was attributed to higher soil exchangeable [Ca] and CEC plus higher temperatures throughout the growing seasons (Table 4.2, Figure 4.2). Ca movement within the canopy is by mass flow of water driven by VPD (Gilliham et al. 2011; Hocking et al. 2016). Relatively higher NT temperatures may have driven VPD to facilitate Ca uptake and transport to fruit (Shivashankara & Mathai 1999).

(K+Mg)/Ca was significantly ($p \leq 0.05$) correlated with FCWP disorder across seasons and regions. Hence, the ratio may prove a useful index for FCWP developing in VHT-treated 'B74' mango (Table 4.5). The initial hypothesis of higher N/Ca ratio causes mango IDs was inconsistent with other results. Rather, (K+Mg)/Ca ratio was most indicative of VHT-induced FCWP disorder in 'B74' mango.

8.3 Crop load effects on mineral nutrients and FCWP incidence in 'B74' mango

Supplementary applications of urea, microfine gypsum, and calcium nitrate for three serial seasons did not improve fruit mineral status nor reduce FCWP disorder (Chapter 5). However, fertiliser treatment effects may have not been evident against the control treatment of no supplementary N or Ca fertiliser due to the basal application of calcium nitrate, potassium nitrate, and gypsum as overarching standard practice on the commercial trial site (Appendix A6). The results are in accordance with Bally (2006), where application of ammonium nitrate and gypsum over three fruiting seasons failed to improve 'Keitt' mango fruit [Ca] and reduce IDs incidence. Ca availability in the soil as %Ca-base saturation in response to fertiliser applications was not correlated to fruit [Ca] at harvest (Appendix A7).

Crop load (fruit per tree) was evidently a factor driving fruit flesh mineral status (Table 5.12). Robinson and Lopez (2010) similarly reported crop load as a major factor affecting apple mineral concentrations and fruit quality compared to N, K, and irrigation applications. Higher crop load in 'Honeycrisp' apple reduced bitter pit disorder and gave lower [K] in flesh (Robinson & Lopez 2010; Serra et al. 2016), which complements findings for 'B74' mango (Tables 5.12, 6.1, and 6.2). Selection of trees based on crop load (fruit number per tree) indicated that high crop load trees (>150 fruit) had lower (K+Mg)/Ca ratio and significantly ($p \leq 0.05$) lower incidence of FWP disorders (9%) compared to trees with low and medium crop loads (Tables 6.1 and 6.2).

The biennial cropping pattern with 'off' and 'on' years on individual trees need be monitored and managed to produce uniform fruit for export (Bally 2006; Zabedah et al. 2008; Isarangkoo et al. 2019). In 'B74' mango, biennial cropping is typically not evident in young trees which, according to Whiley et al. (2006), gave consistent 'on' year cropping with good yield and fruit quality up to 6 years old 'B74' mango. However, as trees age, biennial bearing becomes more evident. Anderson et al. (2019) reported cropping inconsistency within 12 years old 'B74' mango orchard, which complements present results with 17 years old 'B74' trees in three serial seasons (Figure 6.2). Mango biennial bearing is a complex phenomenon (Singh 1969; Prasad & Patak 1970; Rahim et al. 2011; Kaur et al. 2023). Factors reportedly associated with biennial bearing in mango including variety (Singh 1969; Prasad & Patak 1970), mineral nutrition (Singh 1969), carbohydrate deficiency (Singh 1969; Orr et al. 2023), shoot maturity (Singh 1969), environmental fluctuations (Chacko 1989; Sandip et al. 2015), and hormonal imbalance (Chacko et al. 1974; Rahim et al. 2011; Nakagawa et al. 2012). Present results suggested that nutritional deficiency and environmental factors were not involved in 'B74' mango biennial pattern since trees had optimal nutrients range in their leaves (Appendix A4). Moreover, there were no appreciable differences in temperature and relative humidity across seasons (Figure 5.1). Further investigation is warranted to determine whether carbohydrate deficiency (Singh 1969; Orr et al. 2023) or other potential management practices (e.g., pruning) may be involved in

‘B74’ mango biennial cropping.

Fruit position in canopy did not significantly ($p > 0.05$) influence mineral nutrient ratios in ‘B74’ fruit sampled from high crop load trees (>150 fruit/tree) (Table 6.4) and no FCWP after VHT. Upper canopy fruit had significantly ($p \leq 0.001$) higher DM compared to the inner and lower canopy fruit (Table 6.3). Similar results have been reported for ‘Nam Dok Mai’ (Ketsa et al. 1991; Kawphaitoon et al. 2014), ‘Cogshall’ (Joas et al. 2010), and ‘B74’ (Whiley et al. 2006) mango trees. Shaded fruit in the middle and bottom of the canopy had significantly ($p \leq 0.05$) higher flesh [N], [K], and [Mg], and lower [B] (Table 6.4). Sun-exposed fruit had significantly ($p \leq 0.05$) higher weight, DM, and TSS compared to shaded fruit (Table 6.3).

8.4 Mineral nutrient import into developing ‘B74’ mango

Towards determining when fruit should be sampled for at-harvest mineral status, developmental aspects of ‘B74’ mango fruit were studied regarding growth dynamics, mineral nutrient accumulation, and xylem functionality in the lead up to harvest. Serial sampling was adopted from flowering to commercial maturity, and fruit were assessed for mineral nutrient accumulation.

Results showed a trend of gradual decrease in all mineral nutrient concentrations as fruit reached maturity (Table 7.2). Regarding K/Ca and (K+Mg)/Ca ratio, there was no significant ($p > 0.05$) difference after stone hardening at 5-7 weeks before harvest maturity, suggesting a potential sampling point for at-harvest ‘B74’ fruit mineral analysis (Table 7.2). For N/Ca, no significant ($p > 0.05$) differences were observed between inflorescence emergence (IE) stage and at-harvest fruit flesh (Table 7.2). IE stage may be used as an early indication of at harvest flesh N/Ca ratio in ‘B74’ mango fruit as supplemental or alternative tool for diagnosing early nutritional deficiencies. Campisi-Pinto et al. (2017) have suggested cauliflower stage inflorescences as a diagnosis for nutritional deficiencies with regard to ‘Hass’ avocado yield.

Xylem functionality in developing ‘B74’ mango fruit was determined using safranin dye tracer (section 7.3.3). Present results showed gradual decline in xylem functionality as ‘B74’ fruit matured, with dye penetration being limited to the pedicel end of the fruit (Figure 7.7). Song et al. (2018) used safranin to determine xylem functionality in apple, pear, citrus, grape, and litchi fruit at early and full maturity phases and linked it with Ca import in the fruit flesh.

Loss of xylem functionality may limit Ca import and accumulation into fruits (Dichio et al. 2003; Choat et al. 2009; Miqueloto et al. 2014a). Patterns of xylem functionality and Ca import are dynamic and may vary among cultivars. For example, ‘Catarina’ apple showed loss of xylem functionality much earlier in fruit development than ‘Fiji’ apple (Miqueloto et al. 2014a).

Water and mineral import into fruit is linked with xylem functionality and driven by environmental conditions, especially VPD (Montanaro et al. 2014), and fruit physicochemical characteristics, such as transpiration, skin anatomy, and cell growth and development (Bally 1999; Montanaro et al. 2006; Montanaro et al. 2012; Montanaro et al. 2015). For mango fruit, Nordey et al. (2015) linked decline in xylem inflow to xylem emboli in the fruit pedicel during maturation. Bally (1999) reported gradual increase in waxy cuticle thickness during fruit development in 'Kensington pride' mango, along with conversion of stomata into lenticels when fruit reached >38 g weight. Loss of stomatal functioning would lessen fruit transpiration and upstream xylem water inflow. Loss of xylem functionality can also involve physical rupture of vessels in fruit associated with mechanical growth stress and strain during rapid mesocarp cell expansion at later stages (Grimm et al. 2017). Further studies on 'B74' mango are warranted to elucidate driving and/or limiting factors for water and mineral import into the fruit.

The spatial distribution of Ca in 'B74' mango flesh was also mapped by micro-X-ray fluorescence imaging (μXRF) to complement the dye tracing. XRF acquires spatially resolved mineral distribution patterns (Zhao et al. 2014; Kopittke et al. 2018; Wang et al. 2019a; Van Der Ent et al. 2020). Fresh hydrated 2 mm-thick 'B74' slices were mounted on imaging apparatus and imaged for Ca and K spatial distribution at multiple development stages (Table 7.1). Ca rich regions were evident across whole fruit at early growth stages, but 'diluted' as fruit matured (Figure 7.7). A gradual increase in [Ca] was noted in fruit skin throughout the development phase. Higher deposition closer to maturity (Figure 7.7) suggested that fruit skin keep accumulating Ca in contrast to the pattern in fruit flesh.

8.5 Key research questions addressed in this study

The following questions were addressed in the current research project bearing industry interests in mind and as summaries of respective thesis chapters and key outcomes (Figure 8.1).

- What are the major factors causing IDs in mango fruit?

Literature reviews (Chapters 2 and 3) were informative in providing an overall insight into how mango IDs differ among cultivars and across production regions. Focus for IDs was their putative association with fruit mineral concentrations (Chapter 3). This work reflected the working hypothesis that fruit mineral nutrients and their ratios may be key indices towards reducing IDs in 'B74' mango fruit.

- What are the major IDs in export quality 'B74' mango and is there a relationship between 'B74' VHT-induced disorders with flesh minerals?

A variety of disorders are reported in literature. However, 'B74' only expressed FCWP (VHT

induced) and FB (present ± VHT, aggravated by VHT) (Chapter 4). Results showed inconsistency of individual mineral associations with FCWP. It was hypothesised that N/Ca ratio could be the major determining factor (Chapter 2). However, (K+Mg)/Ca ratio showed more consistent correlations with FCWP across different seasons and regions. FB was linked to individual mineral concentration, particularly [B] in fruit flesh (Chapter 4).

- Is there any effect of pre-harvest fertiliser application on IDs and mineral concentrations in 'B74' mango?

Application of supplementary N- and Ca-based fertilisers for three consecutive seasons did not significantly ($p > 0.05$) improve fruit mineral concentrations or reduce incidence of FCWP (Chapter 5). FCWP incidence and fruit mineral concentrations and ratios were primarily driven by crop load rather than fertiliser (Table 5.12). Fruit from trees with high crop load (>150 fruit per tree) had significantly ($p \leq 0.05$) lower (K+Mg)/Ca ratio and FCWP disorder (Chapter 6). When sampling fruit from high crop load trees, fruit position in the canopy (e.g., top, bottom, middle, sun-exposed or shaded) has no significant ($p > 0.05$) effect on mineral nutrient ratios (Chapter 6).

- What is the right time to sample fruit ahead of harvest for mineral analysis and from where in the canopy?

Crop load estimation can be done by flowering estimation or fruit counting after second fruit drop post-stone hardening (Payne et al. 2014). However, flesh mineral concentrations are dynamic throughout development (Joyce et al. 2001). Moreover, fruit mineral analyses by commercial laboratories have a 2-week plus turnaround time, which is hindrance for industry for informed fruit marketing decisions. Serial sampling of 'B74' mango fruit showed that mineral ratios (K+Mg/Ca) do not change significantly ($p > 0.05$) after stone hardening stage (Chapter 7). Thus, this development stage may be used for at-harvest estimation of fruit mineral concentrations.

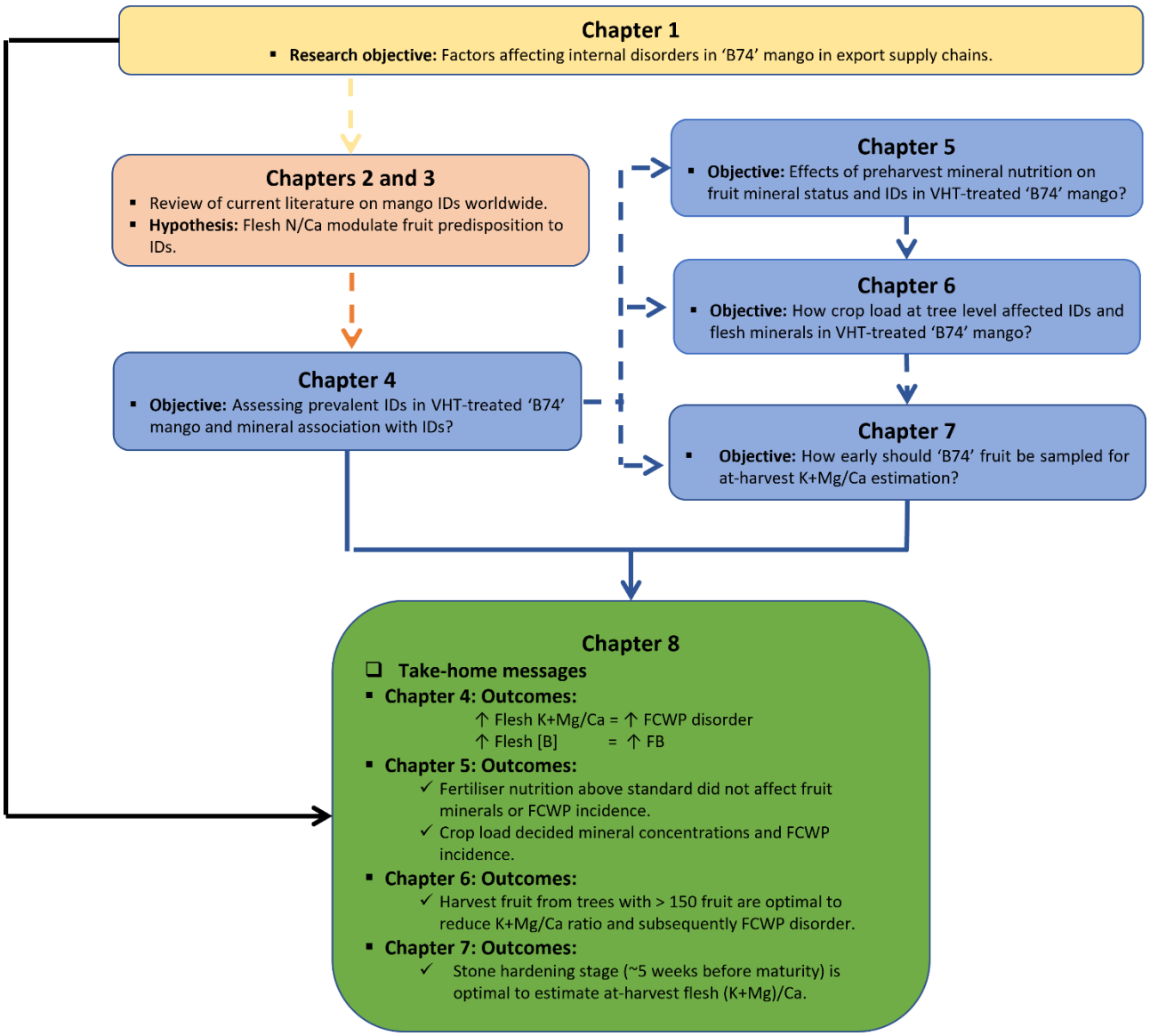


Figure 8.1: Schematic representation linking thesis chapters and outlining overall and individual research objectives in subsequent chapters and take-home messages.

8.6 Future research directions

- The present study investigated absolute mineral concentrations as a function of fruit robustness. However, minerals are present in different physiological forms within the plant. For instance, Ca is available in plant cells in different fractions, viz., soluble Ca (physiologically active form), cell wall-bound, and oxalate crystals. A recent study reported significantly ($p \leq 0.05$) higher [Ca] oxalate and lower soluble [Ca] in severely affected spongy tissue compared to healthy 'Keitt' fruit (Ma et al. 2023). This begs the question as to how these Ca fractions are involved in the predisposition of 'B74' mango to disorders. Future research should consider this aspect as it could inform which type of Ca fertiliser may prove

to be more useful to raise soluble Ca levels in the mesocarp cells during critical fruit growth and development stages.

- An in-depth study on developmental aspects of 'B74' mango (xylem, phloem, and transpiration) is needed to better understand fruit Ca uptake during growth and development, key mechanisms involved in driving or limiting mineral accumulation, genetic variability, as well as environmental and crop management factors (including under varying crop load conditions). Understanding fruit Ca uptake is very important to effectively control the amount of Ca desired in the fruit at harvest.

- Mineral analyses can be very costly with turnaround times of greater than 2 weeks for many commercial laboratories. This encourages sample pooling to reduce analysis cost and leads to loss of variability within data sets. Future research should explore rapid, efficient, and cost-effective benchtop/on-farm analysis tools. These can markedly improve crop quality through timely and efficient monitoring of fertiliser application. It also can help generate extensive datasets to better understand the dynamic roles of these nutrients in horticultural crops.

  A preliminary effort was made to use handheld XRF (Appendix B) as an alternative tool for mineral analysis. Linear regression correlation was obtained between handheld XRF and ICP laboratory methods; for (K+Mg)/Ca: $r^2 = 0.7$, $p < 0.001$ in mango flesh and $r^2 = 0.8$, $p < 0.001$ in avocado skin. However, handheld XRF requires calibration for crop and tissue types. Other potential test methods include ion selective electrodes, pocket ion meters, and portable nutrient analysers as a potentially quick and relatively low-cost means to measure soluble Ca concentrations. Suh techniques are currently being used for plant sap testing (Parks et al. 2012; Buajaila & Miles 2019) and could be optimised for fruit tissue analysis.

- Considerable differences within 'B74' orchards in terms of flesh nutrients and quality were observed. Future research could focus more on site-specific environmental monitoring and precision orchard management strategies to optimise fruit quality for export.

- Towards producing more robust 'B74' mango fruit across Australia for export, a 'systems thinking' approach could be implemented for fruit quality. With quality as the systems output, to map how multiple factors pre-harvest and post-harvest interact to influence robustness status. For example and based on the literature and secondary data, an elementary causal loop diagram (CLD) representing systems thinking is presented in Chapter 3. CLDs can be modified and iteratively improved to effectively depict how complex production systems

work. In turn, this may inform management actions and/or be used as a decision support tool for stakeholders seeking to better understanding their intricate system.

Chapter 9 References

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Appendix A

Appendix A1. Individual mineral nutrients and their ratios in internal disorders of mango. '+' sign indicate direct and '-' sign indicate inverse relationship with expression of disorders. Here internal breakdown refers to the combination of more than one type of disorder in individual mango fruit.

Mineral elements	Flesh concentration (high + or low -)	Disorder type	References
N	+	Spongy tissue Jelly seed Flesh browning Internal breakdown	(Tarmizi et al. 1993; Torres et al. 2004a; Torres & Saúco 2004; Saran & Kumar 2011; Singh & Swati 2011; Ma et al. 2018; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023)
Ca	-	Spongy tissue Soft nose Jelly seed Flesh browning Internal breakdown	(Subraman et al. 1971; Gunjate et al. 1979b; Burdon et al. 1992; Tarmizi et al. 1993; Whangchai et al. 2001; Torres et al. 2004a; Torres & Saúco 2004; Raja 2009; Singh & Swati 2011; Ma et al. 2018; Bitange et al. 2020; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023)
K	+	Spongy tissue Soft nose Flesh browning	(Burdon et al. 1991; Ma et al. 2018; Ma et al. 2022)
	-	Spongy tissue Jelly seed	(Murthy 1981; Raja 2009; Singh & Swati 2011)
B	-	Spongy tissue Flesh browning	(Saran & Kumar 2011; Ma et al. 2018)
P	+	Spongy tissue Soft nose Flesh browning Internal breakdown	(Subraman et al. 1971; Murthy 1981; Burdon et al. 1991; Raymond et al. 1998b; Ma et al. 2022)
Mg	+	Spongy tissue Soft nose	(Burdon et al. 1991; Ma et al. 2018; Ma et al. 2022; Ma et al. 2023)
	-	Spongy tissue Jelly seed Flesh browning	(Andrade et al. 2022)

N: Ca	+	Spongy tissue Jelly seed Flesh browning Internal breakdown	(Tarmizi et al. 1993; Torres & Saúco 2004; Silva et al. 2008; Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023)
K: Ca	+	Spongy tissue Jelly seed Flesh browning Internal breakdown	(Andrade et al. 2022; Ma et al. 2022; Ma et al. 2023)
Mg: Ca	+	Spongy tissue Flesh browning	(Ma et al. 2022; Ma et al. 2023)

Appendix A2. Standard commercial fertiliser application at NT and FNQ farm sites.

Region	Fertiliser	Available nutrient (w/v)	Application method	Rate
NT	NT Horti Trace	Mg 2%, Zn 2.5%, Mn 3%, S 6.2%, Cu 0.5%, Fe 1%, B 0.04%, Mo 0.05%.	Foliar	5 (L/ha)
	CK 55 (S)	Zn 12.8%, Mn 6.4%, Cu 11.9%, Fe 1%, B 14.2%.	Soil	1.3 (kg/h)
	Organibor	B 9.7%, Mg 1.2%, Ca 16%.	Soil	50 (kg/ha)
	Encal	N 13%, Ca 16%, Mg 1.7%, Fe 0.12%, B 0.08%, Zn 0.06%, Cu 0.06%, Mn 0.06%.	Foliar	2.4 (L/ha)
	Bio Gyp	-	Fertigation	100 (L/ha)
	Thio K	K 30%, S 25%.	Fertigation	75 (L/ha)
FNQ	Nitrophoska	N 12%, P 5.2%, K 14.1%, S 8%, Ca 3.2%, Mg 1.2%, B 0.02%, Zn 0.01%.	-	-
	Gypsum	Ca 23.3%, S 18.6%.	-	-
	Thio-sulphate	N 16%, S 35%.	-	-
	Zinc sulphate	Zn 22%, 11.7%	-	-

Appendix A3. The % base saturation of Ca, Mg, and K in response to fertiliser treatments over 3 years period (n = 3).

Depth (cm)	Treatment	% Ca base saturation					% Mg base saturation					% K base saturation				
		Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3
0-30	Control	66.1^{ab}	60.8^{b}	78.5^{a}	79.0	75.2	23.7	30.6	14.3	15.4	16.2	5.3	4.7	5.4	4.2	5.0
	low-N, nil-Ca	65.1^{ab}	61.5^{b}	67.7^{ab}	77.1	69.0	25.3	27.7	18.2	17.1	20.3	4.7	5.8	7.4	4.3	6.6
	high-N, nil-Ca	59.5^{b}	60.9^{b}	73.8^{ab}	76.9	81.6	28.8	29.2	18.3	16.6	11.1	6.1	6.0	5.6	4.9	4.5
	low-N, high-Ca	70.2^{a}	66.6^{a}	63.2^{ab}	86.0	78.3	20.6	24.8	23.0	8.7	12.4	5.5	5.5	6.0	3.9	5.8
	high-N, high-Ca	69.2^{a}	67.4^{a}	73.8^{ab}	81.9	84.9	21.0	23.9	18.6	11.3	8.1	5.5	5.1	5.8	4.7	4.3
	v. high-N, high-Ca	-	60.8^{b}	62.7^{b}	81.3	78.2	-	30.6	25.1	13.0	12.9	-	4.7	6.5	4.3	5.1
	Significance	*	*	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
30-60	Control	67.2	62.5	73.2^{a}	59.3^{b}	65.7^{b}	13.0	22.8^{bc}	18.6^{bc}	22.2	23.0^{a}	6.0	4.5	6.1	7.7	6.4
	low-N, nil-Ca	66.4	61.9	55.3^{b}	61.0^{b}	53.2^{c}	13.7	16.8^{d}	24.8^{d}	28.6	28.0^{a}	5.0	6.1	6.4	6.0	6.3
	high-N, nil-Ca	58.4	60.5	71.5^{a}	59.2^{b}	66.6^{ab}	14.9	21.8^{c}	19.0^{c}	30.2	22.5^{a}	5.4	5.6	6.2	6.4	6.7
	low-N, high-Ca	63.8	60.1	59.7^{ab}	73.8^{a}	65.9^{b}	19.9	26.0^{a}	27.6^{a}	17.0	22.2^{a}	6.6	5.4	6.0	5.9	6.6
	high-N, high-Ca	63.3	60.7	73.2^{a}	60.6^{b}	76.5^{a}	20.3	25.9^{ab}	18.2^{ab}	24.0	14.0^{b}	5.8	5.5	6.0	6.7	5.3
	v. high-N, high-Ca	-	62.5	59.8^{ab}	70.1^{a}	60.1^{bc}	-	22.8^{bc}	25.5^{bc}	21.6	24.0^{a}	-	4.5	5.9	5.4	5.5
	Significance	*ns*	*ns*	**	**	**	*ns*	***	*	*ns*	*	*ns*	*ns*	*ns*	*ns*	*ns*

Here, Pre: pre-flowering, Post: Post-harvest, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

Appendix A4. The optimum range of leaf nutrients for fruiting 'B74' mango trees (Hofman & Whiley 2010). Sampled leaves were mature and collected just prior to the flower bud swelling (n = 3).

Leaf minerals	Results	Recommended	Remarks
Ca (%)	1.23 ± 0.16	2.0-3.5	Low
Mg (%)	0.27 ± 0.02	0.2-0.4	Optimum
K (%)	1.27 ± 0.1	0.4-1.2	Optimum
P (%)	0.20 ± 0.01	0.08-0.18	Slightly high
Na (%)	0.03 ± 0.00	< 0.20	Optimum
S (%)	0.19 ± 0.01	0.2-0.6	Slightly low
Total N (%)	1.14 ± 0.04	1.0-1.5	Optimum
B (mg/kg)	86.02 ± 25.9	40-80	Slightly high
Cu (mg/kg)	19.49 ± 4.66	7-20	Optimum
Fe (mg/kg)	96.62 ± 18.55	50-200	Optimum
Mn (mg/kg)	832.27 ± 231.28	50-500	Slightly high
Zn (mg/kg)	107.97 ± 18.5	02-150	Optimum

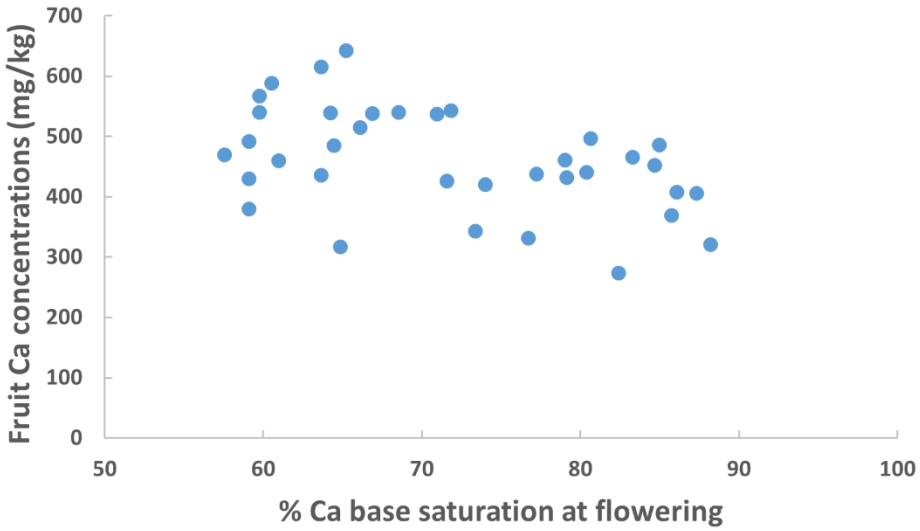
Appendix A5. Mineral analysis of young mature leaves over 3 consecutive seasons. Each value represents the average of 3 analyses.

Treatment	N (% dw)					Ca (%)				
	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3	Post-S-1	Pre-S-2	Post-S-2	Pre-S-3	Post-S-3
Control	1.16	1.13	1.10	1.19	1.14	1.30	1.42	2.17	1.39	1.38
low-N, nil-Ca	1.18	1.11	1.22	1.26	1.25	1.25	1.53	2.02	1.36	1.68
high-N, nil-Ca	1.15	1.08	1.13	1.29	1.19	1.36	1.50	2.09	1.51	1.76
low-N, high-Ca	1.22	1.10	1.08	1.11	1.16	1.49	1.50	2.20	1.44	1.96
high-N, high-Ca	1.22	1.14	1.13	1.26	1.21	1.36	1.58	2.26	1.49	1.81
v. high-N, high-Ca	-	1.13	1.25	1.23	1.18	-	1.40	2.26	1.62	1.89
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

Here, Pre: pre-flowering, Post: Post-harvest, S-1: Season 2020, S-2: Season 2021, S-3: Season 2022.

Appendix A6. Standard commercial fertiliser application at trial site in Childers, Queensland.

Season	Fertilisers	Method	Rate (kg/ha)
2020	BioGyp (Liquid)	Fertigation	90
	Boric Acid	Fertigation	2
	Calcium Nitrate	Fertigation	107
	Fulvic Acid Powder	Fertigation	0
	Inoculant	Fertigation	1
	Iron EDDHA	Fertigation	4
	Magnesium Sulphate	Fertigation	80
	MKP	Fertigation	95
	Potassium Nitrate	Fertigation	25
	Potassium Sulphate	Fertigation	88
	Zinc EDTA	Fertigation	3
2021	Ammonium Sulphate	Fertigation	24
	BioGyp (Liquid)	Fertigation	120
	Calcium Nitrate	Fertigation	50
	Calcium Nitrate & Boric Acid	Fertigation	32
	Easy N	Fertigation	10
	Fulvic Acid Powder	Fertigation	1
	Inoculant	Fertigation	2
	Iron EDDHA	Fertigation	2
	Magnesium Nitrate	Fertigation	12
	Magnesium Sulphate	Fertigation	25
	MKP	Fertigation	100
	Mono. Amm. Phosphate (MAP)	Fertigation	13
	Potassium Sulphate	Fertigation	50
	Urea	Fertigation	10
	Gypsum (Mined)	Spreading	2000
2022	BioGyp (Liquid)	Fertigation	90
	Boric Acid	Fertigation	5
	Easy N	Fertigation	52
	Inoculant	Fertigation	2
	MKP	Fertigation	25
	Gypsum (Mined)	Spreading	2500



Appendix A7. Scatter plot between % Ca base saturation in the soil at the pre-flowering stage and fruit Ca concentrations at harvest.

Appendix B

Comparison of mineral nutrient concentrations using ICP and handheld XRF in fruit flesh

Introduction

The importance of mineral contents especially calcium (Ca), potassium (K), magnesium (Mg), nitrogen (N) and their ratios have previously been documented in the development of different physiological disorders in apples (Pavicic et al. 2004; Miqueloto et al. 2014b), avocado (Witney et al. 1990; Hofman et al. 2002; Willingham et al. 2006), tomato (Brown & Ho 1993; Saure 2001; Tonetto de Freitas et al. 2014; Yoshida et al. 2014) and mango (Tarmizi et al. 1993; Simmons et al. 1996, 1998a; Torres et al. 2004a; Silva et al. 2008; Singh et al. 2013a). Higher ratios of these nutrients have been implicated in modulating fruit predisposition to the development of postharvest disorders and diseases in mango and avocado (Tarmizi et al. 1993; Hofman et al. 2002; Bally 2006, 2009).

Commercial growers and farmers are required to do extensive mineral analysis to timely manage their crop fertilisation regime and eliminate any potentially mineral-related deficiencies. Traditional analytical techniques involve sample digestion and analysis using atomic absorption spectrophotometry for a range of elemental nutrients i.e., Ca, K, Mg, B etc. However, this can be quite expensive, time-consuming and require extensive sample preparation. Such limitations encourage sample pooling which leads to a loss of variability within the data sets. The use of rapid, efficient, and cost-effective benchtop/on-farm analysis tools can significantly improve crop quality through timely and efficient monitoring of fertiliser application and help generate extensive data sets to better understand the dynamic roles of these nutrients in horticultural crops and fruit quality.

Recent technological advances have led to an increased interest in the potential application of handheld portable X-ray fluorescence (hhXRF) spectroscopy for the detection of elements such as Ca and K in plant materials (Kalcsits 2016). In recent years, several studies have demonstrated the usefulness of using hhXRF instruments to analyse minerals in manufactured products (Marguí et al. 2012), archaeological materials (Nazaroff et al. 2010) and soils and plant samples (Zhu & Weindorf 2009; McLaren et al. 2012; Reidinger et al. 2012; Zhou et al. 2020). In plants, Reidinger et al. (2012) used a hhXRF destructively to quantify phosphorus and silica in homogenized and pelletized plant tissue. McLaren et al. (2012) reported significant correlations between traditional digested plant tissue measurements and those made using a hhXRF for analysis of ground, pelletized plant tissue samples across a range of elements including calcium and potassium. Kalcsits (2016) demonstrated the non-destructive potential of hhXRF to determine Ca and K and their role in bitter pit development in apples.

In the present study, the efficiency of hhXRF against the traditional ICP method was compared to determine mineral nutrient concentrations in dried pelletized mango and avocado flesh and avocado skin only.

Materials and Methods

Mango fruit were sourced from a commercial 'B74' mango orchard in Childers South-east Queensland (SEQ), Australia in 2021. Fruit were harvested from the orchard at the mature green stage and subjected to vapour heat treatment (VHT) and later transferred to the postharvest laboratory at The University of Queensland, Gatton campus in an air-conditioned van for postharvest assessment and ripening at 20^{o}C. Later, fruit were cut and a portion of flesh (2 x 2.6 cm) from the middle section of both cheeks was excised at the ripe stage and dried at 65°C in a fan-forced oven for 3 days and then ground to a fine powder using a mini ball mill and homogenised

Avocado fruit were collected from a commercial 'Hass' orchard in Childers, Queensland in 2022. Harvested fruit were properly labelled and immediately transported in an air-conditioned van to the postharvest laboratory. Upon arrival, each fruit was weighed for fresh weight and kept at 20 °C and >90% RH in a shelf life room for ripening. At the eating ripe stage, fruit skin and flesh were separated and dried at 65°C in a fan-forced oven for 3 days and then ground to a fine powder using a mini ball mill and homogenised.

A total of 54 mango flesh samples and 50 avocado flesh and 50 skin samples were used to compare pXRF with the standard laboratory method. A subsample of dried homogenised samples was taken, and Ca, Mg, K, P, and S were measured using Inductively coupled plasma-optical emission spectrometry (ICPOES: Thermo iCAP PRO XP). The samples were open-vessel digested with a 5:1 mixture of nitric and perchloric acids to 200°C. The forward power for the plasma was 1200W and the samples were aspirated into the ICPOES using a concentric nebuliser with a Tracey spray chamber. The instrument was operated in radial mode for the macronutrients and axial mode for the micronutrients. The results were expressed as mg/kg on a dry weight basis.

The remaining samples were used for XRF analysis using TRACER 5 hhXRF (Bruker Elemental, Kennewick, WA, USA). The TRACER 5 was equipped with Rh excitation source to emit X-rays and a silicon diode detector with a maximum voltage of 50kV with operator-adjustable voltage and current to optimise excitation. The detection window diameter was 8mm. The instrument was calibrated with Geo exploration mode under helium conditions which is generally intended for elemental analysis in geological samples. The homogenised, finely ground samples were pelletized using a Parr pellet press in a sample cup covered with 4 µm Prolene film. The pellet was analysed three times by hhXRF and an average value was used for all samples. The results were expressed as ppm.

Statistical analysis

The linear relationship for fruit mineral concentrations between hhXRF and ICPOES methods were developed (Microsoft Excel 365). The variation accounted for by the model was determined using both the significance of fit of the intercept and the slope of the line ($p < 0.05$) and the regression coefficient (r^2).

Results

The linear regression correlation r^2 values were used to compare and determine the strength and significance of the relationship between traditional laboratory ICP method and hhXRF in mango and avocado flesh, and avocado skin samples. In mango flesh, there were strong significant ($p < 0.001$) correlations between hhXRF and ICP for S ($r^2 = 0.76$), K ($r^2 = 0.69$), P ($r^2 = 0.65$) and Ca ($r^2 = 0.53$) (Table B.1), however, the correlation strength was very low for Mg ($r^2 = 0.18$). The mineral nutrient ratios (i.e., K/Ca and K+Mg/Ca) were calculated from the individual elemental concentrations and were also strongly and significantly correlated ($r^2 = 0.69$) (Table B.1, Figure B.1). These mineral ratios play a vital role in mango fruit robustness as discussed in Chapters 4, 5, and 6.

Table B.1. Linear regression correlation (r^2) between laboratory ICP method and handheld XRF in mango flesh, avocado flesh, and skin samples. The level of significance was indicated as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Mineral concentrations (mg/kg D.W.)	Lab-ICP vs hhXRF		
	Mango flesh (n=54)	Avocado flesh (n=50)	Avocado skin (n=50)
Ca	0.53***	0.03	0.09*
K	0.69***	0.07	0.88***
Mg	0.18**	0.02	0.54***
S	0.76***	0.04	0.57***
P	0.65***	0.15*	0.75***
K/Ca	0.69***	0.14*	0.79***
K+Mg/Ca	0.69***	0.14*	0.79***

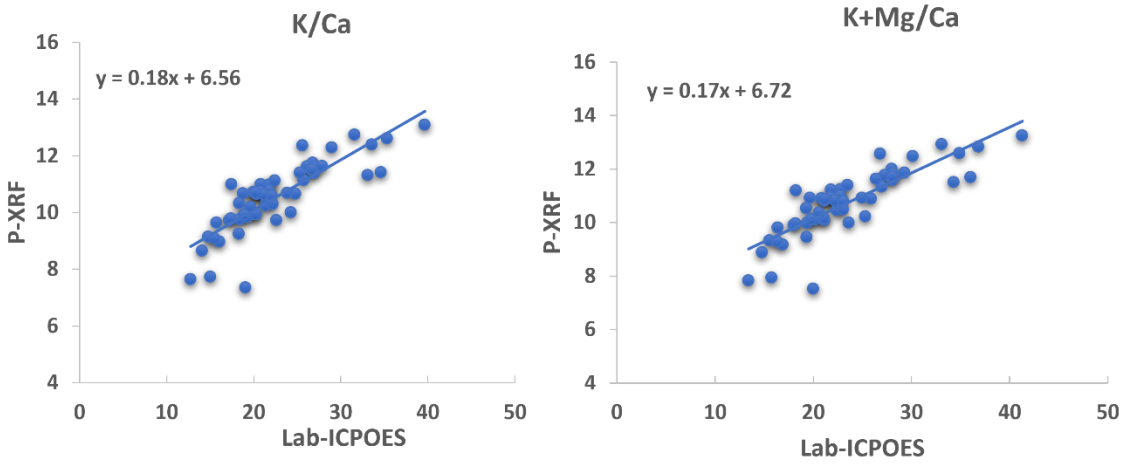


Figure B.1: Linear regression between ICP and hhXRF methods for mineral ratios calculated from individual values in mango flesh (n = 54).

A similar trend was observed in avocado skin tissue samples (Table B.1) with the lowest correlation for Ca (r^2 = 0.09). The highest regression correlation coefficients were observed for K (r^2 = 0.88) and P (r^2 = 0.75) (Table B.1). Mineral ratios of K/Ca and K+Mg/Ca were also significantly strongly correlated with r^2 = 0.79 between ICP and hhXRF (Figure B.2). The difference between sample types was evident between mango and avocado fruit and within different parts of avocado i.e., flesh and skin tissues. Avocado flesh proved to be difficult for hhXRF-based elemental detection. Overall, the regression correlation coefficients were very poor and not significant for any individual elemental concentration except for P (Table B.1). The strength of relationship improved a little with mineral ratios but still were not good for analyses (Figure B.3).

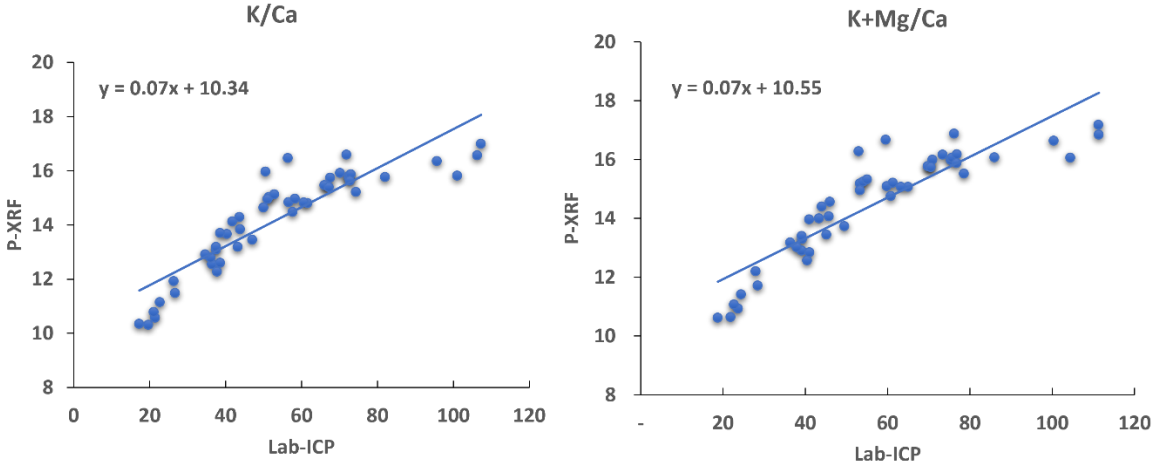


Figure B.2: Linear regression between ICP and hhXRF methods for mineral ratios calculated from individual values in avocado skin (n = 50).

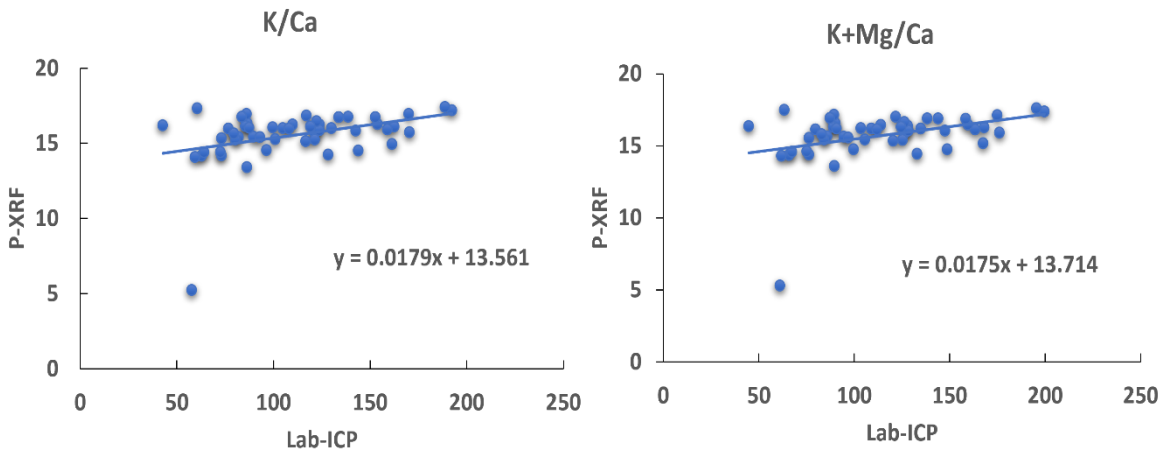


Figure B.3: Linear regression between ICP and hhXRF methods for mineral ratios calculated from individual values in avocado flesh (n = 50).

The mineral detection power of hhXRF may have been higher if the same sample portion, used for hhXRF, was analysed with ICP method. Since a subsample of homogenate was used randomly for hhXRF and for ICP, the variability in the r^2 values can be anticipated. Instrument calibration can also affect mineral detection in a variety of sample types. For example, with single-type calibration, McLaren et al. (2012) reported the differences in r^2 values for Ca were from 0.62 to 0.90 for Wheat and Soybean samples respectively. Hence a custom calibration is needed for a particular sample type to improve hhXRF detection efficiency (Towett et al. 2016).

A visible difference can be seen in our results (Table B.1) where hhXRF showed good mineral detection with strong regression correlation coefficient values for almost all the elements in mango flesh and avocado skin as compared to avocado flesh samples under similar calibration and detection conditions. This difference could be explained by the higher oil content present in avocado flesh as compared to the skin tissue which can likely interfere with elemental detection. Galvão et al. (2014) reported significantly higher oil content in pulp as compared to the skin tissue of three different avocado cultivars ‘Fortuna’, ‘Collinson’ and ‘Barker’. Accounting for tissue differences will likely improve hhXRF elemental detection efficiency.