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**FERTILISER MANAGEMENT STRATEGIES FOR
IMPROVING FRUIT NUTRIENT COMPOSITION AND
QUALITY IN HASS AVOCADO**

**A thesis presented in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy
in
soil science**

at Massey University, Palmerston North, New Zealand.



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2024

Abstract

The avocado industry has the third largest export value of the fruit flesh sector in New Zealand and is focused on exporting premium fruit quality (FQ). However, postharvest internal fruit rots, especially body rots (BR), are a significant issue for fruit harvested late in the season, when the demand from the main export markets is higher. Enhancing fruit nutrient composition, especially increasing Ca concentration and the Ca+Mg:K ratio, and decreasing the N:Ca ratio, has been associated with a lower incidence of internal fruit rots. This study involved a survey of commercial avocado orchards and a fertiliser field trial in the Bay of Plenty (BoP) Region. The study investigated the effects of nitrogen (N), potassium (K) and calcium (Ca) fertiliser practices on fruit nutrient composition and the production of unsound fruit during the late harvest, or fruit affected by any FQ disorder in 5% or more of their surface.

Both components of the study showed a general decline in Ca concentrations in the flesh and skin of late harvest fruit. In the two-year field trial, the average Ca concentration decreased on average by ~50% in fruit flesh and ~16% in fruit skin, between the early (September) and late (January) harvests. The fruit Ca concentration decreased as the flesh dry matter increased from around 23% at the early harvest, to around 33% at late harvest. The decline in fruit flesh Ca concentration was the main factor influencing higher N:Ca ratios and lower Ca+Mg:K ratios in the late harvested fruit. However, in the fruit skin it was the combined effect of the lower Ca concentrations and higher N and K concentrations that resulted in higher N:Ca ratios and lower Ca+Mg:K ratios during the late harvest. Overall, the practice of keeping the avocado fruit on the tree until late harvest results in unfavourable changes in fruit nutrient ratios.

The fruit Ca concentration did not increase with the soil exchangeable Ca concentration at any sampling in the commercial avocado orchards included in the survey study. In the field trial, which had a high initial soil exchangeable Ca concentration, the addition of calcium nitrate fertiliser before the early fruit set also did not increase fruit Ca concentrations at any harvest time. These results support the idea that under conditions of high soil Ca status (over 12 meq Ca/100g), which is common of high-performance

avocado orchards in the BoP Region, additional Ca inputs have a negligible effect on fruit Ca concentration.

Nitrogen fertiliser use was the main factor influencing changes in fruit N:Ca ratios and the production of unsound fruit due to internal fruit rots. In the field trial, the lowest N fertiliser rate of 50 kg N/ha/year decreased the skin N:Ca ratio by 22% and fruit unsoundness by 20% compared to the use of 150 kg N/ha/year, which resulted in the highest N:Ca ratio and fruit unsoundness. It is common for avocado orchards in the BoP Region to use N fertiliser rates greater than 100 kg N/ha/year. Therefore, it is likely that current N fertiliser use practices contribute to the observed unsoundness of fruit harvested late in the season due to internal fruit rots. Additional research is needed to assess lower N fertiliser rates, in the range of 50-100 kg N/ha/year, over an extended period and across different sites, to further validate and refine these N fertiliser recommendations. In addition, the strong correlation observed between the skin N:Ca ratio and fruit unsoundness in late harvested fruit confirmed its potential use as a suitable indicator of internal fruit rots.

In the field trial, two fertiliser practices potentially reduced the severity of BR, the most common FQ disorder of late harvested fruit, compared to the 150 kg/ha/year of N and K that resulted in the highest BR severity. When N and K fertilisers were applied together at their lowest rates (50 kg/ha/year), the severity of highly affected fruit (BR severity >15%) by BR was reduced. In addition, the use of the lowest rate of N combined with the highest rate of K fertiliser (300 kg K/ha/year) reduced the severity of moderately (BR severity >5%) and highly affected fruit by BR. However, in orchards where soil K levels are already optimal, the application of high K rates may be inefficient and costly. In addition, fruit quality assessments in this study were not conducted on treatments that compared the lowest rate of N and more moderate rates of K, between 50 and 300 kg K/ha/year. Therefore, further research is needed to further refine these recommendations, including investigate a benefit from applying a higher proportion of the fertiliser K before fruit set.

Acknowledgements

I would like to express my deepest appreciation to my supervisors James Hanly, Paramsothy (Jeya) Jeyakumar, Chris Anderson, and Nick Roskrige for their unconditional support during this journey, especially during highly uncertain times of the global pandemic. Special thanks to James for his continual help in clarifying my ideas and improving my writing skills in English.

I would like to express my deepest gratitude to the people within the avocado and fertiliser industries for their invaluable support. Special acknowledgement must be given to Danni van der Heiden from AVOCO for her outstanding support in the Bay of Plenty Region and her guidance throughout my PhD research. I have truly learnt from Danni about the vibrant avocado industry in New Zealand.

I'm extremely grateful to the avocado growers who participated in the study, generously sharing their experiences and teachings. Although I cannot mention you by name in these pages, please know that you will always have a special place in my heart. I am profoundly grateful to Fred and Gill Willis for generously providing the site for the field trial, offering unwavering support, and consistently greeting me with warmth and kindness.

Special thanks to Angus Dowson and Harrison Ward from Ballance Agri-Nutrients, Logan Whenua from the Apata packhouse and Hamish McKain from the DMS packhouse, who play crucial roles for the development of this research.

Particularly helpful to me during my endless journeys in the Soil Science Laboratory were Ian Furkert, Peter Bishop, and my office mates Themba Matse, Nilusha Ubeynarayana and May Hedges.

I am deeply honoured and grateful to have been selected as a recipient of the 2020 Massey University Doctoral Scholarship. I would like to express my sincere gratitude for your generous contribution towards the funding of this scholarship, particularly for a mid-

career professional such as myself. Your support has been invaluable to me and my family, especially during these challenging times of a global pandemic.

This PhD research was generously supported by Ballance Agri-Nutrients Limited, covering research costs and a stipend towards one year of the PhD research.

Thanks to AVOCO for supporting this research by providing technical advice, paying for fruit purchases, and helping me with fruit sampling and fruit quality assessment. Special thanks to Apata and DMS packhouses in Tauranga for providing space in their facilities for postharvest cold storage.

This journey has only been possible thanks to the tenacity and patience of my family, who have always encouraged me to carry on, even in the hardest times or when my strength was almost gone. I feel more than privileged to have the love of my wife Juana and my children Ana and Juan. We promised ourselves that together we would make our dreams happen, and we are doing it!

Thanks to my parents, Epimenio and Teresa, and my brothers, Paola, and Rafael. Mum and Dad, you always put our education before your own dreams, this achievement is because of you. Thank you.

Thank you to our friends in Palmy, especially to the big family of Sabados Teatreros, you were our family in Aotearoa!

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List of acronyms and abbreviations

ABI	Alternate bearing index
AIC	Avocado Industry Council of New Zealand
B	Boron
BoP	Bay of Plenty Region
BR	Body rots
Ca	Calcium
Ca+Mg:K	Calcium-and-Magnesium-to-Potassium ratio
CAN	Calcium ammonium nitrate
CBF	Copper based fungicides
CEC	Cation exchange capacity
CHM	Canopy height model
CN	Calcium nitrate
DSM	Digital surface model
DTM	Digital terrain model
DW	Dry weight
FQ	Fruit quality
GCP	Ground control points
GSD	Ground surface distance
ha	Hectares
HSD	Honest significance difference
IQR	Interquartile range
K	Potassium
KNO ₃	Potassium nitrate
LRT	Likelihood-ratio test
meq/100g	Milliequivalents per 100 grams of soil
Mg	Magnesium
N	Nitrogen
N:Ca	Nitrogen-to-Calcium ratio
NZD	New Zealand dollars
P	Phosphorus

PT	Patches at green (external discrete patches and fuzzy patches after cold storage)
RCBD	Randomised complete block design
RSM	Response surface method
S	Sulphur
SER	Stem end rot
SOP	Sulphate of Potash
uBR	Unsoundness due to body rot
uGeneral	General unsoundness
uPT	Unsoundness due to external patches at green
uSER	Unsoundness due to stem end rot
uVB	Unsoundness due to vascular browning
VB	Vascular browning
Zn	Zinc

Chapter 1 - General introduction and literature review

1.1 Background

Avocado (*Persea americana* Mill.) is an evergreen fruit crop cultivated in tropical and subtropical regions worldwide. The Hass cultivar is the leading avocado variety traded worldwide, with approximately 415 thousand hectares planted (Imbert, 2021). In New Zealand, 95% of the total planted area (4912 hectares by the 2022-2023 season) is dedicated to Hass avocados, which is the only cultivar approved for export (New Zealand Avocado, 2024; United Fresh & New Zealand Incorporated, 2023). Although the productive area in the country represents around 1% of the global Hass productive area, the avocado industry is the third most important fresh fruit sector in New Zealand in terms of value (United Fresh & New Zealand Incorporated, 2023). Exports reached a maximum of NZD\$167 million during the 2020-2021 season, primarily to Australia, with a local market around NZD\$60 million (United Fresh & New Zealand Incorporated, 2023).

The avocado industry is concentrated in two northern regions of New Zealand: The Bay of Plenty (BoP) and Northland Regions (Figure 1.1). These locations, situated between 35°S and 39°S latitude, are among the coldest avocado-growing regions worldwide (Everett, 2020), which allows the fruit to remain hanging on the tree for extended periods before being harvested. The extended on-tree storage is commercially beneficial for the New Zealand industry as it allows flexibility in the harvest period to deliver international markets, providing fruit with higher dry matter for fruit harvested late in the season. Consequently, New Zealand avocado growers typically receive better prices for fruit harvested later in the season, driven by the limited supply of Australian-produced fruit and the preference of Australian consumers for avocados with higher dry matter contents (Gamble et al., 2010).

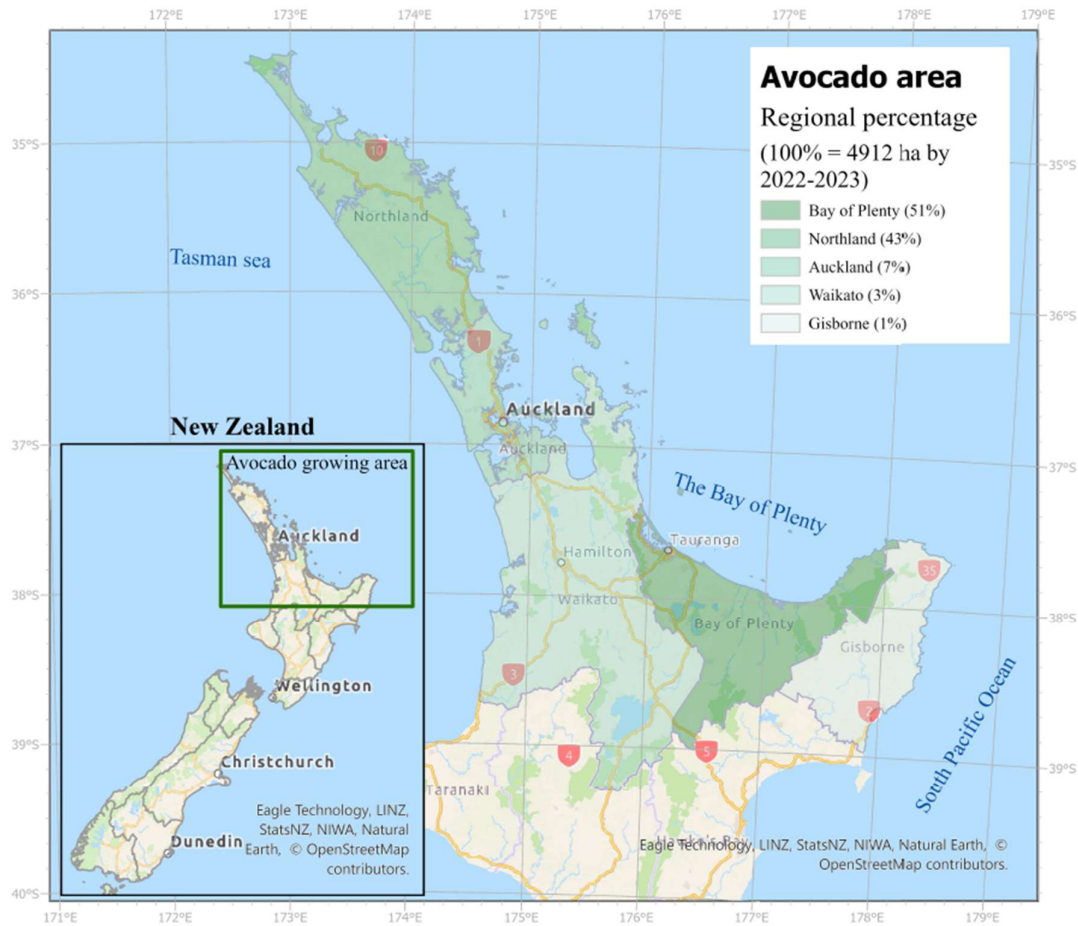


Figure 1.1- Regional distribution map of avocado growing areas in New Zealand

However, the incidence of internal fruit quality (FQ) disorders, particularly fruit rots, is prevalent in fruit harvested late in the season in New Zealand (Dixon et al., 2003; Everett, 2020; Sorensen, 2017; White, 2001). This issue poses a significant challenge for the main market strategy of the New Zealand avocado industry, which focuses on producing avocados with premium FQ. The fruit rots are fungi-caused diseases produced by five fungal species (*Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Botryosphaeria parva*, *Botryosphaeria dothidea* and *Phomosis* spp.) (Everett et al., 2007), and known as anthracnose or body rots (BR) and stem end rots (SER) (White et al., 2005). Fruit rots develop their symptoms during postharvest, diminishing the confidence of consumers to repeat avocado purchases (Gamble et al., 2010) and threatening the growth of the whole avocado industry.

The fruit nutrient composition, specifically an increased Ca concentration in fruit tissues, a higher calcium-and-magnesium-to-potassium (Ca+Mg:K) ratio, and a lower nitrogen-to-calcium (N:Ca) ratio, plays a crucial role in mitigating internal fruit rots. Improved fruit nutrient composition at harvest has been linked to significantly reduced incidences of fruit rots and other FQ disorders at postharvest (Dann et al., 2016; Escobar et al., 2021; Everett et al., 2007; Marques et al., 2003; Thorp et al., 1997; Ullah & Joyce, 2024; Willingham et al., 2006). Three primary pre-harvest factors influence avocado fruit nutrient composition: rootstock/scion genetics, with rootstocks derived from Guatemalan or West Indian genetics being recommended over Mexican-derived genetics due to the tendency of Mexican-derived rootstocks to reduce Ca concentration in scions and upward tissues (Dann et al., 2016; Whiley, 2013a; Willingham et al., 2006); Ca partitioning between vegetative and reproductive tissues, with higher fruit Ca concentration observed in trees with lower leaf-to-fruit ratios (Mullen, 2015; Willingham et al., 2004; Witney et al., 1990b); and orchard fertiliser practices, particularly those aimed at enhancing Ca availability during critical stages and managing N and K fertiliser use to improve fruit nutrient composition (Hofman, 2007; Willingham et al., 2006).

Despite recommendations to improve fruit nutrient composition in Hass avocados through balanced fertiliser practices, few studies have examined practices to achieve the desired composition at harvest. Trials adding Ca sources to soil or leaves have yielded limited results in avocados (Du Plessis & Koen, 1987; Partridge et al., 2002). However, a survey of avocado orchards in the BoP between 2009 and 2011 found increased flesh Ca concentration and Ca+Mg:K ratio following greater use of Ca-rich products by growers during the survey period (Everett et al., 2007). In the survey study, Everett et al. (2007) did not report products or dosages used by growers achieving higher fruit flesh Ca concentration in the BoP. In a replicated fertiliser trial in Queensland, Australia, Hofman (2007) observed reduced Ca concentration in fruit tissues when Ca and K were applied simultaneously compared to the application of Ca fertilisers alone. From this experiment, Hofman (2007) recommended avoiding K fertiliser use during critical periods for Ca translocation into the fruit, suggesting a competitive effect between K fertiliser and fruit Ca concentration. Additionally, Willingham et al. (2006) reported higher fruit skin N concentration and N:Ca ratio using the grower's N fertiliser rate (130 kg N/ha/year) or

doubled that rate compared to the control treatment without N fertiliser use in New South Wales, Australia. Therefore, while N and K fertiliser management has been widely recommended to improve fruit nutrient composition in Hass avocados and support premium FQ, specific fertiliser practices for achieving this goal remain unclear. Additionally, it is unknown how the nutrient composition of avocados changes while they are hanging on the tree after reaching maturity for harvest (around 24% dry matter), and whether fertilizer practices have a differential influence on this composition.

In New Zealand, consultants commonly recommend adjusting N levels in leaves as the key nutritional parameter to ensure optimal tree performance, complemented by adjustments in other leaf mineral concentrations and soil pH. Nitrogen and other fertility parameters are typically adjusted through side-dressing applications of solid fertilisers and amendments. Commonly used products include composite fertiliser blends, calcium ammonium nitrate, sulphate of potash, lime, and gypsum. However, fertiliser programs vary widely based on consultants' target levels of leaf mineral concentration, previous experiences, fruit load, tree health, and grower expectations (West, 2020). Consequently, the impact of fertiliser programs on fruit nutrient composition in high-performance avocado orchards in New Zealand remains to be established.

This thesis study investigated the effects of current and potential fertiliser practices on fruit nutrient composition and FQ outcomes in high-performance avocado orchards. The study analysed the influence of current fertiliser practices on fruit nutrient composition in different avocado orchards under similar agroecological conditions of the BoP by developing a survey study. The analysis included the effects of fertiliser programs on skin and flesh tissues of fruits harvested both at the early and late stage in the season, aiming to identify trends and hypotheses regarding fertiliser practices supporting improved fruit nutrient composition. A second part of the study analysed under controlled conditions, the influence of fertiliser practices on fruit nutrient composition. This component used a two-year replicated trial with twelve treatments. The trial included a control treatment without fertiliser use, nine fertiliser treatments with the combination of low, medium and high N and K fertiliser rates, and two treatments using calcium nitrate as soluble Ca source, while avoiding K fertiliser use during critical periods for Ca translocation into the fruit. This trial investigated the effectiveness of different fertiliser practices in influencing

fruit nutrient composition while maintaining avocado tree performance under the BoP conditions. Additionally, FQ assessments in selected treatments during late harvests were also conducted to establish adequate N and K fertiliser strategies for supporting premium FQ produce and identify directions for further research.

1.2 Research objectives and thesis structure

Based on the research gaps identified in the literature review (Section 1.3), the research objectives of this study were to:

- i. Characterise the change in fruit nutrient composition, namely N, K, and Ca, and their ratios between early and late harvested avocado fruit.
- ii. Evaluate the influence of different fertiliser practices, namely N, K, and Ca soil applications, on avocado fruit nutrient composition in representative high-performance avocado orchards in the BoP.
- iii. Investigate the effectiveness of different fertiliser practices in enhancing avocado fruit nutrient composition, while maintaining avocado tree performance.
- iv. Identify optimal N and K fertiliser strategies for reducing the risk of avocado postharvest fruit rots, especially for fruit harvested late in the season for which rots are more prevalent in New Zealand.

This thesis consists of five chapters. The first chapter includes the literature review, highlighting the main findings and research gaps to improve the fruit nutrient composition by managing fertiliser practices in Hass avocados.

The second chapter assessed the impact of different fertiliser practices on the avocado fruit nutrient composition. This second chapter focused on developing the first thesis objective using a survey study in high-performance avocado orchards with contrasting fertiliser programs in the BoP of New Zealand. The chapter provides insights into the influence of different fertiliser practices in high-performance orchards on fruit flesh and skin mineral composition throughout the harvest season. Besides, this survey study overviews the soil conditions in high-performance avocado orchards in the BoP.

Chapters 3 and 4 were developed based on a two-year fertiliser trial in a high-performance avocado orchard from the BoP. Chapter 3 dives into the influence that fertiliser practices have on fruit nutrient composition and avocado tree performance, having as the main topic the changes generated in flesh and skin mineral concentrations and ratios between Ca, N, K, and Mg across the harvest season. In this chapter, treatments are organised into two fertiliser strategies; the first strategy tests high, medium, and low N and K fertiliser rates evenly applied during the season. While treatments in the second strategy use medium N and K fertiliser rates, soluble Ca was added using calcium nitrate during the early fruit set, delaying K fertiliser after that critical stage for Ca translocation into the fruit. Thus, chapter 3 develops the second thesis objective, investigating the effectiveness of different fertiliser treatments to enhance fruit nutrient composition. Also, this chapter compares the effects of fertiliser practices on fruit mineral composition with tree performance features such as yield, biomass development, and mineral concentration in foliar tissues.

Chapter 4 discusses the findings of the FQ assessment during the late harvest season when disorders such as BR are prevalent in New Zealand. This assessment was developed in selected treatments representing low and high N and K fertiliser treatments, and it also included a treatment assessing the addition of soluble Ca as calcium nitrate. The assessment quantifies the effect on FQ of fruit mineral composition due to changes in fertiliser use, facilitated by the lack of fungicide use during the experimental time. Besides, other preharvest and postharvest factors maximised the expressions of FQ disorders generated by fungi, such as the excessive rainfall during the 2022-2023 season, especially the previous days to harvest in January 2023, and the use of cold storage without a CO₂-controlled atmosphere. Chapter 4 investigated the protective or detrimental effect of different fertiliser treatments on FQ. The chapter developed the third thesis objective, identifying fertiliser practices supporting premium FQ produce.

Finally, Chapter 5 develops the general discussion of the thesis, comparing the effects of fertiliser treatments on fruit composition, FQ, and tree performance.

1.3 Literature review

1.3.1 Avocado (*Persea americana* Mill.)

The avocado (*Persea americana* Mill.) is an evergreen tree of the *Lauraceae* botanical family, domesticated in Mesoamerica since 6400 BC and cultivated in tropical and subtropical regions worldwide (Galindo-Tovar et al., 2008). Their flesh is consumed as a fresh fruit because their flavour, gastronomical versatility and nutritional value (Bhuyan et al., 2019; Chanderbali et al., 2013). Due to their nutraceutical properties such as the high density of monounsaturated fatty acids, their consumption is associated to support cardiovascular health, weight control, and healthy aging in humans (Dreher & Davenport, 2013). The avocado fruit is recognized worldwide for its high nutritional value, with outstanding fibre content, monounsaturated fatty acids that provide most of the fruit's calories, higher K content than bananas, and vitamins such as thiamine, pyridoxine, and carotene, as well as essential micronutrients such as iron, zinc, copper, and selenium, between other nutrients (Bhuyan et al., 2019; Dreher & Davenport, 2013). Besides to the recommended consumption of flesh and oil of avocados, recently their biomass has shown the potential as a source of bioenergy (García-Vargas et al., 2020).

1.3.1.1 Morphological and phenological descriptions

The avocado is a perennial and evergreen tree that grows between 10 and 30 m, although dwarf trees are common in grafted trees (Chanderbali et al., 2013). The trunk can reach a diameter of between 30 and 60 cm, with dense foliage and a variable distribution of the branches (Figure 1.2). The combination of branching pattern and foliage distribution determines the tree shape and canopy volume, which varies from columnar to irregular (Figure 1.3) (IPGRI, 1995). For instance, the trees used in this study, typical in New Zealand avocado orchards from the BoP, had verticillate branching, an irregular shape and average height between 12 to 16 m (Figure 1.4).

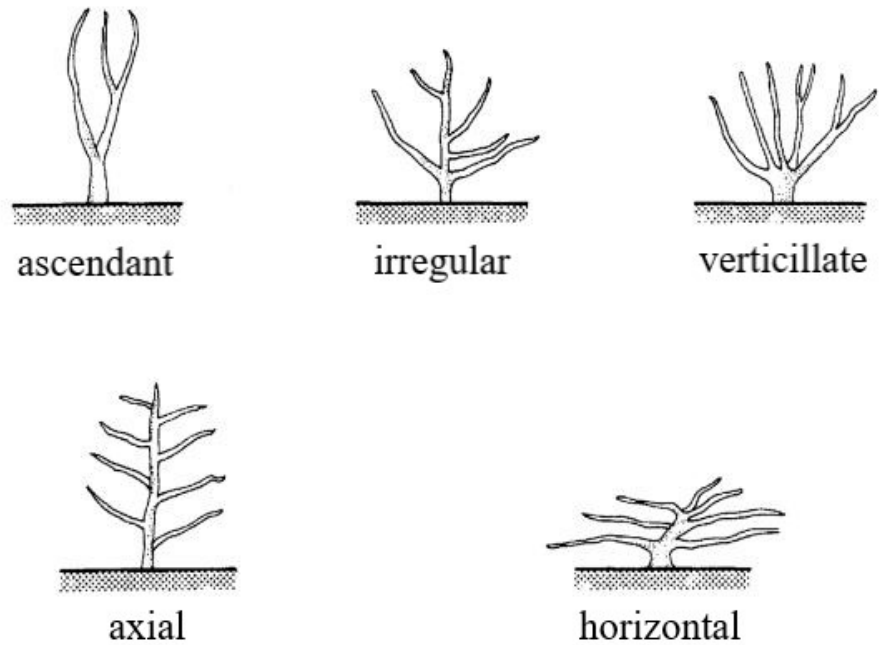


Figure 1.2- Branching pattern observed in avocado (*Persea americana* Mill.) trees. Modified from IPGRI (1995)

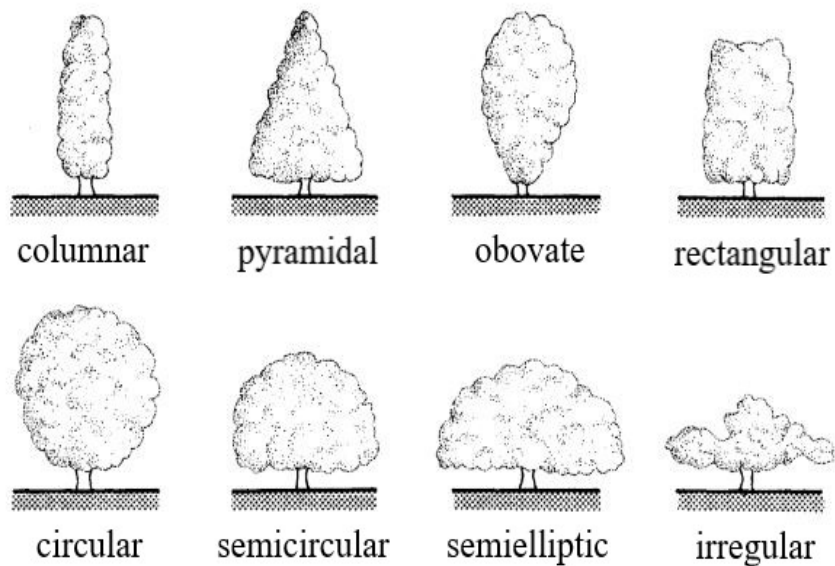


Figure 1.3- Tree shapes commonly observed in avocado (*Persea americana* Mill.) trees. Modified from IPGRI (1995).

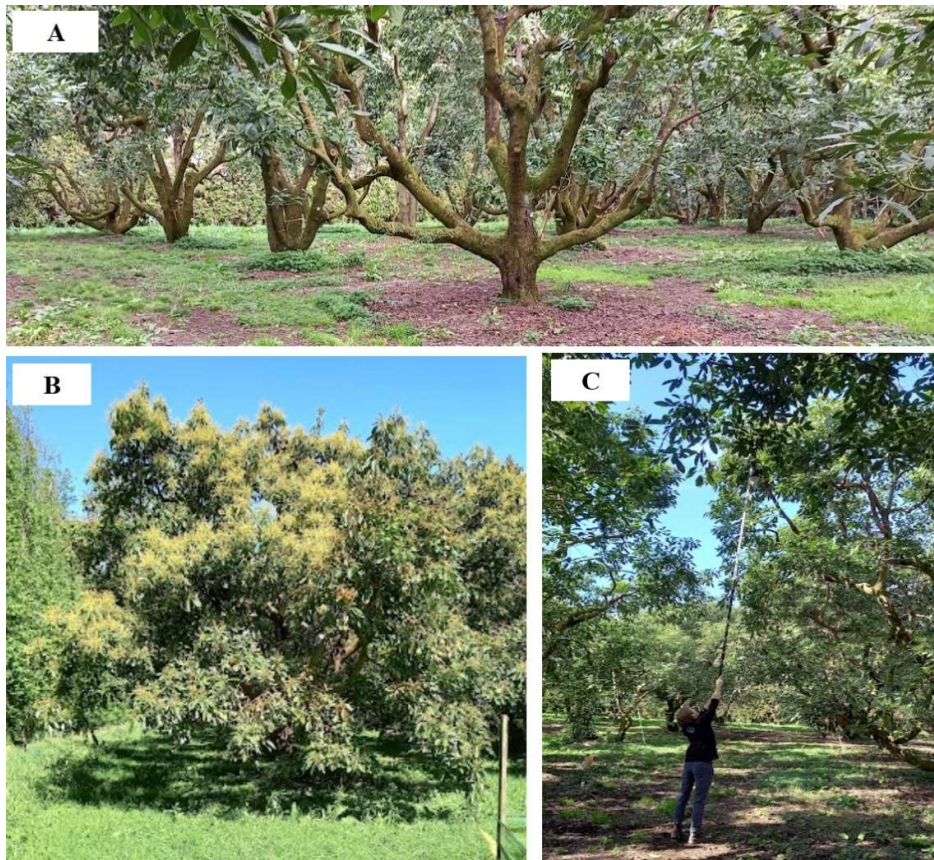


Figure 1.4- Main morphological characteristics of the avocado trees commonly found in the Bay of Plenty region of New Zealand and included in this research. Verticillate branching pattern(A). irregular canopy shape (B). Tree height between 8 to 14 m (C).

The growth of avocado shoots is developed in flushes during the year, typically with two flushes in the subtropic region and between two to six under lowland tropical conditions (Mickelbart et al., 2012; Schaffer et al., 2013). Under subtropical conditions, like in northern New Zealand, two flushes of shoot growth typically occur, the principal during spring and the second during summer (Mickelbart et al., 2012). Avocado shoots can show either indeterminate or determinate flowering, depending on the development of the final bud. If the final bud continues to develop vegetatively, the shoot is considered indeterminate. However, if the final bud develops into an inflorescence, the shoot is considered determinate (Chanderbali et al., 2013). Fruits on different sorts of shoots showed different nutrient compositions with likely effects on fruit quality. While fruits on indeterminate flowering shoots had higher concentrations of Ca and Mg than on determinate flowering, the K concentration is similar between both shoots (Boyd et al., 2007). The determinate flowering is prevalent in sun-exposed positions of the tree

canopy. In this research, most of the adult trees used had a more prevalent flowering of indeterminate type.

During the flushes of shoot growth, old and young leaves are present simultaneously on the shoot, going from being a net sink of nutrients when leaves are young to a net source of nutrients before abscission (Alcaraz et al., 2013). Avocado leaves vary in shape, margin, pubescence, size, and colour (IPGRI, 1995). Despite the avocado being an evergreen tree, the longevity of its leaves is short, lasting between 6 to 8 months, according to the growing site (Alcaraz et al., 2013; Salazar-García et al., 2015).

The growth of avocado roots is more constant than the shoot development during the year. However, the root growth is more active when the soil is warmer or the growth of shoots is less active (Mickelbart et al., 2012). Thus, during Autumn the root growth is more active (Salgado & Cautín, 2008). The root system is shallow, with limited extension from the canopy line, while root hairs are scarce or absent (Chanderbali et al., 2013). The highest density of active non-suberized roots (white colour), principally responsible for water and nutrient uptake in Hass avocados are found between 25 to 30 cm deep (Salgado & Cautín, 2008).

Reproductive bud development in avocados occurs in winter and autumn, promoted by low temperatures, while bud break and inflorescence emergencies occur in spring (Alcaraz et al., 2013; Salazar-García et al., 2013). Then, flowering usually occurs at the same time as the spring flush of growth, peaking at the end of the spring, with a variable length of flowering stage according to the variety and environmental temperature (Alcaraz et al., 2013). As described by Salazar-García et al. (2013), avocado is a cross-pollinated crop, with perfect and complete flowers opening twice a day, each time with a different sex to ensure the cross-pollination. Thus, varieties type A, such as Hass, open in the morning as female (receptive stigma), while in the afternoon, open as male (dehiscent anthers). On the other hand, varieties type B, such as Zutano, open in the morning as male and during the afternoon as female. In commercial avocado orchards of the BoP, a common combination of trees used in each block is of nine commercial Hass trees (Hass scion on Zutano rootstock) by one Zutano tree used as a polliniser. Avocado trees set less than 0.3% of flowers produced during the flowering stage (Salazar-García et al., 2013).

The avocado fruit is a single-seeded berry that, under subtropical conditions, follows a doubled sigmoidal growth pattern lasting around nine months from bud break in the late spring to harvest maturity in early spring the following year (Alcaraz et al., 2013). The fruit growth ceases only during winter with lower dry matter and mineral accumulation (Campisi-Pinto et al., 2017; Rosecrance et al., 2012). However, the fruit could be kept hanging on the tree up to 20 months (Hofman et al., 2013). Avocados ripen only after separation from the tree, being a climacteric fruit (fruit with ethylene production during ripening) (Bower & Cutting, 2011). During the fruit growth, one or two periods of fruitlet drop occur between late spring and early summer (Alcaraz et al., 2013). Despite avocado fruits reaching harvest maturity after approximately 9 months from bud break, fruits are commonly harvested when the dry matter reaches between 23% to 24% to ensure a better fruit quality (Burdon et al., 2008; Hofman et al., 2013). The shape of the mature fruit varies from spheroid to rhomboid, and the weight of the ripe fruit varies from 50 g to 2000 g (Chanderbali et al., 2013; IPGRI, 1995).

1.3.1.2 International avocado market

The Hass cultivar, the most commercialised worldwide, is a hybrid which originated in California (USA) based on cultivars from the Mexican and Guatemalan horticultural races (Rendón-Anaya et al., 2019). Over the last six decades, global Hass avocado production has increased tenfold to approximately 7.2 million tonnes in 2019. Mexico is the largest avocado producer, growing almost a third of the total global figure (Imbert, 2021). The global area planted on Hass avocados by 2021 was around 415 thousand hectares, with 75% growing in Latin America, while in other regions such as California, Africa, or Oceania growing between 4% to 9% (Imbert, 2021). The growing areas has increased exponentially in the last years since 2015, accounting for 130 thousand hectares just between 2015 to 2021 (Imbert, 2021).

The United States and the European Union are large importers of Hass avocado, importing approximately 2.3 and 1.3 millions of tonnes in 2019 respectively (Imbert, 2021). While those two traditional destinations accounted for almost 80% of the global Hass avocado demand, other markets such as Canada, Japan, China, or other Asian markets have participations less than 4% (Imbert, 2021). The dynamic of the avocado

sector worldwide is currently under assessment as prospective analyses of the production and consumption of avocado revealed that towards 2030 is likely to have a general production surplus (Imbert, 2021). This situation is pushing the industry worldwide not only to promote the internal consumption in local and Asian markets, where the avocado consumption is still low, but to develop the industry to produce under more sustainable practices and better fruit quality.

1.3.1.3 Avocado production in New Zealand

In New Zealand, the BoP and Northland regions are the main avocado growing districts, accounting for 92% of the 4912 hectares planted nationally in 2022 (United Fresh & New Zealand Incorporated, 2023). These two regions in the northern zones of the North Island are in a temperate subtropical climate region, between 35° and 39°S latitudes (Figure 1.1), differing in edaphoclimatic conditions. The BoP has well-drained Allophanic soils (Manaaki Whenua, 2019), 14.4°C mean daily temperature (9.9-19°C range), and 1628 mm mean annual rainfall (NIWA, 2023). The Northland region has predominantly granular soils near Whangarei (South Northland) and sandy soils in the Far North (Manaaki Whenua, 2019), 16.2°C mean daily temperature (12.5-20°C range) and 1080 mm mean annual precipitation, and is a warmer and drier region than the BoP (NIWA, 2023).

The growth cycle of avocados in New Zealand is determined by two flushes of growth in spring and autumn and a dormant period during winter (Figure 1.5). Flushes of shoot and root growth are simultaneous in the BoP, region where the present study is focussed, peaking in late spring (October-November) and summer (February-March). During spring, the growth of shoots is prevalent, whereas the root growth is more active in summer (Thorp et al., 1995). The fruiting period in New Zealand from flowering to harvest lasts up to 20 months, as depicted in Figure 1.5. Flowering happens in spring with the flush of growth, with about 50% of flowers opening during the first two weeks of November (Thorp et al., 1995). The fruit development takes between 10 and 11 months from the flowering peak and fruit set to the physiological maturity when the fruit is ready to be harvested between September and October during the following year (Year 1) (White, 2001). However, a premium volume of fruit is retained on the tree until January (Approximately 13 to 15 months after fruit set) (Everett et al., 2007). The fruit harvested

from September to October, considered the early harvest season, has approximately 24% dry matter, while the fruit harvested from January to February contains approximately 33% dry matter and is considered the late harvest season in New Zealand. As a result, the harvest season lasts approximately six months, from September to February (Figure 1.5, Year 1). Some fruit could be maintained on the tree for up to 20 months to supply the local market (White, 2001).

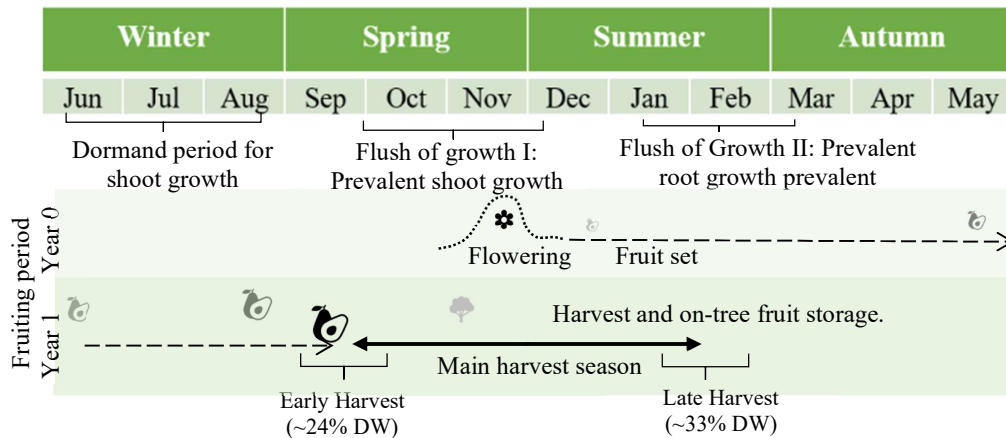


Figure 1.5- Representation of the fruiting cycle in Hass avocados in the Bay of Plenty region of New Zealand. Developed based on Thorp et al. (1995); White (2001); Everett et al. (2007)

1.3.2 Avocado fruit quality

The avocado fruit quality (FQ) could be defined alongside the value chain by different stakeholders according to the suitability of fruits for their intended use (Hofman et al., 2013). While for growers, the skin defects and fruit size could be relevant factors defining the avocado FQ during the pre-harvest stage, for consumers, the flavour and internal FQ disorders could be more relevant (Hofman et al., 2013), even defining the likelihood of repeating the purchase (Gamble et al., 2010). Overall, the most common FQ parameters alongside the avocado value chain include:

- Absence of external and internal FQ disorders/defects (explained in detail in Section 1.3.2.1),
- Organoleptic parameters (size, shape, texture, firmness, and flavour),
- Mineral and biochemical composition (Hofman et al., 2013).

The mineral composition is one of the most important parameters as it not only determines the nutritional value of a nutrient-rich food such as avocado (Bhuyan et al., 2019; Dreher & Davenport, 2013) but it is also related to the occurrence and severity of internal FQ disorders (Dann et al., 2016; Hofman et al., 2002; Marques et al., 2003).

1.3.2.1 Avocado fruit quality disorders and management strategies

Internal FQ disorders are the main challenge for the avocado industry worldwide, as they affect the consumer's decision for future purchases (Gamble et al., 2010). Those disorders are only detected by consumers at the end of the value chain, impacting the sustainability of the avocado industry in moments in which avocado consumption growth has begun to decline (Imbert, 2021). There are five common internal FQ disorders, two of which are fruit rots (i.e., stem-end rot (SER) and body rot (BR), the last known also as anthracnose or body patches under the skin) caused by specific fungi, and three physiological disorders caused by poor postharvest management (i.e. flesh bruising, diffuse flesh discoloration, and vascular browning (VB)) (White et al., 2005). In addition, there are other minor causes of internal quality disorders such as tissue breakdown, uneven ripening, pink staining, flesh adhesion to seed (White et al., 2005).

1.3.2.2 Fruit rots: the prevalent fruit quality disorders for New Zealand avocados

The market strategy for avocados produced in New Zealand is to release a premium quality product, but two fruit-rot types (i.e., stem-end rot (SER) and body rot (BR)) are the main internal FQ disorders threatening this goal of the avocado industry (Thorp et al., 1997). Stem-end rot and BR or anthracnose differs in symptoms but are caused by the same group of pathogenic fungi. Stem-end rot begins at the junction of the pedicel and the fruit flesh (Figure 1.6, A), whereas BR or anthracnose develops randomly throughout the body fruit, affecting the outer flesh and the inner peel with circular stains (Figure 1.6, B) (Everett et al., 2007).

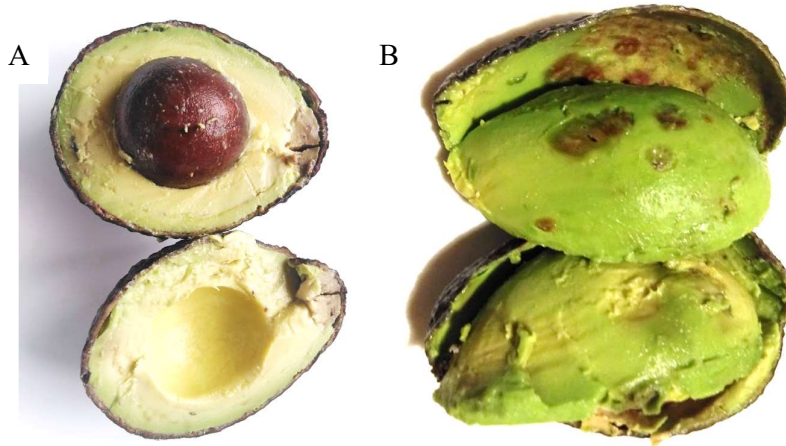


Figure 1.6- Photo of the symptoms for the two most common internal fruit rots in Hass avocados: Stem-end rot (A) and body rots (B)

The fungal inoculum that causes fruit rot is present on the avocado tree, but the disease mainly develops during postharvest. Five fungal species have been identified as the causal agents of both fruit rots: *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Botryosphaeria parva*, *Botryosphaeria dothidea* and *Phomosis* spp. (Everett et al., 2007). The source of inoculum is the dead material generally found in the tree canopy (i.e., leaves, twigs, mummified fruits) (Hartill & Everett, 2002). This inoculum usually reaches the fruit during the harvest period, especially if the fruit is plucked instead of clipped (Hartill & Everett, 2002). Spore germination is inhibited during winter, while the potential active period for spores on the canopy is triggered from September to April (Everett et al., 2007). The fungus *C.gloeosporioides* has shown increased virulence during postharvest on avocado fruits with the alkalization and the availability of ammonia-N in fruit skins (Drori et al., 2003; Wang et al., 2004). After spore germination, during the postharvest ripening stage, avocados begin to show symptoms of internal rot. This epidemiological behaviour for both avocado-fruit rots underscores the need for preventative practices throughout most of the growing season (Everett, 2020).

The incidence of fruit rot increases as the harvest season progresses. For example, avocados exported from New Zealand to USA that were harvested late in the 1999 season had a higher incidence of fruit rot than fruit harvested early in the season (White, 2001). In postharvest trials developed between 2002 and 2003 in the BoP, the incidence of anthracnose or body rot reached up to 43% for avocados harvested late in the season (February 2003), whereas the fruit harvested early in the season had a rot incidence of

only 16% (September 2002) (Dixon et al., 2003). This increase in fruit rot incidence for late harvested fruit is problematic as international demand, mainly from Australia the main external market for avocados in New Zealand, increases from January onwards (Gamble et al., 2010).

1.3.2.3 Preharvest practices to mitigate avocado fruit rots

Practices to reduce avocado fruit rot can be divided into three mitigation strategies: Reduction of fungal inoculum, provision of a suitable environment for tree development, and the improvement of the fruit nutrient composition (Everett, 2020). In the forthcoming sections the most relevant literature is discussed about these strategies.

1.3.2.3.1 Reduction of fungi inoculum of avocado fruit rots

The use of a minimum eight copper-based fungicide (CBF) sprays per season has been shown to be the most effective protection against the development of fruit rots in New Zealand (Everett et al., 2007). Fungicides with other active ingredients to control avocado fruit rot pathogens have either been discontinued (e.g. Benomyl[®]) or are lacking approval for use in avocado orchards in New Zealand (e.g. boscalid/pyraclostrobin, fluazinam) (Everett et al., 2009; Everett & Timudo-Torrevilla, 2007). Pathogenic-biological control agents such as the use of the bacterium *Bacillus subtilis* has not proven to be effective in reducing fruit rot in New Zealand (Everett et al., 2009).

Despite the effectiveness of CBFs for crop protection, increased copper (Cu) accumulation in the topsoil is a major environmental challenge for avocado fruit production worldwide. According to studies performed in avocado orchards in Australia, orchards sprayed annually with CBFs up to 15 times per year to control fruit rots show reductions in earthworm numbers (Van Zwieten et al., 2004) and soil microbial biomass (Merrington et al., 2002). Toxic effects on soil biology have been observed in the topsoil of commercial avocado orchards when the Cu concentrations exceed 270 mg Cu/kg due to the use of CBF (Merrington et al., 2002; Van Zwieten et al., 2004). In New Zealand, a survey of 157 avocado orchards revealed that topsoil Cu levels varied from 4 to 541 mg/kg, with 2% of the orchards exceeding 370 mg/kg (Sorensen, 2017). The Cu-trigger level for further action in New Zealand soils is 100 mg Cu/Kg (NZWWA, 2003), prompting the avocado industry to seek alternative approaches to reduce fruit rots.

1.3.2.3.2 Provision of a suitable environment for tree development

Root protection, soil moisture, and aeration are environmental conditions that support crop health in avocados, and these parameters have both direct and indirect relationships with the incidence of fruit rots. These conditions depend on agronomic practices such as mulching, irrigation, and pruning. Mulch type and depth are directly related to the incidence of fruit rots (Everett et al., 2007). Avocado-leaf litter depth has been shown to have a significant relationship with the incidence of both stem-end and body rots, while stem-end rot is also significantly influenced by the depth of non-leaf litter mulch (Everett et al., 2007). In a study of two orchards in the BoP, avocado-leaf litter was associated with a lower incidence of anthracnose and stem-end rots (8.5 and 4.5% respectively) than pine peel mulch (10.5 and 7.5% respectively) or compost (17.7 and 7.5% respectively), but none of these differences were statistically significant (Dixon et al., 2007). These studies did not analyse mulch or fruit composition, but such results could be important due to the role of avocado leaf litter in improving topsoil nutrient status in avocado orchards (Murovhi & Materechera, 2015; Tamayo-Vélez & Osorio, 2018) and in reducing avocado fruit rot (Dixon et al., 2007; Everett et al., 2007). No other studies have been reported in the literature to understand the role of leaf litter in avocado fruit rots.

Irrigation and pruning have an indirect effect on fruit rot by helping to increase fruit Ca concentration and reducing fungal inoculum. In terms of fruit Ca concentration, irrigation helps to maintain transpiration flux for Ca translocation into the fruit during flowering and fruit set (Hocking et al., 2016). Irrigation regimes that maintain soil moisture tension between 35 and 55 kPa (soil moisture close to field capacity) have been shown to achieve the highest Ca concentration during flowering and fruit set when compared with less frequent irrigation (Bower, 1985). Furthermore, pruning alters the source-sink relationship of mineral partitioning between vegetative and reproductive organs, supporting an adequate mineral balance in avocado fruit (Mullen, 2015) (see section 1.3.3.2). Pruning also removes fungal inoculum on dead twigs, leaves and mummified fruit, and facilitates the penetration of CBF into the canopy (Hartill & Everett, 2002).

1.3.2.3.3 Improvement of fruit nutrient composition

In New Zealand, two surveys in Hass avocado orchards reported low incidences of fruit rots (BR or SER) and vascular browning in fruits with increased fruit flesh Ca

concentration and Ca+Mg:K ratios (Everett et al., 2007; Thorp et al., 1997). Indeed, Thorp et al. (1997) noted that Hass avocados with flesh Ca concentration over 280 mg Ca/kg or Ca+Mg:K ratios exceeding 0.0625 were not affected by internal FQ disorders at postharvest. In addition, a positive correlation between the anthracnose or BR severity and the N:Ca ratio in skins of Hass avocado fruits was reported by Willingham et al. (2001). This ratio analysed in skin of unripe Hass avocados proved to be the most consistent indicator of BR and SER incidence or severity in Australia between 2008 and 2012 (Dann et al., 2016). Moreover, the N:Ca ratio was suggested as a norm for exporting Pinkerton avocados from South Africa, aimed at mitigating internal physiological disorders such as grey pulp and cold injuries. According to Snijder et al. (2002), for Pinkerton avocados produced in South Africa, the optimal flesh N:Ca ratio during early harvest was below 20, while during late harvest it was below 25. Overall, improving fruit nutrient composition with higher Ca concentration, higher Ca+Mg:K, and lower N:Ca ratio has resulted in improved FQ in avocados worldwide (Dann et al., 2016; Escobar et al., 2021; Marques et al., 2003; Ullah & Joyce, 2024; Willingham et al., 2006).

The principal pre-harvest factors influencing fruit Ca concentration include rootstock/scion genetics (Dann et al., 2016; Whiley, 2013a; Willingham et al., 2006), Ca partitioning between vegetative and reproductive tissues (Mullen, 2015; Willingham et al., 2004; Witney et al., 1990b), and the orchard fertiliser practices (Du Plessis & Koen, 1987; Hofman, 2007; Willingham et al., 2006). As detailed in the section 1.3.3.2 Hass avocados produced on trees with Mexican-derived genetics concentrate less Ca and more N and K than avocados produced on trees with West Indian or Guatemalan-derived rootstocks. This effect has been discussed due to the strong filter effect exerted by Mexican-derived rootstocks compared to other rootstock genetics (Dann et al., 2016; Lazare et al., 2020; Willingham et al., 2001, 2006). Furthermore, avocado fruits are strong sinks for N (Zilkah et al., 1987) and weak for Ca (Cutting & Bower, 1989), with greater leaves-to-fruit ratios potentially affecting the fruit Ca concentration relative to N (Mullen, 2015; Willingham et al., 2004; Witney et al., 1990b).

Regarding fertiliser practices, as described in detail in section 1.3.3.3, there is limited literature contributing to understanding how to improve fruit nutrient composition by modifying on-orchard fertiliser practices in Hass avocados. Two replicated experiments

in Hass avocados in Australia have reported the effect of N, K, and Ca fertiliser treatments on the fruit nutrient composition. Willingham et al. (2006) investigated the effect of N fertiliser on fruit nutrient composition in skins using two fertiliser rates applied as either ammonium-N or nitrate-N, compared to a control without N fertiliser use. This experiment found that the two N fertiliser treatments resulted in higher fruit skin N concentrations and N:Ca ratios compared to the control, in two out of four years of the study (Willingham et al., 2006). While Hofman (2007) tested Ca fertilisers alone or in combination with K fertilisers to understand their effects on avocado fruit nutrient composition. This experiment found that Ca concentration and the ratio Ca+Mg:K ratio decreased in fruit skin in treatments with combined use of Ca and K (Hofman, 2007). Additionally, Arpaia et al. (1996) found an increased flesh N concentration with the highest N fertiliser rate used in California (136 kg/ha/year).

Consequently, despite understanding that increased fruit nutrient composition can contribute to producing better FQ in Hass avocados, there is insufficient experimentation aimed at understanding how to improve this composition through fertiliser practices.

1.3.2.4 Understanding the protective role of an improved fruit nutrient composition

The role of high Ca concentration in fruit tissues ensuring better FQ is attributed to its instrumental role in cell wall integrity. This bivalent cation (Ca^{2+}) forms cross-linked structures along with free-negative positions in pectins (a complex family of polysaccharides), providing rigid structures in plant tissues (Hepler & Winship, 2010). During ripening, the softening process in fruits is attributed to the general de-esterification process of pectins by enzymes, in which carboxyl residues that Ca can cross-link are exposed (Hocking et al., 2016). The availability of Ca during the de-esterification process confers the physical properties of cell walls in fruit tissues, such as their strength and elasticity (Hocking et al., 2016). In contrast, during the fruit decay process produced by a fungal attack, the de-esterification process is localised and triggered by the action of fungi-released enzymes (Hocking et al., 2016). Under *in-vitro* conditions, high Ca concentration in the skin of grapefruits inhibits the digestion process by fungi enzymes of *Botrytis cinerea* (Chardonnet et al., 1995). The inhibitory effect of Ca on fungi-released enzymes producing fruit decay is highly variable according to

factors such as the genetic variability of both the pathogen and the fruit plant (Chardonnet et al., 1995; Chardonnet et al., 2000). Ultimately, high Ca concentrations serve as a protective mechanism against the enzymatic degradation promoted by a fungal attack, thereby enhancing FQ and extending shelf life.

Improved relationships between Ca and other nutrients, such as N and K, have consistently resulted in better FQ outcomes in avocados, as discussed in section 1.3.2.3.3. Even the mechanisms behind the protective effect of low N:Ca or high Ca+Mg:K ratio has yet to be widely studied in Hass avocados; the competency between Ca and mobile nutrients such as N and K could contribute to explaining the protective effect of the desired ratios. High N concentration and low Ca concentration could facilitate an excessive availability of N in fruit skins, a co-factor increasing the virulence of *C. gloeosporioides*, the primary causal agent of BR in Hass avocados. A pH of 6.0 and excessive N availability in fruit skin has been identified as the main co-factors facilitating the production of the enzyme pectate lyase by the fungi *C. gloeosporioides*, triggering the cell wall degradation and further development of fruit rot symptoms in avocados (Drori et al., 2003; Kramer-Haimovich et al., 2006).

In terms of the Ca+Mg:K ratio, it highlights the antagonism between the divalent cations Ca and Mg against the monovalent K. An antagonist effect in the transport of K against Ca is of particular interest as avocado trees have an inherent ability for translocating K to fruits, K being the nutrient most extracted by avocado fruits (Lahav & Kadman, 1980; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023; Rosecrance et al., 2012). In avocado fruit tissues, changes in Ca concentration rather than K concentration have generated different Ca+Mg:K ratios in fruit tissues (Everett et al., 2007; Hofman, 2007). For example, as discussed by Hofman (2007) a competitive effect between K fertiliser use and Ca in fruits has been reported, as treatments with K fertilisers decreased the Ca concentration in fruit skin and the Ca+Mg:K ratio.

Despite the general trend of an increased incidence and severity of internal fruit rots with lower Ca+Mg:K ratios (Hofman, 2007; Hofman et al., 2002; Marques et al., 2006; Ullah & Joyce, 2024; Willingham et al., 2006), the protective role of K fertiliser use in reducing fungi-caused diseases in plants has also been recognised. Perrenoud (1990) reported

positive effects of K fertiliser use in 70% of 1549 studies relating to K fertiliser use and incidences of fungal diseases, including fruit diseases. A high K status in plants produced by fertiliser management could ensure the activation and transport of preformed and inducible self-defence mechanisms against pathogenic fungi (Amtmann et al., 2008; Zörb et al., 2014). In avocados, preformed anti-fungal metabolites has been identified to be produced in young leaves and translocated through the phloem conferring resistance to the fungal attack of *C. gloeosporioides* (Drori et al., 2003; Wang et al., 2004), the main causal agent of fruit rots. Thus, there is a requirement to understand the real effects of K fertiliser use in both the fruit K concentration and the incidence of internal FQ disorders in avocados. This understanding could be achieved by quantifying the effect of different K fertiliser rates or exploring K fertiliser management, avoiding the antagonism with Ca, especially during the critical stage for Ca uptake and translocation.

1.3.3 Fertiliser practices in avocado

Nutrient removal by avocado fruits serves as an initial indicator of nutrients that need to be replaced through fertiliser practices in the avocado crop for the next season. Research on Hass avocados worldwide (Huett & Dirou, 2000; Lahav & Kadman, 1980; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023; Rosecrance et al., 2012) demonstrates that K followed by N are the two nutrients most extracted by avocado fruits (Table 1.1). The extraction rate of N and K per tonne of avocados varies between 1 and 6 kg/tonne/year, with K being extracted 20% to 80% more than N. In contrast, the extraction of other mineral nutrients such as P, Ca and Mg barely reaches 1 kg/tonne/year. Despite the simplicity of this indicator, the fertiliser quantity applied each season may vary widely under similar agroecological conditions due to several required local adjustments. Among the factors involved in these adjustments are the orchard's yield potential, tree's age and biomass (Lahav et al., 2013), alternate bearing (Lovatt, 2001), nutrient losses (i.e., leaching, volatilisation, soil retention, and runoff) (Huett & Dirou, 2000), internal nutrient remobilisation before leaves abscission (Salazar-García et al., 2017) and cycling throughout the litterfall (Tamayo-Vélez & Osorio, 2018). Additionally, avocado fruits demand lower quantities of mineral nutrients compared to other plant organs (Witney et al., 1990b), which has led to the adoption of leaf sampling analysis as a decision-making tool to adjust fertiliser practices in avocados. This approach aims to maintain a desired leaf nutrient concentration to support tree performance (Lahav et al., 2013; Salazar-García et al., 2015).

Table 1.1- Quantity of nutrients removed by each tonne of Hass avocados produced in different avocado producing countries

Nutrient	Israel [†] (Lahav and Kadman, 1980)	Australia [†] (Huett and Dirou, 2000)	California, USA [†] (Rosecrance et al., 2012)	Mexico [‡] (Maldonado et al., 2007)	Colombia [‡] (Rebolledo and Burbano, 2023)
--- kg / tonne ---					
N	1.1	4.1	2.2	2.7	2.6
P	0.2	0.8	0.4	0.3	0.2
K	2.0	6.1	3.0	3.3	3.4
Ca	0.2	0.7	0.1	0.2	0.1
Mg	0.5	0.8	0.3	0.3	0.2

[†]Nutrient remotion calculated for a yield potential of 10 tonnes/ha

[‡]Nutrient remotion calculated for a yield potential of 20 tonnes/ha

Mineral nutrient levels in mature leaves are the most common indicator used to adjust fertiliser practices in avocados worldwide (Lahav et al., 1990; Salazar-García et al.,

2015), despite the development of other plant nutrient indicators such as fruits, flowers or pedicels (Campisi-Pinto et al., 2017; Razeto & Salgado, 2004). Leaves sampled from non-fruiting shoots at the end of the summer flush of growth are used as an indicator of the nutrient availability to sustain the fruit load and tree performance during the following season (Lahav et al., 1990; Salazar-García et al., 2015). Thus, ranges of nutrient concentrations, used as norms to make decisions about fertiliser practices, have been established in different countries producing Hass avocados (Dixon, 2008; Lahav & Kadman, 1980; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023). Some of the leaf nutritional norms used for fertiliser consultants in New Zealand are described in the forthcoming section 1.3.3.1 .

Nitrogen status is the most extensively studied aspect of tree nutrition in avocados worldwide (Lahav et al., 2013). Embleton et al. (1959) establish a curvilinear relationship between the N concentration in leaves and yield, recommending the maintenance of N levels in leaves of Fuerte avocados around 1.8% (18 g N/kg). In Hass avocados, the optimal N target is established in several studies; for example, a leaf N concentration of around 2% (20 g N/kg) is established in Mexico (Maldonado-Torres et al., 2007) and Colombia (Rebolledo-Roa & Burbano-Diaz, 2023) to achieve a yield of 20 t/ha/year. Arpaia et al. (1996) found that increasing leaf N concentrations beyond 2% does not significantly enhance the yield of Hass avocados. In New Zealand, Dixon (2008) reports leaf N concentrations between 2.4% and 2.6% for orchards yielding between 20 and 25 tonnes/ha/year in the BoP, while West (2020) notes that fertiliser consultants in the country generally use a wide range of leaf N concentration as a norm (between 2% and 3%). However, Lovatt (2001) emphasizes that there is no correlation between leaf N concentration and yield in several California studies when the experiments are conducted in alternate bearing orchards.

The quantity and timing of N fertiliser use have been reported as relevant factors to ensure an adequate avocado tree performance. Several authors reported N fertiliser rates around 50 kg/ha/year as an adequate fertiliser rate (Lahav & Kadman, 1980; Lovatt & Witney, 2001; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023). In addition, around 80% of that N fertiliser rate was estimated by Huett & Dirou (2000) enough to compensate for different fertiliser application inefficiencies in Australia.

Although these general N fertiliser rates never reach 100 kg N/ha/year, the overall N fertiliser rates used by growers in Australia has been reported as 130 kg N/ha/year (Willingham et al., 2006); between 84 to 168 kg N/ha/year, with some growers exceeding that range in California (Lovatt, 1995); or between 250 and 300 kg N/ha/year in Israel (Silber et al., 2018).

In terms of the N fertiliser timing, Lovatt (2001) compared the total N rate used in California (168 kg N/ha/year) divided into five applications of 28 kg N/ha/year in key phenological stages as a control treatment against doubling the rate to 56 kg N/ha/year in one out of the five applications. This work showed that doubling the N fertiliser rate in April, during the anthesis to early fruit set in California, increased the 4-year yield, especially to produce commercial valuable fruit (fruit weight of 178 to 325 g) compared to the control. Besides, this treatment in April reduced the alternate bearing index (ABI) compared to the control. Meanwhile, doubling N fertiliser use in November, the end of the summer flush of growth (in North latitude regions), increased the 4-year yield and the biggest fruit size production, but without effect on the ABI compared to the control. In further similar research, Salvo & Lovatt (2016) confirmed the relevance of using enough N fertiliser during all the critical phenological stages, especially during summer, to support the multiple phenological processes that occurred at the same time in that season. Thus, the recommended timing for N fertiliser was to apply N fertiliser evenly or in April during the early fruit set to reduce the potential for N leaching (Salvo & Lovatt, 2016).

Foliar N fertiliser use has been a practice with contrasting results but used in subtropical conditions to reduce freezing damage in leaves during winter. Zilkah et al. (1987) demonstrated that urea applied to foliage is actively translocated to young leaves and fruits during the summer flush of growth. However, other studies have reported the challenges of foliar urea application in avocado trees, with insignificant changes in leaves N concentration by their use (Nevin et al., 1990). Indeed, replacing soil-applied N with foliar applied using biuret urea during pre-flowering to early fruit set, resulted in low yield in California, suggesting a toxic effect of urea applied at this stage (Salvo & Lovatt, 2016). However, the application of biuret urea to leaves in winter has been demonstrated to protect against freezing under subtropical conditions (Mandemaker, 2007; Zilkah et al., 1996).

Potassium fertiliser use has received less attention than N fertiliser use, despite K being the mineral nutrient most concentrated in avocado fruits (Huett & Dirou, 2000; Lahav & Kadman, 1980; Rosecrance et al., 2012). Lahav et al. (1976) reported that applying 450 kg K/ha/year increased the height of Hass avocado trees by 20% compared to treatments without K fertiliser, but had no effects on yield or mineral concentration in leaves. Similarly, Koen & Du Plessis (1992) observed an increase in leaf K concentration with the application of K fertiliser, but found no correlation between leaf concentration and yield. Additionally, Hofman (2007) showed that avocado yield remained unaffected when K fertiliser was not used for several seasons, even when leaf K concentration decreased to 0.6%.

Excessive use of K fertiliser is associated with potential reductions in yield and negative effects on fruit quality, though there is insufficient experimental evidence to confirm these trends. Modelling of yield in Hass avocados produced in California predicts yield suppression in trees with leaf K concentrations exceeding 1.0%, potentially inducing alternate bearing in 36% of trees when leaf K reaches 1.4% (Crowley & Campisi-Pinto, 2016), but there is no experimental evidence for those reductions. Besides, the literature suggests that the excessive use of K fertiliser could have a negative effect on FQ through a reduction in fruit Ca concentration, as discussed in section 1.3.3.2 .

1.3.3.1 Fertiliser practices in New Zealand avocado orchards

The aim of fertiliser programs in New Zealand is to maintain threshold levels of fertility based on leaves and soil analyses developed in May (late autumn) after the cessation of the summer flush of growth (Dixon, 2008; West, 2020). These fertiliser programs primarily aim to adjust soil pH, and six nutritional parameters derived from the leaf analyses such as the level of the macronutrients N, K, Ca and Mg and the micronutrients B and Zn (New Zealand Avocado Growers Association, 2000). Fertiliser rates in each orchard are calculated by fertiliser consultants through comparison of annual leaf tests with nutritional norms (Dixon, 2008; West, 2020). Table 1.2 summarises some of the most common ranges of target foliar concentrations used by fertiliser consultants in New Zealand to recommend annual fertiliser programs.

Table 1.2- Ranges of optimal nutrient concentration in leaves used as fertility targets in New Zealand: A- Range levels for high-performance avocado orchards according to New Zealand Avocado Growers Manual; B- Range levels for a yield between 20-25 t/ha in the Bay of Plenty region (Dixon,2008); C- Consolidated range levels used from different fertiliser consultants in New Zealand (West, 2020).

Nutrient	Unit	Range		
		(A)	(B)	(C)
N	%	2.5 - 2.9	2.4-2.6	2.0-3.0
P	%	0.16-0.22	0.13-0.16	0.08-0.22
K	%	1.0-1.2	0.9-1.2	0.75-2.0
S	%	0.3-0.4	0.22-0.31	0.2-0.6
Ca	%	1.8-2.5	1.2-1.8	1.0-3.0
Mg	%	0.5-0.7	0.35-0.44	0.25-1.0
Zn	mg/kg	60-100	35-68	25-150
B	mg/kg	40-60	21-44	25-100

According to the New Zealand avocado growers manual (New Zealand Avocado Growers Association, 2000), the most common fertiliser practices in New Zealand to reach and maintain the fertility parameters set by the industry include:

- The side-dressing application of Ca sources in soil amendments such as lime and gypsum used to adjust soil pH, although gypsum is also commonly applied as a preventative measure against root rots.
- The side-dressing application of fertiliser blends to fulfil the requirements of macronutrients such as N, P, K, S, Mg, and Ca. These applications are made from mid-August (late winter) and early April (early autumn) each season, using fertiliser spreaders or by hand.
- Boron (B) and zinc (Zn) are micronutrients commonly applied by foliar sprays during the season.
- Urea is also sprayed in winter to help maintain winter photosynthesis and reduce the yellowing of leaves during that season (Mandemaker, 2007; Zilkah et al., 1996).

Although most consultants generally set the same fertility parameters, annual fertiliser rates in New Zealand vary widely depending on the target levels of leaves mineral concentration used, the experiences of consultants, fruit load, tree health, and grower's expectations during the season among other considerations (West, 2020). For example, N

and K fertiliser rates in New Zealand avocado orchards using Ballance® products ranged from 50 to 300 kg/ha/year in 2020 (Dowson A. -Ballance Agri-Nutrients, personal communication).

Dixon (2008) analysed leaves mineral nutrient concentrations in New Zealand avocado orchards, classifying them according to different yield categories. However, fertiliser programs used for high-performance avocado orchards in New Zealand have yet to be established. Moreover, the impact of those fertiliser programs on fruit mineral composition and FQ is a gap in the knowledge identified in Hass avocado orchards from New Zealand.

1.3.3.2 Understanding the fruit mineral nutrition

Avocado fruits under subtropical conditions have a doubled-sigmoid growth pattern, reducing their mineral accumulation during winter (Campisi-Pinto et al., 2017; Rosecrance et al., 2012). Calcium has a different pattern of accumulation in fruits than N and K. Calcium moves upward in plants through the xylem using transpiration flow (Hocking et al., 2016; Song et al., 2018). In avocado fruits, Ca enters mainly during the first 12 weeks of the fruit set (Mullen, 2015; Witney et al., 1990a). After that, a reduction in the xylem vessel function at the pedicel of ripe fruits reduces the Ca supply to avocado fruits (Kaiser, 1993; Song et al., 2018). Thus, ensuring Ca availability during the early fruit set is a common goal recommended in literature after Witney et al. (1990a), but with limited evidence based on experimentation, as pointed out by Perkins et al. (2021). In contrast, photoassimilates and mobile minerals such as N and K move actively through the phloem to accumulate in the fruit (Rosecrance et al., 2012).

At least three main factors affect the translocation of nutrients related to Ca concentration up to the fruit: 1. the source-sink relationship between vegetative and reproductive tissues on the scion; 2. the filtering effect of rootstocks, and 3. the uptake of nutrients from the topsoil.

Regarding the source-sink relationship, flower panicles and fruitlets are weaker Ca sinks than leaves in avocado trees (Cutting & Bower, 1989). In addition, the leaf Ca concentration increases steadily over their lifespan (Minchin et al., 2015; Salazar-García

et al., 2015), implying that avocado leaves are net Ca sinks that compete with reproductive tissues for available Ca. In the case of N and K, leaves act as sources of nutrients for fruits from the beginning of summer up to their abscission (Salazar-García et al., 2015). Consequently, more leaves per fruit or higher leaves-to-fruit ratios reduce the Ca concentration in fruits relative to N and K (Mullen, 2015). Moreover, in avocado trees with similar canopy volume, fruits on more productive trees (lower leaf-to-fruit ratio) concentrate higher Ca and less K and N than those on less productive trees (Hofman et al., 2002).

The genetic of rootstocks has demonstrated to exert a filtering effect on Ca flux between the scion and upward tissues, with this effect being stronger for Mexican-derived rootstocks compared to Guatemalan- or West Indian-derived rootstocks (Dann et al., 2016; Lazare et al., 2020; Willingham et al., 2001, 2006). For example, in Australia, Hass avocados grafted on Velvick rootstocks (Guatemalan horticultural race) concentrated more Ca and less N than fruit produced on Hass trees grafted on Duke6 or Duke7 (Mexican race) (Dann et al., 2016; Hofman et al., 2002; Marques et al., 2003, 2006; Mullen, 2015; Willingham et al., 2001, 2006). Consequently, the use of Mexican rootstocks have resulted in a Ca filter effect, leading to higher N:Ca ratios and have increased the production of unsound fruit in Hass avocados (Dann et al., 2016; Hofman, 2005; Marques et al., 2003; Willingham et al., 2001). Overall, the Guatemalan or West-Indian rootstocks have been recommended over Mexican rootstocks to improve the fruit Ca concentration of Hass avocados and the FQ outcomes. This effect could be relevant for New Zealand avocados because most trees are grafted on Zutano rootstocks, a Mexican-derived rootstock.

Avocado trees take up nutrients primarily through non-suberised roots located superficially between the topsoil and the leaf-litter or mulch layer. Partitioning analyses of nutrients in Hass avocado trees have shown that the concentration of Ca and K in non-suberised roots is just exceeded by the concentration of these nutrients in aboveground tissues, which are reported to be net sinks for these elements (i.e. leaves and fruit tissues for Ca and K respectively) (Witney et al., 1990b). Therefore, the availability of nutrients in the litterfall increases with time since the deposition, becoming an important source of

this nutrient in avocado systems (Murovhi & Materechera, 2015; Tamayo-Vélez & Osorio, 2018).

Although the pattern for mineral accumulation in avocados until the harvest maturity has been studied, as described in the previous paragraphs, insufficient attention has been paid to changes in fruit mineral composition of avocados hanging on the tree for longer time. However, in temperate subtropical conditions like those found in the BoP of New Zealand, avocados can hang on the tree for longer periods, leading to an increase in dry matter content from around 24% when avocados reach harvest maturity to 33% or more, approximately 6 months after. It is worth noting that the effects of this prolonged hanging on the tree, on the fruit mineral composition remained unknown.

1.3.3.3 Fertiliser practices to increase calcium concentration in avocado fruits

The leading practices used to increase the Ca concentration in the fruit are the application of soil amendments, solid fertilisers, and foliar sprays. Soil Ca inputs in avocado orchards are mainly related to soil pH correction and aluminium neutralisation. Two specific soil fertiliser use trials have been reported in avocado orchards looking to increase the fruit Ca concentration.

One trial found significant increases, reaching 40% related to the control, of fruit Ca concentration at the highest gypsum and dolomitic lime rates tested (13.7 and 7.1 tonnes/ha/year, respectively). However, other Ca sources, such as Ca silicate and Ca hydroxide, did not increase fruit Ca concentration (Du Plessis & Koen, 1987). In addition, the use of Ca sources other than gypsum increased soil pH and yield but not the fruit Ca concentration in a red clay soil (Soil pH 4.8, 90 mg Al/kg) over the three year experiment, highlighting the challenge of effectively improving fruit Ca concentration (Du Plessis & Koen, 1987).

The other study evaluated Ca and K fertiliser treatments in a ferrosol in Western Australia over four years (Hofman, 2005, 2007) from pre-flowering. It showed four relevant consequences for avocado fertiliser management: 1. K fertiliser made from pre-flowering to fruit set increased K in fruits and reduced Ca concentration. 2. The only treatment

significantly increasing fruit Ca concentration was the Ca fertiliser alone from pre-flowering onwards. 3. Despite treatments with Ca saturated the soil and xylem sap with Ca, the fruit Ca concentration did not increase further. 4. There was no detrimental effect on yield in a scenario where no K fertiliser was used during the season, even at a leaf K level of 0.58% (Hofman, 2005, 2007).

Foliar sprays can also be used to deliver Ca to the fruit. However, the feasibility of foliar Ca fertiliser in avocados is limited due to physiological and anatomical characteristics. As explained previously (refer to Section 1.3.3.2), avocado leaves act as a net Ca sink. Additionally, mature leaves develop a waxy layer on the adaxial surface and wax deposits on the abaxial surface to protect the stomata from dehydration (Whiley et al., 1988). These factors may limit the transport of Ca from leaves to fruit and the uptake of foliar Ca. In fact, a study in Hass avocados evaluating the efficacy of foliar Ca sprays to increase fruit Ca concentration did not find effects on the fruit Ca concentration or FQ in New Zealand (Partridge et al., 2002).

In other crops, such as kiwifruit, CaCl_2 sprays have been shown to increase the Ca concentration when the pH of the spray solution was maintained at 5.5 (Hashmatt et al., 2019). However, there are no reports in the literature on the effect of CaCl_2 sprays on the Ca concentration in avocado fruit. One possible reason is the sensitivity of avocados to chloride toxicity, which may limit the use of CaCl_2 in this crop (Bar et al., 1987; Crowley & Campisi-Pinto, 2016).

In line with the findings of Hofman (2007), alternative Ca fertiliser management looking at increasing Ca concentration and avoiding the K competition should be tested. Thus, one alternative is the use of calcium nitrate (CN) in the soil as a soluble source of Ca during critical times for Ca uptake and translocation to the fruit (i.e., between pre-flowering to early fruit set, according to Witney et al.(1990a)). Besides, delaying K fertiliser use until after the early fruit set to avoid competition with Ca could complement this fertiliser strategy. Using CN has increased fruit Ca concentration in apples, pears, and cherries under high soil Ca concentration (Motesarezadeh et al., 2021).

1.3.3.4 Fertiliser practices to reduce the Nitrogen: Calcium ratio in avocado fruits

Modifications to N fertiliser practices have been recommended to decrease fruit N concentration and N:Ca ratio, which could potentially impact the incidence of internal FQ disorders, but little empirical evidence has been reported so far in avocados. Willingham et al. (2006) reported higher fruit skin N concentration and N:Ca ratio using the grower's fertiliser rate or doubled that rate compared to the control treatment without N fertiliser use. The lower N:Ca ratio for fruit skins in the control treatment was associated with lower incidences of anthracnose or BR in one of the years of the study (Willingham et al., 2006). Arpaia et al. (1996) found an increased flesh N concentration with the highest N fertiliser rate used in California (136 kg/ha/year), increasing the incidence of chilling injuries and reducing the fruit size over time with this treatment. An indirect effect of N fertiliser use on the FQ of Hass avocados could also be associated with the fact that excessive N fertiliser use could induce an increased leaf-to-fruit ratio, which produced a decline in the fruit Ca concentration (Mullen, 2015).

In other fruit crops, higher N fertiliser rates resulted in fruit tissues with high N and N:Ca ratios. For example, in apples, N fertiliser rates between 0-200 kg N/ha/year resulted in an increase in N concentration and a decrease in Ca concentration in the last two seasons out of the three seasons of the study, affecting the N:Ca ratio in fruit tissues (Nava & Dechen 2009). Similarly, in almonds, N fertiliser above 300 kg/ha consistently altered the N concentration in almond fruits over four years of the experiment, even though Ca concentration only reduced in one out of the four years (Muhammad et al. 2015). Besides, the timing of N fertiliser application showed changes in the fruit N concentration. In pears, a reduction in the N:Ca ratio and improved fruit quality occurred when N fertiliser was applied three weeks before harvest (Sugar et al., 1992). The rationale behind this practice was that applying N close to the harvest increased N reserves for the next season but not for the fruit harvested in the same season, as later demonstrated by Quartieri et al. (2002) also in pears. However, the applicability of this practice needs to be assessed in an evergreen fruit crop such as avocados.

Consequently, more research should be conducted under replicated and controlled conditions concerning the effect of N and K fertiliser practices used in Hass avocados on fruit mineral composition. This gap is especially relevant as N and K are the most concentrated nutrients in fruit tissues. Moreover, as highlighted by Perkins et al. (2021), even though improving fertiliser practices is a common suggestion in studies working to achieve a better FQ facilitated by an improved fruit nutrient composition, few studies define how to improve those practices to achieve the desired composition.

1.4 Main findings identified for the present research project

- The pattern of mineral accumulation in avocados up to harvest maturity has been studied, but insufficient attention has been paid to changes in the mineral composition of avocados hanging on the tree after fruit could be harvested. As in New Zealand, fruit could hang on the tree for at least 6 months after this point, there is a need to determine if these fruits change their mineral composition with potential implications for FQ.
- One primary nutritional strategy identified for reducing avocado fruit rots is to enhance fruit nutrient composition. This involves increasing the concentration of Ca in the fruit, increasing the Ca+Mg:K ratio, and reducing the N:Ca ratio. However, there is few experimental evidence aimed at understanding how fertiliser practices can improve the fruit composition.
- There is a significant variability in fertiliser use programs in Hass avocado orchards in New Zealand. There is need to establish fertiliser programs used in high-performance avocado orchards and study their impact on fruit nutrient composition. Consultants employ a variety of criteria to set these programs, including nutrients like N, K, and Ca, which could impact fruit composition and FQ.
- In New Zealand, the incidence of fruit rots increases as the harvest season progresses, especially for avocados harvested late in the season. However, it is unknown whether fertiliser practices in New Zealand have a differential influence on fruit nutrient composition and FQ during the harvest season.
- Nitrogen is the key nutrient studied in the nutrition of avocados. However, there is insufficient empirical evidence supporting the existence of a N fertiliser rate that balances high yield with reduced N:Ca ratios in fruit tissues.
- Increase fruit Ca concentration by adding Ca fertilisers alone has proven to be impractical. However, evidence suggests that Ca concentration can be increased by managing competition with K fertilisers. Testing alternative Ca fertiliser management strategies that avoid K competition is necessary under New Zealand conditions.

- High K concentration in fruits has been found to be detrimental to fruit nutrient composition (resulting in a low Ca+Mg:K ratio) and FQ due to antagonistic effects on Ca concentration. However, K fertiliser use is recognised to have positive effects on quality production in different crops. It is essential to determine whether any fertiliser practice can provide protection against fungi-caused diseases in avocados without altering the Ca+Mg:K ratio in fruit tissues.

Chapter 2 – An orchard survey investigating the influence of fertiliser use regimes on fruit nutrient composition¹

2.1 Introduction

The mineral composition of avocado is vital to achieve premium fruit quality (FQ). Multiple studies worldwide have reported that avocado fruit with high Ca concentration, and reduced N:Ca ratios is less affected by FQ disorders, such as internal FQ disorders (i.e., stem end rot, anthracnose or vascular browning) (Dann et al., 2016; Escobar et al., 2021; Marques et al., 2006; Willingham et al., 2006), chilling injuries (Arpaia, 1994; Barrientos-Priego et al., 2016), or fruit softening during storage (Rivera et al., 2017). In those studies, fruit with improved nutrient composition had higher Ca concentration and improved relationships of Ca with other mineral nutrients such as N and K compared to fruit more affected by FQ disorders (i.e., higher Ca+Mg:K ratios, and lower N:Ca ratios).

Nutritional management in orchards has been identified as a pre-harvest factor influencing fruit nutrient composition (Perkins et al., 2021). However, few studies have reported results on the impact that fertiliser use practices could have on improving fruit Ca concentrations, the N:Ca ratios, or Ca+Mg:K ratios in avocados. In a fertiliser experiment with Hass avocados, Willingham et al. (2006) reported significant reductions

¹ Parts of this chapter were published in:

Monserrate F., Van der Heijden D., Dowson A., Jeyakumar P., Roskrug N., Anderson C., Hanly J., Influence of different fertilization regimes on avocado fruit mineral composition in Bay of Plenty, New Zealand. In: *Proceedings of the tenth world avocado congress*, Auckland, New Zealand. <https://industry.nzavocado.co.nz/wp-content/uploads/2023/05/10th-World-Avocado-Congress-all-full-papers.pdf>, 52-61.

Monserrate F., Hanly J., Van der Heijden D., Dowson A., Jeyakumar J., Roskrug N., Anderson C. 2022. Understanding the influence of nutrient management strategies in high-performance avocado orchards on fruit calcium status: Initial results. In: *Adaptive Strategies for Future Farming*. (Eds. C.L. Christensen, D.J. Horne and R. Singh). <http://flrc.massey.ac.nz/publications.html>. Occasional Report No. 34. Farmed Landscapes Research Centre, Massey University, Palmerston North, New Zealand. 3 pages.

in the N:Ca ratio when no N-fertiliser was used compared to the use of two different N fertiliser rates in northern New South Wales – Australia. Nitrogen fertiliser use has also shown the potential to change the N:Ca ratio in other fruit crops, such as apples and kiwifruit (Nava & Dechen, 2009; Pacheco et al., 2010; Raese & Drake, 1997). In addition to N fertiliser use, K fertiliser use has shown the potential to influence fruit nutrient composition in avocados. Hofman (2007) reported an antagonistic interaction between the concentrations of Ca and K in Hass avocado tissues, for treatments that included K fertiliser use from pre-flowering onwards. However, trials assessing different Ca-sources have found limited evidence of increased Ca concentration in avocados produced in South Africa (Du Plessis & Koen, 1987) and Australia (Hofman, 2007), as discussed in section 1.3.2.3.2.

In New Zealand, the main fertiliser practices used in avocados are the adjustment of N and K, along with the application of Ca-rich amendments to the soil, such as lime and gypsum (New Zealand Avocado Growers Association, 2000). Despite the international evidence about the relationship between fruit nutrient composition and fertiliser practices, limited information is available under New Zealand conditions. A three-year survey reported higher avocado fruit Ca+Mg:K ratios following increased Ca inputs (Everett et al., 2007), but the products and dosages used by growers to improve the ratio were not reported. Moreover, fertiliser plans in New Zealand are developed by consultants based on different criteria (Section 1.3.3.1), resulting in a wide range of N and K fertiliser rates applied each year in different orchards (West, 2020). In this regard, the effects of different fertiliser use regimes on fruit nutrient composition are still not well established.

The aim of this chapter is to evaluate the influence of different fertiliser practices, namely N, K, and Ca soil applications, on avocado fruit nutrient composition in representative high-performance avocado orchards in the BoP. The survey investigates the concentration of nutrients in the skin and flesh of fruit harvested in the early and late harvest seasons. During the late harvest season, the FQ disorders are more prevalent than in the early harvest season in New Zealand. In this way, the survey study also facilitated the identification of further research on fertiliser use practices for improving fruit nutrient composition under New Zealand conditions.

2.2 Materials and methods

2.2.1 Study area

The monitoring of fertiliser practices, soil, and fruit nutrient status was implemented during 2021-2022 in nine commercial avocado orchards from the Western Bay of Plenty district. The Western BoP, located on the north island of New Zealand, is one of the main avocado-growing districts in the country (Horticulture New Zealand & Plant & Food Research, 2019). The orchards were in the towns of Te Puna (2), Aongatete (3), Katikati (3) and Waihi Beach (1), which are located northwest of Tauranga city. Composed of flat to easy-rolling landscapes, the dominant soils in the area are Typic Orthic Allophanic soils (Manaaki Whenua, 2019), dominated by allophane minerals, low-density structure, loam textures, and deep-rooting zone. The climate in the study area is classified as temperate subtropical with an average rainfall of 1180 mm evenly distributed, 15.1 °C annual mean temperature and 2280 hours of average annual sunshine (Chappell, 2013; NIWA, 2023). During summer, in January, the daily average monthly temperature is 19.8°C. While during winter, in July, daily average monthly temperature is 10.5°C (Chappell, 2013; NIWA, 2023).

2.2.2 Orchard selection

Nine commercial avocado orchards with similar horticultural and environmental conditions but different annual N fertiliser rates were selected for this study. Three main criteria were used for the orchard selection:

- Average annual yield higher than 16 tonnes per hectare during the last three harvest seasons (high-performance avocado orchards)
- Mature trees with ages higher than 15 years old
- Trees with Hass Scion grafted on Zutano rootstocks.

Three orchards from each annual N fertiliser level were selected, defined in section 2.2.4 (e.g., high, medium, and low N fertiliser use).

2.2.3 Fertiliser practices in the selected orchards

Fertiliser use records between May 2018 to Apr 2021 were used to calculate the fertiliser use regimes applied at each orchard surveyed. The rates of nutrients (N and K) used in each orchard were calculated from the fertiliser products applied over the period of May in the first year to April the next year (i.e., May 2018 to April 2019). Then, the three annual rates of N and K applied were averaged to provide a single annual average application rate for each nutrient per orchard. The total Ca inputs over the three-year period was also calculated, in the same way, to investigate the cumulative effect of Ca inputs on soil and fruit Ca concentration.

2.2.4 Definition of fertiliser use levels

Orchards in high, medium, and low nitrogen fertiliser use levels used either more than 250 kg N/ha/year, 150 to 250 kg N/ha/year, or less than 150 kg N/ha/year, respectively. According to their N fertiliser use, the orchards were labelled from *Orc1* to *Orc9* in descending order. In the case of K fertiliser use, despite the wide variability in the average K fertiliser use each year, it was not possible to identify clear breakpoints to define three contrasting levels among the orchards. With respect to K, orchards were therefore divided into three groups sorting the average rates in descending order, resulting in three K fertiliser levels with three orchards belonging to each level. The three orchards in each K fertiliser level were from different N fertiliser levels: Orchards *Orc3*, *Orc4*, and *Orc7* were in the high K fertiliser level; orchards *Orc6*, *Orc2*, and *Orc8* were in the medium K fertiliser level; and orchards *Orc1*, *Orc5*, and *Orc9* were in the low K fertiliser level.

2.2.5 In-field monitoring

Five trees with similar biomass and fruit load were randomly selected to monitor from one block of the same age in each orchard. Fruit was collected from each tree and analysed as internal replicates. Four fruits per tree were sampled twice during the harvest season from non-terminal shoots across the canopy, at heights between 2 and 4 m above ground level. The first sampling was during the early harvest season (September 2021), when the avocado flesh dry matter reached 23%, and the second sampling was during the late harvest season (January 2022), when the flesh dry matter reached approximately 33%.

In early winter (July 2021), a soil sample (0-15 cm soil depth) was collected near each of the five monitoring trees on each orchard in accordance with the avocado industry recommendations (New Zealand Avocado Growers Association, 2000). Each soil sample was composed of eight soil cores taken around the tree at a distance between 1.5 - 2 m from the trunk.

Both the fruit and soil samples were transported to the Soil Science Laboratory at Massey University within 24 hours after sampling for analysis.

2.2.6 Laboratory procedures

Avocados were subsampled at the green stage immediately upon arrival at the laboratory, by taking a horizontal slice approximately 2 cm wide located in the equatorial zone of each avocado, as proposed by Boyd et al. (2007). From each slice, the skins and flesh were carefully separated. Composite fruit samples of skin and flesh were obtained from the fruit of each tree. The fruit-flesh samples were minced using a food processor and carefully transferred to an aluminium foil tray for drying, whereas skin samples were packed into paper bags to be dried. Fruit samples were dried in an air-forced oven at 65 °C for 24 h and then finely ground using a 500 W mixer grinder for further analyses (Boyd et al., 2007). The flesh dry matter was calculated in a percentage basis from each sample by weighing the flesh before and after drying in the air-forced oven for 24 hours.

Fruit sub-samples of flesh and skin were acid digested in an aluminium digestion block for cations and total N analyses, following the modified methods from Thorp *et al.* (1997) for cations and a Kjeldahl digestion for total N (Blakemore *et al.*, 1987), respectively. Briefly, for cations, a 0.1 g subsample was digested for 2 h at 120 °C in 2 ml of concentrated nitric acid (69%) using glass funnels on top of the digestion tubes to allow reflux. The samples were then cooled at room temperature, 2 ml of hydrogen peroxide (35%) was added, and then the digestion was continued for 3.5 h at 120 °C with refluxing. The digested subsamples were made up to 25 ml with deionised water after adding 1 ml of 2.5% CsSr as a dispersant, and cation determination was conducted using microwave plasma atomic emission spectroscopy (4200 MP-AES, Agilent, USA). Total N was determined using a Kjeldahl procedure according to Blakemore *et al.* (1987), whereby 0.1 g sub-sample was digested for 8 h at 350 °C using 4 ml of a mixture based on sulphuric acid. The acid mixture was prepared heating 2.5 l of sulphuric acid (95%), 250 g dipotassium sulphate and 2.5 g selenium powder until the mixture turned clear. The digested product was made up to 50 ml and analysed for total N using a Technicon autoanalyzer. The fruit mineral concentration was expressed in a dry weight (DW) basis in mg/kg for Ca and Mg and in g/kg for N and K.

Soil samples were air dried for 48 h at 30 °C, ground in a ceramic mortar and pestle, and sieved through a 2-mm stainless steel mesh to prepare them for analysis. Exchangeable cation concentrations and cation exchange capacity (CEC) were measured in meq/100g of soil after a micro-leaching procedure of 50 ml 1M ammonium acetate (pH 7.0) passing through a mix of 1 g soil and 3 g acid-washed sand (Blakemore *et al.*, 1987). The cations Ca, K, Mg and Na were determined using microwave plasma atomic emission spectroscopy (4200 MP-AES, Agilent, USA) after the addition of 2 ml 2.6% CsSr as a dispersant.

Standard samples with analytical results from two external analytical laboratories (Hill Laboratories Limited and Eurofins Food Analytics NZ Ltd) were used for quality control in the analysis of each set of fruit or soil samples. For fruit tissues, two types of standards were used: one created by bulking together flesh samples and another by combining skin samples from the fruit harvested in 60 individual trees from a commercial avocado

orchard in Katikati by August 2021. Additionally, two internal soil standards, provided by the Soil Science Laboratory at Massey University, were used as quality control samples for soil analyses. Standard samples were included with each set of samples analysed and their analytical results were then checked against the values reported by the two external laboratories.

2.2.7 Statistical analyses

Statistical analyses were performed by fitting linear models with variables related to fruit nutrient composition as dependent variables. Then either individual orchards, orchards grouped in N fertiliser levels, K fertiliser levels or harvest time (e.g., Early and late harvest season) were used as categorical factors. Mean-pairwise comparisons for harvest time, N fertiliser level, or K fertiliser level were developed by a Tukey's honest significance difference (HSD) test using 0.05 as p-value. Data analyses were performed using the statistical language R Version 4.1.1 (R Core Team, 2021), pair-wise comparisons were made using the R package *lsmeans* (Lenth, 2016).

2.3 Results

2.3.1 Regimes of fertiliser use

The nine orchards surveyed exhibited a wide variability in annual N and K fertiliser use. The annual N fertiliser rate per orchard varied from 75 to 315 kg N/ha/year. This annual N fertiliser rate increased significantly ($p < 0.05$) among orchards polled in the low, medium and high N fertiliser level (Table 2.1 – Averages for N fertiliser rates with different upper-case letters). In the case of K fertiliser rates, the annual rate ranged from 32 to 374 kg K/ha, with lower rates ($p < 0.05$) observed in orchards grouped in the low K fertiliser level compared to other levels of K fertiliser (Table 2.1 – Averages for K fertiliser rates with different lower-case letters).

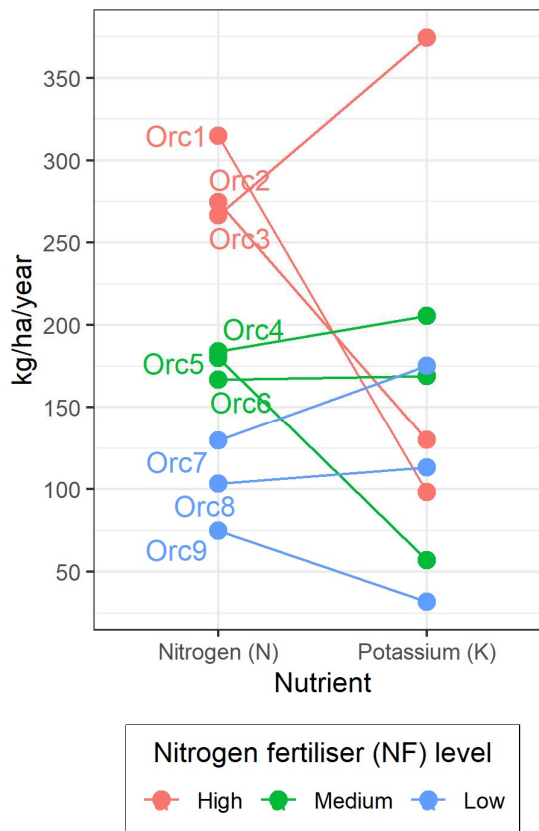


Figure 2.1- Annual average of nitrogen (N) and potassium (K) fertiliser rates in nine commercial avocado orchards from the Bay of Plenty region. The average corresponds to the fertiliser rates data from 2018 to 2021. Orchards are numbered in descending order according to their N fertiliser rates.

The annual N and K fertiliser applications used in the monitored orchards were primarily applied using three main types of fertilisers during the season. Fertiliser blends, with compositions of 12-5-15-8-2-2 (N-P-K-S-Mg-Ca) or similar, were used in all orchards. For orchards using higher N than K each year, this was typically achieved by also using calcium ammonium nitrate (CAN, 27% N and 4% Ca) to increase the N rates (Figure 2.1- *Orc1, Orc2, Orc5, Orc9*). The orchards with higher rates of K typically achieved this by also using sulphate of potash (SOP, 42% K and 17% S) (Figure 2.1- *Orc2, Orc3, Orc7*). Orchards using almost the same N and K fertiliser each year were using fertiliser blends to supply at least 75% of both nutrients, complemented with sporadic applications other products such as potassium nitrate (KNO₃, 12% N and 38% K) (Figure 2.1-*Orc4, Orc6, Orc8*).

The average Ca use per orchard ranged between 29 to 727 kg K/ha/year. Orchards using medium and high N fertiliser rates tended to use higher Ca fertiliser rates than orchards using low N fertiliser levels (Table 2.1 – Averages for Ca fertiliser rates with different upper-case letters). For orchards polled by their K fertiliser rate, there was an insignificant ($p>0.05$) difference among Ca fertiliser rates (Table 2.1 – Averages for Ca fertiliser rates with equal lower-case letters). The primary sources of Ca were lime (36% Ca) and gypsum (22% Ca), a practice reported in eight of the nine orchards during the survey period (2018-2021). These two products together provided between 73% and 97% of the total Ca addition, with the remainder of Ca fertiliser coming from other solid fertilisers.

For magnesium (Mg) fertiliser use, the annual rate ranged from 16 to 67 kg Mg/ha/year without any statistical difference among orchards grouped by N or K fertiliser levels (Table 2.1).

Table 2.1- Annual fertiliser rates and soil related variables in the monitored avocado orchards of the Bay of Plenty region during the season 2021-2022 pooled by nitrogen (Right) and potassium (Left) fertiliser levels (High, medium, and low)

Nutrient [†]	Nitrogen fertiliser level [‡]			Potassium fertiliser level ^{‡‡}		
	High <i>mean ± s.e.</i>	Medium <i>mean ± s.e.</i>	Low <i>mean ± s.e.</i>	High <i>mean ± s.e.</i>	Medium <i>mean ± s.e.</i>	Low <i>mean ± s.e.</i>
-----Annual Fertiliser rates (kg nutrient/ha/year)-----						
Nitrogen	285.3±15.2 A [266.6, 314.8]	176.9±15.2 B [166.8, 184]	102.6±15.2 C [74.9, 129.6]	193.4±15.2 a [129.6, 266.6]	181.6±15.2 a [103.5, 274.6]	189.8±15.2 a [74.9, 314.8]
Potassium	200.8±33.5 A [98.3, 374.3]	143.9±33.5 A [57, 205.5]	106.8±33.5 A [31.8, 175.2]	251.6±33.5 a [175.2, 374.3]	137.4±33.5 ab [113.4, 169.1]	62.4±33.5 b [31.8, 98.3]
Calcium	446.3±114.9 A [367.0, 726.7]	363.5±114.9 A [191.9, 541.2]	90.6±114.9 B [28.8, 141.8]	370.9±114.9 a [28.8, 726.6]	309.4±114.9 a [141.9, 541.2]	220.0±114.9 a [101.0, 367.0]
Magnesium	49.8±8 A [28.6, 66.9]	20.1±8 A [16.9, 22.3]	47.2±8 A [13.5, 64.3]	51±8 a [22.3, 66.9]	46.8±8 a [20.9, 64.3]	19.6±8 a [13.5, 28.6]
-----Soil exchangeable concentration (meq/100g)-----						
Calcium	27.5±1.4 A [20.6, 33.7]	25.3±1.4 A [22.7, 29.8]	18.1±1.4 B [14.8, 20.7]	28.1±1.4 a [20.7, 33.7]	23.5±1.4 ab [18.8, 28.4]	19.3±1.4 b [14.8, 22.7]
Potassium	0.9±0.2 A [0.7, 1.1]	1.2±0.2 A [0.8, 1.6]	1.2±0.2 A [0.9, 1.4]	1.0±0.2 a [0.9, 1.1]	1.2±0.2 a [0.7, 1.6]	1.0±0.2 a [0.8, 1.2]
Magnesium	4.1±0.8 A [2.3, 5.7]	3.4±0.8 A [2.5, 4.4]	3.8±0.8 A [1.9, 6.1]	4.9±0.8 a [4.2, 6.1]	3.9±0.8 a [2.5, 5.7]	2.5±0.8 a [1.9, 3.2]
-----Percentage (%)-----						
Calcium Saturation	68.3 + 4.3 A [59, 82]	68.7 + 4.3 A [65, 74]	60.7 + 4.3 A [55, 63]	71.8 + 4.3 a [60, 82]	65.2 + 4.3 a [63, 68]	60.8 + 4.3 a [55, 67]

[†] Each value represents average of three high-performance avocado orchards by fertiliser level.

[‡]Mean values for N fertiliser levels with different capital letters are statistically different ($p<0.05$) by a Tukey HSD test.

^{‡‡}Mean values for K fertiliser level with different lower-case letter are statistically different ($p<0.05$) by a Tukey HSD test.

2.3.2 Soil calcium status

The soil exchangeable Ca concentration per orchard ranged from 14.8 to 33.7 meq Ca/100g (Table 2.1), which was 1.2 to 2.8 times higher than the soil fertility target (12 meq Ca/100g) proposed by the avocado industry in New Zealand for high-performing avocado orchards (New Zealand Avocado Growers Association, 2000). This soil Ca concentration exhibited a positive correlation with the annual average Ca fertiliser use per orchard ($R^2 = 0.71$, $p < 0.05$). In general, Ca dominated the cation exchange capacity (CEC) with saturations ranging from 55% to 82% per orchard, irrespective of the N fertiliser or K fertiliser level (Table 2.1- Use equal upper-case letters for N fertiliser and lower-case letters for K fertiliser). However, the total Ca use, rather than the average Ca fertiliser per orchard, demonstrated a high correlation with the soil Ca concentration ($R^2 = 0.77$, $p < 0.05$, See Figure 2.2), indicating a cumulative effect of Ca fertiliser in the monitored orchards. In fact, the total Ca use was also strongly correlated with the Ca saturation in soils when compared to the average Ca fertiliser ($R^2 = 0.72$, $p < 0.05$ for the correlation between total Ca use and Ca saturation, compared to $R^2 = 0.65$, $p > 0.05$ for the correlation between average Ca fertiliser use and the Ca saturation).

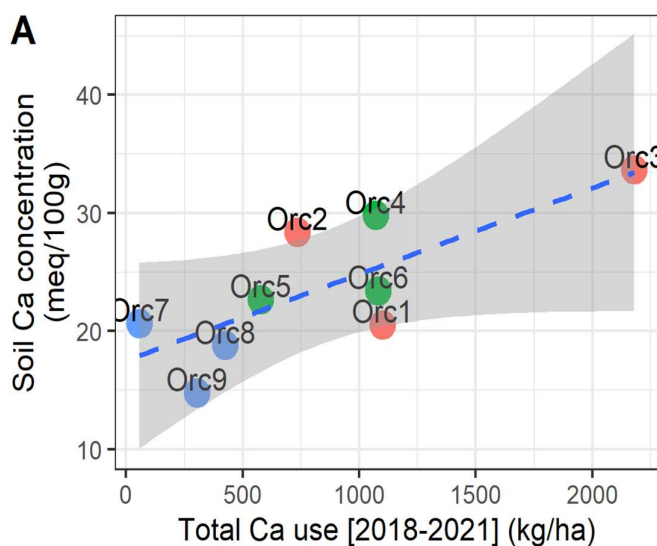


Figure 2.2 – Linear relationship between the total calcium (Ca) use and the exchangeable soil Ca concentration in the monitored high-performance avocado orchards of the Bay of Plenty region. Orchards are represented by coloured dots according to their nitrogen fertiliser level. Orchards in pink are high N fertiliser users, orchards in green are medium N fertiliser users, orchards in blue are low N fertiliser users.

For the orchards surveyed based on their N fertiliser level, the soil Ca concentration was higher in orchards applying high and medium N fertiliser levels compared to those using low N fertiliser levels ($p < 0.05$), as summarised in Table 2.1 and illustrated in Figure 2.2. Additionally, the average soil Ca concentration in orchards with a high K fertiliser level was statistically higher than the average for orchards with a low K fertiliser level ($p < 0.05$) (Table 2.1). Therefore, the average Ca concentration for orchards in the high and medium N fertiliser or K fertiliser levels reached at least two times the recommended soil fertility target for the avocado industry in New Zealand, while orchards in the low N fertiliser or K fertiliser levels reached only 1.5-times the same target of 12 meq Ca/100g.

Regarding other exchangeable cations such as potassium (K) and magnesium (Mg), there were no significant differences among averages based on N or K fertiliser levels. These cations ranged from 0.7 to 1.6 meq K/100g for K and between 1.9 and 5.7 meq Mg/100g for Mg (Table 2.1).

2.3.3 Fruit nutrient composition

2.3.3.1 General changes in the fruit nutrient composition during the harvest season

The Ca concentration in avocado fruit skin and flesh decreased between the early (when the dry flesh matter was ~23%) and late harvest (when the dry flesh matter was ~33%) during the 2021-22 season (Figure 2.3A and B). The average Ca concentration decreased 16%, from 762.9 mg Ca/kg in the skins and 58% from 471.3 mg Ca/kg in the flesh, between the early and late harvest, respectively (Table 2.2A). The variability in fruit Ca concentration represented by the interquartile range (IQR) was lower at the late harvest, especially in the fruit flesh (Figure 2.3B).

The N, K, and Mg concentrations in fruit flesh also decreased between the early and late harvest, but the concentrations in fruit skins showed the opposite trend (Figure 2.3 and Table 2.2A). On average, avocado flesh N and K concentrations at late harvest decreased

by 13% and 10%, respectively, compared to the early harvest (13.2 g N/kg and 18.2 g K/kg during the early harvest) (Figure 2.3D and F; Table 2.2A). In contrast, avocado skins harvested late, increased N and K concentration by 40% and 74%, respectively, compared to skins harvested early (10.4 g N/kg and 15.5 g K/kg during the early harvest) (Figure 2.3C and E; Table 2.2A). The Mg concentration followed the same trends described for N and K, with a reduction of 28% in fruit-flesh concentration and an increase of 23% in fruit-skin concentration for the late harvest compared to the early harvests (During the early harvest the Mg concentration was 1072.9 mg Mg/kg in fruit skins and 994.3 mg Mg/kg in fruit flesh) (Table 2.2A).

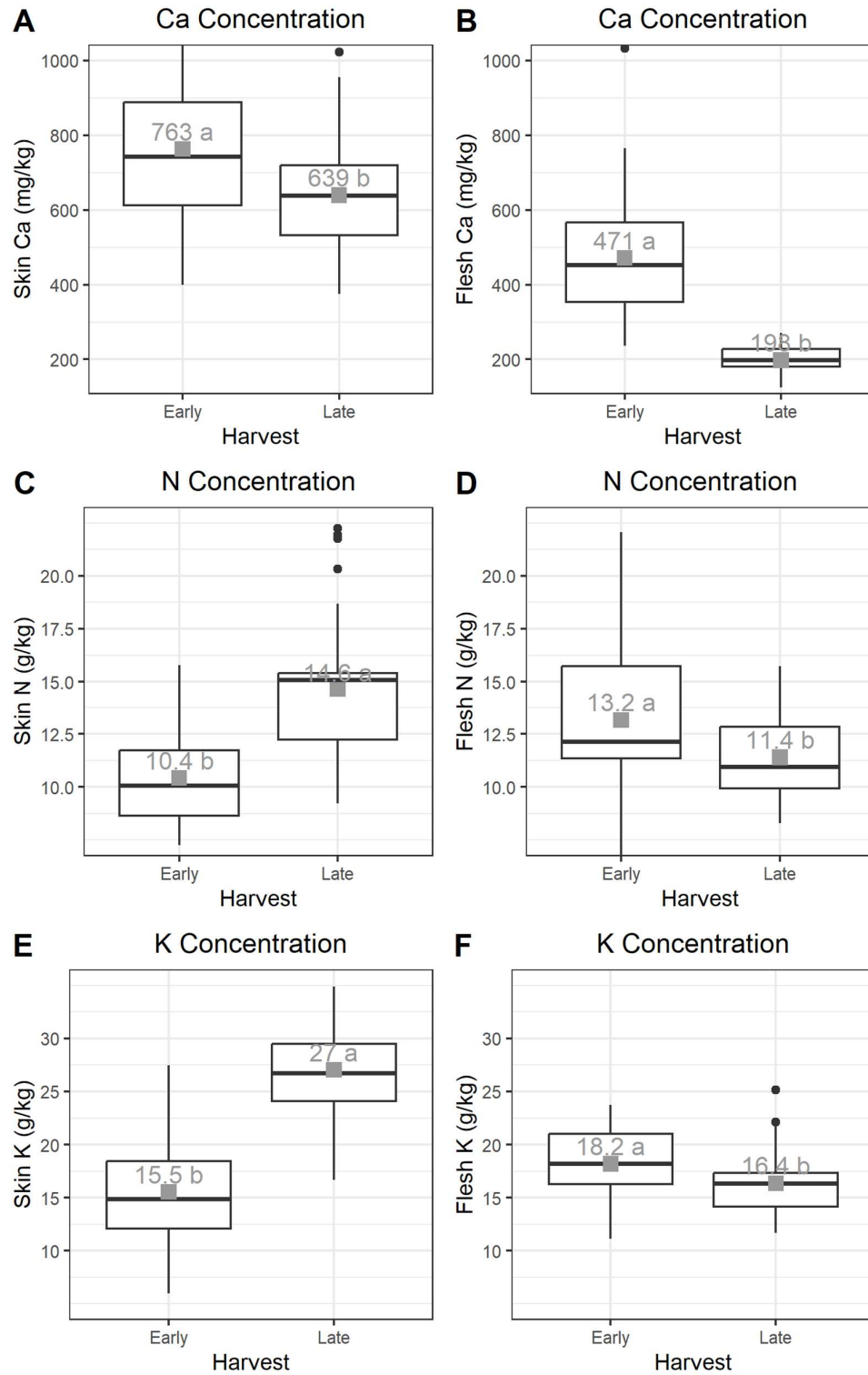


Figure 2.3- Boxplots for the mineral nutrient concentrations of calcium (Ca), nitrogen (N), and potassium (K) in fruit skin (Left: A, C, E) and flesh (Right: B, D, F) harvested early and late harvest during the 2021-2022 season in nine commercial avocado orchards of the Bay of Plenty. Grey squares and numbers represent the mean value for each nutrient and harvest time. Means with different letters are significantly different by the Tukey HSD test ($p < 0.05$).

Table 2.2 - Mean comparison for the mineral nutrient concentrations of nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg) in fruit skins and flesh in nine high-performance avocado orchards of the Bay of Plenty region by: harvest time (A), interaction between harvest time and nitrogen fertiliser levels (B), as well as harvest time and potassium fertiliser levels (C)

A- Comparison by the time of harvest						
	N (g/kg)	Ca (mg/kg)	N:Ca (ratio)	K (g/kg)	Mg (mg/kg)	Ca+Mg:K (ratio*100)[§]
<i>Avocado Skin</i>						
<i>Harvest</i>	----[mean ± s.e.] [†] ----					
Early	10.4 ± 0.4 b	762.9 ± 24.2 a	14.7±1 b	15.5 ± 0.6 b	1072.9 ± 35.3 b	12.7 ± 0.4 a
Late	14.6 ± 0.4 a	639.5 ± 24.2 b	24.5 ± 1 a	27 ± 0.6 a	1315.3 ± 35.3 a	7.3 ± 0.4 b
Change(%) [‡]	40	-16	67	74	23	-43
<i>Avocado Flesh</i>						
Early	13.2 ± 0.4 a	471.3 ± 15.4 a	29.9 ± 1.9 b	18.2 ± 0.4 a	994.3 ± 14.6 a	8.2 ± 0.2 a
Late	11.4 ± 0.4 b	198.1 ± 15.4 b	59.4 ± 1.9 a	16.4 ± 0.4 b	720.8 ± 14.6 b	5.7 ± 0.2 b
Change(%) [‡]	-13	-58	99	-10	-28	-31
B- Comparison by harvest time and level of nitrogen fertiliser						
	N (g/kg)	Ca (mg/kg)	N:Ca (ratio)	K (g/kg)	Mg (mg/kg)	Ca+Mg:K (ratio*100)[§]
<i>Avocado Skin</i>						
<i>Harvest: N fertiliser</i>	----[mean ± s.e.] [†] ----					
Early:Low	10.2 ± 0.6 d	782 ± 41.8 a	13.9 ± 1.7 c	16.4 ± 1.1 b	1184.7 ± 61.1 abc	12.8 ± 0.7 a
Early:Medium	10.4 ± 0.6 d	786.8 ± 41.8 a	14.5 ± 1.7 c	16.6 ± 1.1 b	980.5 ± 61.1 c	11.6 ± 0.7 a
Early:High	10.7 ± 0.6 cd	720 ± 41.8 a	15.9 ± 1.7 bc	13.6 ± 1.1 b	1053.5 ± 61.1 bc	13.7 ± 0.7 a
Late:Low	13 ± 0.6 bc	639.2 ± 41.8 a	20.9 ± 1.7 bc	28.8 ± 1.1 a	1303 ± 61.1 ab	6.8 ± 0.7 b
Late:Medium	14.1 ± 0.6 b	659.8 ± 41.8 a	22.7 ± 1.7 b	25.8 ± 1.1 a	1266.4 ± 61.1 ab	7.5 ± 0.7 b
Late:High	16.7 ± 0.6 a	619.3 ± 41.8 a	30 ± 1.7 a	26.5 ± 1.1 a	1376.5 ± 61.1 a	7.6 ± 0.7 b
<i>Avocado Flesh</i>						
Early:Low	11.9 ± 0.6 b	453.4 ± 26.7 a	28.4 ± 3.2 c	17.9 ± 0.7 ab	968.1 ± 25.2 a	8 ± 0.3 a
Early:Medium	12.4 ± 0.6 b	442.8 ± 26.7 a	29.1 ± 3.2 c	17.6 ± 0.7 ab	1010.7 ± 25.2 a	8.6 ± 0.3 a
Early:High	15.2 ± 0.6 a	517.7 ± 26.7 a	32.2 ± 3.2 c	19 ± 0.7 a	1004.1 ± 25.2 a	8.2 ± 0.3 a
Late:Low	10.5 ± 0.6 c	203.2 ± 26.7 b	53.8 ± 3.2 b	16.3 ± 0.7 ab	742.8 ± 25.2 b	5.8 ± 0.3 b
Late:Medium	11.2 ± 0.6 b	198.5 ± 26.7 b	57.3 ± 3.2 ab	15.5 ± 0.7 b	711 ± 25.2 b	6 ± 0.3 b
Late:High	12.5 ± 0.6 b	192.7 ± 26.7 b	67.1 ± 3.2 a	17.2 ± 0.7 ab	708.7 ± 25.2 b	5.4 ± 0.3 b

To be continued next page

Table 2.2 – Continuation

C- Comparison by harvest time and level of potassium fertiliser						
	N (g/kg)	Ca (mg/kg)	N:Ca (ratio)	K (g/kg)	Mg (mg/kg)	Ca+Mg:K (ratio*100)[§]
<i>Avocado Skin</i>						
----[mean ± s.e.] [†] ----						
<i>Harvest: K fertiliser</i>						
Early:Low	10.1 ± 0.6 b	818.8 ± 41.8 a	13.4 ± 1.7 c	16.1 ± 1.1 b	1137 ± 61.1 bc	13.4 ± 0.7 a
Early:Medium	10.3 ± 0.6 b	736.8 ± 41.8 ab	15.1 ± 1.7 c	13.6 ± 1.1 b	1013.3 ± 61.1 c	13.2 ± 0.7 a
Early:High	10.8 ± 0.6 b	733.2 ± 41.8 ab	15.8 ± 1.7 c	17 ± 1.1 b	1068.3 ± 61.1 c	11.4 ± 0.7 a
Late:Low	13.4 ± 0.6 a	732.2 ± 41.8 ab	18.9 ± 1.7 bc	27.3 ± 1.1 a	1345.7 ± 61.1 ab	7.7 ± 0.7 b
Late:Medium	14.5 ± 0.6 a	638 ± 41.8 bc	23.7 ± 1.7 b	26.1 ± 1.1 a	1210 ± 61.1 abc	7.2 ± 0.7 b
Late:High	15.9 ± 0.6 a	548.2 ± 41.8 c	31 ± 1.7 a	27.8 ± 1.1 a	1390.2 ± 61.1 a	7 ± 0.7 b
<i>Avocado Flesh</i>						
Early:Low	12.3 ± 0.6 ab	426.4 ± 26.7 a	31.4 ± 3.2 b	15.9 ± 0.7 bc	937.5 ± 25.2 a	8.8 ± 0.3 a
Early:Medium	13 ± 0.6 ab	534.7 ± 26.7 a	26.9 ± 3.2 b	19 ± 0.7 a	1009.3 ± 25.2 a	8.3 ± 0.3 a
Early:High	14.1 ± 0.6 a	452.8 ± 26.7 a	31.5 ± 3.2 b	19.7 ± 0.7 a	1036 ± 25.2 a	7.7 ± 0.3 ab
Late:Low	10.5 ± 0.6 b	199 ± 26.7 b	53.8 ± 3.2 a	14.5 ± 0.7 c	736.8 ± 25.2 b	6.5 ± 0.3 bc
Late:Medium	12.1 ± 0.6 ab	210.3 ± 26.7 b	59.4 ± 3.2 a	17.5 ± 0.7 ab	698.7 ± 25.2 b	5.3 ± 0.3 c
Late:High	11.6 ± 0.6 ab	185 ± 26.7 b	65.1 ± 3.2 a	17.1 ± 0.7 abc	727 ± 25.2 b	5.4 ± 0.3 c

[†] Mean values and standard errors modelled after fitting linear models using as predictor factors: harvest time (A), interaction between harvest time and N fertiliser level (B), and interaction between harvest time and potassium fertiliser level (C). Means with different letters are statistically different (*p*-value <0.05) by a Tukey HSD test.

[‡] Percentual change between the mineral concentration at the early and late harvest.

[§] Ca+Mg:K ratio expressed in percentage (ratio*100)

The ratios N:Ca and Ca+Mg:K showed opposite trends, with the N:Ca ratio increasing in both fruit skins in flesh between the early and late harvest, while the Ca+Mg:K ratio decreased during the same period (Table 2.2A). The N:Ca ratio in skins increased by 67%, while for flesh almost doubled between early and late harvest. For the skins this increase was caused by a 40% increase in N concentration and 16% decrease in Ca concentrations. Whereas for the flesh the increase in N:Ca ratio resulted from a 13% decrease in N concentration and a 58% decrease in Ca concentration. The Ca+Mg:K ratio decreased between the early and late harvests by 43% in fruit skins and 31% in fruit flesh (Table 2.2A).

2.3.3.2 Influence of the nitrogen fertiliser level on fruit calcium status

Orchards in the high N fertiliser level produced avocados with higher skin N concentrations during the late harvest and higher flesh N concentrations during the early harvest (Figure 2.4A, B; Table 2.2B). The skin N concentration at early harvest remained around 10 g N/kg for all three N fertiliser levels. In contrast, during the late harvest the skin N concentration significantly increased from 13 g N/kg for low N fertiliser orchards, to 16.7 g N/kg for the high N fertiliser orchards (Figure 2.4A; Table 2.2B). For fruit flesh, the N concentration was higher in orchards using a high N fertiliser level and lower in orchards using low N fertiliser at both harvest times. The fruit-flesh N concentration was 15.2 and 12.5 g N/kg for orchards using high N fertiliser at the early and late harvest, respectively. Whereas for orchards using low N fertiliser, the flesh N concentration was 11.9 and 10.5 g N/kg at the early and late harvest, respectively (Figure 2.4B and Table 2.2B).

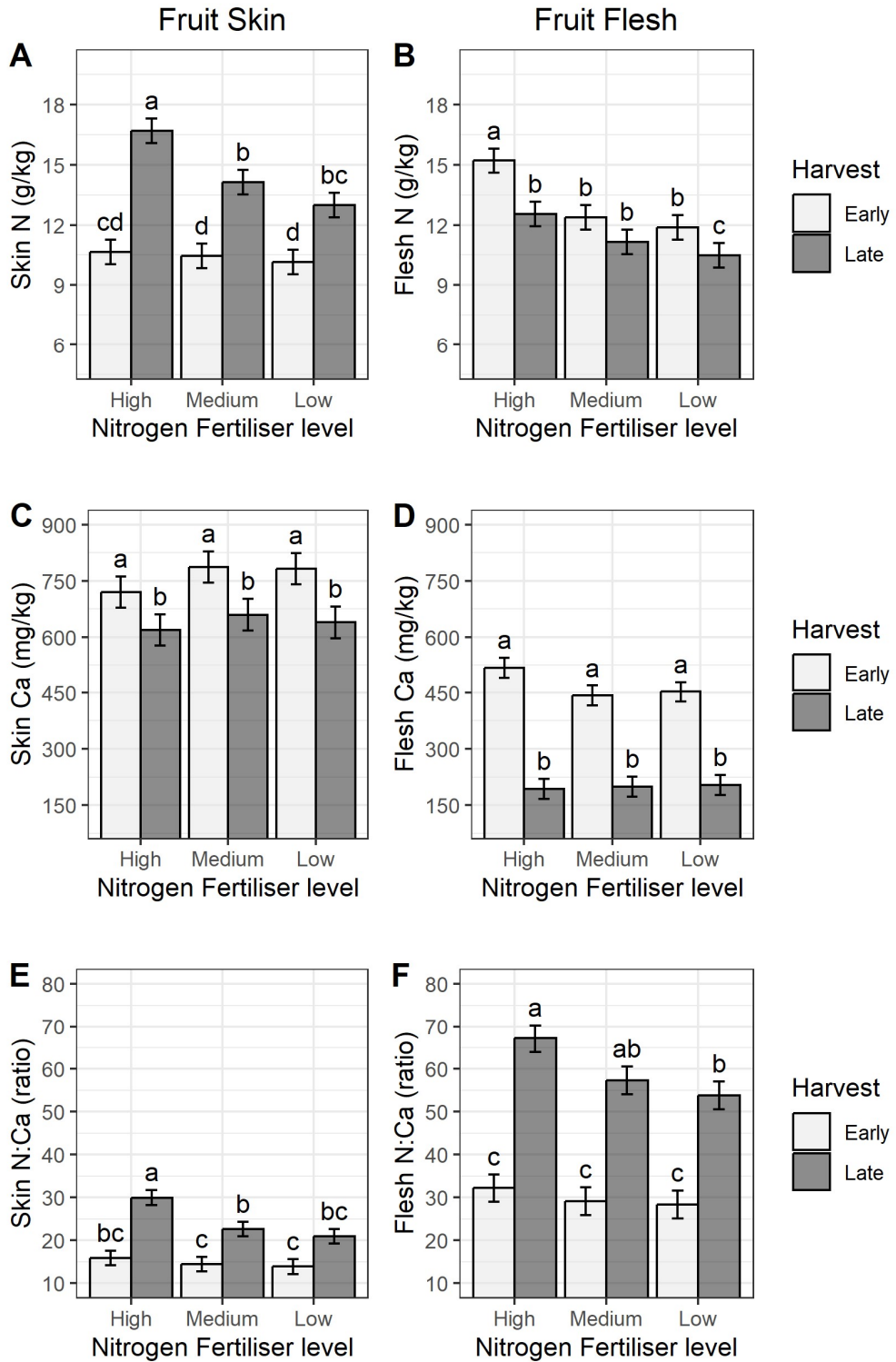


Figure 2.4 - Mean comparison for the mineral concentrations of nitrogen (N), calcium (Ca), and the N:Ca ratio in fruit skin (Left: A, C, E) and flesh (Right: B, D, F) in nine commercial avocado orchards of the Bay of Plenty region pooled by nitrogen fertiliser level and harvest time. Bars represent means by harvest time and nitrogen fertiliser level, and vertical lines represent the standard error of the mean. Means with different letters are significantly different by the Tukey HSD test ($p < 0.05$).

Calcium concentration in skins and flesh was not influenced by N fertiliser level for either the early or late-harvested fruit ($p>0.05$), with harvest time being the primary influence for Ca concentration in fruit tissues (Figure 2.4B, C; Table 2.2B – equal letters for orchards in each harvest time independently of N fertiliser level). However, the effect of N fertiliser level on N concentration had a significant influence ($p<0.05$) on the N:Ca ratio in both types of fruit tissues, with a higher ratio for late-harvested fruit in orchards using high N fertiliser levels compared to the lower two N fertiliser levels (Figure 2.4E, F and Table 2.2B). The N:Ca ratio in fruit skins at the late harvest was 30 for orchards using high N fertiliser, which was significantly ($p<0.05$) higher than the ratios of 22.7 and 20.9 for the orchards using medium and low N fertiliser, respectively. The fruit flesh N:Ca ratio followed the same trend as in skins, but the ratio was more than twice as high for the skins. The fruit-flesh N:Ca ratio during the late harvest was 67.1 in orchards using high N fertiliser, while in orchards using medium and low N fertiliser levels was 57.3 and 53.8, respectively. At early harvest, the N:Ca ratio for fruit skin and flesh was lower than at late harvest, and the effect of N fertiliser levels was insignificant ($p>0.05$).

2.3.3.3 Influence of potassium fertiliser level on fruit calcium status.

Potassium fertiliser levels influenced the fruit flesh K concentration during both harvest times, with a trend toward lower K concentration for orchards in the low K fertiliser compared to orchards in the others two K fertiliser levels (Table 2.2C and Figure 2.5B). The average fruit flesh K concentrations did not reach 16.0 g K/kg for orchards in the low K fertiliser level at both harvest times. However, the other two higher rates of K fertiliser achieved flesh K concentrations at or above 19.0 g K/kg during early harvest and at or above 17.1 g K/kg at late harvest (Table 2.2C). For fruit skins, K fertiliser levels did not have any significant influence ($p>0.05$) on K concentration during the early or late harvests (Figure 2.5A; Table 2.2C), despite the differences in K concentration between both harvest times as discussed in section 2.3.3.1 .

There was a trend of lower fruit skin Ca concentration at the high K fertiliser level, especially at late harvest (Figure 2.5C; Table 2.2C). The average skin Ca concentration at late harvest in orchards with the high K fertiliser level was 548.2 mg Ca/kg, which was

statistically lower than the values for low K fertiliser levels at the same harvest (732.2 mg Ca/kg for low K fertiliser levels). Despite the difference in skin Ca concentration at late harvest, the Ca+Mg:K ratio in fruit skin during the same harvest was similar for the three K fertiliser levels (Figure 2.5E; Table 2.2C), which was partly due to the insignificant difference in fruit skin K concentration during that period (Table 2.2C).

Ca+Mg:K ratio in fruit flesh and skins was primarily influenced by the harvest time rather than the K fertiliser level. Changes in K fertiliser levels did not have a significant impact on the Ca+Mg:K ratio, whether considering changes in flesh K concentration or skin Ca concentration. In fruit flesh, the trend of higher ratios in orchards using low K fertiliser was insignificant. This was in part due to the influence of higher Mg concentrations in orchards using medium and high K fertiliser levels, despite the difference in Mg concentration among K fertiliser levels being insignificant ($p>0.05$) (Table 2.2C). In fruit skins, the influence of the lower skin Ca concentration at late harvest with high K fertiliser levels did not result in different Ca+Mg:K ratios, which is mainly due to higher Mg concentrations (Table 2.2C).

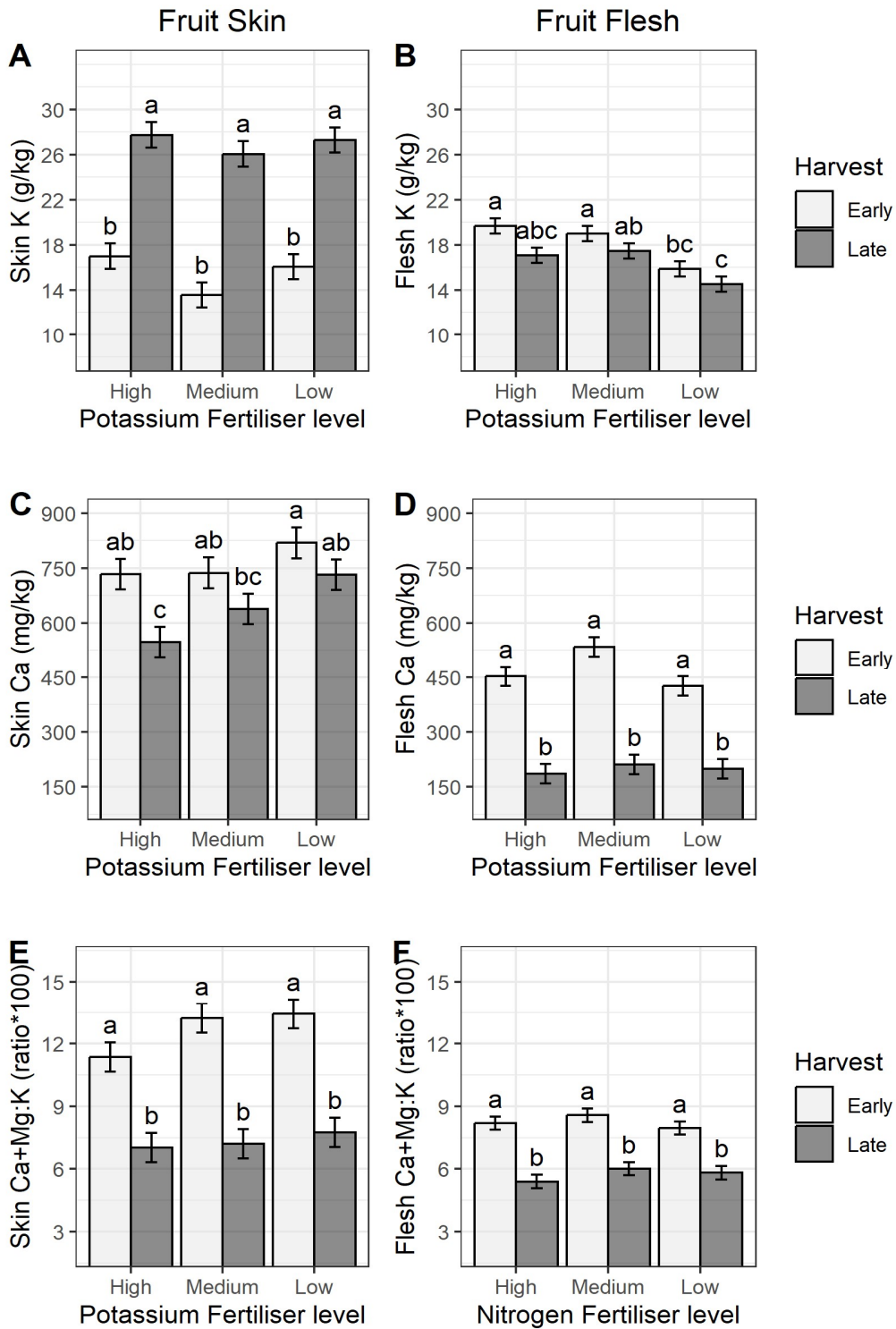


Figure 2.5 - Mean comparison for the mineral concentrations of potassium (K), calcium (Ca), and the Ca+Mg:K ratio in fruit skin (Left: A, C, E) and flesh (Right: B, D, F) in nine commercial avocado orchards of the Bay of Plenty region pooled by potassium fertiliser level and harvest time. Bars represent means by harvest time and potassium fertiliser level, and vertical lines represent the standard error of the mean. Means with different letters are significantly different by the Tukey HSD test ($p < 0.05$).

2.4 Discussion

2.4.1 General changes in the fruit nutrient composition during the harvest season

The results from this study show two main trends in fruit nutrient composition of Hass avocados produced in high-performance orchards in the BoP. First, nutrient composition decreased at late harvest, as the flesh dry matter content increased. Overall, fruit harvested late in the season, when the fruit rots are prevalent, exhibited decreased Ca concentration in both fruit tissues compared to fruit harvested early. Additionally, N, K, and Mg concentrations decreased in the fruit flesh and increased in the skin between the two harvests in the season. With the most significant changes seen in decreasing Ca concentrations between harvests, both the N:Ca ratio increased and the Ca+Mg:K ratio decreased in both tissues (Section 2.3.3.1). A similar trend towards a reduction of fruit N:Ca ratio with increasing dry matter was reported for flesh of Hass avocados produced in Colombia, as the dry matter changed from 22% to 30% through a season (Escobar et al., 2021). Moreover, the N:Ca ratio in fruit flesh of Pinkerton avocados, produced in South Africa, increased 1.3 times between the harvest in November (~ 22% dry matter) and the fruit harvested five months later with dry matter around 30% (Snijder et al., 2002).

The second trend observed in the current study, was a consistently lower N:Ca ratio and higher Ca+Mg:K ratio in fruit skins compared to flesh. This study is the first to report the N:Ca and Ca+Mg:K ratios in both fruit tissues throughout the harvest season in avocados. A compilation of works measuring Ca and N concentrations in fruit tissues of Hass avocados worldwide found that the N:Ca ratio in skins varied from 9 to 47 and in the flesh from 8 to 61 (Perkins et al., 2021), but any work compiled results from skins and flesh when the harvest season advanced. The results presented in the current work can therefore become a benchmark for changes in the nutrient concentrations and ratios related to the fruit nutrient composition during the harvest season in Hass avocados. The influence of fertiliser management on those two trends discussed here warrants further exploration,

and the coming section introduces a first insight based on the selected orchards of this study.

2.4.2 Influence of different fertiliser use regimes on the fruit nutrient composition

In the survey of selected orchards in the BoP, avocados produced in orchards using high N fertiliser levels (>250 kg N/ha/year) had higher N:Ca ratios in fruit flesh and skins during late harvest (Figure 2.4E and F). This result highlights the critical role that N fertiliser management has on fruit mineral composition. Although a number of studies (Dann et al., 2016; Escobar et al., 2021; Marques et al., 2006; Rivera et al., 2017; Willingham et al., 2006) have identified a high N:Ca ratio in avocado fruit as a consistent indicator for FQ disorders during postharvest, the effect of N fertiliser use on this ratio has not been widely assessed in avocados. To date, the research conducted by Willingham et al. (2006) in New South Wales, Australia, had been the only reporting on the influence of N fertiliser use on fruit mineral composition of Hass avocados. In contrast to the results discussed here for the current survey during the late harvest season in the BoP, the N:Ca ratio was similar for two treatments using either traditional or doubled the traditional grower rates in New South Wales (N:Ca ratio between 16 to 23 with N fertiliser rates of 160 and 320 kg N/ha/year) (Willingham et al., 2006). However, the Australian study only analysed the fruit nutrient composition during the early harvest season (July to August in New South Wales). In that sense, the results of Willingham et al. (2006) are comparable with the results during the early harvest season in this study, showing no differences among the N fertiliser rates and highlighting the influence of the harvest time factor on fruit mineral composition.

Potassium fertiliser levels had two main effects on fruit nutrient composition in the survey of selected orchards in the BoP. First, the flesh K concentration decreased with the K fertiliser level, but not enough to generate changes in the Ca+Mg:K during any harvest time (Figure 2.4E and F). Second, the fruit skin Ca concentration during the late harvest

reduced when the K fertiliser level increased from low to high (Figure 2.4A). Similar interactions between K fertiliser and Ca concentration in Hass avocados were seen in Queensland-Australia with fertiliser treatments using only Ca, only K, or Ca and K fertilisers (Hofman, 2007). In the Queensland trial, treatments using K fertiliser (only K and K+Ca treatments) resulted in less Ca in skins compared to treatments using only Ca fertiliser (Hofman, 2007).

2.4.3 Influence of soil exchangeable calcium concentration on fruit nutrient composition

The soil exchangeable Ca concentration in orchards using medium and high N fertiliser levels was between 1.4 and 1.5 times greater than in orchards using low N fertiliser (Table 2.1). However, the fruit Ca concentration did not change among N fertiliser levels (Table 2.2). Thus, orchards with higher soil Ca concentration and total Ca fertiliser did not produce fruit with higher fruit Ca concentrations, contrary to reports from previous surveys in avocado orchards in New Zealand (Everett et al., 2007; Thorp et al., 1997). However, these earlier studies included orchards with lower soil Ca concentrations (2.5 to 6.3 meq Ca/100g; Thorp et al., (1997)) compared to values for the current study (14 to 33 meq Ca/100g).

The results from this survey in the BoP suggest that when the soil in orchards concentrated at least 14 meq Ca/100 g (1.2 times the proposed soil fertility target in New Zealand), further increases in soil exchangeable Ca levels may have limited influence on increasing fruit nutrient composition. This limited influence could be related to the filter effect exerted by the Zutano rootstocks used in the orchards surveyed in the study. Mexican-derived rootstocks as Zutano have demonstrated an increased filter effect against Ca translocation into the scion compared to Guatemalan- or West Indian-derived rootstocks in multiple studies (Dann et al., 2016; Hofman et al., 2002; Marques et al., 2003, 2006; Mullen, 2015; Willingham et al., 2001, 2006).

An alternative to lime and gypsum, which are the primary Ca sources used in New Zealand, is calcium nitrate (CN) which present benefits in similar situations of high soil Ca concentrations. For instance, Motesarezadeh et al. (2021) reported that CN increased Ca concentration in other tree crops, such as apples, pears, and cherries produced under conditions of high soil Ca concentration. Thus, the use of CN during critical stages for Ca uptake and translocation, such as the early fruit set warrant further investigations under conditions of the BoP.

2.5 Conclusions

The survey in high-performance avocado orchards in the BoP found that avocado fruit left on the tree for longer, harvested late (January) in the season rather than early (September), experienced a decrease in Ca concentrations in both fruit skin and flesh, with more pronounced changes in the flesh. Concentrations of other nutrients, namely N, K, and Mg, also decreased in the fruit flesh, but increased in the skin between the two harvest times. The reduction in fruit Ca concentrations also influence the fruit nutrient ratios, leading to a decrease in the Ca+Mg:K ratio and an increase in the N:Ca ratio in both fruit tissues. These changes were observed when the dry matter increased from around 24% in September to around 33% in January. The changes in fruit nutrient composition at late harvest, in particular the lower fruit Ca concentration and Ca+Mg:K ratio, and higher N:Ca ratio, are risk factors for higher incidence of postharvest internal fruit rots.

In the survey, orchards with higher soil exchangeable Ca concentrations, which also tended to have the higher N fertiliser use, did not appear to result in higher fruit Ca concentrations. However, all orchards in the study had soil exchangeable Ca concentrations above the proposed soil fertility target level for avocados, which is likely to explain the lack of a response. Therefore, further research is needed to identify other approaches to improving fruit nutrient composition.

The survey helped identify trends relating to the influence of fertiliser practices on fruit nutrient composition. A main finding is that higher N fertiliser use appeared to be associated with higher N:Ca ratios in both fruit flesh and skins, particularly in late harvested fruit.

In addition, high rates of K fertiliser tended to be associated with lower Ca concentrations in fruit skins of late harvested fruit. These findings suggest that managing N and K fertiliser use may play an important role in influencing fruit nutrient composition, which has potential to help mitigate the higher incidence of postharvest disorders seen in late harvested fruit from the BoP.

Therefore, the associations between N, K and Ca fertiliser use and avocado fruit nutrient composition were investigated further in a field trial, and the results are reported in the Chapter 3.

Chapter 3 – Effect of fertiliser strategies on soil fertility and fruit nutrient composition

3.1 Introduction

Fertiliser use practices have been identified as one preharvest factor contributing to defining the fruit quality during postharvest throughout their influence on fruit nutrient composition (Hofman, 2007; Perkins et al., 2021; Willingham et al., 2006). The survey study discussed in Chapter 2 signalled the potential effects of fertiliser use practices on fruit nutrient composition from high-performance avocado orchards in the BoP. Those fertiliser effects were mainly expressed during the late harvest season when the dry matter increased to 33% from the early harvest in which the dry matter was around 24%. The changes were associated with changes in N concentration and N:Ca ratio in fruit tissues due to N fertiliser use and changes of K and Ca concentration in fruit tissues due to K fertiliser use. In addition, Ca concentrations did not increase in orchards with the highest concentrations of exchangeable soil Ca concentration, which, in turn, used the highest quantities of Ca sources such as lime and gypsum.

Despite the widespread use of N and K fertilisers, replicated studies in Hass avocado have only investigated the separate impacts of N or K fertiliser use on avocado fruit mineral composition (Hofman, 2007; Willingham et al., 2006). For instance, Willingham et al. (2006) investigated the effect of N fertiliser on avocado skin nutrient composition using two fertiliser rates applied as either ammonium-N or nitrate-N, compared to a control without N fertiliser. That experiment found that the two N fertiliser treatments resulted in higher fruit skin N concentrations and N:Ca ratios compared to the control, in two out of four years of the study (Willingham et al., 2006). Hofman (2007) tested K and Ca fertiliser treatments to understand their effects on avocado fruit nutrient composition, finding an antagonistic effect between K fertiliser use and fruit skin Ca concentration. Thus, replicated experiments, including a range of N and K fertiliser rates commonly used in

New Zealand, are needed to properly understand their effect on fruit nutrient composition in Hass avocados.

Another gap in the knowledge about improving the fruit nutrient composition through fertiliser use in Hass avocados lies in exploring the effects that alternative Ca fertiliser practices could have to increase the Ca concentration in fruits. One alternative could be the use of CN in the soil during critical times for Ca uptake and translocation to the fruit (i.e., between pre-flowering to early fruit set, according to Witney et al.(1990a)). According to Perkins et al. (2021), using CN in critical times is a practice followed by some growers in Australia to increase the Ca concentration, with results still unquantified. However, CN has increased fruit Ca concentration in other fruit crops, such as apples, pears, and cherries, under high soil Ca concentration (Moteszarezhadeh et al., 2021). Then, it bears logic to test CN applications between pre-flowering and early fruit set under New Zealand conditions, withdrawing K fertiliser during that stage to avoid the antagonist K effect on Ca concentration as discussed by Hofman (2007).

Along with the effects of fertiliser practices on fruit nutrient composition, it is also necessary to compare their impact on soil fertility parameters and tree performance. Understanding fertiliser practices that potentially improve fruit nutrient composition without affecting yield or fruit size could facilitate the adoption of practices identified to support the premium FQ produce. Moreover, understanding the effect of N and K fertilisers on leaf mineral concentrations, particularly at the end of the summer flush of growth in May, is crucial, as these concentrations are used by consultants to adjust fertiliser programs annually (Dixon, 2008; West, 2020). Therefore, a comprehensive analysis of the effects of fertiliser practices on fruit nutrient composition and other aspects of tree performance is imperative to identify optimal fertiliser practices that support premium FQ produce.

The aim of this chapter is to investigate the effectiveness of different fertiliser practices at enhancing avocado fruit nutrient composition, while maintaining avocado tree performance. Therefore, this experiment provides evidence based on a controlled and replicated experiment for the combined effects of N and K fertiliser use on fruit nutrient

composition and other effects on soil fertility and tree performance. This evidence explores the N and K fertiliser effect throughout the harvest season, giving results for the fruit mineral composition during the early and late harvest time, which is pertinent for the New Zealand avocado market. In addition, the experiment was also designed to test an alternative Ca fertiliser strategy to the traditional lime and gypsum application developed in high-performance avocado orchards of the BoP. Finally, the analyses of other effects on avocado trees complements the discussion about the changes that different N and K fertiliser practices produce on fruit nutrient composition.

3.2 Materials and methods

3.2.1 Field site setup

The fertiliser field trial was conducted during two consecutive seasons: 2021-2022 and 2022-2023, in one commercial avocado orchard located near the town of Katikati, Western Bay of Plenty, New Zealand (Latitude 37.59°S; Alt 12 m.a.s.l.). The climate in the area is characterised as temperate subtropical, with an annual precipitation of 1607 mm and an average temperature of 16°C. The soil is classified as Typic Orthic Allophanic Soils (Manaaki Whenua, 2019), characterised by allophane minerals, low-density structure, loam textures, and a deep rooting zone extending beyond a meter. The orchard is situated on a flat landscape with a slope of < 2%.

The selected orchard is a high-performing avocado orchard with an average annual fruit yield of 20.5 t/ha. The orchard has an average ABI of 32%, from 2018 to 2022, which indicates an approximately 32% change in yield across seasons. Before the experiment began, the orchard had been using high N and K fertiliser inputs of an average of 267 kg N/ha/year and 374 kg K/ha/year.

The experiment was conducted on a productive block covering 1.2 hectares with 120 mature-productive trees (>20-year-old trees). The trees in the block were planted with a tree spacing of 10 m between rows and 10 m between trees in each row. Within the block, there is approximately one pollinator tree for a group of nine commercial trees producing Hass avocados (Trees with Hass scion grafted on Zutano rootstocks). Sixty visually similar Hass trees were selected within the plot to establish the fertiliser treatments, as outlined in the subsequent section. This selection excluded 38 trees located along the borders of the block, 11 pollinator trees, and 11 young commercial trees.

The plot was managed using standard commercial practices, except for fertiliser use and the use of copper-based fungicides. During the experimental period, from May 2021 to May 2023, no foliar sprays containing N-based fertilisers or Cu-based fungicides were

applied. Other horticultural practices were developed following the recommendations of the New Zealand Avocado Industry (New Zealand Avocado Growers Association, 2000). In late autumn, trees were injected in their trunks with 20% di-potassium phosphonate to prevent root rots. The tree canopy was pruned in February, following late harvest in January each season, to maintain a maximum of 60% canopy shading. Pest and disease management practices were adjusted as needed based on the monitoring during the season. Soil moisture was monitored using a soil moisture probe (Irrimax®) and maintained above 60% of field capacity throughout the experimental period with a micro-sprinkler irrigation system.

3.2.2 Nutrient management strategies

Twelve fertiliser treatments, encompassing two different nutrient management strategies, were tested on the 60 experimental Hass on Zutano trees selected (treatments and products used are described in Table 3.1). The experimental design employed a randomised complete block design (RCBD) with five blocks, each consisting of twelve consecutive treatment trees. Treatment 1 was a control, receiving no mineral fertiliser addition (N0K0). Treatments 2 to 10 represented nine combinations of low (50 kg/ha/year), medium (150 kg/ha/year), and high (300 kg/ha/year) N and K fertilisers inputs, as outlined in Table 3.1 (referred as strategy 1 in Table 3.1). In these nine treatments, one-seventh of the total seasonal fertiliser rate was applied every six weeks from August to March. In addition, Treatments 11 and 12 had medium N and K fertiliser rates (150 kg N and K/ha/year) but incorporated 70 or 90 kg/ha/year of additional soluble Ca from pre-flowering to early fruit set (September to January). Treatments 11 and 12 used Ca in the form of CN. Furthermore, K fertiliser was withheld between September to January, as detailed in Table 3.1, following the recommendation made by Hofman (2007) (referred in Table 3.1 as Strategy 2).

Table 3.1- Treatments applied in the fertiliser trial developed in a commercial avocado orchard of the Bay of Plenty region testing two fertiliser use strategies

Treatment	Label	Nutrient Strategy	Annual fertiliser (kg/ha/year)			Fertiliser app. [†]	Products used each fertiliser app. [‡]				
			N	K	Ca**		Blend	CAN	SOP	CN	
T1	N0K0	Control	0	0	0	1 st to 7 th	0	0	0	0	
T2	N50K50	Strategy 1:	50	50	10	1 st to 7 th	554	48	0	0	
T3	N150K50	Influence of annual N and K fertiliser rates on fruit nutrient composition	150	50	25	1 st to 7 th	554	576	0	0	
T4	N300K50		300	50	47	1 st to 7 th	554	1370	0	0	
T5	N50K150		50	150	10	1 st to 7 th	670	0	302	0	
T6	N150K150		150	150	30 (0)	1 st to 7 th	1661	138	0	0	
T7	N300K150		300	150	52	1 st to 7 th	1661	931	0	0	
T8	N50K300		50	300	10	1 st to 7 th	670	0	813	0	
T9	N150K300		150	300	31	1 st to 7 th	2010	0	401	0	
T10	N300K300		300	300	60	1 st to 7 th	3322	275	0	0	
T11	N150K150Ca7		Strategy 2:	150	150	100 (70)	1 st	1000	0	0	0
			Addition of				2 nd to 4 th	0	0	0	1500
		CN fertiliser				5 th to 7 th	2200	0	400	0	
T12	N150K150Ca9	during early fruit set	150	150	122 (90)	1 st	1000	0	0	0	
						2 nd to 4 th	0	0	0	2000	
						5 th to 7 th	1500	0	600	0	

* Nitrogen fertiliser; Potassium fertiliser; and Ca fertiliser applied by treatment.

** Soluble calcium (Ca) fertiliser applied as calcium nitrate (CN) by each treatment using 150 kg of N and K/ha/year.

† Fertiliser application made from mid-August to mid-April every 45 days.

‡ Quantity of fertiliser spread to each treated tree by application. Blend (Advantage Avogain®); Calcium ammonium nitrate - CAN (YaraBela CAN®); Calcium nitrate (CN) (YaraLiva NitraBor®).

3.2.3 Fruit samplings

Fruit was sampled twice during the harvest season; in September and January, when the dry matter was approximately 24% and 33%, respectively. These two harvest times are referred to hereafter as early and late harvest, respectively. In addition, a baseline fruit sampling was conducted in August of 2021, prior to the application of fertiliser treatments, when fruit dry matter was around 20%.

At each sampling time, 10 fruits were sampled per tree, which were of a commercial export size (over 200 g/fruit). The fruit were harvested from non-terminal shoots located in different directions across the canopy at heights of between 2 and 4 m above the

ground. Then, fruit was immediately weighted and transported within 24 hours after sampling to the Soil Science Laboratory at Massey University for analyses.

3.2.4 Leaf samplings

Leaves samplings on each experimental tree were developed during May each year after the summer flush of growth has ceased (autumn season in New Zealand in May 2021 and May 2022), as recommended by the avocado industry worldwide to adjust the fertiliser management (Campisi-Pinto et al., 2017; New Zealand Avocado Growers Association, 2000; Salazar-García et al., 2015). In addition, leaves were sampled during the flowering peak in summer (mid-November in the BoP in November 2021 and November 2022). During this time in the season, the growth of shoots is active, being the previous stage of the early fruit set, in which Witney et al. (1990a) reported the maximum Ca translocation into the fruit. Therefore, the leaf sampling in November was performed to assess an antagonism between K fertiliser use and Ca concentration in vegetative tissues of fruiting shoots.

For leaves during the first sampling in May, twenty adult leaves were harvested around the tree canopy at shoulder height in non-fruiting shoots, as recommended by Dixon (2008). Leaves harvested were the second to the fourth from the terminal bud, collecting leaves and petioles without any physical damage. During the second leaf sampling in November, twenty leaves of two types were harvested from fruiting shoots, in maximum flowering at that moment: Mature-hardened leaves and young or non-hardened leaves. Leaves sampled were packed into paper bags and transported to the Soil Science Laboratory of Massey University for further analyses.

3.2.5 Soil samplings

Soil samples were taken under each experimental tree in May each year (May 2021 and May 2022), as recommended by the avocado industry to adjust fertility parameters in orchards (autumn season in New Zealand). Additional soil samples were also taken in

January 2022 and January 2023, after the late fruit harvest had developed and the critical period for Ca uptake had happened. By this time, five of the seven soil fertiliser applications had been developed. The soil sampling involved extracting eight soil cores from the top 15 cm layer around the drip irrigation zone of each tree (approximately 1.5 to 2.0 m from the trunk) after carefully removing the leaf litter covering the soil. Then, soil cores were packed into plastic bags kept cool and transported to the Soil Science Laboratory of Massey University for further analyses.

3.2.6 Yield estimation

The yield estimations by each experimental tree were developed by a local avocado expert in September before the early harvest of 2022-2023 season. The estimation was based on the canopy inspection of each tree facilitated by a mobile lifting platform, following the procedure to estimate the production volume by tree blocks by the New Zealand industry (New Zealand Avocado Growers Association, 2000). Briefly, the production volume is estimated by avocado experts using the potential number of 5.5-kg trays produced in each block. As a reference, the number of avocados packed in a tray of 5.5 kg from the main commercial sizes for export purposes in New Zealand are 18 with a weight between 290 and 324 g, 20 with a weight between 257 and 289 g, and 23 with a weight between 219 and 256 g. Despite the subjectivity of this methodology, the avocado expert forecasted the yield with a deviation between 10 and 15% related to the actual production in the block. Then, the number of trays by the tree was transformed into kg of fruit by the tree for further statistical analyses. The yield estimation by tree during the 2021-2022 season was not undertaken because the fruit had already set in November 2020, prior to the commencement of treatment application.

3.2.7 Biomass estimation

A comparative canopy biomass estimation by tree was developed only during the 2022-2023 season, after an initial qualitative observation during the 2021-2022 season for an increased biomass production in some treatments. The biomass produced by trees during

the season was calculated by the difference between the biomass estimated in January 2023 (After the late harvest in the season 2022-2023) and the estimation in April 2022 (After the general tree pruning in February 2022). Each time, The tree biomass was estimated following a procedure in three stages: First, images were taken using a DJI Phantom 2.0 drone following the flight planning recommended by Tu et al. (2020) to optimise the measurement of avocado tree structure. Briefly, the flight followed a doubled grid mission over the canopy, parallel to the trees in a two-directional grid 60 m above the ground level. The overlap between flight lines was 85%, and the ground surface distance (GSD) was approximately 2.3 cm. The images were acquired with a clear sky at midday during the flights. Each time, six to seven ground control points (GCP) with known coordinates, including latitude, longitude, and altitude, were used to facilitate the georectification and orthorectification processes.

Second, the ortho-mosaic, digital surface model (DSM), and digital terrain model (DTM) for April 2022 and January 2023 were reconstructed based on the drone images and the GCP used each time. This reconstruction was developed using the OpenDroneMap™ software (OpenDroneMap Authors, 2020). The spatial resolution for the ortho-mosaic was 5 cm by pixel (each pixel representing an area of 5 cm by 5 cm on the ground or 0.0025 m²), while for the DSM and DTM the spatial resolution was 10 cm by pixel or 0.01 m².

Third, the canopy volume in m³/tree in April 2022 and January 2023 was calculated using three steps in ArcGIS Pro 3.2.1® (ESRI, 2023). During both estimations, three datasets were overlaid to facilitate the digitalisation of the footprint for each tree: the DSM, the ortho-mosaic, and the in-field GPS coordinates taken underneath each experimental tree. Then, the canopy height model (CHM) was generated by subtracting the DTM from the DSM. Then, a zonal statistic of heights based on the CHM was generated using the footprint of trees as zones. Finally, the estimated canopy volume by tree was obtained by multiplying the sum of heights in the trees' footprint area by 0.01 m² (the pixel area in the CHM). The difference in canopy biomass was also calculated by tree to be analysed.

3.2.8 Laboratory procedures

3.2.8.1 Plant tissue analyses

For fruit tissues, four avocados randomly selected out of the ten sampled were subsampled at green stage immediately upon arrival at the laboratory, by taking a horizontal slice approximately 2 cm wide located in the equatorial zone of each avocado, as proposed by Boyd et al. (2007). From each slice the skins and flesh were carefully separated. The fruit flesh samples were minced using a food processor and carefully transferred to an aluminium foil tray, whereas skin samples were packed into paper bags to be dried. Fruit tissues were dried in an air-forced oven at 65 °C for 24 h and then finely ground using a 500 W mixer grinder for further analyses (Boyd et al., 2007). The flesh dry matter was calculated in a percentage basis from each sample by weighing the flesh before and after drying in the air-forced oven for 24 hours. In the case of leaves, the drying process lasted 72 h until leaves reached a constant weight before continuing with the grinding process.

Plant tissues (i.e., fruit flesh, skin, and leaves) subsamples were acid digested for cations and total N analyses, following the modified methods from Thorp *et al.* (1997) and a Kjeldahl digestion, respectively. Briefly, for cations, a 0.1 g subsample was digested for 2 h at 120 °C (in an aluminium digestion block) in 2 ml concentrated nitric acid (69%) using glass funnels on top of the digestion tubes to allow reflux. The samples were then cooled at room temperature, 2 ml of hydrogen peroxide (35%) was added, and then the digestion was continued for 3.5 h at 120 °C with refluxing. The digested subsamples were made up to 25 ml with deionized water, after adding 1 ml 2.5% CsSr as a dispersant for cation determination using microwave plasma atomic emission spectroscopy (4200 MP-AES, Agilent, USA). Total N was determined using a Kjeldahl procedure according to Blakemore et al. (1987). Whereby 0.1 g sub-sample was digested for 8 h in an aluminium digestion block at 350 °C using 4 ml of a mixture based on sulphuric acid. The acid mixture was prepared by heating 2.5 l of sulphuric acid (95%), 250 g dipotassium sulphate and 2.5 g selenium powder until the mixture turned clear. The digested product was made up to 50 ml and analysed for total N using a Technicon autoanalyzer. The fruit mineral

concentration was expressed in a dry weight (DW) basis in mg/kg for Ca and Mg and in g/kg for N and K.

Internal standard samples with nutrient concentration results from two commercial analytical laboratories were used for quality control in the analysis of each set of fruit or leaf samples. The standard used in the digestion of each plant tissue were created by bulking samples of fruit flesh, fruit skins, or leaves sampled from the 60 experimental trees in the orchard by August 2021. Standard subsamples of the corresponding tissue analysed were included with each set of samples. The standard analytical determination was then verified against the average analytical results obtained for those standards through determinations performed by two external laboratories: Hill Laboratories Limited and Eurofins Food Analytics NZ Ltd.

3.2.8.2 Soil sample analyses

Soil samples were air dried for 48 h at 30 °C, ground in a ceramic mortar and pestle, and sieved through a 2-mm stainless steel mesh to prepare for analysis. Soil pH, exchangeable cations and mineral nitrogen were analysed following standard procedures used in New Zealand soil laboratories (Blakemore et al., 1987). Briefly, soil pH was measured in deionised water with a 1:2.5 soil-to-solution suspension. Exchangeable cation concentrations and cation exchange capacity (CEC) were measured in meq/100g of soil after a micro-leaching procedure of 50 ml 1M ammonium acetate (pH 7.0) passing through a mix of 1 g soil and 3 g acid-washed sand (Blakemore et al., 1987). The cations Ca, K, Mg, and Na were determined using microwave plasma atomic emission spectroscopy (4200 MP-AES, Agilent, USA) after adding 2ml 2.6% CsSr as a dispersant. Soil mineral N (NH_4^+ -N and NO_3^- -N) was extracted by shaking 3 g air-dried soil subsamples with 30 ml 2M KCl in an end-over-end shaker for 1 h. Then, samples were centrifugated for 10 min at 5000 rpm, and the supernatant was analysed in a Technicon autoanalyzer after filtering through a Whatman 41 filter paper.

3.2.9 Statistical analyses

3.2.9.1 Statistical analyses for fruit tissue mineral concentrations

Linear models for each combination of mineral nutrient concentration and fruit tissue were developed using different factors as predictors, according to three levels of analysis. In the first level, general trends for mineral concentration in fruit skin and flesh were analysed using the sampling time as a categorical factor (e.g., early 2021-2022, late 2021-2022, early 2022-2023, late 2022-2023). In the second level of analysis, linear models were fitted for the treatments in each strategy. For the first strategy, treatments T1 to T10 were analysed according to their level of N fertiliser or K fertiliser (e.g., N0, N50, N150, N300 for N fertiliser; K0, K50, K150, K300 for K fertiliser). For the second strategy, linear models for treatments T1 (N0K0), T6 (N150K150), T11 (N150K150Ca70) and T12 (N150K150Ca90) were analysed using treatment as a categorical factor. In the third level of analysis, the interaction between N and K fertiliser rates for treatments T2 to T10 was assessed using two different statistical approaches: first, by fitting linear models with N fertiliser, K fertiliser, and their interaction as categorical factors; and second, by modelling the interaction using response surface method (RSM) models.

For the interaction analysis, the linear models employed the three levels of N fertiliser and K fertiliser and their interactions as categorical factors (e.g., N50, N150, and N300 for N fertiliser and K50, K150, and K300 for K fertiliser). In the case of the RSM models, the annual N fertiliser and K fertiliser rates were coded using two linear functions to provide the RSM method with the quantitative and standardized independent values required to adjust the models: $x \sim (N \text{ fertiliser} - 150)/150$ and $y \sim (K \text{ fertiliser} - 150)/150$. In this case, the dependent variable were the N:Ca and Ca+Mg:K ratios, using the data of both seasons grouped by harvest time (early or late harvest). Two statistical parameters assessed the adjustment of the RSM models according to Lenth (2009) and Younis et al. (2022): The significance of the model adjustment and the non-significance of the lack of fit in both cases using a p-value of 0.05 as a threshold.

The statistical analyses were developed in the statistical language R Version 4.1.1 (R Core Team, 2021). The R packages *lsmeans* (Lenth, 2016) and *rsm* (Lenth, 2009) were used to

develop the linear models along their pairwise comparisons and the response surface method (RSM) models, respectively.

3.2.9.2 Statistical analyses for other variables

Linear models were also developed for other dependent variables related with mineral concentrations in leaves and soils, as well as variables related to the tree performance such as the yield estimation, fruit weight, and tree biomass. The models were developed, including the treatments in each strategy, with treatments T1 to T10 to analyse the strategy 1 (Influence of annual N and K fertiliser rates on fruit nutrient composition) and the treatments T6, T11 and T12 to analyse the strategy 2 (addition of CN fertiliser during the early fruit set). For the strategy 1, two statistical analyses were performed. First, linear models were fitted with N fertiliser or K fertiliser rates as categorical factors and mineral concentrations in soils, leaves or estimations of yield, fruit weight or biomass as dependent variables. Then, pairwise comparisons between fertiliser rates were developed by a Tukey HSD test using 0.05 as p-value. Then, interaction analyses were analysed with treatments T2 to T10, using linear models with both N fertiliser and K fertiliser rates as categorical factors and the response variables as dependent variables. For strategy 2, linear models using treatments as categorical factors were fitted for the three treatments included in this strategy. The statistical analyses were developed in the statistical language R Version 4.1.1 (R Core Team, 2021). The R package *lsmeans* (Lenth, 2016) was used to develop the linear models along their pairwise comparisons.

3.3 Results

3.3.1 Soil tests and climate description for the experimental site

Initial soil samples were collected from the experimental site in May 2021, just before treatment application in the orchard (Table 3.2). Most of the soil test results were either within or above the optimum range recommended by the New Zealand avocado industry for achieving high yields (New Zealand Avocado Growers Association, 2000). Soil pH, Olsen P, exchangeable K, and boron were within the optimum range. Whereas exchangeable Ca and Mg concentrations were more than double the target level for those nutrients. Exchangeable Ca was the dominant cation on the soil cation exchange sites, making up 82% of exchangeable cations. Consequently, the soil fertility parameters in the orchard at the beginning of the trial were not considered to be limiting factors for achieving optimal crop yields.

Table 3.2- Soil characteristics for the experimental site (0-15 cm) in the Bay of Plenty region sampled in May 2021 before the beginning of the experiment and optimum fertility target ranges defined by the New Zealand avocado industry for a high-performance in avocados.

Soil characteristic	Test	Optimum
	Experimental site	Range ‡
pH (1:2.5)	6.5	6.2 - 6.6
Cation exchange capacity (CEC) (meq/100g)	41.2	n.a. ‡
Exchangeable Ca (meq/100g)	33.6	7.5-12.0
Exchangeable K (meq/100g)	1.1	0.6-1.1
Exchangeable Mg (meq/100g)	4.2	0.6-1.9
Exchangeable Na (meq/100g)	0.2	n.a. ‡
Ca Saturation (%)	81.8	n.a. ‡
Olsen-P (mg/l)	61.0 †	30.0-60.0
Boron (mg/kg)	4.5 †	2.0-6.0
Bulk density (g/cm ³)	0.9	n.a. ‡
Organic matter (%)	14.8 †	n.a. ‡

† Laboratory analyses performed in May 2021 by Hill Laboratories. Other analyses were performed in the soil science laboratory of Massey University as part of the present study. ‡ n.a. not available fertility range. Ranges defined by the avocado industry in New Zealand (New Zealand Avocado Growers Association, 2000).

The weather conditions in the BoP district varied between the experimental seasons compared to the 30-year average (Table 3.3). The 30-year average precipitation in the BoP is 1607 mm, and the temperature is 15.1°C. Notably, in the second season of

experimentation, the precipitation increased by 40% compared to the long-term average, while the temperature increased roughly by 1°C during both seasons. In the first season (2021-2022), cumulative precipitation was higher than the 30-year average only during the spring, being 36% higher than the long-term average. The seasonal temperatures were more than 1°C higher in spring and summer compared to the 30-year averages. In the second season, precipitation exceeded the 30-year average between June 2022 and January 2023, being 34% higher in winter, 110% higher in spring, and 58% higher in summer compared to the 30-year average. The mean temperature increased between 0.3 and 1.6°C related to the average normal temperature, with temperatures above 1°C in autumn and winter. The weather conditions in 2022-2023 seasons were influenced by two tropical storms in February 2022 and December 2022, the first event in February with strong winds, while the second with precipitations around 300 mm. These extreme weather events had implications for the experiment. Firstly, the higher precipitations and soil saturation may have facilitated higher N loss via leaching for the treatments in the experiment. Secondly, the wet and warm conditions create an ideal condition for rots expressions in the fruit, aiding in quantifying the impact of fruit mineral composition on FQ.

Table 3.3- Comparison of the weather conditions during the experimental time and the 30-year average climatological data in the Bay of Plenty region by season

Weather season †	Average (1990-2020)		2021-2022		2022-2023	
	Prec.‡ (mm)	Temp.§§ (°C)	Prec.‡ (mm)	Temp.§§ (°C)	Prec.‡ (mm)	Temp.§§ (°C)
Autumn (Mar-Apr-May)	435	16.0	233	16.7	314	17.2
Winter (Jun-Jul-Aug)	501	10.9	461	11.7	672	12.5
Spring (Sep-Oct-Nov)	332	14.3	450	15.4	697	15.1
Summer (Dec-Jan-Feb)	339	19.4	283	21.1	537	19.7
Annual Average	1607	15.1	1427	16.2	2220	16.1

† Seasonal data calculated based on monthly weather data recovered from NIWA (<https://niwa.co.nz/our-science/climate>, date of retrieval June 2023).

‡Precipitation (Prec.) is the cumulative seasonal value.

§§ Temperature (Temp.) is the average seasonal value.

3.3.2 Fertiliser influence on topsoil mineral nutrient concentrations

The topsoil (0-15 cm soil depth) mineral nutrient concentrations and pH levels were influenced by fertiliser treatments at the three different sampling times (January 2022,

May 2022, and January 2023) following the commencement of treatments applications (Table 3.4). The highest K fertiliser rate (300 kg K/ha/year) significantly ($p < 0.01$) increased the soil exchangeable K concentrations, compared to lower K fertiliser rates, and maintained the level above the optimum range of 0.6-1.1 meq K/100g (Table 3.2). Compared to the nil K fertiliser treatment, the 300 kg K/ha/year increased the soil exchangeable K concentrations by 50, 100 and 133% at the January 2022, May 2022, and January 2023 sampling times, respectively. While the K fertiliser treatments did not significantly influence the soil exchangeable Ca concentration during any sampling in the study, the increase in exchangeable K concentrations for the highest K fertiliser rate (300 kg K/ha/year) influenced a reduction in soil Ca saturation for K300 treatments, reaching between 12 to 16%, compared to the nil K fertiliser treatment. However, even with the highest K fertiliser rate, the Ca saturation stayed above 66%, so still maintained a high proportion of the cation exchange site.

Nitrogen fertiliser treatments influenced both soil mineral N and soil pH. The effect of N fertiliser treatments on soil mineral N was not consistent at all sampling times, but there was a general trend of mineral N being higher for the N added treatments at the two summer sampling times, compared to the nil-N fertiliser treatment. Overall, the differences in the amount of mineral N present in the soil at the three posttreatment sampling times was relatively small, compared to the amount of N applied in fertiliser, reflecting the high mobility of mineral N in the soil. For example, at the January 2022 sampling time the 300 kg N/ha/year treatment significantly increased mineral N, compared to the nil-N fertiliser treatment, but only by 30.5 kg N/ha/year (i.e. about 10% of what was applied). The more consistent effect of highest N fertiliser rate was the acidification of the topsoil. The 300 kg N/ha/year treatment significantly ($p < 0.05$) reduce soil pH by 0.33, 0.42 and 0.31 units at the January 2022, May 2022 and January 2023 sampling times, respectively. This acidification effect was due to the N fertiliser quantity and form of N applied, with the majority (~56%) of the N fertiliser applied as ammonium N, which is an acidifying fertiliser.

Table 3.4- Mineral nutrient concentrations of potassium (K), calcium (Ca), and mineral nitrogen (N), as well as Ca saturation and pH in the topsoil (0-15 cm) during the experimental time in samplings in May and January. Potassium, Ca, and Ca saturation averaged by K fertiliser rate and N concentration and soil pH averaged by N fertiliser rate.

Factor	2021-2022		2022-2023	
	Autumn (May 2021) [†]	Summer (Jan 2022)	Autumn (May 2022)	Summer (Jan 2023)
K concentration (meq/100g) [mean ± S.E.]				
<i>Potassium fertiliser</i>				
K0	1.1 ± 0.1	1.0 ± 0.1 b	1.0 ± 0.2 b	0.6 ± 0.1 b
K50	1.2 ± 0.1	1.0 ± 0.1 b	1.1 ± 0.1 b	0.7 ± 0.1 b
K150	1.0 ± 0.1	1.2 ± 0.1 b	1.4 ± 0.1 b	0.9 ± 0.1 b
K300	1.0 ± 0.1	1.5 ± 0.1 a	2.0 ± 0.1 a	1.4 ± 0.1 a
sig. code [‡]	ns	**	**	**
Ca concentration (meq/100g) [mean ± S.E.]				
<i>Potassium fertiliser</i>				
K0	30.3 ± 2.5	32.8 ± 1.9	27.4 ± 2.2	36.1 ± 2.4
K50	27.7 ± 2.8	28.1 ± 1.1	26.9 ± 1.3	31.1 ± 1.4
K150	33.2 ± 2.5	28.6 ± 1.1	27.9 ± 1.3	29.1 ± 1.4
K300	28.0 ± 2.5	28.2 ± 1.1	27.6 ± 1.3	30.4 ± 1.4
sig. code [‡]	ns	ns	ns	ns
Ca saturation (%) [mean ± S.E.]				
<i>Potassium fertiliser</i>				
K0	82.0 ± 1.3	77.7 ± 2.8 a	77.2 ± 2.7 a	79.0 ± 2.4 a
K50	81.1 ± 1.5	68.8 ± 1.6 b	70.6 ± 1.5 ab	74.1 ± 1.4 ab
K150	83.1 ± 1.3	72.7 ± 1.6 ab	71.7 ± 1.5 ab	72.2 ± 1.4 ab
K300	79.5 ± 1.3	67.4 ± 1.6 b	66.4 ± 1.5 b	71.2 ± 1.4 b
sig. code [‡]	ns	**	**	*
Mineral N (kg/ha) [mean ± S.E.]				
<i>Nitrogen Fertiliser</i>				
N0	57.7 ± 12.2	67.0 ± 9.2 b	33.3 ± 3.4	56.3 ± 5.7 b
N50	42.7 ± 14.1	92.3 ± 5.3 ab	32.0 ± 1.9	76.0 ± 3.3 a
N150	68.5 ± 12.2	75.4 ± 5.3 b	30.3 ± 1.9	64.3 ± 3.3 ab
N300	82.7 ± 12.2	97.5 ± 5.3 a	36.3 ± 1.9	72.8 ± 3.3 ab
sig. code [‡]	ns	**	ns	*
Soil pH (1:2.5) [mean ± S.E.]				
<i>Nitrogen Fertiliser</i>				
N0	6.50 ± 0.08	6.48 ± 0.09 a	6.75 ± 0.07 a	6.53 ± 0.06 a
N50	6.52 ± 0.09	6.39 ± 0.06 a	6.58 ± 0.04 a	6.39 ± 0.03 a
N150	6.56 ± 0.08	6.37 ± 0.06 a	6.61 ± 0.04 a	6.41 ± 0.03 a
N300	6.47 ± 0.08	6.15 ± 0.06 b	6.33 ± 0.04 b	6.22 ± 0.03 b
s.c. [‡]	ns	*	**	**

[†] Sampling in May 2021 represents a baseline before the beginning of treatments application.

[‡] s.c.- Significance codes: '**' $p < 0.01$; '*' $p < 0.05$; 'ns' $p > 0.05$

3.3.3 Seasonal influences on fruit mineral nutrient concentrations

When avocado fruit mineral nutrient concentrations were averaged for each harvest time during the season, concentrations between the early and late harvest varied, depending on the nutrient and fruit tissue assessed. The Ca concentration (Table 3.5) in the fruit flesh decreased significantly ($p<0.01$) between the early and late harvest by 57% in the 2021-2022 season and by 43% in the 2022-2023 season. However, the Ca concentration in fruit skin only decreased significantly ($p<0.01$) between the early and late harvests during the 2022-2023 season, with a decrease of 23%. There were also differences in Ca concentration between seasons, with higher fruit flesh and skin concentrations in the first season compared to the second season.

Table 3.5- Mean comparison for fruit mineral concentration of calcium (Ca), nitrogen (N), potassium (K), magnesium (Mg), as well as the N:Ca ratio and Ca+Mg:K ratio in fruit flesh and skin according to the sampling time during the fertiliser trial in the Bay of Plenty region. Means modelled after fitted a linear model with the sampling time as categorical factor and the mineral concentrations of ratios as dependent variables. Means with different letters are statistically different by the Tukey HSD test ($p<0.05$).

	Ca (mg/kg)	N (g/kg)	K (g/kg)	Mg (mg/kg)	N:Ca (ratio)	Ca+Mg:K (ratio)
<i>Harvest</i>						
<i>Avocado Flesh</i>						
---- [mean ± SE] ----						
2021-2022						
Early	455.1±11.6 a	14.1 ± 0.4 a	24.2±0.6 a	1112±18.9 a	31.5 ± 4 d	0.066±0.002 a
Late	193.8±6.7 c	11.9 ± 0.2 b	17.4±0.3 c	718.8±10.9d	66.4 ± 2.3 b	0.054±0.001 b
2022-2023						
Early	303.8±6.7 b	13.3 ± 0.2 a	23.2±0.3 a	911.4±10.9b	45.9 ± 2.3 c	0.053±0.001 b
Late	173.9±6.7 c	13.9 ± 0.2 a	21.3±0.3 b	839.4±10.9c	82.9 ± 2.3 a	0.048±0.001 c
sig. code †	**	**	**	**	**	**
<i>Harvest</i>						
<i>Avocado Skin</i>						
---- [mean ± SE] ----						
2021-2022						
Early	530.6±22.1 a	10.1±0.5 c	13.7±0.6 c	1047.6±31 b	20.6 ± 3 c	0.118±0.003 a
Late	483.6±12.7 a	15.9±0.3 a	23.7±0.3 a	1280±17.9 a	35.2 ± 1.7 b	0.075±0.002 b
2022-2023						
Early	315.9±12.7b	9.8±0.3 c	14.1±0.3 c	731.2±17.9d	33.8 ± 1.7 b	0.076±0.002 b
Late	244.3±12.7 c	13.7±0.3 b	19.7±0.3 b	806.8±17.9 c	58.6 ± 1.7 a	0.054±0.002 c
s.c. †	**	**	**	**	**	**

† s.c.- Significance codes: *** $p<0.01$; ** $p<0.05$; . $p<0.1$; 'ns' $p>0.1$

Nitrogen concentrations in fruit flesh decreased significantly ($p<0.01$) by 16% between the early and late harvests during the 2021-2022 season but remained statistically similar during the 2022-2023 season (Table 3.5). In contrast, N concentration in fruit skin increased significantly ($p<0.01$) between the early and late harvest during both seasons,

with increases of 57% during the 2021-2022 season and 40% during the 2022-2023 season.

Potassium concentration in fruit flesh decreased significantly ($p < 0.01$) between the early and late harvests by 28% in the 2021-2022 season and by 8% in the 2022-2023 season. Meanwhile fruit skin K concentration exhibited the opposite trend, with significant ($p < 0.01$) increases between the early and late harvests of 73% in the 2021-2022 season and 40% in the 2022-2023 season. Magnesium concentrations in fruit showed a similar trend as K in both seasons, decreasing in fruit flesh and increasing in fruit skins between the early and late harvests. Magnesium concentration between the early and late harvest, decreased in fruit flesh by 35% in 2021-2022 and 8% in 2022-2023, and increased in fruit skin by 22% in 2021-2022 and 10% in 2022-2023.

In general, all four nutrients investigated mostly showed a trend of decreasing concentrations in fruit flesh between the early and late harvests, except for N in the 2022-2023 season. However, fruit skin N, K and Mg concentrations showed a different trend between harvest times, compared to Ca. The skin concentrations of these three nutrients increased between early harvest and late harvest, whereas Ca concentrations decreased.

The N:Ca ratio in fruit flesh exhibited an increase between the early and late harvests of 111% during the first season and 81% during the second season. These differences were mainly attributed to the lower Ca concentrations observed during the late harvests in both seasons. Similarly, in fruit skins the N:Ca ratio increased between harvests by 71% in the 2021-2022 season and 73% in the 2022-2023 season. In this case, the change in the ratio between harvests was primarily due to the higher N concentrations at the late harvest. The N:Ca ratios showed statistically significant increases ($p < 0.01$) between the first and second seasons for both fruit tissues, which was mainly attributed to the generally lower Ca concentration observed in fruit tissues during the second season.

Fruit flesh Ca+Mg:K ratios decreased between the early and late harvests by 18% and 9% in the first and second seasons, respectively. Fruit skins Ca+Mg:K ratios showed a larger decrease between the early and late harvests, being 36% lower in the first season and 29%

lower in the second season. The lower Ca+Mg:K ratio values at the late harvest were mainly due to increases in the K concentration between the early and late harvests. The Ca+Mg:K ratios showed statistically significant decrease ($p<0.01$) between the first and second seasons for both fruit tissues.

3.3.4 Influence of annual N and K fertiliser rates on fruit nutrient status

This section presents the effect of N fertiliser and K fertiliser rates on fruit nutrient concentrations (e.g., Ca, N, K, Mg) and on N:Ca and Ca+Mg:K ratios. The results are organised by each nutrient or ratio, as a dependent variable, and the N fertiliser rate, K fertiliser rate, and the interaction between them are used as factors.

Overall, N fertiliser rate had a significant ($p<0.05$) influence on fruit nutrient concentrations and ratios, except for Mg concentration, during the study. However, this influence was not consistent for all sampling times, and was infrequent for some nutrients. As expected, the effect of N fertiliser rate was most frequently significant for fruit N concentration. In comparison, the effect on fruit Ca concentration was only significant at a single sampling time and only for fruit skin. Potassium fertiliser, used as an independent factor, did not influence the nutrient concentrations during the study. However, the interaction between N and K fertiliser became significant ($p<0.05$) only during late harvest in the 2022-2023 season for N concentration in the flesh, for K concentration in flesh and skins, and for the ratio Ca+Mg:K in both fruit tissues. The following sections describe in more detail the results for each nutrient or ratio analysed.

Table 3.6- Mean comparisons for the mineral nutrient concentration of calcium (Ca), nitrogen (N), potassium (K), magnesium (Mg), N:Ca ratio and Ca+Mg:K ratio in fruit flesh and skin from the early and late harvest of the 2021-2022 and 2022-2023 seasons according to the N fertiliser on used in the fertiliser trial of a commercial avocado orchard of the Bay of Plenty region. Averages are expressed in a dry weight (DW) basis.

NF	Season							
	Harvest				2022-2023			
	Early		Late		Early		Late	
Flesh	Skin	Flesh	Skin	Flesh	Skin	Flesh	Skin	
Ca concentration (mg Ca/kg) [mean \pm s.e.]								
N0	402 \pm 32	410 \pm 61	202 \pm 28	482 \pm 61ab	271 \pm 31	329 \pm 40	169 \pm 15	224 \pm 22
N50	491 \pm 24	603 \pm 61	210 \pm 17	544 \pm 35a	305 \pm 18	327 \pm 23	185 \pm 9	263 \pm 13
N150	445 \pm 24	472 \pm 61	181 \pm 17	408 \pm 35b	303 \pm 18	289 \pm 23	183 \pm 9	238 \pm 13
N300	500 \pm 24	534 \pm 61	199 \pm 17	509 \pm 35ab	270 \pm 18	305 \pm 23	171 \pm 9	253 \pm 13
s.c. †	ns	ns	ns	*	ns	ns	ns	ns
N concentration (g N/kg) [mean \pm s.e.]								
N0	14.0 \pm 0.5a	8.9 \pm 0.9	11.7 \pm 0.7	13.8 \pm 1.2b	10.9 \pm 0.9b	9.6 \pm 0.7	11.8 \pm 0.7b	12.4 \pm 0.6b
N50	13.0 \pm 0.5b	9.4 \pm 0.7	11.7 \pm 0.4	15.9 \pm 0.9ab	13.2 \pm 0.5ab	9.6 \pm 0.4	12.1 \pm 0.4b	12.0 \pm 0.4b
N150	15.6 \pm 0.5a	11.2 \pm 0.7	12.9 \pm 0.4	18.2 \pm 0.9a	13.6 \pm 0.5ab	9.8 \pm 0.4	15.0 \pm 0.4a	14.4 \pm 0.4a
N300	15.7 \pm 0.5a	11.1 \pm 0.7	12.0 \pm 0.4	15.9 \pm 0.9ab	13.7 \pm 0.5a	9.9 \pm 0.4	14.6 \pm 0.4a	14.3 \pm 0.4a
s.c. †	**	ns	ns	*	*	ns	**	**
K concentration (g K/kg) [mean \pm s.e.]								
N0	26.0 \pm 1.5 a	13.6 \pm 1.9	17.1 \pm 1.1 b	23.4 \pm 1.4	22.0 \pm 1.2	13.5 \pm 0.9	22.0 \pm 1.0	20.7 \pm 1.2ab
N50	22.0 \pm 0.8 b	13.5 \pm 1.0	16.8 \pm 0.7 b	23.1 \pm 0.8	22.6 \pm 0.7	13.4 \pm 0.5	20.2 \pm 0.6	18.5 \pm 0.7b
N150	26.6 \pm 0.8 a	15.0 \pm 1.0	19.5 \pm 0.7 a	23.9 \pm 0.8	25.0 \pm 0.7	14.4 \pm 0.5	22.2 \pm 0.6	21.2 \pm 0.7a
N300	26.1 \pm 0.8 a	13.7 \pm 1.0	17.0 \pm 0.7 b	24.0 \pm 0.8	22.8 \pm 0.7	14.8 \pm 0.5	21.1 \pm 0.6	19.5 \pm 0.7ab
s.c. †	*	ns	*	ns	ns	ns	ns	**
Mg concentration (mg Mg/Kg) [mean \pm s.e.]								
N0	1067 \pm 88	913 \pm 186	712 \pm 44	1189 \pm 134	875 \pm 54	746 \pm 42	847 \pm 50	834 \pm 40
N50	1071 \pm 44	1016 \pm 93	722 \pm 22	1309 \pm 67	900 \pm 27	738 \pm 21	845 \pm 25	796 \pm 20
N150	1115 \pm 44	1102 \pm 93	731 \pm 22	1218 \pm 67	940 \pm 27	709 \pm 21	874 \pm 25	803 \pm 20
N300	1248 \pm 44	1111 \pm 93	725 \pm 22	1298 \pm 67	895 \pm 27	713 \pm 21	848 \pm 25	801 \pm 20
s.c. †	ns	ns	ns	ns	ns	ns	ns	ns
N:Ca (ratio) [mean \pm s.e.]								
N0	35.4 \pm 4.4	22.7 \pm 5.2	60.8 \pm 11.0ab	29.9 \pm 5.7b	41.1 \pm 6.6	29.1 \pm 7.1	70.7 \pm 6.1 b	55.7 \pm 3.9ab
N50	26.8 \pm 2.2	15.9 \pm 2.6	59.1 \pm 6.4b	30.7 \pm 3.3b	45.7 \pm 3.8	31.6 \pm 4.1	67.6 \pm 3.1 b	47.1 \pm 1.9b
N150	35.3 \pm 2.2	24.1 \pm 2.6	80.2 \pm 6.4a	47.7 \pm 3.3a	46.3 \pm 3.8	36.1 \pm 4.1	83.3 \pm 3.1 a	62.9 \pm 1.9a
N300	31.7 \pm 2.2	21.9 \pm 2.6	64.1 \pm 6.4ab	32.7 \pm 3.3b	53.6 \pm 3.8	37.3 \pm 4.1	87.4 \pm 3.1 a	58.9 \pm 1.9a
s.c. †	ns	ns	.	**	ns	ns	*	**
Ca+Mg:K (ratio) [mean \pm s.e.]								
N0	0.057 \pm 0.008	0.100 \pm 0.16	0.053 \pm 0.006	0.074 \pm 0.003ab	0.053 \pm 0.002	0.081 \pm 0.008	0.046 \pm 0.002	0.052 \pm 0.003ab
N50	0.071 \pm 0.004	0.120 \pm 0.08	0.057 \pm 0.003	0.080 \pm 0.002a	0.054 \pm 0.001	0.080 \pm 0.004	0.052 \pm 0.001	0.058 \pm 0.002a
N150	0.059 \pm 0.004	0.105 \pm 0.08	0.048 \pm 0.003	0.068 \pm 0.002b	0.050 \pm 0.001	0.070 \pm 0.004	0.048 \pm 0.001	0.049 \pm 0.002b
N300	0.067 \pm 0.004	0.120 \pm 0.08	0.055 \pm 0.003	0.076 \pm 0.002ab	0.051 \pm 0.001	0.070 \pm 0.004	0.049 \pm 0.001	0.055 \pm 0.002ab
s.c. †	ns	ns	ns	*	ns	ns	ns	*

†s.c. -Significance codes: '**' $p < 0.01$; '*' $p < 0.05$; '.' $p < 0.1$; 'ns' $p > 0.1$

3.3.4.1 Fruit Ca concentration

The effect of N fertiliser rates on fruit Ca concentration was only significant ($p < 0.05$) for fruit skins during late harvest in the 2021-2022 season (Table 3.6). During this season, the Ca concentration in the N150 treatments decreased significantly ($p < 0.05$) compared to the value in N50 treatments. Fruit skin in avocados from trees receiving the N50 treatments on average concentrated 33% higher Ca than trees receiving N150 treatments. The Ca concentration in skins for N150 was statistically like N300 during this sampling. The N fertiliser had no significant influence ($p > 0.05$) on fruit flesh Ca concentration at any sampling time in the 2021-2022 season. During the 2022-2023 season, no trend was identified for changes in fruit Ca concentration due to N fertiliser rates. These results indicate that while the N150 fertiliser rate can potentially reduce fruit Ca concentration, it was only observed for fruit skins at late harvest, and only in one out of two seasons sampled.

3.3.4.2 Fruit N concentration

Nitrogen fertiliser rate was the main factor influencing changes in fruit flesh and skin N concentrations in both seasons of the field trial (Table 3.6). During the 2021-22 season, there was a trend of fruit flesh and skin N concentrations increasing with N fertiliser rate. However, this trend was only significant for fruit flesh at the early harvest and fruit skins at the late harvest. At the early harvest, the fruit flesh N concentrations for the two highest N fertiliser rates (i.e., N150 and N300) were significantly ($p < 0.01$) higher than for the N50 treatment but not different from the N0 treatment. At late harvest, unexpectedly only the N150 treatments had significantly ($p < 0.05$) higher fruit skin N concentration, compared to the treatment without N fertiliser (N0) treatment. However, fruit skins N concentration in N150 treatments did not increase significantly compared to others N fertiliser rates. The N150 treatment increased fruit skin N concentration by 31.9% compared to the N0 treatment value of 13.8 g N/kg.

During the 2022-2023 season, the trend for higher fruit flesh and skin N concentrations with the two highest N fertiliser rates was consolidated, especially during the late harvest (Table 3.6). Nitrogen concentration in flesh increased significantly with N fertiliser at early harvest, like in the previous season, but also for both fruit flesh and skin at late

harvest. During the early harvest, the N300 rate significantly ($p<0.05$) increased fruit-flesh N concentration by 21% compared to the concentration for the N0 treatment of 10.9 g N/kg. However, the difference in flesh-N concentration between the N300 rate and lower N fertiliser rates were not statistically different at this sampling. During the late harvest, the N150 and N300 rates significantly ($p<0.01$) increased the N concentration in fruit flesh and skins compared to the N50 and N0 treatments. The N150 rate exhibited the highest N concentrations in both fruit flesh and skin, which were 27% higher than the N0 value of 11.8 g N/kg for flesh and 20% higher than the N50 value of 12.0 g N/kg for fruit skin.

The effect of the interaction between N fertiliser and K fertiliser rates on fruit N concentration was only statistically significant ($p<0.05$) during the late harvest of the 2022-2023 season (Figure 3.1). Therefore, there is an influence of the K fertiliser rates on how N fertiliser rates influenced the fruit N concentrations at this sampling time. For treatments with the two highest K fertiliser rates (K150, K300), flesh N concentrations increased until the N150 rate, and then ceased increasing for N300 rates. The highest flesh and skin N concentrations for treatments using the two highest K fertiliser rates were found in N150K150 and N150K300. Surprisingly, the N300K300 treatment did not concentrate higher N in skins or flesh. In the case of treatments using K50, the lowest K fertiliser rate, the fruit N concentration constantly increased with N fertiliser rates, from N50 to N300. The maximum N concentration for treatments using K50 rates was found in the treatment N300K50. Thus, N concentrations in both fruit flesh and skin showed the largest concentration increases between treatments using N50 and N150.

During the late harvest of the 2022-2023 season, the N150K150 treatment increased the N concentration in fruit flesh by 46% and in skins by 36% compared with the N50K50 treatment, which had the lowest fruit N concentrations the during the season of 9.4 g N/kg in flesh and 9.5 g N/kg in skin. Overall, treatments using N50 did not reach 13 g N/kg during the 2022-2023, while the averages for treatments with higher N fertiliser rates surpassed that value.

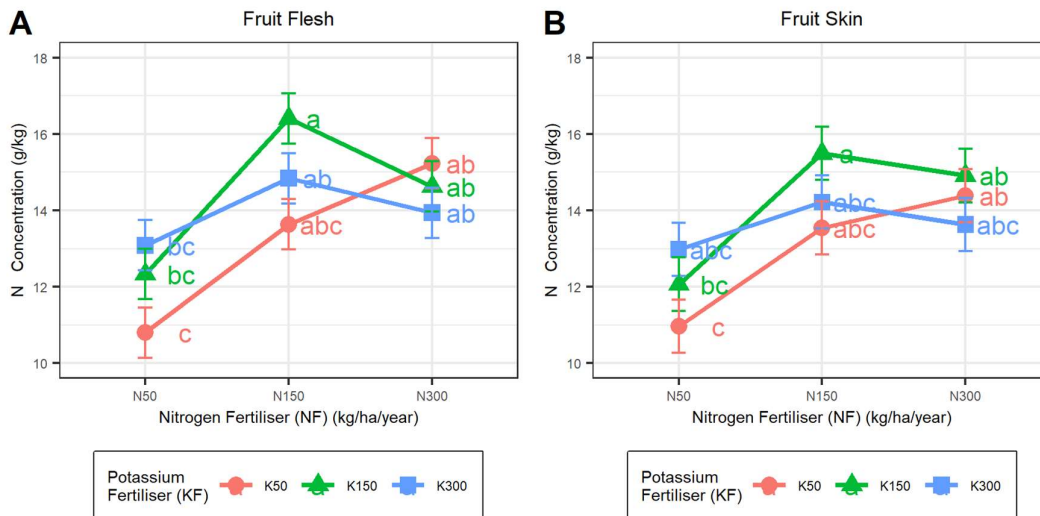


Figure 3.1- Interaction plot for fruit nitrogen (N) concentration in flesh (A) and skin (B) during the late harvest of the 2022-2023 season: This plot illustrates the Interaction among treatments using 50, 150 and 300 kg/ha/year of N and potassium (K) fertiliser rates. The points represent the modelled mean values of N concentration (g/kg) in each fruit tissue after fitting linear models, with N concentration in flesh or skin as the dependent variable and the interaction between N and K fertilisers as a categorical factor. Vertical lines indicate the standard error of the mean. Means with different letters are statistically different according to the Tukey HSD test ($p < 0.05$).

3.3.4.3 Fruit K concentration

During the 2021-2022 season, there was a trend of higher fruit K concentration from the N150 treatments compared to the N50 treatments (Table 3.6). However, this trend was only statistically significant ($p < 0.05$) for fruit flesh during both harvest times in the 2021-2022 season. At early harvest, fruit flesh K concentration for the N150 treatment was 21% higher than the value for the N50 treatment of 22.0 g K/kg. At late harvest, fruit flesh-K concentration for the N150 treatment was 16% higher than the value for the N50 treatment of 16.8 g K/kg. The interaction between N fertiliser and K fertiliser was not statistically significant ($p > 0.05$) for the K concentration in fruit tissues during this season.

During the 2022-2023 season, despite the same trend towards higher K concentration in N150 compared to N50 rates, the effect of N fertiliser rates was only statistically significant ($p < 0.05$) in fruit skins at late harvest. At this harvest, the N150 rate increased fruit skin K concentration by 15% compared to the N50 treatment value of 18.5 g K/kg. Additionally, during the same late harvest of this season the interaction between N fertiliser and K fertiliser was significant for flesh ($p < 0.05$) and skin ($p < 0.01$) (Figure 3.2).

This interaction exhibited a greater influence of low K fertiliser rates (K50) reducing the K concentration only when the N fertiliser was the lowest (N50) in both fruit tissues. Thus, the N50K50 was the only treatment consistently reducing fruit flesh and skin K concentration among the three treatments using K50 rates. Unexpectedly, in fruit skins, the highest K concentration was found in N150K50.

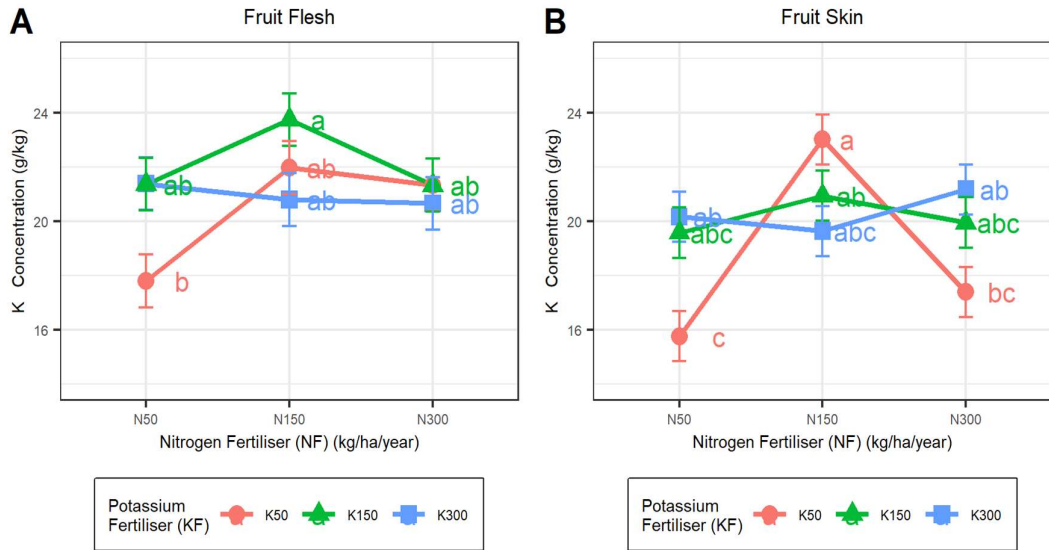


Figure 3.2- Interaction plot for fruit potassium (K) concentration in flesh (A) and skin (B) during the late harvest of the 2022-2023 season: This plot illustrates the Interaction among treatments using 50, 150 and 300 kg/ha/year of nitrogen (N) and K fertiliser rates. The points represent the modelled mean values of K concentration (g/kg) in each fruit tissue after fitting linear models, with K concentration in flesh or skin as the dependent variable and the interaction between N and K fertiliser as a categorical factor. Vertical lines indicate the standard error of the mean. Means with different letters are statistically different according to the Tukey HSD test ($p < 0.05$).

3.3.4.4 Fruit Mg concentration

The concentration of Mg in fruit skin and flesh was not significantly influenced ($p > 0.05$) by the levels of N fertiliser (Table 3.6), K fertiliser, or their interaction during the experimental period.

3.3.4.5 Fruit N:Ca ratio

An influence of N fertiliser rates on the N:Ca ratio in fruit tissues was observed in both seasons but was only statistically significant at the late harvests (Table 3.6). At the late harvests in both seasons, N fertiliser rates had a statistically significant ($p < 0.01$) effect

on fruit skin N:Ca ratios. In the first season, the N150 rates increased the late harvest skin N:Ca ratio by 60%, compared to the N50 treatment value of 30.7. In the second season, N fertiliser increased the N:Ca ratio in skins for N150 and N300 rates compared with N50 treatments, reaching increases of 34% of N150 and 25% of N300 compared to a 47.1 value for the ratio in N50 rates. In fruit flesh, the influence of N fertiliser rates on N:Ca ratio at late the harvests was not as strong as the effect on fruit skins in both seasons. In the first season, despite the N150 treatment increasing the flesh N:Ca ratio by 36%, compared to the N50 treatment value of 59.1 in N50, the difference was only marginally significant ($p < 0.1$). In the second season, the N150 and N300 rates significantly ($p < 0.05$) increased the N:Ca ratio in fruit flesh compared to N0 and N50 rates. The ratio increased by 23% with N150 rates and 29% with N300 rates respectively, compared to the N50 value of 67.6.

The changes in the N:Ca ratio described for the late harvest were related to changes in different nutrient concentrations each season. During the 2021-2022 season, the trend for the highest skin N:Ca ratio for the N150 rate was due to both the highest fruit skin N concentration and lowest Ca concentrations occurring in the N150 rate. Meanwhile, during the 2022-2023 season, the changes in the ratio were principally associated with increases in N concentrations in fruit flesh and skin for the N150 and N300 rates, rather than changes in both N and Ca concentrations, as was seen in the previous season.

3.3.4.6 Fruit Ca+Mg:K ratio

The effect of N fertiliser rates on the Ca+Mg:K ratio was only statistically significant for fruit skins from the late harvest of both seasons (Table 3.6). This ratio was significantly lower ($p < 0.05$) for the N150 rate by 15% compared to the N50 rate, during the late harvest in both seasons (ratio values for the N50 rate were 0.08 and 0.058 in 2021-2022 and 2022-2023 seasons, respectively). In the first season, the higher fruit skin Ca+Mg:K ratios for the N50 rate was due to the lower fruit skin Ca concentration achieved with the N150 rate during the late harvest. However, in the second season the difference between the influence of the N50 and N150 rates on fruit skin Ca+Mg:K ratios was principally due to the higher skin K concentrations for the N150 rate.

The Ca+Mg:K ratio during the late harvest of the 2022-2023 season had a significant ($p < 0.05$) interaction between N fertiliser and K fertiliser (Figure 3.3). This interaction was defined by the changes in K concentrations during the season. Thus, the interaction represented the inverse behaviour related to the K concentration in both tissues as compared to Figure 3.2, except for the ratio in the flesh of the treatment N300K150, in which the ratio was lower than expected. Although there was insufficient statistical evidence ($p > 0.05$) for a lower Ca concentration in the N300K150 treatment during the late harvest of the 2022-2023 season, the treatment had the lowest Ca concentration in fruit flesh among treatments (158 mg Ca/kg), which could explain it as having the lowest Ca+Mg:K ratio. Thus, as discussed for the K concentration, K50 rates influenced the Ca+Mg:K ratio only at the N50 rates, increasing the ratio in the N50K50 treatment compared with other treatments using higher N fertiliser rates and N50 rates.

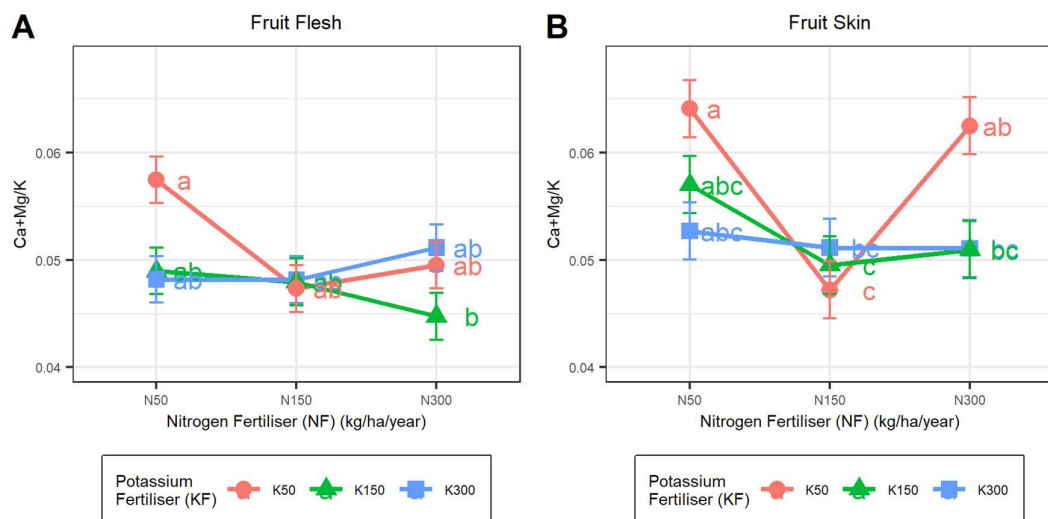


Figure 3.3- Interaction plot for the Ca+Mg:K ratio in fruits in flesh (A) and skin (B) during the late harvest of the 2022-2023 season: This plot illustrates the Interaction among treatments using 50, 150 and 300 kg/ha/year of nitrogen (N) and potassium (K) fertiliser rates. The points represent the modelled mean values of the Ca+Mg:K ratio in each fruit tissue after fitting linear models, with the ratio in flesh or skin as the dependent variable and the interaction between N and K fertiliser as a categorical factor. Vertical lines indicate the standard error of the mean. Means with different letters are statistically different by according to the Tukey HSD test ($p < 0.05$).

3.3.4.7 Response surface method models for the N:Ca and Ca+Mg:K ratios in fruit tissues

Response surface method (RSM) models for N:Ca ratios, at the late harvest in flesh and skin, were fitted with significant statistical adjustment ($p < 0.05$), and insignificant ($p > 0.05$) lack of fit between the surface and the empirical data (Figure 3.4). In these

models, as seen in the Table 3.7, two trends were statistically significant ($p < 0.05$): the positive linear effect of N fertiliser rate and the negative quadratic effect of N fertiliser rate. The RSM models for the late harvest season in both tissues showed surfaces with similar trends (Figure 3.4). The N:Ca ratio increased linearly with N fertiliser rate due to the positive linear effect, with the greatest increase between 50 and 150 kg N/ha/year. Then, between 150 and 300 kg N/ha/year the ratio reached a maximum, forecasted by the significant negative quadratic effect of N fertiliser. Thus, the maximum N:Ca ratio in both fruit tissues was forecasted by the RSM models in the upper-right quadrant of the experimental space in fertiliser ranges of 189-205 kg N/ha/year and 147 -172 kg K/ha/year. Both RSM models, especially the model for the skin, forecast isolines (lines with equal N:Ca ratio) with shorter distances in the N fertiliser direction, suggesting quicker changes in this direction related to K fertiliser rates. Moreover, the RSM models for the N:Ca ratio in fruit flesh and skins predicted the lowest ratios in the direction of the N50K50 and the N50K300 treatments, while the highest ratios were predicted for treatments with 150 and 300 kg N/ha/year rates. Surprisingly, the N300K300 treatment, exhibited lower ratios than treatments with N150 rates. However, the lower values in the modelled N:Ca surface for the N300K300 treatment compared to the values for the N150 treatments coincided with the averages for the N concentration in both tissues, even those values were not statistically lower as discussed in section 3.3.4.2 . It is not clear why there is apparent reduction in N:Ca at the highest rates of N and K combined, but the advantage of these RSM models lies in their ability to identify trends and directions for further investigation. This will be explored more in subsequent chapters, in terms of the effects of these treatments on fruit quality and tree biomass.

Table 3.7- Equations for the RSM models of the N:Ca ratio at the late harvest season in fruit tissues

Equation[†]

$$\text{N:Ca}_{\text{flesh}} = 85.97 + 14.73 \text{NFT} - 20.21 \text{NFT}^2 \quad (1)$$

$$\text{N:Ca}_{\text{skin}} = 58.76 + 11.50 \text{NFT} - 20.47 \text{NFT}^2 \quad (2)$$

[†] NFT is the transformed N fertiliser rate using the linear function $\text{NFT} \sim (\text{N fertiliser} - 150)/150$

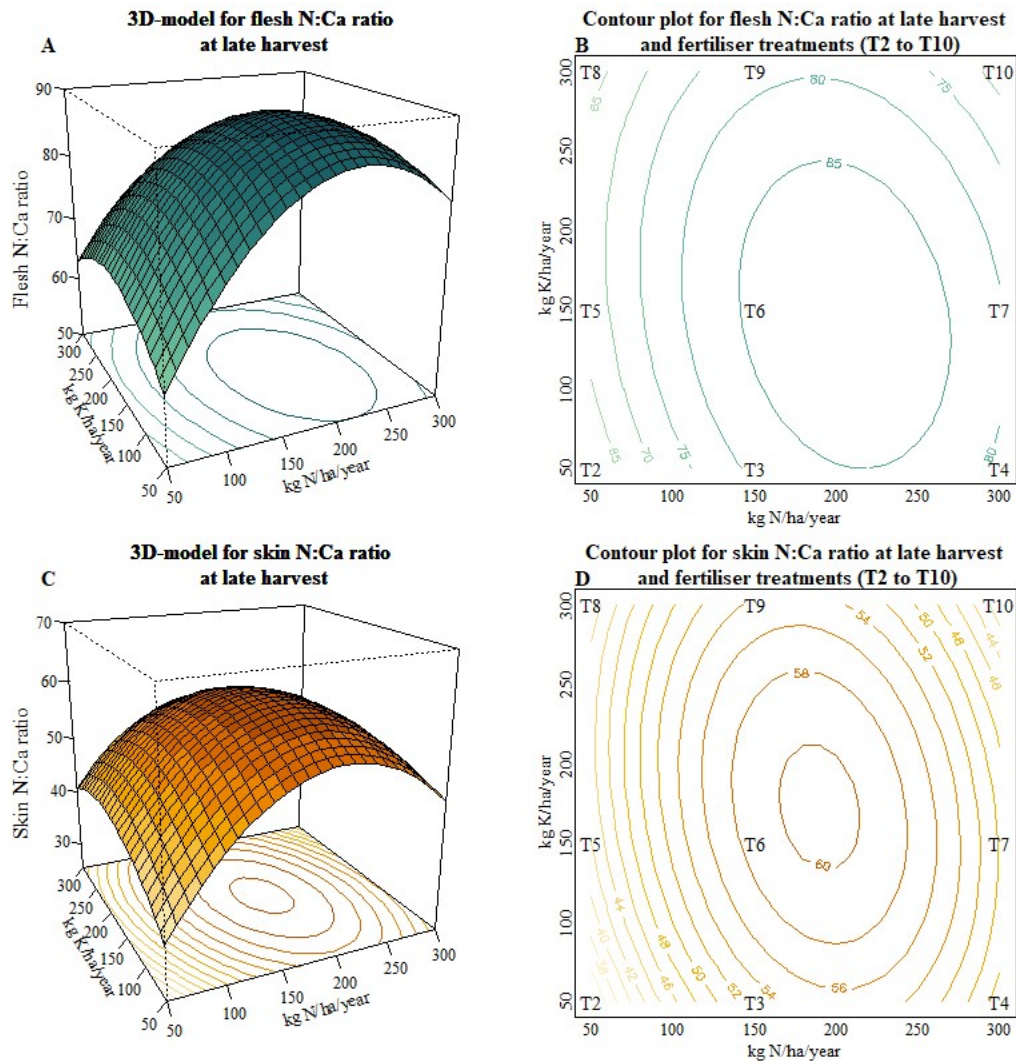


Figure 3.4- Response surface method (RSM) models for the nitrogen-to-calcium (N:Ca) ratio in fruit flesh (Top: A, B) and skin (Bottom: C, D) at the late harvest. The surfaces were modelled using as dependent variables, the data for both the N:Ca ratio during the late harvest of the 2021-2022 and 2022-2023 seasons, and as independent predictor variables, the nitrogen (N) and (K) fertiliser rates in kg/ha/year. In the contour plots (Right: B, D), the labels T2 through T10 represent the N and K fertiliser rates for treatments T2 to T10 in the trial.

3.3.5 Effect of adding calcium nitrate fertiliser during early fruit set on fruit nutrient composition

The application of treatments with CN (T11-N150K150Ca70 and T12-N150K150Ca90; Strategy 2 in Table 3.1) at early fruit set did not show a statistically significant change in fruit flesh or skin Ca concentrations at either the early or late harvest during both seasons, compared to the same treatment without CN addition (T6-N150K150 treatment). For instance, the skin Ca concentration at late harvest in the 2022-2023 season was 230.3 ± 19.8 mg Ca/kg for the treatment using CN during fruit set (T12-N150K150Ca90) and 241.82 ± 19.8 mg Ca/kg for the treatment without CN applied (T6-N150K150). The application of CN at fruit set also did not appear to have an influence on the fruit concentrations of any of the other nutrients assessed at any sampling time.

3.3.6 Fertiliser effect on the performance of avocado trees

3.3.6.1 Yield estimation and fruit weight

The yield estimation (September 2022) was not significantly influenced ($p > 0.05$) by N or K fertiliser rates during the 2022-2023 season, however, there was a trend of yield decreasing with increasing N fertiliser rate with the two highest K fertiliser rates (300 and 150 kg K/ha/year) (Figure 3.5A). For example, when K was applied at a rate of 300 kg K/ha/year, increasing the N fertiliser rate from 50 to 300 kg N/ha/year decreased yield estimation by 50 kg/tree/year (42%). A similar reduction in yield estimation was observed with increasing N fertiliser rate at the 150 kg K/ha/year rate. In contrast, at the lowest K fertiliser rate of 50 kg K/ha/year, increasing the N fertiliser from 50 to 300 kg N/ha/year tended to increase yield estimation by 40 kg/tree/year (55%). The high intra-treatment variability in yield estimates during the 2022-2023 season was partly due to the impact of the ex-tropical cyclone Dovi in February 2022, which reached winds of up to 120 km/h when it hit the BoP. After Dovi, between 5 and 20 fruits/m² were counted on the ground across the experimental area, affecting yield during the season. Although the variability generated by the extreme weather conditions on yield estimation, the fruit

weight of avocados with commercial sizes followed a similar pattern to the yield estimation during the season 2022-2023, except for the treatment N150K300.

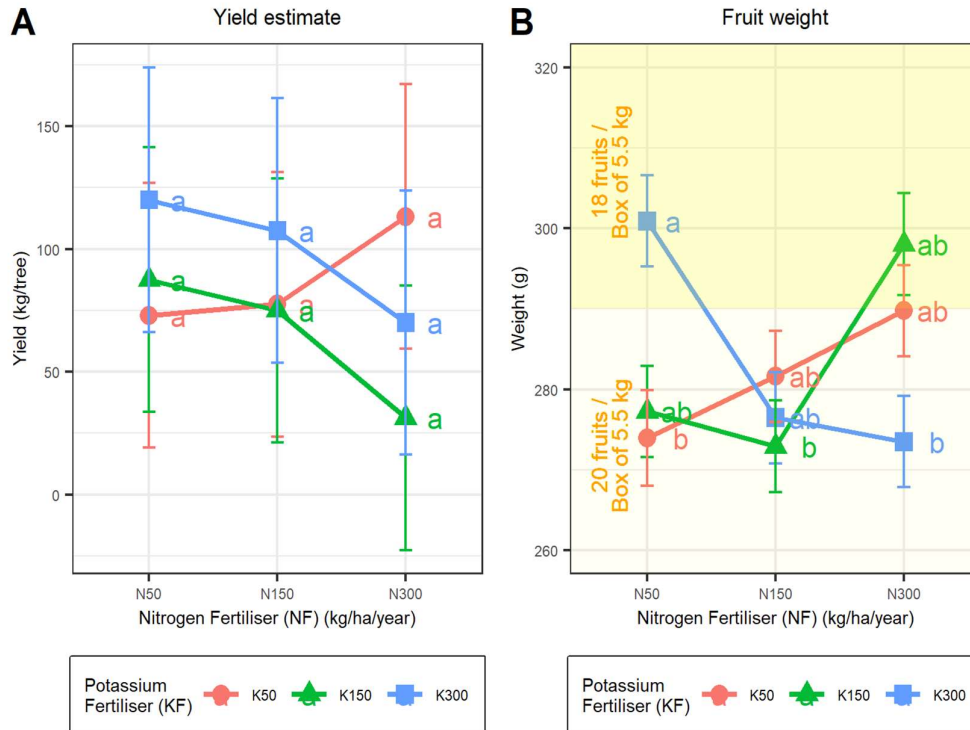


Figure 3.5- Interaction plot for yield estimate per tree (A) and the fruit weight of commercially exportable fruit (B) estimated in September 2022, before the early harvest during the 2022-2023 season: This plot illustrates the interaction among treatments using 50, 150 and 300 kg/ha/year of nitrogen (N) and potassium (K) fertiliser rates. The points represent the modelled mean values after fitting linear models, with yield estimation (kg/tree) or fruit weight (g) as the dependent variables and the interaction between N and K fertilisers as a categorical factor. Vertical lines indicate the standard error of the mean. The shaded yellow areas in graph B represent the two weight ranges commonly exported from New Zealand (18-count: 18 fruits in a 5.5 kg carton; 20-count: 20 fruits in a 5.5 kg carton). Means with different letters are statistically different according to the Tukey HSD test ($p < 0.05$).

The fruit weight during 2022-2023 season was significantly affected by the interaction between N and K fertiliser rates ($p < 0.05$), following a similar pattern to the yield estimation, except for treatments using 150 kg K/ha/year (Figure 3.5B). For treatments using 300 kg K/ha/year, increasing the N fertiliser rate from 50 to 300 kg N/ha/year decreased fruit weight significantly ($p < 0.05$) by 28 g/fruit (9%). The N50K300 treatment produced avocados with average fruit weight of 301 g/fruit, typically falling within the 18-count size category for the New Zealand industry (18 fruits packed in a 5.5 kg box). In contrast, for treatments using 50 kg K/ha/year, increasing the N fertiliser from 50 to 300 kg N/ha/year, tended to increase the fruit weight by 16 g/fruit (6%), but the difference

was not significant among treatments ($P>0.05$). For treatments using 150 kg K/ha/year, the fruit weight was lower in the N150K150 treatment (273 g/fruit) and higher in the N300K150 treatment (298 g/fruit), changing no significantly ($p>0.05$) between these two treatments. It is worth noting that fruit produced with low N fertiliser rates showed no trend towards reduced yield or fruit weight, with fruit produced in N50K300 showing the highest fruit weight of the fertiliser treatments.

3.3.6.2 Mineral nutrient concentrations in leaves

Mineral nutrient concentrations in mature leaves of non-fruiting shoots were not influenced significantly ($p>0.05$) by any fertiliser treatments at the May 2022 sampling (Autumn), which followed one full year of fertiliser treatment applications (Table 3.8). This includes insignificant differences between the control without fertiliser use and the fertiliser treatments. During this sampling in Autumn, which is used by consultants to guide fertiliser practices for the upcoming fruiting season, the concentrations of N, K, Ca, and Mg were similar or higher than the baseline averages for leaves sampled in May 2021, and generally fell within the typical range reported by Dixon (2008) for trees from high yielding (20 - 25 tonnes/ha/year) avocado orchards during the Autumn sampling in the BoP.

Table 3.8- Leaf mineral concentrations of nitrogen (N), potassium (K), calcium (Ca), and magnesium (Mg) during the Autumn sampling in May 2021 (Baseline) and May 2022, after one year of fertiliser treatment applications. In addition, the range of leaf mineral concentrations in orchards of the Bay of Plenty region yielding between 20 and 25 tonnes/ha according to Dixon (2008).

Nutrient	May 2021 Baseline	May 2022		Range (Dixon, 2008)
		Non-Fertilised treatments	Fertilised treatment	
		<i>g/ kg [mean ± S.E.]</i>		
N	27.3 ± 0.7	27.4 ± 1.1	28.6 ± 0.4	24 – 27
K	9.2 ± 0.6	10.3 ± 0.7	10.2 ± 0.2	9 – 12
Ca	12.4 ± 0.6	14.4 ± 1.2	15.8 ± 0.4	12 – 18
Mg	3.0 ± 0.2	3.7 ± 0.3	3.8 ± 0.1	3.5 - 4.4

For leaf samples from fruiting shoots collected in November 2021 and November 2022, which is a time of maximum flowering, treatments using 300 kg K/ha/year significantly ($p<0.05$) increased the leaf K concentrations, except for mature leaves in November 2021 (Figure 3.6). Overall, the effect was observed after the application of three out of the seven (i.e. 43%) annual K fertiliser applications during the season, as described in Table

3.1 (e.g. 130 kg K/ha for the treatments using 300 kg K/ha/year). At the November 2021 sampling, K fertiliser significantly influenced the K concentration in young leaves, but not the K concentration in mature leaves. During this sampling, the 300 kg K/ha/year treatments increased the K concentration in young leaves by 18% compared to the control (0 kg K/ha/year) treatment value of 17.5 g K/kg. At the November 2022 sampling, K300 treatments consistently increased the leaf K concentration in both young and mature leaves, compared to lower K fertiliser rates. In mature leaves, K300 treatments significantly increased the K concentration in the Summer of 2022, compared to the K150 and K50 treatments. The increase in K concentration in mature leaves with the K300 treatment reached 20%, compared to 8.8 g K/kg in K50 and 25% compared to 8.5 g K/kg in K150 rates. In young leaves, like in mature leaves, the K concentration increased significantly ($p < 0.05$) by 10% in K300, compared to the K concentration of 18 g K/kg in K150 rates.

In terms of Ca concentration, the leaf concentrations did not change significantly ($p > 0.05$) with K fertiliser rates at any of the two samplings undertaken either in May or November (Data not shown). All treatments had an adequate Ca concentration in leaves by May (12 to 18 g Ca/kg) regardless of the K fertiliser rate, ensuring an adequate Ca supply for the upcoming season. Similarly, the quantity of K applied by November each year (~130 kg K/ha for the K300 treatments) neither influenced the Ca concentration in leaves of fruiting shoots. These results suggest that under the trial conditions of high soil exchangeable Ca concentration, there does not appear to be significant antagonist effect between the use of high K fertiliser rates and the Ca concentration in leaves during the season.

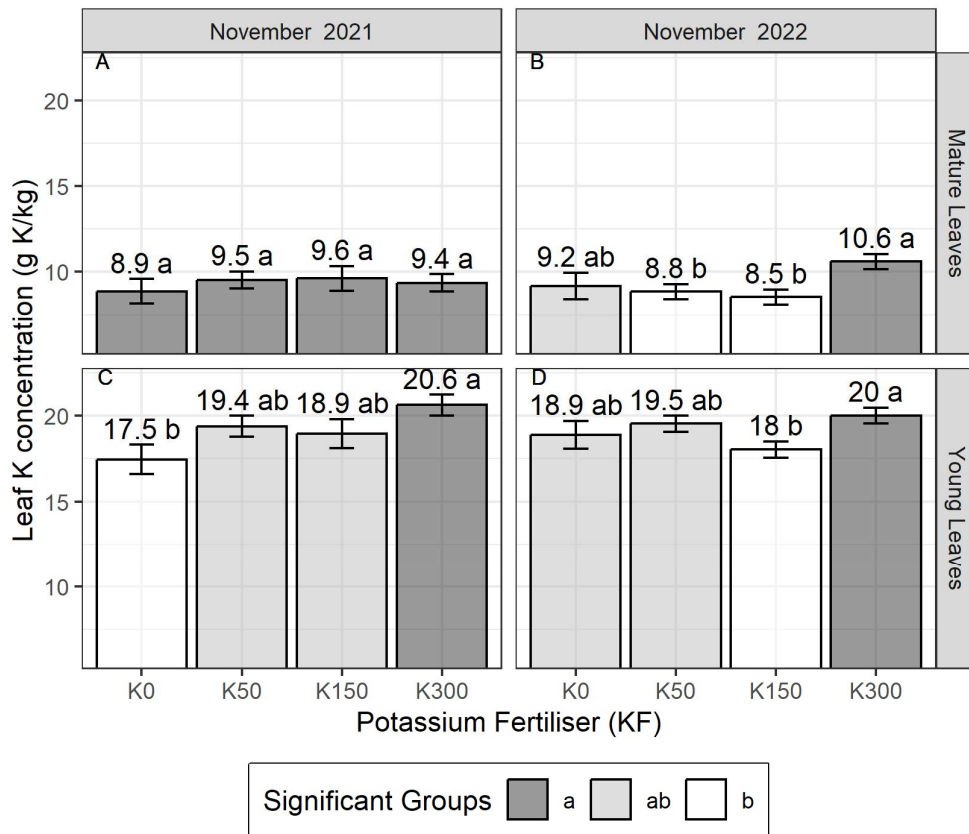


Figure 3.6 – Mean potassium (K) concentration in mature and young leaves by potassium fertiliser (KF) rate during early fruit set in November of 2021 and 2022 in the fertiliser trial: The bars represent the mean values after fitting linear models for the K concentration (g/kg) in each leaf sampling as the dependent variable, with KF rates as a categorical factor. Vertical lines indicate the modelled standard error of the mean. The numbers above the bars are the mean values. Means followed by different letters are statistically different according to the Tukey HSD test ($p < 0.05$) and belong to different significant groups.

3.3.6.3 Biomass production

The N fertiliser rate influenced the development of the canopy biomass during the 2022-2023 season (Figure 3.7), confirming qualitative observations during the previous season on the experimental trees. Despite the canopy biomass estimation during April 2022 (Baseline) and January 2023 not showing statistical differences ($p > 0.05$) among N fertiliser rates, the difference between the canopy biomass estimation by tree during the two samplings showed the effect of N fertiliser use on biomass production. During the biomass estimation in April 2022 (Baseline) (Figure 3.7A), developed right after the general pruning of the trees in the trial in February 2022, the trend was a decline in the canopy biomass with the N fertiliser rate, which was not statistically significant ($p > 0.05$).

Thus, the tree canopy biomass reduced from 477 ± 71 m³/tree in trees with no N fertiliser use (N0) to 374 ± 37 m³/tree in trees with N300 rates. A likely explanation for this result is the more intense pruning of the tallest trees of N300 treatments after one year of N fertiliser application compared to the trees in the N0 treatment.

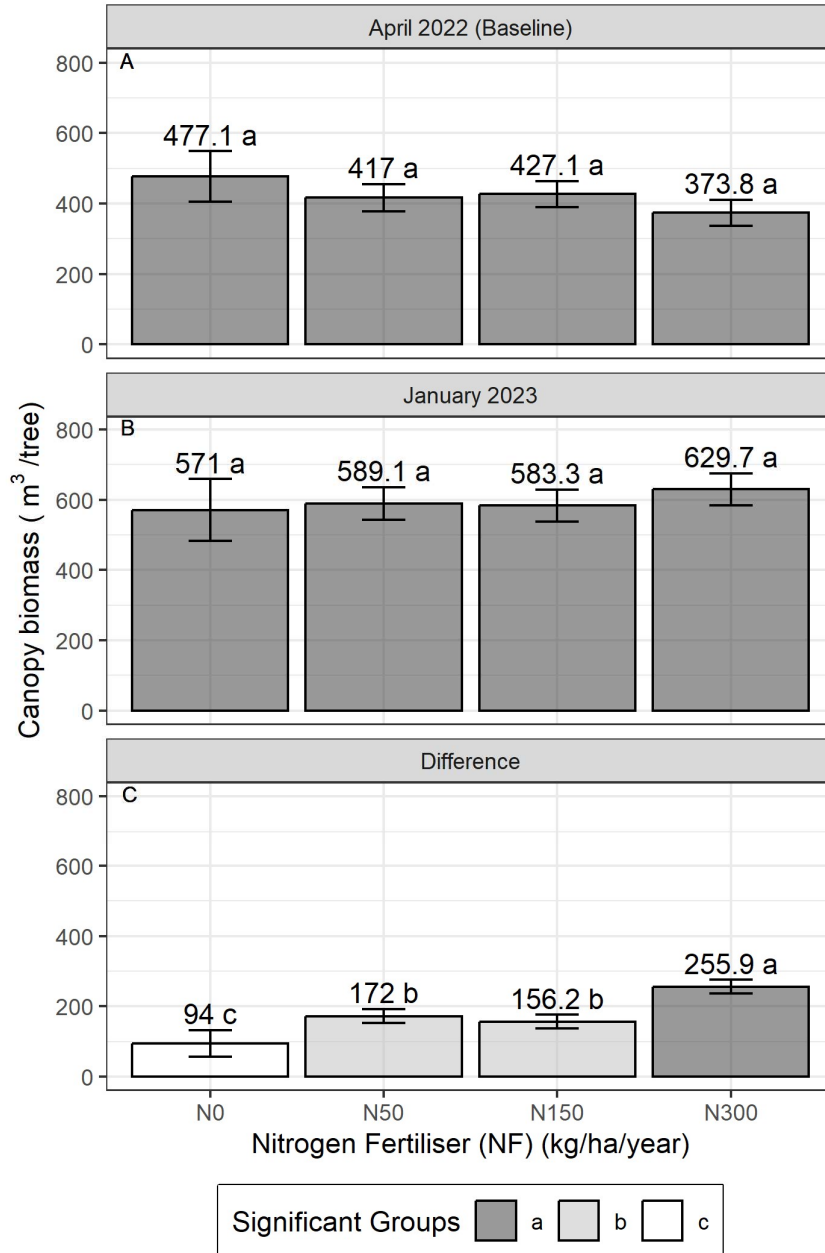


Figure 3.7- Mean tree canopy biomass by nitrogen fertiliser (NF) rate throughout the 2022-2023 season: The bars represent the mean values after fitting linear models with tree biomass estimations in April 2022 (A) and January 2023 (B), as well as the difference between the two samplings (C), as dependent variables, with NF rates as categorical factors. Vertical lines indicate the modelled standard error of the mean. The numbers above the bars are the mean values. Means followed by different letters are statistically different according to the Tukey HSD test ($p < 0.05$) and belong to different significant groups.

Nine months after the baseline biomass estimation, in January 2023 (Figure 3.7B), the general trend was an increment in the canopy biomass with the N fertiliser rate. This trend was insignificant ($p>0.05$), like for the canopy biomass estimation in April 2022. However, the biomass increased from 571 ± 87 m³/tree in trees of the N0 treatment to 630 ± 45 m³/tree in trees with N300 fertiliser rates, highlighting a differential growth rate of canopies in trees using the highest N fertiliser rates.

Therefore, the difference in the canopy biomass estimation between April 2022 and January 2023 increased significantly ($p<0.05$) with the N fertiliser rate (Figure 3.7C). The difference in canopy biomass estimation between both samplings increased significantly ($p<0.05$) from 94 ± 38 m³/tree in trees without N fertiliser (N0) to 256 ± 20 m³/tree in trees with N300 rates. These differences in canopy biomass between April 2022 and January 2023 represented 19% increase for trees without N fertiliser use, around 40% for trees using N50 and N150 rates, and 68% for trees using N300 rates.

The interaction between N fertiliser and K fertiliser rates was insignificant for the difference in canopy biomass, with a clear trend towards higher changes between April 2022 and January 2023 in treatments with higher N and K fertiliser inputs (Figure 3.8). Then, the highest differences in the canopy biomass were for trees in the N300K300 treatment, while the lowest differences were for trees on the N50K50 and N150K50 treatments. These differences in the N300K300 treatment were at least twice the increments for the N50K50 and the N150K50 treatment. The results discussed here showed that the biomass change in the tree is proportional to the N and K fertiliser use.

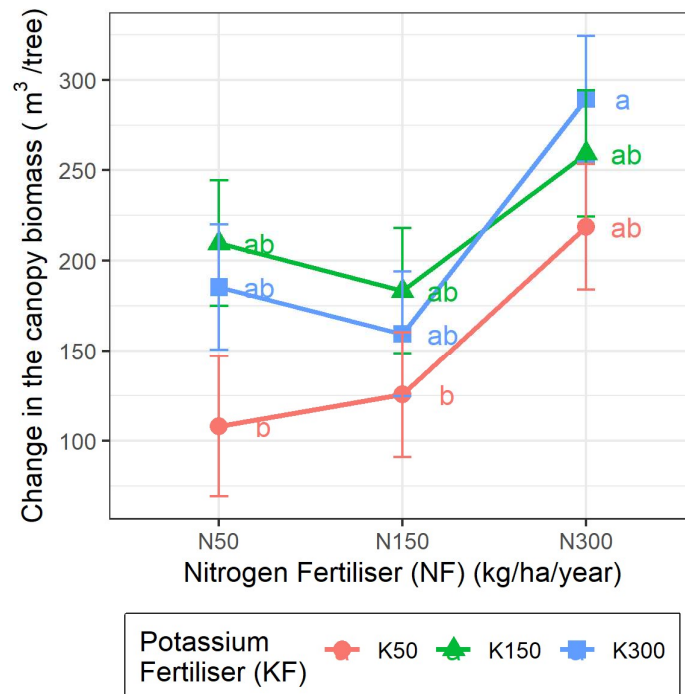


Figure 3.8- Changes in the canopy biomass estimation in m³/tree between April 2022 and January 2023 in treatments with different nitrogen (N) and potassium (K) fertiliser rates during the 2022-2023 season. Dots represent the mean after fitting linear models for the difference in tree biomass between April 2022 and January 2023. Means with different letters are statistically different by a Tukey HSD test ($p < 0.05$). Vertical lines represent the standard error of the mean.

3.4 Discussion

3.4.1 Influence of fertiliser practices on topsoil mineral nutrient concentrations

Regarding the effect of K fertiliser use, high K fertiliser rates (300 kg K/ha/year) increased the topsoil 0-15 cm exchangeable K concentration compared to other treatments, surpassing the value of 1.1 meq K/100g for optimal production (Table 3.4). Even this elevated soil K concentration, soil exchangeable Ca concentration did not change significantly with K fertiliser rate, suggesting a non-significant antagonism between both minerals in the topsoil 0-15 cm under the trial conditions. In the Allophanic soils where the trial was conducted, the results contradicted the decrease in exchangeable Ca concentrations observed when K fertilisers were applied at rates between 83 and 332 kg K/ha/year in Ferrosols cropped with Hass avocados in Australia (Hofman, 2007). The higher strengths of Allophanic soils to retain Ca and K in the cation complex compared to the Ferrosols in Australia could contribute to explaining the results for the exchangeable Ca and K concentration in the trial. In addition, because of the continued use of Ca-rich soil products, the exchangeable Ca concentration in the current study for K300 treatments (~28 meq Ca/100g) was four times higher than in the Australian soils. However, a prolonged use of 300 kg K/ha/year could represent a risk of decreasing yield if the leaf K concentrations by May (autumn) increased also over the optimal range as reported by Crowley & Campisi-Pinto (2016).

In addition, high N fertiliser rates (300 kg N/ha/year) consistently acidified the topsoil 0-15 cm and during one out of the two seasons increased the topsoil mineral N concentration. This consistent acidification effect of high N fertiliser rates could generate a reduction in the soil Ca availability. This result suggests that 300 kg N/ha/year could trigger the acidification of the topsoil, especially with 56% out of the total N being ammonia-N as in the current study. In terms of the topsoil mineral N, the lack of increase in topsoil mineral N concentration despite using more N fertiliser suggests excessive use,

possibly leading to N leaching into the subsoil. Therefore, further studies are needed to quantify the effects of N fertiliser on N leaching in avocado.

3.4.2 General changes in fruit mineral concentration between the early and late harvests

The Ca concentration in fruit tissues showed a different trend compared to the concentrations of N, K, and Mg, between the early and late harvests. While Ca concentration decreased between the two harvests in fruit skins, the concentrations of N, K, and Mg increased over the same period, as shown in Table 3.5. However, the concentration of all four nutrients in fruit flesh decreased between the early and late harvests. The exception to this trend was the N concentration in fruit flesh during the second season, especially in trees receiving the highest N fertiliser rates, as discussed in later sections. The same trend for changes in fruit mineral composition between the early and late harvest was discussed for the survey in the current study (section 2.4.1). Similar results, showing decreases in the flesh N and Ca concentrations, as the harvest season progresses from the early to late season, were observed for avocados in Colombia and South Africa (Escobar et al., 2021; Snijder et al., 2002). This decrease in fruit flesh mineral concentrations has been attributed to a dilution effect in other fleshy fruits, such as apples (Nachtigall & Dechen, 2006), kiwifruits (Clark & Smith, 1988), and oranges (Paramasivam et al., 2000), resulting from cellular division and expansion during fruit development. In avocados, the dilution of nutrients in fruit flesh tissues, as the harvest season progresses, is also likely related to cellular division and expansion, which continue even after the fruit is physiologically matured on the tree, and the vascular system of the fruit pedicel is no longer functional (Blumenfeld & Shmuel, 1974).

In this study, only the concentration of mobile nutrients (e.g., N, K, Mg) in fruit skin exhibited increases between the early and late harvests, while Ca concentration decreased. Because N, K and Mg are usually transported in the plant phloem, a potential remobilisation from the flesh to the skins between the early and late harvests is possible. This hypothesis about internal remobilisation of mobile nutrients within the fruit, was demonstrated for K in tomatoes (Ramesh et al., 2021). Internal remobilisation of K from

the inner to the outer fruit pericarp was reported in tomato fruit between the green mature and red ripe stage, whether the fruit was still on the plant or harvested at a green mature stage (Ramesh et al., 2021). Thus, if the remobilisation mechanism were responsible for changes in nutrient concentrations during the late harvest, avocado fruit with high N, K, or Mg concentrations in fruit flesh during early harvest could potentially result in higher concentrations of those nutrients in skins during late harvest. In contrast, Ca is less mobile within the plant and is transported primarily via the plant xylem (Hocking et al., 2016). Therefore, there is limited potential for fruit skins to gain additional Ca between the early and late harvests.

Overall, keeping fruit on the tree for a longer duration until the late harvest season seems to negatively affect the fruit Ca concentration in flesh and skins, affecting the ratios N:Ca and Ca+Mg:K as the season progresses. The changes discussed in this study refer to the comparison between the fruit mineral composition in September or early harvest, when the dry matter was around 24%, and the late harvest in January with dry matter around 33%. Further research is needed to better understand the mechanisms underlying changes in fruit mineral concentrations between the early and late harvests in avocado, particularly under subtropical conditions like those found in New Zealand, where late harvest may be incentivised by international markets. Besides, there is need to estimate the magnitude of changes along the harvest season, which could have further implications for the fruit rots incidence.

3.4.3 Effect of fertiliser practices on fruit nitrogen concentration

The results of this replicated field experiment provided strong evidence that N fertiliser rate is an important factor driving changes in fruit mineral nutrient concentrations, especially in fruit harvested late in the season, as discussed in section 3.3.4 . As expected, N fertiliser rates produced consistent changes in fruit N concentration, and the N:Ca ratio. However, the N fertiliser rates produced inconsistent changes in fruit K and Ca concentrations, and Ca+Mg:K ratios. This result highlights the critical role that N fertiliser management has on fruit nutrient composition, which agrees with similar

observations identified in the orchard survey study, discussed in Chapter 1, in which high N fertiliser rates increased the fruit skin and flesh N:Ca ratio during late harvest. Although not always significant, the N fertiliser increased the flesh N concentration since the early season. Several studies have proposed N fertiliser management as a critical factor influencing fruit quality in Hass avocados, because of its effect on fruit N:Ca ratio (Dann et al., 2016; Marques et al., 2003, 2006). Indeed, the avocado industry in South Africa proposed using the skin N:Ca ratio of fruit harvested early in the season, as a criteria to modify N fertiliser practices for orchards producing fruit for export (Snijder et al., 2002). However, as highlighted by Perkins et al. (2021), only a limited number of studies have tested specific N fertiliser management strategies to assess their effect on influencing avocado fruit skin and flesh nutrient composition.

Nitrogen fertiliser rate significantly influenced the N concentration in fruit flesh and skin of avocados harvested late in the season (Table 3.5). The higher rates of N (150 and 300 kg N/ha/year) showed a trend towards increasing N concentration compared to the 50 kg N/ha/year rate. This finding is consistent with results in Hass avocados in New South Wales, Australia, where N fertiliser rates of between 130 and 260 kg/ha/year significantly increased the N concentration in fruit skin (Willingham et al., 2006). In the current study, the N concentration in fruit tissues of the 150 and 300 kg N/ha/year treatments remained more consistently similar throughout the study compared to the concentrations observed in the 0 and 50 kg N/ha/year treatments. This suggests that the increase in N concentration in fruit was not linear, and after N150 tended to plateau in both tissues. Similar results indicating a plateau effect of N fertiliser rate on fruit N concentration were reported for 'Fuji' apples. In that case, N concentration increased in a quadratic trend, with 160 kg N/ha/year being the stabilisation point for concentration increase in fertiliser use evaluated up to 200 kg N/ha/year (Nava & Dechen, 2009).

3.4.4 Effect of fertiliser practices on fruit calcium concentration

For Ca concentration in fruit tissues, the 150 kg N/ha/year rates significantly decreased Ca concentration in fruit skin only during the late harvest season of 2021-2022. This also

coincided with the highest N concentration in fruit skins. However, an antagonist effect between the concentration of both nutrients could not be confirmed in fruit skin. This is because during the late harvest season of 2022-2023, when the trend towards higher N concentration was more pronounced in the two highest N fertiliser rates, compared to 2021-2022, the Ca concentration remained unchanged. The inconsistent influence of N fertiliser rates on fruit Ca concentration observed in this study in avocados, is not an unusual result in other fleshy fruits. In the studies conducted by Nava & Dechen (2009) on apples, lower fruit Ca concentrations coincided with higher N concentrations in fruit tissues in only two out of the four years of the study. Meanwhile, Muhammad et al. (2015) studying almonds reported the same effect in one out of the four years of a study in almonds. These experiments, which used various N fertiliser rates to analyse fruit nutrient concentrations, showed similarities to the present research with avocados. In all cases, the observed decline in Ca concentration occurred alongside elevated N levels. One likely factor influencing Ca nutrition in the 2022-2023 season of the current study, could be the high rainfall described in the Section 3.3.1 of this chapter. This was also an observation of Muhammad et al. (2015) in almonds. Higher rainfall and humidity could lead to reduced transpiration rates in fruit, thereby weakening the sink strength of fruit relative to leaves, which could reduce the fruit transpiration and subsequent Ca translocation (Blanco et al., 2021; Winkler et al., 2020).

In the current study, several factors could help explain the observed lower Ca concentrations in fruit skins in treatments with higher N concentrations, during the late harvest season 2021-2022. Firstly, there is a differential sink strength between reproductive and vegetative tissues for both nutrients. Research by Zilkah et al. (1987) indicated that avocado fruit are equally strong sinks for N compared to leaves during the early stage of fruit development. This suggests a higher N concentration with higher N pools in the tree derived from higher N fertiliser rates. Secondly, Cutting & Bower (1989) reported a weaker sink strength of avocado fruit for Ca compared to vegetative tissues. Thus, vegetative growth stimulated by N fertiliser use competed for Ca with reproductive tissues, decreasing Ca concentration in fruit as reported by several authors in avocados (Mullen, 2015; Willingham et al., 2004; Witney et al., 1990a).

Calcium concentrations in fruit skin or flesh was not influenced by either different K fertiliser rates or the use of CN, used as a soluble source of Ca during early fruit set. The outcome for the use of different K fertiliser rates challenges previous research findings in Hass avocados, such as those reported by Hofman (2007), who observed a decrease in Ca concentration in fruit skins with the application of K fertilisers alone or in combination with Ca sources. In terms of the CN use, the result from the current study differs from findings with other fruit crops, such as apples, pears, and strawberries. In these crops, Motesharezadeh et al. (2021) observed that CN addition increased fruit Ca concentration without an increase in the fruit N concentration. In the current study, the lack of a response to different K fertiliser rates or Ca addition, from CN use, could be due to the very high exchangeable Ca (33.6 meq/100g) levels already present in the soil at the start of the experiment (Table 3.2).

Delaying K fertiliser use until after early fruit set, also did not result in higher fruit Ca concentrations in this study, contrary to what Hofman (2007) suggested for Hass avocados produced in Australia. A likely explanation for this lack of response under the BoP conditions compared to the Australian study, could be the lower soil exchangeable Ca and K concentrations in Australian soils. As discussed in Section 3.3.1, exchangeable Ca and K concentrations in the present study were above the optimum range in soils, but concentrations were below the optimum range in the Australian study. Taken together, the lack of an influence of K or Ca fertiliser addition on fruit Ca concentration underscores the challenge of influencing fruit Ca concentration under conditions of very high soil Ca status, which is common among high-performance avocado orchards in the BoP.

3.4.5 Effect of fertiliser practices on the fruit N:Ca ratio

The N:Ca ratio in avocado fruit tissues tended to increase with the 150 and 300 kg N/ha/year fertiliser rates during the late harvest season (Table 3.5). These changes in the N:Ca ratio, produced by the higher N fertiliser rates, were primarily driven by changes in the fruit N concentration rather than for changes in Ca concentration. These findings agree with earlier replicated studies conducted on Hass avocados, where manipulating N concentration to influence the N:Ca ratio in fruit skin was more consistently achieved

than changes in Ca concentration (Willingham et al., 2006). Similar increases in fruit N:Ca ratio, primarily due to higher N fertiliser rates increasing fruit N concentrations rather than decreasing Ca concentration, were reported in apples and almonds (Muhammad et al., 2015; Nava & Dechen, 2009; Raese & Drake, 1997).

The RSM model for the N:Ca ratio during the late harvest, identified directions for further research, including the unexpected decrease in the N:Ca ratio in the direction of the N300K300 treatment, which was insignificantly different from the N150K150 treatment ($p>0.05$). The lowest N:Ca ratios in flesh and skins were predicted in the direction of the N50K50 and the N50K300 treatments, indicating that the ratio could be manipulated towards reductions by using low N fertiliser rates, regardless of the K fertiliser rate used. There was a steep increase in the N:Ca ratio as the N fertiliser rate is increased from 50 to 150 kg N/ha/year, and further evaluations within this range may help to better identify if the changes in the ratio are linear or curvilinear. According to the multiple studies relating to the fruit mineral composition and prevalence of internal FQ disorders (Dann et al., 2016; Marques et al., 2006; Willingham et al., 2006), higher incidence of internal FQ disorders could be expected for fertiliser rates increasing the N:Ca ratio, such as fertiliser rates between 150 kg N/ha/year and towards 200 kg N/ha/year. However, any response to N fertiliser use will also be dependent on the background soil mineral supply, which can vary from orchard to orchard and between years at the same orchard. The results of this study are specifically related to conditions of a high-performance avocado orchards in the BoP, with high soil organic matter content and mineral N availability. Therefore, the trends observed in the current study should also be further validated with additional experiments, and other sites over multiple seasons. Also, higher rates of N are also associated with higher environmental impacts, including nitrate leaching and greenhouse gas emissions, as well as higher costs. Therefore, higher rates of N, above what is required for economic optimum yields, should be avoided.

The N:Ca ratio, particularly in the avocado skin, reaches a maximum in the upper-right quadrant of the experimental space (fertiliser rates of N and K >150 kg/ha/year), where the effect becomes less predictable. Opting for fertiliser practices with very high N and K rates should be avoided due to their higher cost, increased environmental impacts, and

the resulting uncertainty in achieving the desired fruit mineral composition. Instead, a more sustainable approach involves utilising lower fertiliser rates, closer to 50 kg N and K per hectare, which both reduces the N:Ca ratio, and increases the Ca+Mg:K ratio.

The influence of N fertiliser rates on the N:Ca ratio was found to be stronger in fruit skin than in flesh, suggesting that the nutrient analysis of skin could be more effective in discriminating the effects of different fertiliser practices on fruit mineral composition. This result offers two advantages for avocado stakeholders involved in predicting internal FQ disorders based on the fruit mineral composition. Firstly, multiple authors have reported that the N:Ca ratio in skins is the most reliable indicator for internal fruit quality disorders (Dann et al., 2016; Escobar et al., 2021; Marques et al., 2003; Snijder et al., 2002; Ullah & Joyce, 2024; Willingham et al., 2001). Secondly, preparing avocado skin for analysis is easier compared to flesh tissues, which are prone to quick oxidation and require extra attention during sampling to prevent cross-contamination. Additionally, as reported by Kämper et al. (2020), non-destructive methodologies for estimating nutrient concentrations in skin could be developed, facilitating the analysis process.

3.4.6 Effect of fertiliser practices on the fruit potassium concentration and Ca+Mg:K ratio

There was some evidence of fruit K concentrations being lower at the lowest N fertiliser rate (N50), but this effect was not consistent at all samplings. Indeed, the N50K50 treatment was the only treatment consistently decreasing K concentration in fruit tissues during the late harvest. This result highlights that decreases in K concentration could only be achieved by minimising both N and K fertiliser use under the trial conditions. This finding is not in agreement with the results of Willingham et al. (2006), who did not observe any response in skin K concentration in Hass avocados using different N fertiliser rates, including the control without N fertiliser use.

In the current study, fruit Ca+Mg:K ratio varied in response to changes in fruit K concentration, showing significant differences in fruit skin at late harvest. In survey studies in Hass avocado orchards conducted in New Zealand by Everett et al. (2007) and

Thorp et al. (1997), the Ca+Mg:K ratio in flesh changed with Ca additions to the soil; however, these surveys were conducted under concentrations of soil exchangeable Ca between 2.5 and 6.3 meq/100g, which were below the optimum range of 7.5-12.0 meq/100g and less than 20% the concentrations of exchangeable Ca measure in the current study. The current study represents the first results showing the effects of fertiliser use on changes in the Ca+Mg:K ratio when soil exchangeable Ca concentrations are high, which is more typical of high-performance avocado orchards in the BoP. This suggests that reducing both N and K fertiliser rates closer to 50 kg/ha could increase the ratio in fruit skins. Implications for this on FQ disorders are investigated in the following chapter.

3.4.7 Fertiliser effects on avocado tree performance

In addition to its impact on fruit mineral composition, as discussed earlier, N fertiliser use significantly increased the canopy biomass production, as described in the Section 3.3.6.3 . This result is contrary to Arpaia et al. (1996) who found no significant increase in canopy biomass with varying N fertiliser between 0 and 168 kg N/ha/year in young Hass avocado trees. The current study, focusing on mature trees (22 years old) with full ground cover, demonstrates increased canopy biomass production during the season with increased N fertiliser use. An increased canopy development faces several challenges for avocado growers. Firstly, higher biomass development requires a more intense tree pruning every year, increasing production cost, and elevating occupational risk for the orchard operation during pruning and harvesting. Secondly, higher leaf-to-fruit ratios could generate higher N and lower Ca concentration in fruits as demonstrated by Mullen (2015), potentially inducing internal FQ disorders.

The results for fruit mineral concentrations and yield estimates suggest that low N fertiliser (50 kg N/ha/year) could simultaneously produce fruit with lower N:Ca ratios and maintain yield under the high fertility conditions of the trial in the BoP. Although the results about the yield estimation in this study should be carefully interpreted due to the high internal variability by treatment, likely produced by extreme weather conditions, the lowest N fertiliser rate did not tend to reduce yield or fruit weight. Indeed, yield and fruit weight in the treatment N50K300 was the highest among the treatments assessed.

Therefore, under the conditions of high soil fertility, as in the present study, the use of 50 kg N/ha/year represents an opportunity to improve the fruit mineral composition, by reducing the N:Ca ratio, while maintaining high yield. This finding is also supported by the fact that leaf N concentration never falls below the critical level of 24 g N/kg for high yielding avocado orchards in the BoP (Dixon, 2008), even in the no N fertiliser treatment.

Further research is needed to assess the effects of N fertiliser rates in the range of 50 to 100 kg N/ha/year as in that range the N:Ca ratio was forecasted to decrease rapidly (Figure 3.4). Although the effect of N fertiliser rates below 100 kg/ha/year on tree performance is unknown, evidence suggests that N fertiliser in that range meet the long-term N requirements of avocado trees without depleting soil reserves. While soil N supply could change between orchards and seasons due to factors in addition to N fertiliser applications, evidence suggest that using less than 100 kg N/ha/year is enough to maintain high-performance in Hass avocados. For example, a nutrient balance model developed in the New Zealand OverseerFM^{®2} software predicted for the experimental conditions in the BoP that 75 kg N/ha is required for a 20-tonne avocado production to minimise soil N depletion over time. This model agrees with various studies suggesting that applying between 80 to 100 kg N/ha/year could simultaneously replenish fruit extraction and address multiple fertiliser inefficiencies in Hass avocados (Huett & Dirou, 2000; Lahav & Kadman, 1980; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023). These findings represent a relevant insight into an improved fertiliser practice in New Zealand, as N fertiliser rates over 100 kg N/ha/year are common in the country.

Although the over-optimum soil K concentrations in treatments using 300 kg K/ha/year, the leaf K concentration and yield estimate during the 2022-2023 season (Figure 3.5) were not influenced by K fertiliser. However, using high K fertiliser rates in Hass avocados could have detrimental effects on tree performance when the leaf K levels surpassed the optimum range by the sampling in May (autumn in New Zealand). The leaf K concentrations during the trial varied around 10.2 g K/kg (1.02%) regardless of the K

² OverseerFM is an on-line software designed to estimate the nutrient use and transfer of nutrients within agricultural systems in New Zealand. The software includes models for 6 animal systems and 8 crop systems including avocados <https://www.overseer.org.nz/overseerfm>

fertiliser rate, concentrations above 1% (10 g K/kg) have been reported to have a negative effect on yield in Hass avocados (Crowley & Campisi-Pinto, 2016; Dixon, 2008). Crowley & Campisi-Pinto (2016) predicted yield suppression when leaf K concentration exceeded 1% in Hass avocados, with 36% of trees not bearing fruit when leaf K concentrations were above 1.4% at autumn sampling. In addition, Dixon (2008) found a negative relationship between yield and K concentration in mature leaves sampled between April and May in the BoP, with yield reduction when leaf K concentration exceeded 1.05% (10.5 g K/kg). Therefore, the continued use of 300 kg K/ha/year under the conditions of the trial is not advisable.

High K fertiliser rates (300 kg K/ha/year) did not influence the leaf Ca concentrations at any sampling, but it did affect leaf K concentrations on fruiting shoots at the November sampling (peak of flowering during summer in New Zealand). The leaf Ca results seem to discard an antagonism between K fertiliser use and leaf Ca concentrations under the trial conditions of high soil exchangeable Ca concentration, either from fruiting shoots in November or non-fruiting shoots in May. In terms of K concentrations, by November, when 43% (~130 kg/ha) out of the 300 kg K/ha/year had been applied (Table 3.1), K300 treatments consistently increased the leaf K concentrations on fruiting shoots compared to treatments using lower K fertiliser rates, especially of young leaves (Figure 3.6). Taken together, these results indicate that the application of about 130 kg K/ha up to November (before the early fruit set) in the K300 treatments increased K concentration in young leaves of fruiting shoots without affecting Ca translocation to this type of shoots. This supports the idea that under the trial conditions of high soil Ca concentration, the use of K fertiliser before the early fruit set is not antagonist with the Ca concentration in leaves or fruits.

3.5 Conclusions

This field trial demonstrated changes in avocado fruit nutrient composition between the early and late harvests. Fruit harvested late in the season had lower Ca concentrations in both fruit flesh and skin. For N, K and Mg, the change from early to late harvest resulted in reduced concentrations in the fruit flesh and increased concentrations in the skin. The lower Ca concentrations in both tissues contributed to higher N:Ca ratios and lower Ca+Mg:K ratios in the late harvested fruit, compared to the early harvested fruit. In addition, the higher concentrations of N and K in fruit skins during the late harvest also contributed to higher N:Ca and lower Ca+Mg:K ratios in this tissue, which are associated with an increased risk of postharvest internal fruit rots.

In this field trial, there was limited to negligible influence of N and K fertiliser use on avocado fruit Ca concentrations. While higher N fertiliser rates were associated with a decrease in Ca concentrations in fruit skin during the late harvest of the 2021-2022 season, the effect was not consistent across seasons. Fruit Ca concentrations were also not significantly affected by K fertiliser use or the use of CN before the early fruit set. The high soil exchangeable Ca levels present in the orchard, which is typical of high-performance avocado orchards in the BoP, may have contributed to fertiliser use having limited influence on fruit Ca concentrations.

The use of N and K fertilisers influenced the concentrations of these two nutrients in fruit harvested late and, therefore, also the N:Ca and Ca+Mg:K ratios. In general, N fertiliser had the most significant influence on the observed changes in fruit nutrient composition. High N fertiliser rates, of 150 kg N/ha/year, increased N concentrations and N:Ca ratios in the flesh and skin of late harvested fruit. Reducing the N fertiliser rate from 150 to 50 kg N/ha/year was effective at lowering both fruit N concentrations and N:Ca ratios, especially in the fruit skin of late harvested fruit. Further research is needed to validate the impact of N rates ranging from 50 to 150 kg N/ha/year. However, models for the fruit N:Ca ratio during the late harvest suggest that the most significant reduction is likely to occur between 50 and 100 kg N/ha/year. In addition, the treatment with the lowest N and K fertiliser rates (50 kg N and K/ha/year) achieved higher Ca+Mg:K ratios in both fruit tissues, mainly because of lower fruit K concentrations. Therefore, low rates of both N

and K fertiliser support improved fruit nutrient composition, which is potentially beneficial for reducing the risk of postharvest internal fruit rots.

In contrast, high fertiliser use, namely N fertiliser, increased the N:Ca ratio and the risk of internal FQ disorders. The effect of fertiliser use on the incidence of postharvest internal fruit rots is investigated in Chapter 4. High N fertiliser use also raises orchard input costs, can result in more pruning to manage higher biomass production, and increases the associated environmental risks, such as nitrate leaching and nitrous oxide emissions. Therefore, further research is needed to assess the long-term tree performance at lower rates of N fertiliser.

Chapter 4 - Effect of fertiliser strategies on fruit quality of avocado harvested late in the season

4.1 Introduction

The primary market strategy for New Zealand avocados is to produce premium fruit quality (FQ) for export purposes. This strategy aims to minimise the quantity of fruit with more than 5% of their internal or external surface area affected by any FQ disorder, which is considered unsound fruit. Avocados from New Zealand are mainly exported to Australia (Burdon et al., 2008) and, more recently, to Asian markets. However, the main constraint for exporting premium FQ remains the production of unsound fruit due to internal fruit rots during postharvests, such as body rots (BR) and stem-end rots (SER), generated by several fungi species: *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Botryosphaeria parva*, *Botryosphaeria dothidea* and *Phomopsis* spp (Hartill, 1991). Avocado rots and other internal and external FQ disorders, such as vascular browning (VB), or external patches after cold storage (PT) are more prevalent in fruit harvested late in the season (January onwards in New Zealand), as the dry matter increases (Dixon et al., 2003; Everett et al., 2007). However, the fruit harvested late in New Zealand is highly demanded in international markets, especially in Australia, where fruit with higher dry matter is preferred (Gamble et al., 2010) and the Australian domestic avocado season has finished by January.

Over the last four decades, various practices throughout the production chain have been identified to minimise the severity of internal fruit rots and other quality disorders in avocados. Several compilations of practices, spanning from pre-planting to fruit handling on retail shelves, have been developed to improve FQ outcomes (Everett, 2002; Hofman et al., 2013; Perkins et al., 2021; Sorensen, 2017). In the pre-harvest stage, practices can be categorised according to two primary goals. Firstly, there are practices aimed at reducing the inoculum of fruit rots, such as the fungicides use (Everett et al., 2007) or the removal of dead twigs from trees and dropped fruits from the ground (Everett, 2020).

Secondly, practices focus on increasing the fruit Ca concentration, and improving the ratios between Ca and other mineral nutrients such as N and K (i.e., lower N:Ca ratio and higher Ca+Mg:K ratio). The focus on improving the ratios between Ca and other nutrients in fruit is based on the essential role that Ca plays in cell wall integrity and the activation of self-defence mechanisms in fruit tissues during the postharvest period (Hocking et al., 2016).

Even though several studies (Dann et al., 2016; Everett et al., 2007; Marques et al., 2003, 2006; Thorp et al., 1997) have demonstrated that Hass avocados produced with improved fruit nutrient composition exhibit better FQ, the specific on-orchard practices required to achieve this goal remain poorly understood. The most consistent practice to produce Hass avocados with better fruit nutrient composition and FQ outcomes involves the use of Guatemalan and West Indian rootstocks at planting, as opposed to Mexican rootstocks (Dann et al., 2016; Hofman, 2005; Marques et al., 2003; Willingham et al., 2001). Workers have reported a Ca filter effect in Mexican rootstocks, contributing to both a high N:Ca ratio and the production of poor-quality Hass fruit. Although fertiliser practices were identified as critical factors to ensure a fruit nutrient composition supporting a better FQ in Hass avocados, only two previous controlled trials directly addressed their influence on fruit composition. Willingham et al. (2006) studied the influence of N fertiliser use, reporting significant increases in the fruit N:Ca ratio and severity of SER and BR by using N fertiliser rates of 130 or 260 kg N/ha/year compared to no-N fertiliser use. In a study investigating the effects of K fertiliser on FQ, Hofman (2007) reported a reduced fruit Ca concentration by using K fertilisers between 83 and 322 kg K/ha/year, compared to using only Ca fertilisers, but did not find an effect on FQ. Therefore, there is a need to establish a consensus on the best fertiliser practices to achieve the fruit nutrient composition that supports premium FQ, meaning a minimal quantity of unsound fruit.

The aim of this chapter is to identify optimal N and K fertiliser strategies for reducing the risk of avocado postharvest fruit rots, especially for fruit harvested late in the season for which rots are more prevalent in New Zealand. The current field study, described in Chapter 3, demonstrated that while N and K fertiliser use did not appreciably influence fruit Ca concentrations, there was an influence on the fruit N:Ca and (Ca+Mg)/K ratios during the late harvest season. In general, increasing N fertiliser use increased the fruit N:Ca ratios, while increasing either N or K fertiliser use decreased the fruit (Ca+Mg)/K

ratio. The changes in these ratios resulting from high N or K fertiliser use are associated with the potential for higher incidence of fruit rots affecting postharvest FQ. In this chapter, fruit harvested late from different fertiliser treatments were assessed after cold storage at green and ripe stages for FQ disorders. These assessments were employed to develop models quantifying the predicted probabilities to produce unsound fruit or fruit without premium FQ by selected fertiliser treatments. Further, correlation analyses were developed between the fruit unsoundness and the fruit mineral composition by treatments, as well as a severity analysis for the most prevalent disorder in New Zealand, known as body rots (BR), were also developed by treatment assessed. In this manner, the chapter aims to quantify the effect of fertiliser use practices on the FQ of Hass avocados harvested late in New Zealand.

4.2 Materials and methods

The FQ assessment was conducted in January 2023, during the late harvest season when the mean avocado-flesh dry matter was approximately 33%. Before the late harvest in January 2023, an initial harvest was developed in September 2022, ensuring that enough fruit were available for the late harvest season. This timing was selected because FQ disorders are known to be prevalent in New Zealand during that specific stage of the harvest season (Everett et al., 2007). The avocado trees in the trial were not treated with any copper or other fungicides throughout the experiment, aiming to assess the impact on FQ resulting from various fertiliser practices. The FQ assessment during the late harvest of the 2021-2022 season (January 2022) was not analysed, prioritising the fruit mineral determinations during the season. This decision arose because during the first year of experimentation, the harvest during the early season in October 2021 was highly intense on the trees from the commercial orchard, resulting in insufficient fruit of commercial export size and quality by January 2022 for all trees of the experiment.

The FQ assessment was conducted in six out of the twelve fertiliser treatments from the trial described in Section 3.2.2 due to logistical and financial limitations for this PhD research study (Table 4.1). Four treatments represented the combinations between the lowest and highest N and K fertiliser rates (50 and 300 kg of N or K/ha/year) evenly applied in seven applications between mid-August and mid-April. Two treatments represented mid N and K fertiliser rates (150 kg N and K/ha/year): the N150K150 treatment with seven even applications between mid-August and mid-April and the N150K150Ca90 treatment with 90 kg Ca/ha/year applied as calcium nitrate during the early fruit set, and the K applied after the early fruit set from January onwards.

Table 4.1- Treatments from the fertiliser trial included in the fruit quality assessment during late harvest of the 2022-2023 season.

Treatment	Label	Annual fertiliser use		
		N	K	Ca
(kg/ha/year)				
T2	N50K50	50	50	10
T4	N300K50	300	50	47
T6	N150K150	150	150	30
T8	N50K300	50	300	10
T10	N300K300	300	300	60
T12	N150K150Ca90	150	150	122 (90) [†]

[†] T12 is a treatment with 90 kg Ca/ha/year applied as calcium nitrate (CN) out of the 122 kg applied in total during the year. Potassium fertiliser in T12 was delayed until after the early fruit set stage as detailed in section 3.2.2 .

4.2.1 Fruit sampling

Twenty avocados per tree from each of the 30 trees in the six selected treatments (five tree/replicates by treatment), were harvested for postharvest FQ assessment during late harvest in January 2023 (Season 2022-2023). Avocados free of visual external damage, caused by either sunburn, insects, rots, or physical damages, were taken in different directions across the canopy at heights of between 2 and 4 m above ground level. Each avocado harvested was of a commercial export size, ranging between 257 and 324 g. The pedicel was carefully trimmed during the harvest, leaving a length of 2 to 3 mm on the fruit. Subsequently, the fruit from each individual tree were packed into a carton box with a fruit cavity tray to protect the fruits during the package and transport. Then, the 30 carton boxes with the harvested avocados were transported to the packhouse within 2 hours of harvest for cold storage. No fungicide was applied to the fruits after harvest.

4.2.2 Postharvest management and fruit quality assessments

The avocado trays were put into cold storage with temperatures between 4 to 5°C for 28 days. The storage conducted involved the use of air, without a controlled atmosphere of CO₂ or O₂, which increase the expression of postharvest disorders (Burdon et al., 2008). Subsequently, the trays were shifted to a room with a temperature of 18°C until each avocado reached the eatable ripe stage (5 to 7 days without ethylene use). The ready-to-eat ripe stage was defined as when a gentle hand pressure caused flesh deformation without denting (Dixon & Parton, 2014; White et al., 2005).

The FQ assessment is a subjective activity, highly reliant on the expertise of the assessor (Dixon & Parton, 2014). Therefore, to provide consistent results, an experienced FQ assessor, from the New Zealand avocado industry, conducted the fruit quality assessments for this study. The FQ assessments were performed on all avocados at both the green and ripe stages of avocado fruit maturity, using the procedure outlined in the New Zealand avocado industry council fruit assessment manual (Dixon & Parton, 2014). Each FQ disorder was rated from 0 to 100, based on the percentage of avocado area affected by the symptoms of each disorder assessed in each ripe stage. Four prevalent FQ disorders in the BoP were assessed, one at green stage and three at the ready-to-eat ripe stage. During the green stage assessment, after cold storage and before the ripening into the room

temperature, were assessed the external patches (PT- discrete and fuzzy patches). Subsequently, once each fruit reached the ready-to-eat ripe stage at room temperature, there were assessed the most common internal FQ disorders: stem end rot (SER), vascular browning (VB), and body rots (BR) (Dixon & Parton, 2014; White et al., 2005).

During the assessment of internal FQ disorders at the ready-to-eat ripe stage, each fruit was longitudinally cut using a sharp knife, and the SER and VB were assessed over the internal flesh area after separation from the seed. Then, the skin was carefully separated from the flesh, and the assessment of BR was conducted on the inside area of the avocado skins as indicated in Figure 4.1.

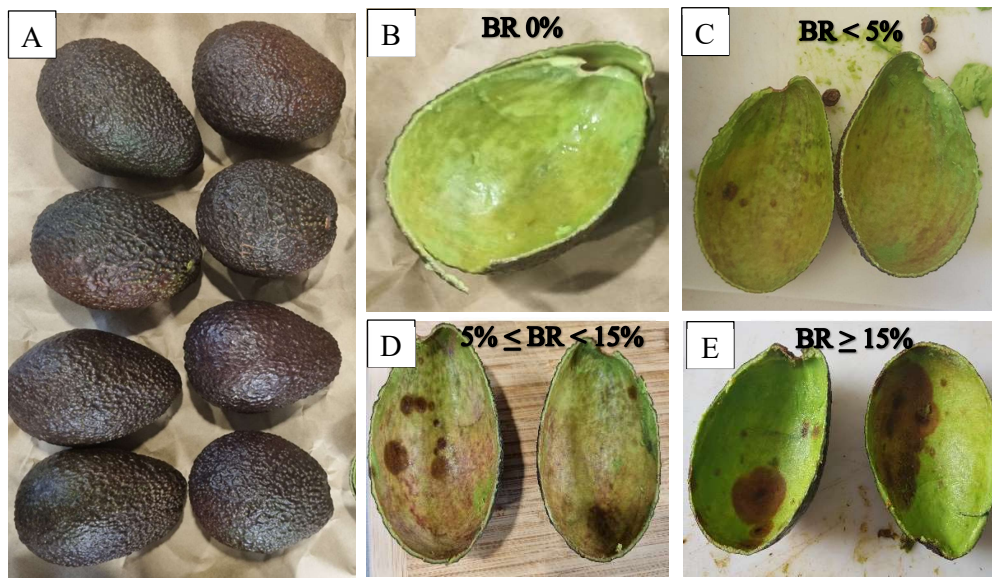


Figure 4.1- Example of photos showing different severity levels of body rots (BR) during the fruit quality (FQ) assessment. Ready-to-eat ripe Hass avocados to be assessed (A). Fruit skins of sound avocados with BR severity lower than 5% (B and C). Fruit skins of an unsound fruit moderately affected by BR (BR severity between 5% and 15%) (D). Fruit skins of an unsound fruit highly affected by BR (BR severity greater than 15%) (E).

4.2.3 Statistical analyses

4.2.3.1 Fruit unsoundness

Logistic binomial regression models were fitted to analyse which fertiliser treatment was associated with a higher probability of producing unsound fruit, or fruit with 5% or more of their flesh surface area affected by any FQ disorder. This analysis aims to support the avocado industry, as unsound avocados diminish consumer confidence in future purchases, regardless of their rating in the FQ assessment (Gamble et al., 2010). The

logistic models were fitted as they are an appropriate statistical method for evaluating factors influencing the production of different quality categories in other fruit crops (Díaz-Pérez et al., 2019a, 2019b).

Five logistic binomial models were fitted using the fertiliser treatment as a categorical explanatory variable and the classification of the FQ rating in unsound/sound categories (1 - unsound fruit; 0 - sound fruit) as a dependent binomial variable. For general unsoundness (uGeneral), the highest rating for each avocado was used as a binomial variable. Four additional models were developed for the individual rating of each FQ disorder assessed (e.g., BR, SER, VB, PT).

After fitting the models, a likelihood-ratio test (LRT) was implemented in R to perform mean separation of the odds ratios per treatment, using a significance level of 0.05 ($p < 0.05$). This LRT test was similar to the LRPAIR test implemented in GenStat by Goedhart & Thissen (2018). The logistic binomial regression models and mean separation analyses were conducted in the statistical language R Version 4.1.1 (R Core Team, 2021) using the core packages of R and the packages *emmeans* (Lenth, 2022) and *multcomp* (Hothorn et al., 2008).

4.2.3.2 Correlation between fruit unsoundness and fruit nutrient composition

Linear correlations were developed between the general unsoundness and the mineral concentrations of Ca, N, and K, as well as with the N:Ca and Ca+Mg:K ratios in both tissues fruit flesh and skins. The determination coefficients (R^2) and significance of the correlation were obtained by using the function *ggpairs* from the R package *GGally* (Schloerke et al., 2021).

4.2.3.3 Severity analyses of body rots

A multinomial linear regression model was fitted for the severity analysis of BR, the prevalent FQ disorder during the study. In this model, the rating for the BR assessment, different from the binomial classification in the unsoundness analysis due to BR (uBR) (section 4.2.3.1), classified the fruits into three categories of severity. The categories were as follows: sound fruit or fruits with BR incidence less than 5%, unsound fruit

moderately affected by BR with a severity between 5% and less than 15%, and unsound fruit highly affected by BR with a severity equal to or higher than 15%. The threshold of 5% to define an unsound fruit was defined according to the criteria used by the New Zealand avocado industry to define the fruit unsoundness, while the threshold of 15% for fruit highly affected was defined in the international avocado quality manual for non-commercial fruit (White et al., 2005). The independent categorical variable was the fertiliser treatment. Although fruit with ratings higher than 5% (unsound fruit) is non-marketable according to the New Zealand standard, the severity analysis aims to understand if fertiliser treatments could potentially mitigate the development of BR symptoms in unsound fruit. This analysis could be relevant, especially under the current experimental conditions in which other complementary mitigation strategies, such as fungicides, were not used.

The multinomial model compares the odds ratio of each treatment for producing fruits in a specific severity classification level related to both a specific reference category, and a specific reference level (UCLA: Statistical Consulting Group, 2021a, 2021b). The reference category used in the analysis (N150K150 treatment) was selected because it exhibited significantly higher unsoundness levels, as indicated in section 4.3.1 . A significant negative log-odd ratio implies that the evaluated treatment has a lower propensity of producing fruit in the BR severity category compared to the reference category of the N150K150 treatment, suggesting a differential protective effect of the fertiliser treatment. The odds values in the multinomial analysis indicate that for every hundred avocados produced under the reference category (N150K150 treatment), the proportion represented by the odd would be produced under the reference level (BR severity less than 5%) if the avocados were produced with the treatment being assessed (UCLA: Statistical Consulting Group, 2021a, 2021b). The multinomial logistic regression model was fitted using the *multinom* function from the R package *nnet* (Venables & Ripley, 2002).

4.3 Results

4.3.1 Fruit unsoundness analyses by fertiliser treatment

4.3.1.1 General unsoundness

Annual fertiliser rates of 150 kg N and K/ha/year (i.e. N150K150 and N150K150Ca90 treatments) significantly ($p < 0.05$) increased the predicted probabilities of producing unsound avocado fruit during the late harvest of the 2022-2023 season (Figure 4.2). The addition of CN as a soluble source of Ca did not produce any differences, compared to using the same N and K fertiliser rate without using CN or delaying K fertiliser use until after early fruit set. For treatments with the 150 kg N and K/ha/year rates, the predicted probabilities of producing unsound fruit reached over 70%, representing between 20% and 26% higher predicted probabilities ($p < 0.05$) compared to treatments either using lower N and K fertiliser rates (i.e., N50K50, N50K300 treatments) or higher K fertiliser rates (i.e., N50K300, N300K300 treatments).

For treatments using the lowest N fertiliser rate of 50 kg N/ha/year (i.e. N50K50 and N50K300 treatments), the predicted probabilities of producing unsound fruit significantly decreased ($p < 0.05$) compared to 150 kg N/ha/year treatments (Figure 4.2). In contrast, in the case of treatments using the highest N fertiliser rate of 300 kg N/ha/year, the only treatment with significantly lower predicted probabilities of producing unsound fruit compared to 150 kg N/ha/year treatments was the N300K300 treatment. Therefore, a reduced predicted probability of producing unsound avocado fruit was achieved in treatments with either the lowest N fertiliser rate (50 kg N/ha/year) or the highest K fertiliser rate (300 kg K/ha/year).

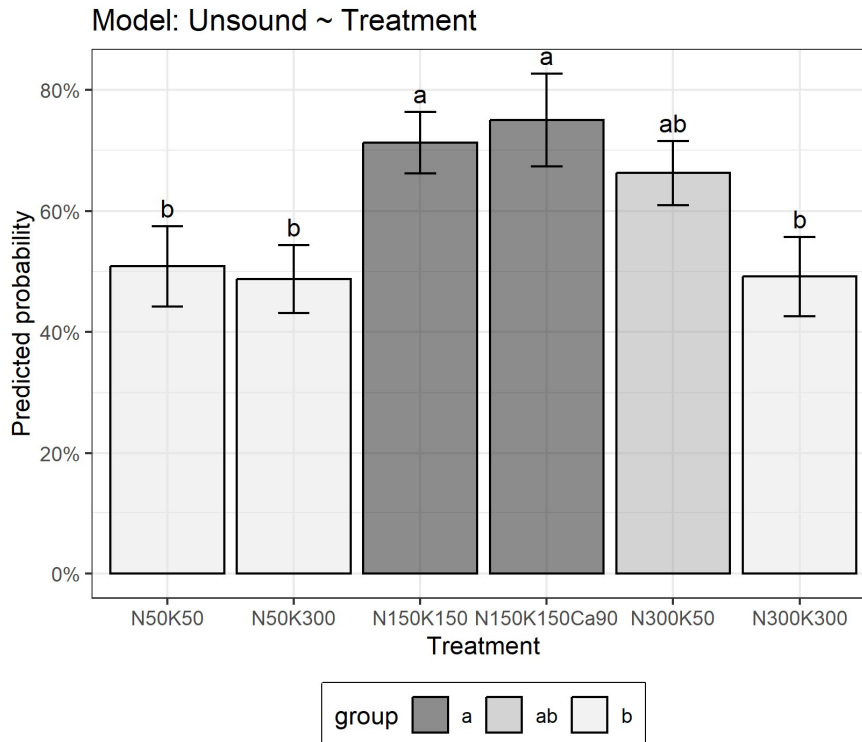


Figure 4.2- Mean predicted probabilities of producing unsound fruit by assessed fertiliser treatment during the late harvest of the 2022-2023 season in Hass avocados in the Bay of Plenty region. The probabilities were estimated using logistic binomial regression models with the binary variable "unsound/sound" fruit as the dependent variable and fertiliser treatment as categorical factor. Predicted probabilities with different letters indicate statistical differences determined by a likelihood-ratio test (LRT) on the odds ratios per treatment ($p < 0.05$).

4.3.1.2 Unsoundness due to each fruit quality disorder

Body rots at the ripe stage of fruit ripening, emerged as the FQ disorder with the highest severity in 91% of the avocados assessed. Vascular browning was the most severe disorder in 7% of the avocados, while both SER and PT were the disorders with highest severities in only 1% of the avocados assessed. Therefore, BR was the FQ disorder defining the fruit unsoundness for most of the avocados assessed during the study.

As BR was the individual FQ disorder causing most of the fruit unsoundness, the predicted probabilities of producing unsound fruit considering only BR followed a similar trend as the general unsoundness (Table 4.2). Therefore, treatments with N and K fertiliser rates of 150 kg/ha/year, exhibited a greater probability of producing unsound

fruit due to BR ($p < 0.05$), compared to treatments using fertiliser rates of either 50 kg N/ha/year or 300 kg K/ha/year.

Table 4.2- Predicted probabilities of producing unsound fruit during the late harvest 2022-2023 in Hass avocados according to the selected fertiliser treatment in the trial in the Bay of Plenty Region.

Unsoundness [†]	uGeneral	uBR	uSER	uVB	uPT
Predicted probability [mean ± S.E.]					
Treatment [‡]					
N50K50	50.9 ± 6.6 b	49.1 ± 6.6 b	8.8 ± 3.7 ab	12.3 ± 4.3 ab	1.8 ± 1.7
N300K50	66.2 ± 5.3 ab	61.2 ± 5.4 ab	20.0 ± 4.5 a	27.5 ± 5.0 a	2.5 ± 1.7
N150K150	71.2 ± 5.1 a	67.5 ± 5.2 a	15.0 ± 4.0 a	32.5 ± 5.2 a	3.7 ± 2.1
N150K150Ca90	75.0 ± 7.7 a	68.7 ± 5.2 a	18.8 ± 6.9 a	28.1 ± 7.9 a	6.2 ± 4.3
N50K300	48.8 ± 5.6 b	43.8 ± 5.5 b	12.5 ± 3.7 a	16.3 ± 4.1 ab	1.3 ± 1.2
N300K300	49.2 ± 5.5 b	47.5 ± 5.5 b	1.7 ± 1.7 b	6.8 ± 3.3 b	1.7 ± 1.7
s.c. ^{‡‡}	*	*	*	*	ns

[†] Probabilities were estimated from logistic binary regression models using fertiliser treatments as predictor factor and unsound/sound fruit as dependent variable. The model for the general unsoundness fruit (uGeneral) was calculated based on the highest FQ disorder by each avocado assessed; Models for the unsoundness due to body rots (uBR), stem-end rot (uSER), vascular browning (uVB), and patches at green (uPT) are based on the unsoundness due to the assessed individual FQ disorder.

[‡] Predicted probabilities with different letters are statistically different by a likelihood-ratio test (LRT) on the odd ratios per treatment ($p < 0.05$).

^{‡‡} Significance codes: '*' $p < 0.05$; 'ns' $p > 0.05$.

For SER and VB, the highest predicted probabilities for producing unsound fruit were with the N150K150 treatments compared to N300K300 treatment ($p < 0.05$) (Table 4.2). The 150 kg N/ha/year treatments reached unsoundness probabilities of between 15% to 18% for SER and between 28% to 33% for VB. In comparison, the treatment with the highest rates of N and K had predicted probabilities of 1.7% for producing unsound fruit due to SER and 6.8% due to VB. Despite the N300K300 treatment having reduced the probabilities of producing unsound fruit due to SER and VB compared to the N150K150 treatments, the probabilities for those two disorders were less than a half compared to BR.

In the case of PT, the unsoundness model did not predict any significant ($p > 0.05$) differences among fertiliser treatments.

4.3.2 Correlation between fruit unsoundness and fruit nutrient composition

There were highly significant ($p < 0.001$) positive linear relationships between fruit unsoundness and both the N concentration and N:Ca ratios in skin and flesh (Table 4.3).

The strongest relationship was observed between fruit unsoundness and the N:Ca ratio in fruit skins, reaching an R^2 of 0.831 ($p < 0.001$, $n = 30$). Thus, avocados produced on trees with higher fruit N:Ca ratios, particularly in fruit skins, increased the probability of fruit unsoundness during the late harvest. The correlation between the unsoundness and the N concentration in both tissues was also significant ($p < 0.01$), being stronger for skins than in flesh.

In the case of fruit Ca concentration, the correlation with the fruit unsoundness was negative and only significant in skins ($p < 0.01$).

Table 4.3- Determination coefficients and significance codes for the linear relationships between the general fruit unsoundness and the concentration of the mineral nutrients: nitrogen (N), calcium (Ca), potassium (K), and magnesium (Mg) during the late harvest of the 2022-2023 season in the selected treatments assessed for fruit quality (FQ).

Correlation	Skin		Flesh	
	R^2	s.c. †	R^2	s.c. †
Unsoundness vs N:Ca ratio	0.831	***	0.671	***
Unsoundness vs N concentration	0.674	***	0.604	**
Unsoundness vs Ca concentration	-0.581	**	-0.403	.
Unsoundness vs K concentration	0.104	ns	0.302	ns
Unsoundness vs Ca+Mg:K ratio	-0.193	ns	-0.36	.

†s.c.- Significance codes: '***' $p < 0.001$; '**' $p < 0.01$; '*' $p < 0.05$; '.' $p < 0.1$; 'ns' $p > 0.1$

4.3.3 Severity analyses of body rots by fertiliser treatment

For fruit highly affected by BR (BR > 15%) (Table 4.4), the log-odd ratios were significant ($p < 0.05$) for the treatments N50K50, N50K300, and N300K300 with values of -1.382, -0.772, and -1.785, respectively. For this level of BR severity, either treatments using the lowest N fertiliser (50 kg N/ha/year e.g., N50K50, N50K300 treatments), or the highest K fertiliser (300 kg K/ha/year e.g., N50K300, N300K300 treatments) had significantly lower odds of producing unsound fruit with BR severity higher than 15% compared to the N150K150 treatment (reference category³). The negative log-odd ratio implies that for every hundred avocados with BR severity over 15% produced using the N150K150 treatment, over 46 avocados would be sound fruit if were produced with N50K300 treatment (odd of 0.462); over 25 avocados would be sound if were produced

³ The N150K150 treatment was defined as the reference category and the BR severity <5% was defined as the reference level according to the section 4.2.3

with N50K50 treatment (odd of 0.251); and over 17 avocados would be sound if were produced with N300K300 treatment (odd of 0.168). These results highlight the relevance of using fertiliser practices reducing the fruit N:Ca ratio to support a reduction in the severity of highly affected fruit by BR, especially using low N fertiliser that could support the nutritional aim in a sustainable way.

Table 4.4 – Multinomial logistic regression model for the severity levels of body rots (BR) and assessed fertiliser treatment at the late harvest of the 2022-2023 season.

BR severity [†] (Severity level)	Treatment (Category)	Log-odd ratio	SE	p - value [‡]	Odd
(Unsound fruit highly affected by BR)	>15% (Intercept)	-0.039	0.280	0.889	0.962
	N50K50	-1.382	0.506	0.006**	0.251
	N300K50	-0.504	0.408	0.216	0.604
	N50K300	-0.772	0.388	0.047*	0.462
	N300K300	-1.785	0.557	0.001**	0.168
	N150K150Ca90	-0.066	0.538	0.902	0.936
(Unsound fruit moderately affected by BR)	5% -15% (Intercept)	0.109	0.270	0.686	1.115
	N50K50	-0.432	0.394	0.273	0.649
	N300K50	-0.109	0.371	0.768	0.897
	N50K300	-1.208	0.402	0.003**	0.299
	N300K300	-0.408	0.386	0.290	0.665
	N150K150Ca90	0.153	0.500	0.759	1.166

[†] The three levels of BR severity included in the model are: BR severity <5% (sound fruit-reference level); BR severity between 5% and 15% (unsound fruit moderately affected by BR); BR severity ≥ 15% (unsound fruit highly affected by BR). The reference category is the treatment N150K150 (treatment with the highest prevalence of unsound fruit by BR according to the section 4.3.1). Significance codes: ** $p < 0.01$; * $p < 0.05$.

[‡] A significant p -value and a negative log-odd ratio means that using the specific treatment is less propense to produce unsound fruit at the severity level compared to using N150K150 treatment. The odd for the treatment with a significant p -value represents the proportion of unsound fruit produced with N150K150 that would be sound if the fruit were produced with the assessed treatment.

For fruit moderately affected by BR (BR severity between 5% and 15%) (Table 4.4), the N50K300 treatment had a significant ($p < 0.05$) log-odd ratio of -1.208 and odd of 0.299. The negative log-odd ratio implies that, when avocados are produced using the N50K300 treatment, the propensity of producing fruit moderately affected by BR is lower than producing the fruits using the N150K150 treatment. In addition, for every hundred avocados produced using the N150K150 treatment with BR severity between 5% and 15%, about 30 less avocados (odd of 0.299) are expected to be unsound fruit (reference level) if the fruit were produced with the N50K300 treatment. Log-odd ratios for other fertiliser treatments were statistically similar ($p > 0.05$) to the N150K150 treatment at the same level of BR severity.

Therefore, using the N50K300 treatment was less likely to produce unsound fruit at any level of severity compared to the N150K150 treatment. However, caution must be taken to use 300 kg K/ha/year, as it would be an inefficient approach to produce premium FQ for orchards with already high soil exchangeable K levels in the BoP. Meanwhile, the N50K50 or N300K300 treatments resulted in lower probabilities to produce fruit severely affected by BR (BR severity > 15%) than the N150K150 treatment, but not to produce moderately affected fruit. Additionally, the N150K150 treatment had statistically similar odds of producing unsound fruit, regardless of whether extra soluble Ca was applied, and the K applications were delayed until after late fruit set.

4.4 Discussion

4.4.1 Influence of fertiliser practices on fruit unsoundness

Treatments with different annual N and K fertiliser rates, rather than additional Ca using soluble sources such as CN, influenced the predicted probabilities of producing unsound fruit due to fruit rots at late harvest (Figure 4.2). Regarding N fertiliser use, the principal protective effect against fruit rots was the use of the 50 kg N/ha/year rate. Using this low N fertiliser rate decreased the predicted probability of producing unsound fruit between 20% and 30% compared to using 150 kg N/ha/year. This trend of producing a higher proportion of marketable fruit (less unsound fruit) with lower N fertiliser rates was already reported in Hass avocados. For example, in Australia, the fruit marketability associated with low severity of fruit rots, such as BR and SER, reached 80% when nil-N fertiliser was used compared to 56% and 60% marketability by using 133 and 266 kg N/ha/year, respectively (Willingham et al., 2006). Therefore, there is an opportunity of improving the crop fertiliser practices supporting the avocado FQ by using lower rates of N fertiliser, especially as is common among for high-performance avocado orchards in the BoP, to use N fertiliser greater than 100 kg/ha/year (Figure 2.1)

The influence of N fertiliser on FQ, expressed as the predicted probability of producing unsound fruit (fruit with the severity of any FQ disorder equal to or greater than 5%) is directly related with the fruit skin N:Ca ratio. The stronger correlation between fruit unsoundness and the N:Ca ratio in fruit skins (Table 4.3), emphasizes the relationship between higher skin N:Ca ratios and increased fruit unsoundness. This positive correlation is consistent with findings from other research studies on Hass avocados (Dann et al., 2016; Escobar et al., 2021; Marques et al., 2006; Ullah & Joyce, 2024). Fruit produced with 50 kg N/ha/year exhibited the lowest unsoundness compared to those produced with 150 kg N/ha/year, indicating the significance of reducing N fertiliser rates to decrease the N:Ca ratio in fruit skin, as discussed in Section 3.4.5 . High N:Ca ratios show the availability of N in fruit skins, a co-factor favouring the virulence of *C. gloeosporioides*, the primary causal agent of BR (Drori et al., 2003; Kramer-Haimovich et al., 2006), the prevalent FQ disorder in this study. Thus, fertiliser practices decreasing the N:Ca ratio represent an opportunity to support the premium FQ.

The correlation between N:Ca ratio in fruit skin and fruit unsoundness observed in this study (Table 4.3) opens the possibility of using this ratio as an indicator of internal FQ disorders during postharvest. The primary advantage lies in the option of employing non-destructive methodologies for its estimation, compared measuring the ratio in fruit flesh. For example, Kämper et al. (2020) used remote sensing technologies during postharvest in Hass avocados to estimate the mineral concentration in skins. Additionally, unlike other FQ indicators such as the Ca+Mg:K ratio, the N:Ca ratio directly involves N concentration, the nutrient with the highest influence on fruit nutrient composition during the present study, as discussed in section 3.3.4 . This ratio was found to be more influenced by N fertiliser practices than by changes in Ca or K, which already have high soil availability under the conditions of high yielding avocado orchards in the BoP.

The positive correlation between skin N:Ca ratio and fruit unsoundness (Table 4.3), suggests that promoting lower rates of N fertiliser rates would be an effective practice to produce premium FQ. This range is supported by the fact that the RSM model for the skin N:Ca ratio decreased steadily below 100 kg N/ha/year as described in Figure 3.4. In addition, as discussed in the section 3.4.7 , N fertiliser rates under 100 kg N/ha/year would be sufficient to support a high avocado yield without mining N reserves in the long term.

When high N fertiliser use was combined with high K fertiliser use, in the N300K300 treatment, lower unsoundness compared to 150 kg N/ha/year treatments were observed (Figure 4.2). Besides, this treatment resulted in lower unsoundness due to SER and VB. However, the unsoundness due to these two disorders reached less than a half compared to the unsoundness generated by BR, representing a limited advantage for the use of this treatment, compared to the use of low N fertiliser rates. As mentioned above, BR is the most prevalent FQ disorder, therefore reducing unsound fruit affected by BR will have a higher impact on FQ outcomes. Moreover, adopting 300 kg of N/ha/year to support the reduction of BR involves higher costs in fertilisers and pruning management, as well as the potential for increasing negative environmental impacts (e.g. nitrate leaching and nitrous oxide emissions).

4.4.2 Influence of fertiliser practices on the severity of body rots

Although the N50K50 treatment proved to be the most sustainable fertiliser treatment, reducing the severity of highly affected fruit by BR compared to N150K150, the potential benefits of using low N with increased K fertiliser rates should not be overlooked. The increased K fertiliser rate is supported by the fact that the N50K300 treatment consistently reduced the severity of highly and moderately affected fruit by BR compared to N150K150 (Table 4.4). However, the use of 300 kg K/ha/year under conditions of already high soil exchangeable and leaf K concentrations represents an inefficient practice to support premium FQ. Further evaluations of different K fertiliser rates in combination with low N fertiliser rates should be conducted, as this FQ assessment only included the rates of 50 and 300 kg K/ha/year with the rate of 50 kg N/ha/year. In addition, the use of 50 kg N/ha/year in combination with 150 kg K/ha/year did not increase the skin N:Ca ratio (Figure 3.4), indicating that lower K fertiliser rates than 300 kg K/ha/year could be enough to reduce the predicted probabilities to produce unsound fruit highly and moderately affected by BR.

In addition, the use of part of the K fertiliser from the N50K300 treatment should be further tested as a potential fertiliser practice to reduce the odds of fruit production moderately and severely affected by BR. The use of 130 kg K/ha before early fruit set is suggested because this amount of K fertiliser in the K300 treatments increased leaf K concentration in fruiting shoots by November (summer in New Zealand), especially in young leaves (Figure 3.6). The positive effect of increasing K fertiliser before early fruit set on reducing BR severity may be related to the role of K in plants. High K status in plants has been linked to the activation and phloem transport of self-defence mechanisms against pathogenic fungi (Amtmann et al., 2008; Zörb et al., 2014), as detailed in section 1.3.2.4. It is therefore hypothesised that around 130 kg K/ha prior to early fruit set under BoP conditions could be involved in the activation and transport of self-defence compounds in avocado. For example, *dienes*, the principal antifungal compounds in Hass avocados controlling the virulence of *C. gloeosporioides*, the causal agent of BR, are mainly produced in young leaves (Wang et al., 2004).

Increasing K fertiliser before early fruit set challenges the initial hypothesis of competition between K fertiliser use and Ca concentration in fruits proposed after the Hofman (2007) report. However, as discussed in section 3.4.7 , K fertiliser did not show antagonistic effects with Ca under the high exchangeable Ca concentration of the trial. Therefore, testing around 130 kg K/ha/year before early fruit set combined with low N fertiliser rates during the season warrants further investigation. This approach may produce fruit with a low skin N:Ca ratio and potentially trigger increased antifungal production, reducing the odds of highly and moderately affected fruit by BR. This practice could also mitigate the risks to reduce tree performance compared to using 300 kg K/ha/year under the conditions of high leaf and soil K concentrations.

4.5 Conclusions

The findings of the field trial identified that BR emerged as the fruit disorder with the highest severity in late harvested (January) avocados. Treatments with different annual N and K fertiliser rates, rather than additional Ca, influenced the predicted probabilities of producing unsound fruit due to fruit rots. The N:Ca ratio in the skin of fruit harvested late in the season, resulted in the most reliable indicator of postharvest internal fruit rots, the main fruit quality disorder in New Zealand avocados. Therefore, this research supports the potential use of the fruit skin N:Ca ratio as a predictor for fruit rots. The lowest rate of N fertiliser (50 kg N/ha/year) resulted in lower N:Ca ratios in fruit skin, which related to a reduced unsoundness due to internal fruit rots. Using this low N fertiliser rate decreased the predicted probability of producing unsound fruit by up to 26% compared to using 150 kg N/ha/year, which is closer to a more typical rate used by avocado growers in the BoP.

The use of the lowest rates (50 kg/ha/year) of N and K combined reduced the severity of fruit highly affected by BR, compared these two nutrients both applied together at 150 kg/ha/year. However, there was some evidence to support that the highest rate of K (300 kg K/ha/year) applied with the lowest rate of N, achieve additional benefits in achieving lower severity of BR, compared to when both nutrients were applied at the lowest rate. However, for orchards with high soil K concentrations the use of 300 kg K/ha/year may be an inefficient approach to further reducing BR severity. In addition, the fruit quality assessments in this study were not conducted on treatments that compared the lowest rate of N and more moderate rates of K, between 50 and 300 kg K/ha/year. Therefore, further research is required to further refine these recommendations.

Chapter 5 – General discussion and future research recommendations

5.1 Introduction

Postharvest internal fruit rots, which are more prevalent in fruit harvested late in the season, remain a significant challenge for the avocado industry in New Zealand (Everett, 2020; Sorensen, 2017). Improved fruit nutrient composition, characterised by higher Ca concentration in fruit tissues, a higher Ca+Mg:K ratio and a lower N:Ca ratio, has been associated with reduced incidences of fruit rots and other fruit quality (FQ) disorders (Dann et al., 2016; Escobar et al., 2021; Everett et al., 2007; Marques et al., 2003; Thorp et al., 1997; Ullah & Joyce, 2024; Willingham et al., 2006). Practices that have been shown to reduce the risk of postharvest fruit rots in New Zealand, include the application of Ca-rich products and fertilisers to enhance the exchangeable soil Ca concentration (Everett et al., 2007). However, in the BoP, it is typical for established high performing avocado orchards to have elevated soil exchangeable Ca levels due to regular inputs of Ca-rich soil amendments like lime and gypsum. Despite this, fruit rots remain prevalent during the late harvest. Consequently, the potential to enhance fruit nutrient composition through additional applications of lime and gypsum is limited. Therefore, fertiliser management should prioritise either alternative nutrient additions or preventing antagonisms between Ca and other nutrients, especially N and K fertilisers. Few studies have investigated the impact of managing N or K fertiliser inputs on fruit nutrient composition (Hofman, 2007; Willingham et al., 2006). Additionally, research on the combined effects of N and K on avocado fruit nutrient composition and the risk of postharvest internal fruit rots has been limited.

This thesis investigated the effects of N and K fertiliser practices on avocado fruit nutrient composition and FQ, specifically the incidence of postharvest fruit rots in the BoP, New Zealand. The research objectives of this study were to:

- i. Characterise the change in fruit nutrient composition, namely N, K, and Ca, and their ratios between early and late harvested avocado fruit (Chapter 2 and 3).
- ii. Evaluate the influence of different fertiliser practices, namely N, K, and Ca soil applications, on avocado fruit nutrient composition in representative high-performance avocado orchards in the BoP (Chapter 2).
- iii. Investigate the effectiveness of different fertiliser practices in enhancing avocado fruit nutrient composition, while maintaining avocado tree performance (Chapter 3).
- iv. Identify optimal N and K fertiliser strategies for reducing the risk of avocado postharvest fruit rots, especially for fruit harvested late in the season for which rots are more prevalent in New Zealand (Chapter 4).

5.2 Seasonal changes in fruit nutrient composition

The survey study in Chapter 2 identified trends in the nutrient composition of avocado fruit flesh and skin as the harvest season progressed. In particular, there was a general decrease in Ca concentration in flesh and skin of late harvest fruit, being greater the decrease in flesh Ca concentration. This trend was supported by the two-year fertiliser field trial (Chapter 3), which showed that the average Ca concentration decreased on average by about 50% in the fruit flesh and about 16% in the skin, between the early and late harvests. This decrease in flesh Ca concentration aligns with previous studies, showing a consistent pattern that is influenced by an increase in flesh dry matter content between the early and late harvest (Escobar et al., 2021; Snijder et al., 2002). In the current study, fruit flesh dry matter increased from around 23% at the early harvest (September), to around 33% at late harvest (January).

The general trend in fruit N, K, and Mg concentrations between the early and late harvest was also consistent between the survey study (Chapter 2) and the field trial (Chapter 3). In the field trial, the concentrations of N, K and Mg in the skin increased by 49%, 56% and 16% respectively between the two harvests. Meanwhile, the concentrations of N, K and Mg in the flesh decreased by 6%, 18% and 22% respectively. In the fruit skin, the higher N and K concentrations combined with lower Ca concentrations resulted in higher N:Ca ratios and lower Ca+Mg:K ratios during the late harvest, compared to the early harvest. However, in fruit flesh the modest decreases N and K concentrations relative to the more pronounced decline in the Ca concentrations also resulted in higher N:Ca ratios and lower Ca+Mg:K ratios in the late harvested fruit. Overall, the practice of keeping the avocado fruit on the tree until late harvest results in unfavourable changes in fruit nutrient ratios, which is related to an increased risk internal FQ disorders as fruit rots.

5.3 Effect of fertiliser practices on fruit nutrient composition and fruit quality.

5.3.1 Effect of calcium fertiliser

In the orchard survey (Chapter 2), orchards with higher soil exchangeable Ca concentrations, did not appear to result in higher fruit Ca concentrations. However, all orchards in the survey had exchangeable soil Ca concentrations above the proposed soil fertility target level for avocados, of 12 meq Ca/100g, which is likely to explain the lack of a response (New Zealand Avocado Growers Association, 2000). Similarly, in the field trial (Chapter 3), which also had a high initial soil exchangeable Ca concentration, the addition of calcium nitrate before the early fruit set also did not increase fruit Ca concentrations at any harvest time (Section 3.3.5). These results support the idea that under conditions of high exchangeable soil Ca concentrations, additional Ca inputs have a negligible effect on fruit Ca concentration. In addition, Mexican-derived rootstocks, like the Zutano rootstock used in the study, can also limit Ca uptake which has been reported in multiple studies overseas (Dann et al., 2016; Hofman et al., 2002; Marques et al., 2003, 2006; Mullen, 2015; Willingham et al., 2001, 2006).

5.3.2 Effect of nitrogen fertiliser

Nitrogen fertiliser use was the main driver influencing changes in fruit nutrient composition, particularly for avocados harvested late in the season. In both components of this research, the survey study (Chapter 2) and the fertiliser field trial (Chapter 3), higher N fertiliser use was associated with higher N concentrations and N:Ca ratios in late harvested fruit. For instance, during the 2022-2023 season of the field trial, increasing the N fertiliser rate from 50 to 150 kg N/ha/year increased the fruit N concentration from ~12 g N/kg in both fruit tissues, to 14.6 g N/kg in flesh and 14.3 g N/kg in skin during the late harvest (Table 3.6). At the same time, treatments using 50 kg N/ha/year produced fruit with N:Ca ratios of 68 in flesh and 47 in skins during the late harvest, which were at least 22% lower than for treatments with higher rates of N fertiliser.

During the same late harvest of the 2022-2023 season, the FQ assessment demonstrated that decreasing N fertiliser from 150 to 50 kg N/ha/year reduced the probability of producing unsound fruit by around 20% (Table 4.2). This reduction coincided with a lower N:Ca ratio in fruit skins at the late harvest, which was strongly correlated with fruit unsoundness (Table 4.3). Therefore, the use of low rates of N are associated with improvements in both fruit nutrient composition and a reduction of unsoundness in late-harvested fruit, primarily caused by BR.

While it is common for avocado growers in the BoP to use rates of N fertiliser higher than 100 kg N/ha/year, the field trial in the current study do not find evidence of yield being negatively affected by the lowest rate of N fertiliser (50 kg N/ha/year). In addition, other studies on Hass avocados (Huett & Dirou, 2000; Lahav & Kadman, 1980; Maldonado-Torres et al., 2007; Rebolledo-Roa & Burbano-Diaz, 2023) and nutrient budget modelling using OverseerFM[®] support rates between 75 and 100 kg N/ha/year as adequate to replace N losses from high-yielding avocado orchards. The current study also demonstrated that low rates of N (50 kg N/ha/year) were adequate for maintaining leaf N concentrations above the critical level proposed by Dixon (2008) of 24 g N/kg for avocados in the BoP. Therefore, between 50 and 100 kg N/ha/year is likely to be adequate for avocado tree requirements, while the current study demonstrated that 50 kg N/ha reduced the risk of postharvest fruit rots in late harvested fruit, compared to rates of 150 kg N/ha/year or greater. Therefore, more research that compares lower rates of N fertiliser, in the range of 50-100 kg N/ha/year, over an extended period and across various sites, is needed to further validate and refine these N fertiliser recommendations.

5.3.3 Effect of potassium fertiliser

The survey study (Chapter 2) showed the influence of a possible antagonism effect of K fertiliser use on the Ca concentration in fruit skins at late harvest (Figure 2.5), which has also been previously reported in Hass avocados (Hofman, 2007). However, in the fertiliser field trial (Chapter 3), there was no evidence of K fertiliser use, even at rates as high as 300 kg K/ha/year, affecting the Ca concentration in fruit skin nor flesh. The trial site had a very high initial exchangeable soil Ca level of 33.6 meq Ca/100g (2.7-times the

target level of 12 meq Ca/100g), which could explain why fruit Ca was maintained at a similar level irrespective of K fertiliser use.

The FQ assessment during the late harvest of the 2022-2023 season, highlighted the need to investigate the effect of different K fertiliser rates combined with low N fertiliser rates as a strategy for reducing the severity of BR (Table 4.4). When N and K were applied together at their lowest rates (50 kg/ha/year) of N and K, they resulted in a reduced BR severity of highly affected fruit (BR severity >15%), compared the applying these nutrients together at 150 kg N/ha. However, when the lowest N fertiliser rate was applied in combination with highest K fertiliser rate (300 kg N/ha/year), there was a reduction in the BR severity of both highly (BR severity > 15%) and moderately ($5\% < \text{BR} < 15\%$) affected fruit. A possible mechanism explaining the effect of the high K fertiliser rate, could be an increase in the expression of antifungal compounds in young leaves during summer (Wang et al., 2004), providing a protective effect against BR in late-harvested fruit. However, this was not investigated in the current study and, therefore, needs further research. For orchards with high exchangeable soil K concentrations, the use of high rates of K fertiliser may be an inefficient approach to further reducing BR severity. In addition, the FQ assessments in this study were not conducted on treatments that compared the lowest rate of N and more moderate rates of K, between 50 and 300 kg K/ha/year. Therefore, further research is necessary to further refine these recommendations and test them over a range of sites and over several seasons.

5.4 Summary and practical implications

A key finding of this research is that the N:Ca ratio in avocado fruit skin is a useful indicator of the risk of postharvest internal fruit in late harvested fruit. In addition, changing N fertiliser use was shown to be a more effective way of influencing this ratio, than through altering Ca inputs. This is especially the case for orchards which already have high soil Ca status, as in common in the BoP.

Another main finding is that the risk of producing unsound fruit by fruit rots and the severity of these rots, principally BR, can be decreased by using a low rate of N fertiliser of 50 kg N/ha/year. This is below the N fertilisers rates commonly used in avocado orchards in the BoP, which are typically greater than 100 kg N/ha/year. Therefore, it is likely that current N fertiliser practices are contributing to the higher observed incidence of fruit rots in late harvested fruit. Accordingly, programmes focused on improving the quality of late harvested fruit need to include an emphasis on supporting growers to move toward lower N fertiliser use. This is especially relevant for growers that intend to retain a portion of the season's crop on the tree to harvest late. The use of lower rates of N will also help to mitigate some of the associated negative impacts of N fertiliser use, such as nitrate leaching and nitrous oxides emissions, which will help lower the environmental footprint of the industry.

There was also evidence in this study that the use of a low rate of N in combination of a high rate of K fertiliser (300 kg K/ha/year) may further reduce the risk of BR, compared to when both nutrients were applied together at a low rate. However, for orchards already with optimum soil K status, the use of high rates of K may be an inefficient and costly approach to further reducing fruit BR severity. In addition, fruit quality assessments in this study were not conducted on treatments that compared the lowest rate of N and more moderate rates of K, between 50 and 300 kg K/ha/year. Another alternative is testing increased K fertiliser rates before the early fruit set with K fertiliser quantities around 130 kg K/ha (the quantity applied in treatments using 300 kg K/ha/year by summer each season). Therefore, further research is needed to refine these recommendations and test them over a range of sites and over several seasons.

Practical implications for growers of the main finding of this research are:

- i. The use of additional Ca fertiliser, beyond the traditional applications of lime and gypsum, does not improve fruit Ca status, particularly for orchards with high exchangeable soil Ca concentration (>12 meq Ca/100 g).
- ii. When soil exchangeable Ca concentration was high, an antagonistic effect between K fertiliser use and fruit Ca concentration was not observed, even at K rates as high as 300 kg K/ha/year. Therefore, K fertiliser recommendations can be based on crop requirements, without needing to consider the effect on fruit Ca status, for orchards with high soil exchange Ca concentrations.
- iii. High rates of N fertiliser (>100 kg N/ha/year) are not recommended because they increase the risk of producing unsound fruit, especially for late harvested fruit.
- iv. Monitoring N:Ca ratio in avocado fruit skins, particularly in late-harvested fruit, has potential as a monitoring tool to assess the risk of postharvest FQ disorders.

5.5 Study limitations and future research recommendations

The main limitations of this study and areas for future research include:

- i. The survey study (Chapter 2) involved a small number of orchards. While this survey was primarily intended as an exploratory assessment to inform the design for the field trial (Chapters 3 and 4), using a larger number of orchards is expected to have improved the ability to identify relationships between fertiliser practices and fruit nutrient status.
- ii. The replicated field trial (Chapters 3 and 4) was conducted on only one orchard, which had a history of high N and K fertiliser inputs. Because the results observed in the study will be influenced by previous nutrient management practices, the results may have differed if the research had been conducted on an orchard with lower previous N and K fertiliser use.
- iii. Financial and logistic constraints limited the FQ assessment to six treatments out of the twelve used in the field trial (Chapter 4). Using all of the treatments is expected to have improved the FQ assessment comparisons.
- iv. The current study helped to narrow down the rates of N and K that showed improvements in fruit nutrient composition and lowered the risk of postharvest internal fruit rots in avocados. Additional research is required to further refine these rates over a number of years and at multiple different locations in the BoP and Northland Regions. Specifically, future research should focus on whether rates of N fertiliser between 50 and 100 kg N/ha/year are adequate for both maintaining high crop yields and achieving lower fruit unsoundness caused by rots, like those observed with the 50 kg N/ha/year rate used in the current study. While determining the exact N fertiliser rates may pose challenging due to fluctuations in soil N supply influenced by various factors beyond fertiliser applications, such as site and seasonal variations, it is important to conduct additional experiments with lower N fertiliser rates to demonstrate the potential benefits to growers.

- v. Future research should also focus on refining the K fertiliser rates, between 50 and 300 kg K/ha/year, that are optimum for reducing the severity of BR, while maintaining high crop yields. It would also be useful to confirm whether applying a higher proportion of the K fertiliser before early fruit set has any additional benefits in improving reducing fruit rots.

- vi. Research should be conducted to demonstrate the environmental benefits of lower rates of N fertiliser, specifically on nitrate leaching and nitrous oxide emissions. This would help to quantify the benefits of growers to lower N use systems and support the decision-making process, contributing to greater sustainability of the avocado sector in New Zealand. Following completion of the current field trial, the Avocado Industry Council of New Zealand (AIC) started a project called “Understanding Nutrient Leaching to Improve Fertiliser Efficiency”, which uses the same site to measure the impact of the N fertiliser rates used on the potential for N leaching.

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Appendices

Appendix 1 - Full paper for oral presentation in the tenth world avocado congress 2023

Monserrate F., Van der Heijden D., Dowson A., Jeyakumar P., Roskrige N., Anderson C., Hanly J., Influence of different fertilization regimes on avocado fruit mineral composition in Bay of Plenty, New Zealand. In: *Proceedings of the tenth world avocado congress*, Auckland, New Zealand. <https://industry.nzavocado.co.nz/wp-content/uploads/2023/05/10th-World-Avocado-Congress-all-full-papers.pdf>, 52-61.

Influence of different fertilization regimes on avocado fruit mineral composition in *Bay of Plenty*, New Zealand.

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Summary

Fruit mineral composition in avocados has been identified as an indicator of fruit quality (FQ) disorders during postharvest. Fruit with high calcium (Ca) concentration, low nitrogen (N) concentration, and low potassium (K) concentration are desirable to support the production of premium FQ. A lower N:Ca ratio in skins consistently indicates better FQ. However, the influence of different soil fertilization regimes on fruit skin N:Ca is not well established in New Zealand. Nine high-performance avocado orchards from the Bay of Plenty (New Zealand) with different N and K fertilization use were monitored during the season 2021-2022. The soil-Ca concentration surpassed the soil-fertility target level set by the industry (12meq Ca/100g). Fruit from 5 similar mature trees (Hass/Zutano) were sampled early and late in the season (September 2021 and January 2022, respectively). Calcium concentration decreased by 16% between the early and late harvest (from 762.9 to 639.5mg Ca/kg), whereas N and K concentration increased by 40% and 74%, respectively (from 10.4 to 14.6g N/kg for N and from 15.5 to 27.0g K/kg for K). The skin N:Ca in early-harvested fruits was approximately 14, independent of the N fertilization level, while for late-harvested fruits, the ratio reached approximately 29 when N fertilization was higher than 250kg N/ha. High N fertilizer rates resulted in higher skin N concentrations, whereas high K fertilizer rates showed a trend of decreasing skin Ca concentrations. Therefore, an optimization of N and K fertilization in avocados is needed to minimize FQ disorders, particularly with late-harvested fruit.

Key words: Calcium; Nitrogen; Potassium; N:Ca; Fruit Quality

Influencia de diferentes regímenes de fertilización en la composición mineral de aguacates producidos en *Bay of Plenty*, Nueva Zelanda.

Resumen

La composición mineral en aguacates ha sido identificada como indicador de calidad del fruto (CF) en poscosecha. Frutos con alta concentración de calcio (Ca), bajo nitrógeno (N) o potasio (K) ayudan a producir CF premium. Relaciones N:Ca bajas son un indicador consistente de adecuada CF.. Sin embargo, la influencia de diferentes regímenes de fertilización sobre la relación N:Ca no está bien establecida en Nueva Zelanda. Nueve huertos de alto rendimiento en Bay of Plenty con diferentes fertilizaciones de N y K fueron monitoreados durante la cosecha 2021-2022. Los huertos tuvieron Ca edáfico arriba del recomendado por la industria (12meq Ca/100g). Se muestrearon frutas de 5 árboles similares (Hass/Zutano) por huerto durante la cosecha temprana y tardía (septiembre 2021 y enero 2022). La concentración de Ca se redujo en 16% entre la cosecha temprana y tardía (De 762.9 a 639.5mg Ca/kg), mientras las concentraciones de N y K incrementaron en 40% y 74%, respectivamente (De 10.4g N/kg a 14.6g N/kg para N y de 15.5 a 27.0g K/kg para K). La relación N:Ca en cascaras de cosecha temprana estuvo alrededor de 14 independientemente del nivel de fertilización, mientras en la cosecha tardía la N:Ca alcanzo 29.9 en cascaras de huertos usando más de 250kg N/ha/cosecha. Altas fertilizaciones de N resultaron en alta concentración de N en las cascaras, mientras altas fertilizaciones de K redujeron la concentración de Ca. La optimización de la fertilización con N y K en aguacate es necesaria para minimizar problemas de CF, especialmente en la cosecha tardía.

Palabras clave: Calcio; Nitrógeno; Potasio; N:Ca; Calidad de fruta

Introduction

Avocado mineral composition plays a vital role in achieving good fruit quality outcomes. Multiple studies worldwide have reported that fruit with high Ca status were less affected by fruit quality disorders such as internal fruit rots (Willingham *et al.*, 2006; Dann *et al.*, 2016; Escobar *et al.*, 2021), chilling injuries (Arpaia, 1994; Barrientos-Priego *et al.*, 2016), fruit softening at storage (Rivera *et al.*, 2017), or vascular browning (Marques *et al.*, 2006). In those studies, a better Ca status was achieved when Ca concentration increased and their relationship with other mineral nutrients such as N and K improved (i.e., high ratio Ca+Mg:K, and low ratio N:Ca). The principal pre-harvest factors known to influence fruit Ca status have been the rootstock/scion-tree genetic (Willingham *et al.*, 2006; Dann *et al.*, 2016), the calcium partitioning between vegetative and reproductive tissues (Witney *et al.*, 1990; Willingham *et al.*, 2004; Mullen, 2015), and the orchard nutritional management (Willingham *et al.*, 2006; Hofman, 2007).

In New Zealand, previous studies reported that avocado orchards improved the fruit Ca status by increasing the fruit Ca concentration when growers increased the application of soil Ca amendments (Everett *et al.*, 2007). In those studies, the incidence of internal fruit quality disorders was low when the ratio Ca+Mg:K was higher than 0.065 (Thorp *et al.*, 1997; Everett *et al.*, 2007). However, fruit quality disorders related to reduced fruit Ca concentration continue challenging the avocado industry. A higher incidence of internal fruit rots and external black patches at late harvest still affects fruit quality outcomes each season. Even though the orchard nutritional management is a component of the New Zealand avocado industry to reduce the incidence of fruit rots affecting the FQ (Sorensen, 2017), the industry's recommendations are based on overseas experimentation. Besides, there are different criteria used to recommend nutritional management by fertilizer consultants (West, 2020), and the effect of those recommendations on fruit Ca status is not well established.

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This survey study explored the influence of fertilization regimes of high-performance avocado orchards in the Bay of Plenty (BoP) on fruit Ca status. In this way, the study also facilitated the identification of further research required under New Zealand conditions.

Methods

The monitoring of fertilization practices, soil, and fruit Ca status was developed during 2021-2022 in commercial avocado orchards from the western BoP, one of the traditional avocado productive districts in New Zealand. Nine commercial avocado orchards with similar horticultural and environmental conditions but different N fertilization use were selected. The orchards monitored had three main horticultural characteristics: Yield over 16t/ha in the last three seasons (high-performance orchards), mature trees (>15 years old), and trees with the combination Hass (Scion) on Zutano (Rootstock) varieties. Orchards were located on flat to easy-rolling landscapes, *Typic Orthic Allophanic* soils, and temperate-subtropical climate with 1180mm annual precipitation evenly distributed, 14.8°C annual mean temperature (Maximum 24°C in January -summer-, Minimum 5°C in July -winter-).

Three sets of orchards based on different levels of historical N fertilization were selected: High N fertilization orchards using more than 250kg N/ha/season, medium N fertilization orchards using between 150 to 250kg N/ha/season, and low N fertilization orchards using less than 150kg N/ha/season. Fertilization records between 2018 and 2021 were used to summarize the nutrient fertilization by season, then to calculate the fertilization regime with the 3-season average of N and K. The maximum seasonal Ca fertilization was used instead of the average Ca fertilization, as Ca-rich amendments such as lime and gypsum are applied every two or three years.

Five similar trees were chosen in each selected orchard based on a visual examination of biomass and fruit load during the harvest season 2021-2022. Four fruits per tree were sampled from non-terminal shoots across the canopy twice during the harvest season: Early in September 2021 (~23% DM) and late in January 2021 (~33% DM). Soil samples from the topsoil (0-15cm) were sampled in early winter (July 2021), referring to the avocado industry recommendations (NZ Avocado growers association, 2000). The soil was sampled by taking eight cores in the drip irrigation zone underneath the tree canopy (1.5- 2m from the trunk).

Fruit and soil samples were transported to the Massey University laboratories within 24h after sampling for analyses. Avocados were kept at room temperature (~16°C) for two weeks until ripe and then sub-sampled. An equatorial belt approximately 2cm wide was taken from each avocado with a kitchen knife, the skins were carefully separated from the flesh, and composite fruit samples of skin and flesh were obtained from each tree. The fruit-skin samples were dried in an air-forced oven at 65°C for 24h before finely ground for further analyses (Boyd *et al.*, 2007). Subsamples of fruit skins were acid digested in an aluminum digestion block for cations and total N, following modified methods from Thorp *et al.* (1997) and a Kjeldahl digestion, respectively. Briefly, for cations, a 0.1g subsample was digested for 2h at 120°C in 2ml concentrated nitric acid (69%) using glass funnels on top of the digestion tubes to allow reflux. The samples were then cooled at room temperature, and 2ml hydrogen peroxide (35%) was added, and the digestion continued for 3.5h at 120°C with refluxing. The digested samples were made up to 25ml with deionized water after adding 1ml 2.5% CsSr as a dispersant for cation determination in a 4200 MP-AES (Agilent, USA). Fruit-skin samples were also digested to determine total N using a Kjeldahl digestion procedure of 0.1g subsample for 8h at 350°C. The digested product was analyzed for total N in a Technicon autoanalyzer. Soil samples were air dried for 48h at 30°C, ground in a ceramic pestle and mortar, and

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sieved through a 2-mm stainless steel mesh for further laboratory analysis. The exchangeable cations (e.g., Ca, K, Mg, Na) were quantified after the extraction from soil using a micro-leaching procedure with 1M ammonium acetate (pH7) (Blakemore *et al.*, 1987) and then determined in a 4200 MP-AES (Agilent, USA).

The statistical data analyses were performed by fitting linear models having as categorical factors the individual orchards or the orchards grouped by nitrogen fertilization level (NF) and harvest time (e.g., Early and late harvest season). Mean-pairwise comparisons were developed using the Fisher's LSD test. The data analyses were performed using the statistical language R Version 4.1.1 (R Core Team, 2021), and for the pair-wise comparisons were used the R package *agricolae* (de Mendiburu, 2021).

Results

Fertilization regimes in the monitored orchards:

The orchards surveyed had a wide variability of fertilizer nutrient inputs. The 3-season average N fertilization varied from 74 to 314kg N/ha, whereas K fertilization went from 32 to 374kg K/ha. The orchards monitored did not consistently use high, medium, and low N and K fertilization (Figure 1). This variation in N and K fertilization was due to the nutritional composition of the fertilizer products used at each orchard. The orchards surveyed used three main fertilizers to supply most of the N and K fertilization:

- All orchards used fertilizer blends of compositions 12-5-15-8-2-2(N-P-K-S-Mg-Ca) or similar. Those fertilizers fulfilled between 23% and 73% of the seasonal N fertilization and between 45% and 92% of the seasonal K fertilization.
- Five orchards used calcium ammonium nitrate (CAN). Four of them used CAN to provide most of the applied N, which were the orchards using more N than K seasonally (Figure 1- Orc1, Orc2, Orc5, Orc9).
- Three orchards complemented the K fertilization with sulfate of potash (SOP). Two of them used roughly 40% more K than N seasonally (Figure 1- Orc3, Orc7).

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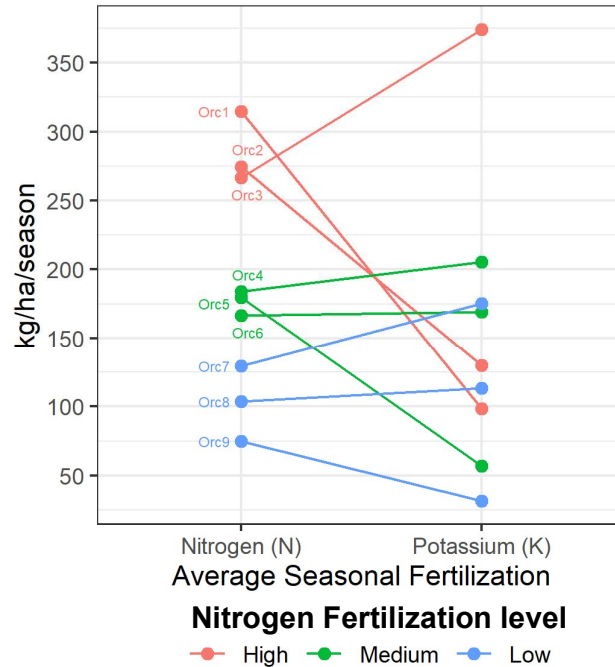


Figure 1- Average seasonal fertilization of nitrogen (N) and potassium (K) in nine commercial avocado orchards surveyed in BoP. The average corresponds to the fertilization data from 2018 to 2021. Orchards are numbered in descendent order according to their N fertilizer rates.

Calcium inputs were mostly from using lime and gypsum, a practice reported in eight of nine orchards the survey period (2018-2021). The maximum seasonal Ca fertilization ranged from 1181 to 34kg Ca/ha. The latter value was reported in the only orchard that did not use lime or gypsum during the survey period (Orc7). However, prior to the survey the grower used lime and gypsum at rates of 1t/ha in that orchard. Those two Ca amendments represented between 71% to 92% of the maximum seasonal Ca fertilization for orchards using them. Orchards using medium and high N fertilization levels (NF) had higher Ca fertilization than orchards using low (NF). Indeed, orchards in the medium and high NF used at least 1t/ha of lime, and four used lime and gypsum during the same season. The maximum Ca fertilization directly impacted the soil Ca concentration as is shown in the next section.

Soil Ca status

The soil Ca concentration and the maximum seasonal Ca fertilization were positively correlated ($R^2=0.74$, $p<0.05$) (Figure 2A). This concentration varied from 14.8meq Ca/100g in Orc9 (Low NF) to 33.7meq Ca/100g in Orc3 (High NF). During the monitoring, two orchards with contrasting soil Ca inputs had similar soil Ca concentrations (~20meq Ca/100g for Orc1 and Orc7). However, lime and gypsum were applied in Orc7 at rates higher than 1t/ha before the monitoring period. As orchards using medium and high NF applied higher dosages of soil Ca amendments, their soil Ca concentrations were higher (27.6 and 25.3meq Ca/100g for high and medium NF) compared with orchards using low NF (18.1meq Ca/100g) (Figure 2B). Overall, the soil Ca concentration by orchard was 1.2 to 2.8-times the soil fertility target proposed by the avocado industry in New Zealand for high-performance avocado orchards of 12meq Ca/100g (NZ Avocado growers association, 2000).

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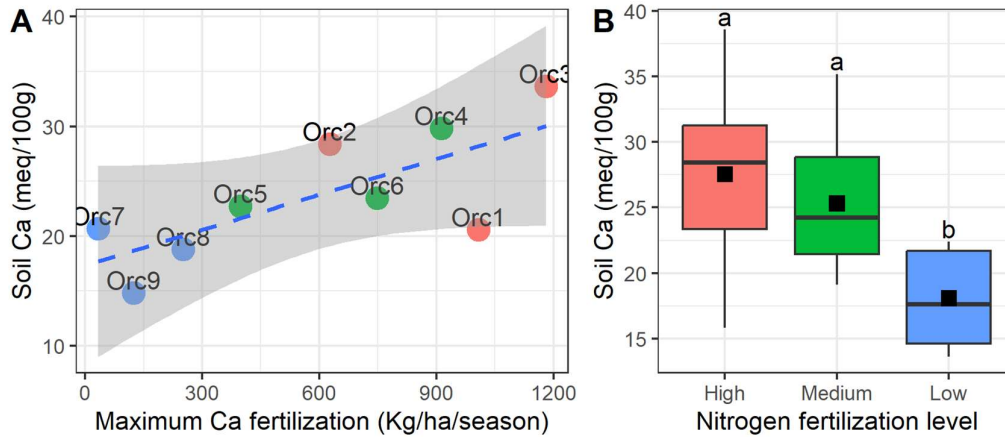


Figure 2- Relationship between soil Ca concentration and total Ca fertilization by orchard (A) and boxplot for the soil Ca concentration by level of nitrogen fertilization (B). Black squares in B represent mean values by NF and letters the pairwise-mean comparison among levels. Means with different letters are significantly different by the Fisher's LSD-test ($p < 0.05$).

Changes in fruit mineral concentration between the early and late harvest:

The Ca concentration in avocado fruit skin and flesh decreased between the early and late harvest during the 2021-22 season (Figure 3A and C). The average concentrations reduced from 762.9 to 639.5mg Ca/kg (16.2%) in the skins, and from 471.3 to 198.1mg Ca/kg (58%) in the flesh between early and late harvest, respectively. The N and K concentrations in fruit flesh also decreased between the early and late harvest, but the concentrations in fruit skins showed the opposite trend (Figure 3B and C for N and Figure 3D and E for K). On average, avocado skins harvested late in the season had 40.4 and 74.2% higher concentrations of N and K, respectively, compared to fruit harvested early with concentrations of 10.4g N/kg and 15.5g K/kg. This trend of higher skin N and K concentrations in late harvested fruit warrants further exploration to determine influence that fertilizer management has on these values.

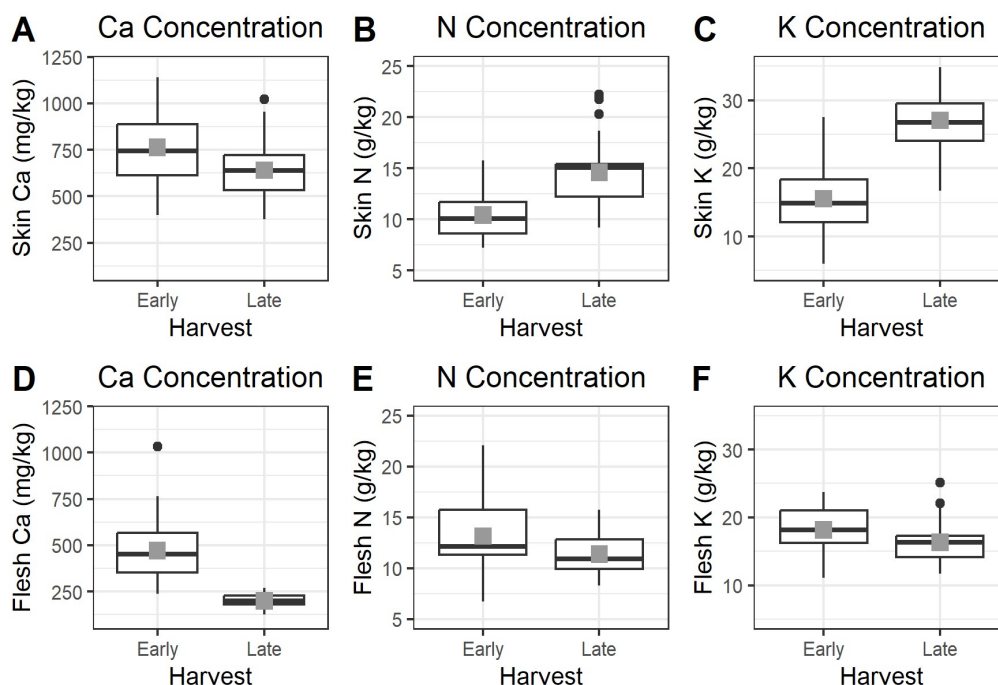


Figure 3- Boxplots for the mineral composition of fruit skin and flesh harvested early and late during the harvest season 2021-2022 in nine commercial orchards of BoP (Grey points represent the mean value for each mineral and harvest time).

Influence of N fertilization levels on N and Ca status of fruit skins:

The N fertilization (NF) level increased the N concentration in fruit skins during the late harvest, especially for orchards with high NF (Figure 4A). The N concentration in fruit skins during the early harvest remained about 10g N/kg. In contrast, during the late harvest, the N concentration in fruit skins significantly ($p < 0.05$) increased from 12.9 and 14.1g N/kg, in orchards using low and medium NF levels respectively, to 16.6g N/kg in orchards using a high NF level. However, NF did not significantly ($p > 0.05$) change the Ca concentration in fruit skins for either the early or late harvested fruit (Figure 4B). The influence of NF level on N concentration in late-harvested fruit skins was also reflected in the N:Ca ratio in fruit skins. The N:Ca ratio in fruit skins at the late harvest was 29.9 for orchards using high NF, which was significantly ($p < 0.05$) higher than the values of 22.6 and 20.9 for the orchards using medium and low NF, respectively (Figure 4C). The skin N:Ca ratio in late-harvested fruit was also higher than early harvested fruit at all NF levels.

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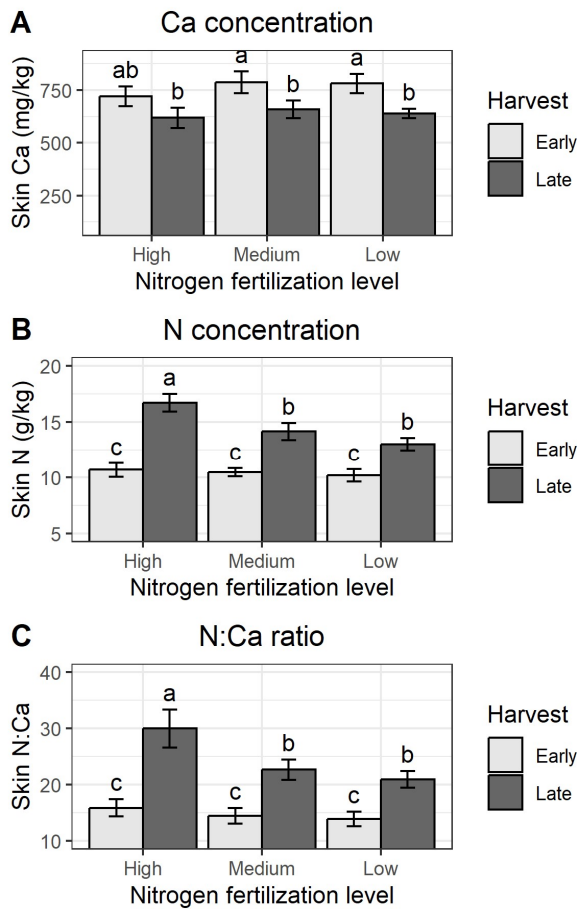


Figure 4- Mean comparison for fruit skin Ca status in nine commercial avocado orchards of BoP pooled by nitrogen fertilization level and harvest time (Bars represent means by harvest time and nitrogen fertilization level, and vertical lines the standard error. Means with different letters are significantly different by the Fisher's LSD-test ($p < 0.05$)).

Influence of K fertilization levels on Ca status of fruit skins:

The primary criteria for orchard selection in this study was based on N fertilizer use. However, because the orchards also used a range of K fertilizer rates, the relationship between K fertilization (KF) level and fruit-skin Ca concentration was also investigated. After classifying the orchards into three levels of K fertilization (three orchards by level), the fruit skin Ca concentration was the lowest on average in the three orchards using the higher K fertilization rates, being 548.2mg Ca/kg, compared to values of 638.0 and 732.2mg Ca/kg for orchards using the medium and low KF, respectively. Thus, the highest fruit skin Ca concentration at late harvest was obtained in orchards using the lowest K fertilization (Figure 5). While this result shows an interesting trend that warrants further investigation, it needs to be used with caution because the K fertilizer rates at the different KF levels were not as well separated, as was the case for N.

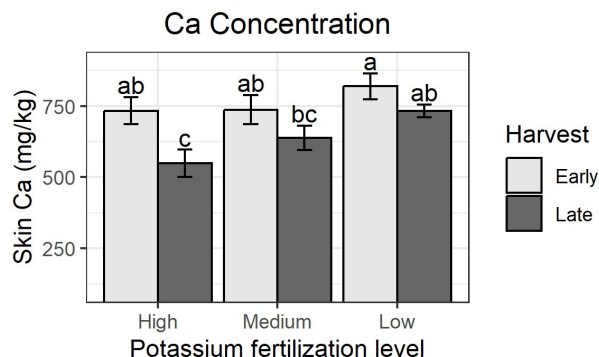


Figure 5- Mean comparison for fruit-skin Ca concentration in nine commercial avocado orchards of BoP pooled by potassium fertilization level and harvest time (Bars represent means by harvest time and potassium fertilization level and vertical lines the standard error. Means with different letters are significantly different by the Fisher's LSD-test ($p < 0.05$)).

Discussion

The fertilization regimes surveyed in the monitored avocado orchards of BoP influenced the mineral composition of fruit skins harvested late in the season, which is also when avocado rots are higher. Skins of avocados harvested late in orchards using high N fertilization levels (>250 kg/ha/season) had higher N:Ca ratios (Figure 4C). This result highlights the critical role that N fertilization management could have on fruit mineral composition, as has been reported internationally (Wolstenholme, 2004). High N fertilization increased N concentration and the N:Ca ratio in fruit skins for one year in a multiyear-controlled experiment in northern New South Wales, Australia (Willingham *et al.*, 2006). In that experiment, the ratio N:Ca in skins differed between the nil-N and the high-N fertilization treatment (~ 300 kg/ha/season). However, the experiment tested the fruit skins during the early harvest each year.

In the current study, high KF showed a trend of reducing Ca concentration in fruit skins at the late harvest, despite the high soil Ca status in all orchards. The competition between K and Ca uptake, especially during critical periods of fruit Ca accumulation, was identified as affecting fruit Ca concentration in southeast Queensland, Australia (Hofman *et al.*, 2005; Hofman, 2007). That study recommended the improvement of Ca availability during the critical period for Ca translocation into the fruit (early fruit set) by applying fertilizers rich in soluble Ca and withdrawing K. However, for orchards with high soil Ca status, like in the current study in the BoP, a focus on reducing K fertilizer use during critical periods may be a useful strategy that warrants further research.

Additional research is needed to improve Ca status of fruit harvested late in high-performance avocado orchards, which assesses different combinations of N and K fertilization. The migration from a strategy to increase Ca in soils to one looking to optimize N and K fertilization is justified in BoP for at least two reasons: The wide variability observed in both N and K fertilizer use; and the current common use of high Ca inputs, such as lime and gypsum. Soils with higher Ca concentrations did not produce fruit with higher fruit Ca concentrations, contrary to reports from previous surveys in BoP (Thorp *et al.*, 1997; Everett *et al.*, 2007). However, these earlier studies included orchards with lower soil Ca concentrations. For example, during the survey in the early 90s, Thorp *et al.*, (1997) reported soil Ca concentration ranging from 2.5 to 6.3meq Ca/100g, which is a much lower range compared to the current study (14 to 33meq/100g). Moreover, previous trials overseas had limited results in increasing fruit

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Ca status, even using very high Ca inputs or saturating the soil and xylem flow with Ca (Du Plessis & Koen, 1987; Hofman, 2007).

As part of the current study, research in a replicated field trial has been conducted to assess the effect on avocado fruit nutrient status and fruit quality of two main nutritional strategies: The use of different combinations of N and K fertilizers rates and the reduction of K use during critical periods for Ca translocation into the fruit.

Acknowledgements

This work is being developed thanks to the support of Massey University Doctoral Scholarship, Ballance Agri-Nutrients, and AVOCO. Special thanks to the avocado growers who decided to participate in the monitoring and shared their fertilizer records for this analysis. Besides, thanks to the New Zealand horticultural science advancement trust for funding the participation in the conference.

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Appendix 2- Extended summary for poster presentation at the 34th Farmed landscapes research centre seminar

Monserrate F., Hanly J., Van der Heijden D., Dowson A., Jeyakumar J., Roskruge N., Anderson C. 2022. Understanding the influence of nutrient management strategies in high-performance avocado orchards on fruit calcium status: Initial results. In: *Adaptive Strategies for Future Farming*. (Eds. C.L. Christensen, D.J. Horne and R. Singh). <http://flrc.massey.ac.nz/publications.html>. Occasional Report No. 34. Farmed Landscapes Research Centre, Massey University, Palmerston North, New Zealand. 3 pages.

UNDERSTANDING THE INFLUENCE OF NUTRIENT MANAGEMENT STRATEGIES IN HIGH-PERFORMANCE AVOCADO ORCHARDS ON FRUIT CALCIUM STATUS: INITIAL RESULTS

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Abstract

Nutrient management strategies vary widely in New Zealand avocado orchards because the use of diverse approaches used to recommend fertilisers by consultants. Nutrients traditionally associated with fruit quality, such as calcium (Ca), nitrogen (N), and potassium (K) are applied at a wide range of rates, even to orchards with similar soil types, tree age, yield, and environmental conditions. Given the high variability in fertilisation practices, there is a need to understand the influence of these nutrient management strategies on fruit mineral composition to help identify impacts on avocado fruit quality in New Zealand. This work shows the results of seasonal fertiliser use and soil Ca status in ten high-performance avocado orchards of the Bay of Plenty Region.

Methodology

Ten avocado orchards with differing fertiliser practices and high performance (Yield > 16 t/ha/season) were monitored in the Bay of Plenty Region, the main avocado productive district in New Zealand. The orchards had similar horticultural conditions (Tree age, Scion/rootstock variety). Mineral determinations (i.e. N, K, Ca, and Mg) on soils, leaves and fruit were performed and analysed in ten trees of each orchard monitored along with fertiliser use records. The orchards were classified according to the N fertiliser use in three categories: High N fertilization (> 250 Kg N/Ha/season), medium N fertilisation

(150 – 250 Kg N/Ha/season), and low N fertilisation (<150 Kg/Ha/season). This poster is about the initial results for the fertiliser use and soil Ca status.

Fertilization by season

The Figure 1 displays the multi-seasonal average of N and K fertilization in ten orchards of Bay of Plenty. The total fertiliser use varied from 74 kg/Ha/Season to 314 kg/Ha/season of N, whereas the K use went from 31 kg/Ha/Season to 374 Kg/Ha/Season. The ratio between the total N and K applied seasonally at each orchard was defined by the combination of fertilisers used. In the case of orchards using more N than K, the main fertiliser used during the season was calcium nitrate (CAN) with occasional fertilizations using blended fertilisers. For other orchards the main fertiliser used were blended fertilisers combined with occasional applications of CAN, when the quantity of N and K used was similar, and with sulphate of potash (SOP), when the use of K was prevalent during the season. Even though the variation in fertiliser use in the orchards monitored, one common practice is the use of blended fertilisers generally with the nutrient partitioning 18-7-22-12-2.5 (N-P-K-S-Ca) or similar. Another common practice is the use of soil amendments with high Ca content as lime and gypsum (data not shown).

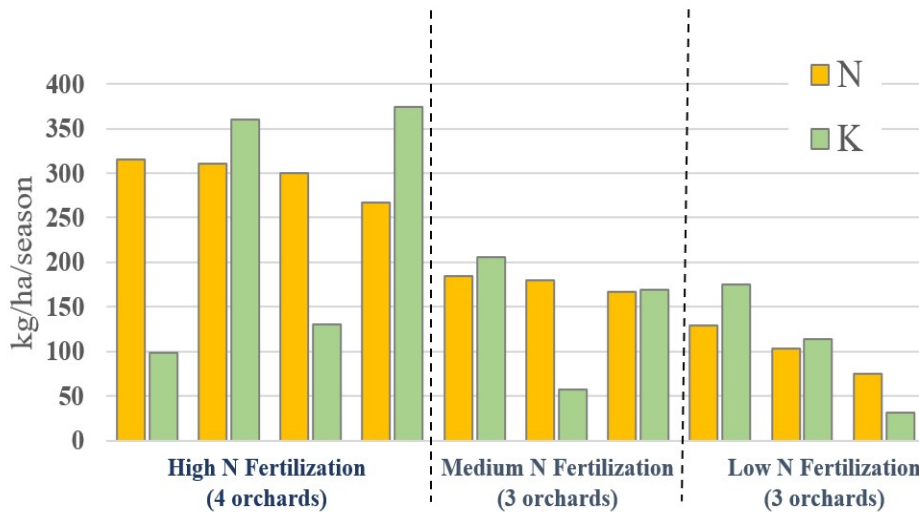


Figure 1- Fertilisation by season in 10 high performance orchards of Bay of Plenty (Kg/Ha/Season). (Average three seasons: 2020-2021, 2019-2020, 2018-2019)

Soil mineral status according to the fertilization level

The soil pH, Ca, K and Mg levels for ten trees in the ten orchards monitored pooled according to their N fertiliser use are shown in the Figure 2. Besides, the industry recommended guidelines for each parameter in high-performance avocado orchard was added for comparison purposes (Figure 2, blue line) (NZ Avocado growers association, 2000). The level of Ca and Mg at topsoil for all orchards were higher than the industry recommendation, and for some orchards, especially for those using high N fertilisers, the Ca and Mg levels were up to the recommended values. In the case of Ca (data not shown), the average Ca saturation was 62% going up to 85% in some cases. For K, the average levels at topsoil were almost in the recommended values specially for orchards in the

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Medium and low category of N fertilization. The pH levels were below the recommended guideline and close to 6.0. Overall, these soils showed a trend to be saturated with Ca, especially considering that the guideline used in this poster for comparison is the highest among the reference values used by consultants in New Zealand (West, 2020). However, some pH adjustments could be recommended, adding more Ca to the saturated soils under study. The effect of this soil-Ca saturation and their relationship with the Ca status in fruits are being assessed on these orchards.

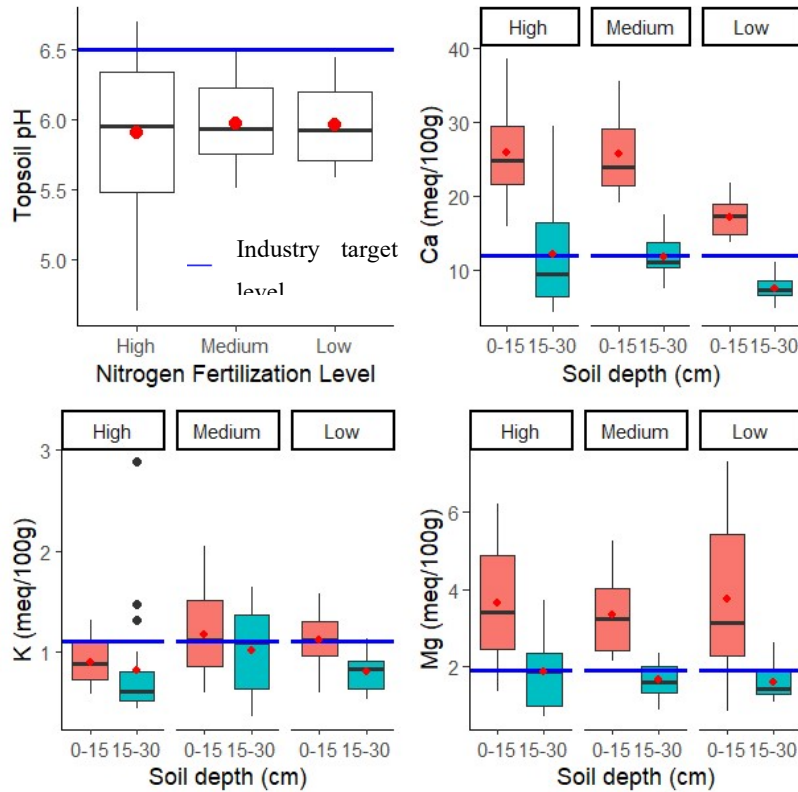


Figure 2- Soil mineral status in ten high-performance orchards according to the N fertilisation by season

Conclusions

The orchards monitored in this study constitutes a baseline to assess the influence of different fertilisation practices on fruit calcium status in avocados produced in high-performance orchards of Bay of Plenty. A wide range of variability was found in terms of quantities applied by season, as well as fertilisers used, having the composed blends as common fertiliser used in different combinations mainly with CAN and SOP.

A common characteristic for the soil Ca status in the orchards monitored, independently of their fertilisation level, is the trend to be saturated in Ca and with high levels of Mg and K. Besides, the pH in all orchards trend to be around 6.0, suggesting some further adjustments using lime or other Ca sources could be proposed by fertilisers consultants. The influence of the soil-Ca saturation status is currently being assessed.

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Appendices

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Acknowledgments

This work is being developed thanks to the support of Massey University Doctoral Scholarship, Ballance Agri-Nutrients, AVOCO, and the avocado growers in Bay of Plenty. Especial thanks to NZ Avocado industry Limited.